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EFFECTS OF SELECTED MULTIPURPOSE, MEDICINAL AND AROMATIC PLANTS ON INVITRO METHANE PRODUCTION AND MICROBIAL DIVERSITY

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Abstract

The study was carried out to evaluate the effects of selected multipurpose, medicinal and aromatic plants on the in vitro methane production and microbial diversity. The plants include multi-purpose trees; *Pterocarpus santalinoides*, *Leucaena leucocephala*, *Albizia lebbek*, *Albizia saman*, *Enterolobium cyclocarpum*, *Gliricidia sepium*, *Milletia griffoniana*, *Ficus thonningii*; Aromatic plants: *Ocimum basilicum*, *Vernonia amygdalina*, *Aspilia africana* and Medicinal plants: *Moringa oleifera*, *Cymbopogon citratus* and *Alternanthera repens*. Leaf samples were collected for determination of chemical analysis, in vitro gas production and microbial analyses. Data on chemical composition were subjected to one-way analysis of variance, while data on in vitro microbial analyses was subjected to phylogeny analysis using the parsimony software. Methanogen-specific primers Met86F and Met1340R were designed to identify methanogens. There were significant differences ($P < 0.05$) in dry matter (DM), crude protein (CP), crude fibre (CF) ether extract (EE), ash contents, neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin contents of plants. The DM values ($P < 0.05$) ranged from 168 g/kg in *V. amygdalina* to 395 g/kg in *P. santalinoides* and the lowest ($P < 0.05$) CP value of 23.6 g/kg was obtained in *O. basilicum*. Least ($P < 0.05$) methane production was obtained from *A. repens* and *P. santalinoides* with 4.45% and 12.62% respectively. Polymerase chain reaction analyses of DNA extracts revealed that *Methanobrevibacter* spp. were dominant. Other detected methanogens include *Methanobacteriales archaeon* spp. and *Methanoplasmatales* spp. Eight samples of the methanogenic archaea were at least 78 to 99% similar to *Methanobrevibacter* spp. Three samples were at least 83 to 97% similar to *Bacterioidetes bacterium* clone. Two samples were at least 85 to 98% similar to *Methanobacteriales archaeon* spp. *Moringa oleifera* sample revealed 99% similarity with 16S rDNA in *Methanobrevibacter* spp. It is concluded that *Methanobrevibacter* spp. is the predominant methanogen and *A. repens* suppressed methanogenesis.

Keywords: Multipurpose, medicinal, aromatic plants, in vitro, methanogens, phylogeny

EFFETS DE CERTAINES PLANTES POLYVALENTES, MEDICINALES ET AROMATIQUES SUR LA PRODUCTION IN VITRO DE METHANE ET LA DIVERSITE MICROBIENNE

Résumé

La présente étude a été réalisée dans le but d'évaluer les effets de certaines plantes polyvalentes, médicinales et aromatiques sur la production in vitro de méthane et la diversité microbienne. Les plantes comprenaient des arbres à usages multiples, dont *Pterocarpus santalinoides*, *Leucaena leucocephala*, *Albizia lebbek*, *Albizia saman*, *Enterolobium cyclocarpum*, *Gliricidia sepium*, *Milletia griffoniana*, *Ficus thonningii*; des plantes aromatiques, notamment *Ocimum basilicum*, *Vernonia amygdalina*, *Aspilia africana* et des plantes médicinales - *Moringa oleifera*, *Cymbopogon citratus* et *Alternanthera repens*. Des échantillons de feuilles ont été recueillis pour la détermination de l'analyse chimique, la production de gaz in vitro et les analyses microbiennes. Les données sur la composition chimique ont été soumises à une analyse de variance à un facteur, tandis que les données sur les analyses microbiennes in vitro ont été soumises à une analyse phylogénique à l'aide un logiciel basé sur le principe de la parcimonie. Les amorces spécifiques au méthanogène Met86F et Met1340R ont été conçues pour identifier les méthanogènes. On a relevé des différences significatives (P

<0,05) au niveau de la matière sèche (DM : Dry Matter), de la protéine brute (CP : Crude Protein), de la cellulose brute (CF : crude fibre), de l'extrait à l'éther (EE), de la teneur en cendres, de la fibre de détergent neutre (NDF : neutral detergent fibre), de la fibre au détergent acide (ADF : acid detergent fiber) et de la teneur en lignine des plantes. Les valeurs DM (P <0,05) variaient de 168 g / kg pour *V. amygdalina* à 395 g / kg pour *P. santalinoides*, et la plus faible valeur (P <0,05) CP - soit 23,6 g / kg - a été notée pour *O. basillicum*. La plus faible (P <0,05) production de méthane a été observée pour *A. Repens* et *P. santalinoides*, respectivement avec 4,45% et 12,62%. Des analyses de réaction en chaîne par polymérase d'extraits d'ADN ont révélé que le *Methanobrevibacter* spp était dominant. D'autres méthanogènes détectés comprenaient *Methanobacteriales archaeon* spp et *Methanoplasmatales* spp. Huit échantillons d'archaea méthanogènes étaient au moins à 78 - 99% similaires aux *Methanobrevibacter* spp. Trois échantillons étaient au moins à 83 - 97% similaires au clone de la *Bacterioidetes bacterium*. Deux échantillons étaient au moins à 85 - 98% similaires au *Methanobacteriales archaeon* spp. L'échantillon de *Moringa oleifera* a révélé une similarité de 99% avec 16S rDNA chez le *Methanobrevibacter* spp. Il a été conclu que le *Methanobrevibacter* spp est le méthanogène prédominant, et *A. repens* a supprimé la méthanogénèse.

Mots-clés : plantes polyvalentes, médicinales, aromatiques, in vitro, méthanogènes, phylogénie

Introduction

Ruminant animals suffer loss of approximately 2-12% of ingested feed-derived energy, depending upon the diet (Johnson and Ward, 1996). Animal agriculture generates greenhouse gas emissions as methane (CH₄) from enteric fermentation and manure, nitrous oxide (N₂O) from the widespread use of nitrogenous fertilizers and animal manure, and carbon dioxide (CO) from the fossil fuels for energy usage plus land use change. Methane, however, is not only an environmental hazard but is also associated with a loss of carbon from the rumen and therefore a waste of energy. Conversion of feed material into methane in the rumen involves the integrated activities of several different microbial species, the final step being carried out by methanogenic archaea. Therefore, efficiency of energy utilization by ruminants is affected, more also this energy loss contributes significantly to environmental pollution in form of methane emitted by the animal. Several approaches had been deployed into mitigating methane production: either a direct effect on the methanogens or an indirect effect caused by the impact of the strategy on substrate availability for methanogenesis, usually through an effect on the other microbes of the rumen.

There has been increasing interest in the use of plants and plant extracts to mitigate

enteric ruminal methane emissions (Martin *et al.*, 2010). For example, the use of browse plants containing secondary compounds as feed supplement for ruminants in many parts of the tropics is increasing in order to improve animal performance (Abdurazak *et al.*, 2000). Plant secondary metabolites such as tannins are particularly attractive as rumen modifiers as these compounds are natural products, which are generally accepted to be environmental friendly and safe in food production systems. Tannins and phenolic monomers have been found to be toxic for some of the rumen microbes, especially ciliate protozoa, fiber degrading bacteria and methanogenic archaea, and as a result methanogenesis in the rumen can also be reduced (Carlos and Edgar, 2010). Recent studies have shown that many plant secondary metabolites (PSM) have potential to modify rumen fermentation favourably, at relatively low concentrations. At appropriate doses, saponins, or saponin containing plants, have suppressed protozoal populations, increased bacterial and fungal populations, propionate production, microbial yield and efficiency of microbial protein synthesis (EMPS), and decreased methanogenesis to improve growth in ruminants (Hristov *et al.*, 1999, Hess *et al.*, 2003, Bhatta *et al.* 2012, 2013). Tropical plants containing tannins/ saponins have been shown to suppress or eliminate protozoa from the rumen and reduce

methane and ammonia production (Patra and Saxena 2010; Bodas et al. 2012). This study demonstrates that plant phytochemicals can have important effects on rumen methanogens, either by affecting methanogens directly and/or indirectly by affecting rumen protozoa. However, effectiveness of plants having high content of saponins, flavonoids and tannins varied depending upon the source, type and level of secondary metabolite present in it. Therefore, in the present study, the plants were selected on the basis of the presence of secondary metabolites. The objective of this study is to evaluate the effects of some browse, medicinal and aromatic plants that are available in southwestern Nigeria, possessing secondary compounds on in vitro methanogenesis and rumen methanogen diversity.

Materials and Method

Experimental plants

The selection of these plants was based on earlier reports. The selected forages for this study are; *Pterocarpus santalinoides*, *Leucaena leucocephala*, *Albizia lebbek*, *Albizia saman*, *Enterolobium cyclocarpum*, *Gliricidia sepium*, *Milletia griffoniana* and *Ficus thonningii*, *Ocimum basilicum*, *Aspilia Africana*, *Vernonia amygdalina*, *Moringa oleifera*, *Cymbopogon citratus* and *Alternanthera repens* were collected in and around Federal University of Agriculture, Abeokuta, Ogun State. Harvesting of the foliage were carried out at late wet season

Preparation of the Samples

Harvested leaves of the selected plants were pooled from each individual branch of plant species and sub-samples were taken and oven dried at 65°C to constant weight to determine the dry matter. Dried samples were milled and sieved to 2.0mm particles size, bulked individually and stored in an air tight container till required for chemical analysis and determination of in vitro gas production.

Chemical Analyses

Crude protein (CP), crude fibres (CF), ether extract (EE) and ash contents of the

selected forages were determined according to A.O.A.C (2005). Neutral detergent fibre (NDF), Acid detergent fibre (ADF) and Acid detergent Lignin (ADL) were determined according to Van Soest et al., (1991). Determination of trypsin inhibitor was carried out by procedure of Prokopet and Unlenbruck (2002). Oxalate was determined according to Munro (2000). Alkaloids was analysed according to Harborne (1973). Saponin content was determined according to Obadoni and Ochuko (2001). Tannin content was determined according to Jaffe (2003) and phytate (phytic acid) was analysed according to Russel (1980).

In Vitro Gas Production Procedure

For the in vitro gas production test, rumen liquor was collected after morning feeding using suction tube (Babayemi and Bamikole, 2006) from two Ndama bulls fed mixture of guinea grass and concentrate diets consisting of wheat offal, dried brewers grains and salt. The laboratory handling of rumen fluid was carried out under a continuous flow of CO₂. The rumen liquor strained through a muslin cloth was pooled and used as inoculum. About 200 mg air-equilibrated sample was accurately weighed into a nylon bag (12 x 12 mm, pore size: 45 µm). Each nylon bag was put into a 100 ml glass syringe fitted with a plunger and incubated with 30 ml buffered rumen inoculum (Menke et al. 1979) consisting of 10 ml of rumen fluid and 20 ml of buffered solution and were placed in a shaking water bath (DS H2-300; Taicang, Jiangsu, China) with 50 movements per minute at 39°C. Leaf samples were incubated in triplicate for each samples and repeated three times. Incubations without leaf sample served as the blanks for each set. Incubations were run for 24 h with recording of gas production at 4, 8, 12 and 24 h. After 24 h of incubation, the volume of fermentation gas produced was recorded. The average of the volume of gas produced from the blanks was deducted from the volume of gas produced by any other sample at that time. After the incubation period, 4ml of NaOH (10M) was introduced into the digesta to estimate the methane production as reported

by Fievez et al. (2005). The average gas volume produced from the blanks was deducted from total gas volume and methane gas produced per sample to determine the net gas and net methane respectively.

Total DNA extraction and PCR amplification

The DNA microbial analysis was carried out at DNA Analysis Facility at the Biotechnology Centre, International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. Incubation liquid was collected and centrifuged at 14,000 x g for 5 min at 40°C. The obtained pellet of approximately 0.2ml were subjected to DNA extraction according to a bead-beating method and followed by phenol-chloroform extraction according to Zoetendal et al. (1998). DNA extracts (three replicates for each sample) were diluted 10 times in ddH₂O prior to polymerase chain reaction (PCR) and 1 µl of the diluted DNA solutions was used as templates. Methanogen 16S rRNA genomic sequences were amplified from purified extracted microbial DNA by polymerase chain reaction using the methanogen-specific primers Met 86f (GCTCAGTAACACGTGG) and Met 1340r (CGGTGTGTGCAAGGAG) (Wright and Pimm, 2003). Primer pair Met 86f and Met 1340r was designed from the conserved region of the 16S rRNA genes from 82 methanogens and can amplify 26 diverse strains of methanogens (Wright and Pimm, 2003). Therefore, these primers were used in this experiment. Polymerase chain reactions were performed with Taq polymerase on a C1000 Thermal Cycler under the following conditions: hot start (4 min, 95°C), followed by 35 cycles of denaturation (30s, 95°C), annealing (30s, 58°C) and extension (2 min, 72°C), and ending with a final extension period (10 min, 72°C).

Sequencing analysis

PCR-amplified products from each extracted DNA samples were kept in tubes placed in a thermal cycler screened by colony-PCR with the M13 Forward and M13 Reverse primers. Sequencing was performed with Big Dye Terminator v 3.1 Cycle sequencing kit under

the following condition; initial denaturation (Rapid thermal ramp to 96 °C, 1 min), followed by 25 cycles (10s, 96 °C), annealing (5s, 50°C) and extension (4min, 60 °C). PCR-amplified products from each extracted DNA sample were screened by colony-PCR with the M13 Forward and M13 Reverse primers. PCR products from positive bacterial clones were used directly as templates for Sanger DNA sequencing with the new forward and reverse primers Met643F (5'-GGA CCA CCW RTG GCGAAG GC-3') and Met834R (5'-CTT GCG RCC GTA CTT CCC AGG-3').

Phylogenetic Analysis of Methanogenic Archaea

The 16S rRNA sequences from this study were used to query GenBank. 16S rRNA gene sequences from GenBank were included in the analysis to place these sequences within a phylogeny of representative methanogenic archaea. The alignment was generated with ClustalW (Thompson, et al., 1994). The neighbour joining tree was constructed using Phylogenetic Analysis Using Parsimony and Other Methods software (PAUP_ 4.0b) (Swofford, 2002) employing a distance matrix calculated with the Jukes-Cantor correction model. The tree was subjected to 1,000 replicates of bootstrapping, and the percentages of replicates supporting a given node.

Statistical Analysis and Model

Regression Analysis for Gas Production
The data obtained from determined net gas and net methane produced were fitted to the non-linear equation model of France et al. (2002):

$$A = b (1 - e^{-c(t-L)})$$

Where:

A = the volume of gas produced at time t,
b = the potential/asymptotic gas production (ml/g DM) from the fermentable fraction of forage,

c = the fractional rate of gas production (/h) from the slowly fermentable feed fraction b, and

L = the discrete lag time prior to gas production.
Analysis of Variance (ANOVA)

Data obtained on chemical analyses and in vitro gas parameters were subjected to one-way analyses of variance while data on in vitro microbial analyses was subjected to phylogeny analysis using the parsimony software.

The model for the study is:

$$Y_{ij} = \mu + T_i + \epsilon_{ij}$$

Where

Y_{ij} = observation from in vitro experiment

μ = population mean

T_i = mean effect of selected browse plants

ϵ_{ij} = Residual error

Results

There were significant differences ($P < 0.05$) in dry matter (DM), crude protein (CP), crude fibre (CF) ether extract (EE), ash contents, neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin contents of selected plants (Table 1). The DM values significantly ($P < 0.05$) ranged from 168 g/kg in *V. amygdalina* to 395 g/kg in *P. santalinoides*. Lowest significant ($P < 0.05$) value of 23.6 g/kg DM for CP was obtained in *O. basillicum* and was highest in *M. oleifera*. NDF values ranged from 420 g/kg in *A. repens* to 550 g/kg in *A. saman*. The highest ($P < 0.05$) gas production was of 32.3 ml/200mg was obtained in *A. lebeck* while *M. griffoniana* recorded lowest ($P < 0.05$) gas volume of 12.7 ml/200mg. Net methane production of 68.3% was significantly ($P < 0.05$) highest in *G. sepium* and was significantly ($P < 0.05$) suppressed to 4.50% by *A. repens*. The concentration of trypsin inhibitor was significantly ($P < 0.05$) highest with value of 16.6 g/kg in *A. repens* and lowest in *V. amygdalina* having a value of 1.0 g/kg. Tannin concentration ranged from 0.30 g/kg in *L. leucocephala* to 2.7 g/kg *M. griffoniana*. Saponin values significantly ($P < 0.05$) ranged from 7.30 in *O. basillicum* to 102 g/kg in *M. griffoniana*. A schematic representation of phylogeny of partial 16S rRNA sequences from incubated rumen liquor is depicted in Fig. 1. Methanogen-specific primers Met86F and Met1340R were designed to identify methanogens in

the in vitro gas supernatant. The polymerase chain reaction analyses of the DNA extracts revealed that methanobrevibacter spp. were dominant. Other detected methanogens include Methanobacteriales archaeon spp. and Methanoplasmatales spp. Amplicons generated from primers targeting the 16S rRNA gene to describe the phylogenetic diversity of rumen methanogen archaea indicated that the introduction of different plants type into the rumen did not alter methanogen populations. Clustering of the methanogen sequences showed grouping into two clades. The clade A had the higher number of methanogen compared to clade B. In clade A, methanogen as a result of *Verononia amygdalina* (medicinal plant) were closely related with that of *Cymbopogon citratus* (Aromatic plant) while in clade B, *Bacterioidetes bacterium* clone as a result of *Enterolobium cyclocarpus* diet were closely related to Methanobacteriales archaeon spp from *Ficus thonningii*. The clade B had a strong bootstrap value (100) which implied good reliability of the members of cluster B.

The results of closely related sequences to denaturing gradient gel electrophoresis (DGGE) bands based on forage plants nucleotide sequence database is shown in Table 2. Eight samples of the Methanogenic archaea were at least 78 to 99% similar to Methanobrevibacter spp. Three samples were at least 83 to 97% similar to *Bacterioidetes bacterium* clone. Two samples were at least 85 to 98% similar to Methanobacteriales archaeon spp. *Moringa oleifera* sample revealed 99% similarity with 16S rDNA in Methanobrevibacter spp. The lengths of the individual lines in phylogenetic tree reflect the amount of sequence change (Table 2).

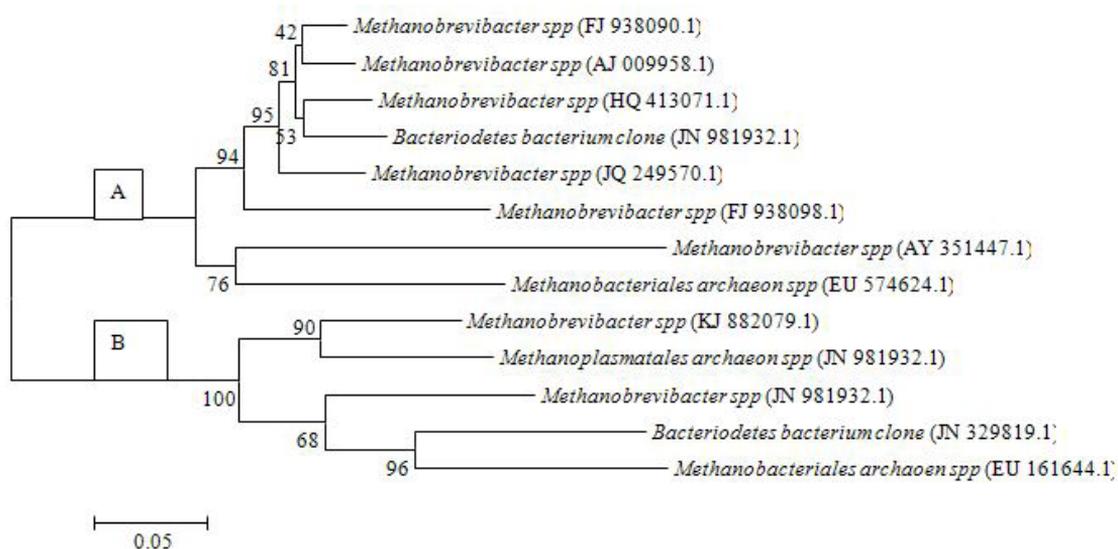
Table 1: Chemical composition (g/kg DM) of leaves of MPTS, aromatic and medicinal plants

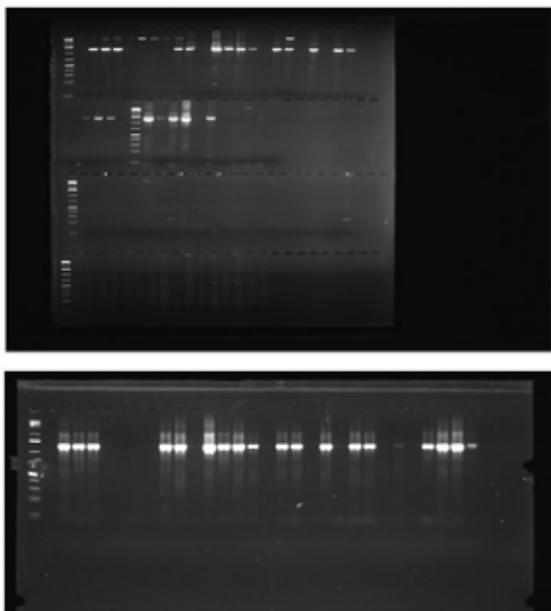
Plant	Dry Matter (g/kg)	Crude Protein (g/kg)	Neutral Detergent Fibre (g/kg)	Gas Production (ml/200mg DM)	Net Methane Production (%)	Trypsin inhibitors (mg/kg)	Saponin (g/100g)	Alkaloids (g/100g)	Tannin (mg/kg)
MPTS									
<i>Pterocarpus santalinoides</i>	395 ^a	151 ^{de}	530 ^a	19.7 ^{abc}	12.6 ^{bc}	5.5 ^{bc}	77.4 ^d	113 ^d	1.2 ^d
<i>Enterolobium cyclocarpum</i>	357 ^b	254 ^{ab}	540 ^a	21.3 ^{abc}	29.1 ^{abc}	1.7 ^{de}	32.6 ^g	78.0 ^j	0.7 ^g
<i>Milletia griffoniana</i>	351 ^b	210 ^{bcd}	510 ^{abc}	12.7 ^c	49.3 ^{ab}	2.7 ^{cde}	102 ^a	64.8 ^k	2.7 ^a
<i>Leuceana leucocephala</i>	200 ^f	189 ^{bcd}	500 ^{abc}	28.3 ^{ab}	31.3 ^{abc}	4.7 ^{bcd}	10.5 ^k	91.3 ^g	0.3 ^m
<i>Albizia lebbeck</i>	272 ^{de}	302 ^a	440 ^d	32.3 ^a	38.4 ^{abc}	7.6 ^b	31.7 ^g	87.1 ^h	1.1 ^e
<i>Ficus thoningii</i>	358 ^b	190 ^{bcd}	500 ^{abc}	20.7 ^{abc}	33.7 ^{abc}	4.1 ^{cde}	76.0 ^e	71.5 ^j	0.4 ^k
<i>Albizia saman</i>	318 ^c	234 ^{abc}	550 ^a	13.7 ^{bc}	30.6 ^{abc}	5.6 ^{bc}	28.8 ^h	122 ^a	1.2 ^c
<i>Gliricidia sepium</i>	261 ^e	210 ^{bcd}	520 ^{ab}	25.3 ^{abc}	68.3 ^a	2.2 ^{de}	87.4 ^c	120 ^b	0.4 ^l
Aromatic Plants									
<i>Ocimum basillicum</i>	167 ^g	23.6 ^{abc}	530 ^a	22.3 ^{abc}	20.4 ^{bc}	2.2 ^{de}	7.3 ^l	59.2 ^l	0.7 ^h
<i>Cymbopogon citratus</i>	291 ^d	127 ^d	530 ^a	16.0 ^{bc}	51.0 ^{ab}	4.7 ^{bcd}	22.2 ^j	117 ^c	0.6 ^j
<i>Aspilia Africana</i>	207 ^f	166 ^{cd}	470 ^{bcd}	16.0 ^{bc}	24.4 ^{bc}	2.0 ^{de}	32.8 ^g	77.8 ⁱ	0.6 ⁱ
Medicinal plants									
<i>Vernonia amygdalina</i>	168 ^g	215 ^{bc}	470 ^{bcd}	22.0 ^{abc}	26.2 ^{bc}	1.0 ^e	89.1 ^b	90.7 ^g	0.6 ^j
<i>Moringa oleifera</i>	227 ^f	306 ^a	460 ^{cd}	24.3 ^{abc}	26.1 ^{bc}	3.5 ^{cde}	18.2 ^j	113 ^e	2.0 ^b
<i>Alternanthera repens</i>	213 ^f	232 ^{abc}	420 ^d	23.3 ^{abc}	4.45 ^c	16.6 ^a	40.2 ^f	97.0 ^f	0.9 ^f
SEM	11.24	9.98	7.12	1.30	3.70	0.63	4.86	3.10	0.10

^{abcdefghi} - Means on the same column having different superscripts are significantly different ($P < 0.05$)

Table 2: Closely related sequences to denaturing gradient gel electrophoresis (DGGE) bands based on forage plants nucleotide sequence database

Forage sample	Methanogens	Identity %	E value	Length	Accession
MPTs					
<i>Pterocarpus santalinoides</i>	Nil				
<i>Enterolobium cyclocarpus</i>	<i>Bacteriodes</i> bacterium clone	97	1e-123	1261	JN 329819.1
<i>Milletia griffoniana</i>	<i>Methanobrevibacter</i> spp,	78	0.0	1263	KJ 882079.1
<i>Leuceana leucocephala</i>	<i>Bacteriodes</i> bacterium clone	83	0.0	1256	JN 981932.1
<i>Albizia lebbeck</i>	<i>Methanobrevibacter</i> spp	96	1e-177	1256	HQ 413071.1
<i>Ficus thonningii</i>	<i>Methanobacteriales</i> archaeon spp	85	0.0	1263	EU 161644.1
<i>Albizia saman</i>	<i>Methanobrevibacter</i> spp	87	3e-164	1263	JQ 249570.1
<i>Gliricidia sepium</i>	<i>Methanobacteriales</i> archaeon spp	98	1e-127	1263	EU 574624.1
Aromatic plants					
<i>Ocimum basilicum</i>	<i>Methanobrevibacter</i> spp	98	0.0	1262	AY 351447.1
<i>Cymbopogon citratus</i>	<i>Methanobrevibacter</i> spp	96	0.0	1263	AJ 009958.1
<i>Aspillia Africana</i>	<i>Bacteriodes</i> bacterium clone	95	0.0	1257	JN 981932.1
Medicinal plants					
<i>Verononia amygdalina</i>	<i>Methanobrevibacter</i> spp	95	0.0	1263	FJ 938090.1

**Figure 1:** Phylogeny of partial 16S rRNA sequences from incubated rumen liquor placed within the context of several methanogenic species within the Archaea. Sequences harvested from GenBank are followed by accession numbers in brackets. Numbers at the nodes are percentages supported by bootstrap evaluation. The scale bar represents the nucleotide substitution rate.



Figures 2 & 3: PCR methanogen ladder of 16S rRNA of selected MPTS, aromatic and medicinal plants

Discussion

The DM content of leaves of *P. santalinoides*, *E. cyclocarpus*, *M. oleifera*, *M. griffoniana*, *A. repens*, *V. amygdalina*, *O. basilicum* and *G. sepium* were within the ranges reported in earlier studies (Fasae *et al.*, 2010; Arigbede *et al.*, 2012). The CP content of the leaves of *P. santalinoides*, *E. cyclocarpus*, *M. oleifera*, *M. griffoniana*, *L. leucocephala*, *G. sepium*, *A. repens* and *A. saman* were comparable to earlier reports (Arigbede *et al.*, 2012; Afolabi *et al.*, 2012). Earlier report (Norton, 2003) suggested that 7 - 9% CP will provide ammonia required by rumen microorganism to support optimum microbial activity, and 10 - 12% for lactation. It seems likely that all forage species evaluated in this study would be good protein supplements or as sole feed provided they were adequately degraded and non-toxic to the rumen microbes and host animal maintenance and lactation. However, the difference in CP content between species can be explained by inherent characteristics of each species related to the ability to extract and accumulate nutrients from soil and/or to fix atmospheric nitrogen,

which is the case for legumes plants (Njidda, 2010).

NDF values recorded in the current study was higher than the values of 362 g/kg reported by Oni *et al.*, (2008) for *E. cyclocarpus* and 286 g/kg reported by Murro *et al.* (2003) for *M. oleifera* but comparable with the values of 565g/kg for *A. Saman* (Korbut *et al.*, 2009), 450g/kg *A. lebbeck* (Lowry *et al.*, 1994), 591g/kg (*G. sepium*) and 660g/kg (*L. leucocephala*) reported by Ly *et al.* (2001). Aynalem and Taye (2008) reported higher value of 582.1g/kg for *V. amygdalina*. The range of NDF contents in our samples was below the range of 600 – 650 g/kg DM suggested as the limit above which intake of tropical feeds by ruminant would be limited (Van Soest *et al.*, 1991).

The in vitro gas volume produced by *E. cyclocarpus*, *L. leucocephala*, *M. oleifera*, *G. sepium*, *P. santalinoides*, *V. amygdalina* and *M. griffoniana* were comparable to those reported in earlier studies (Daodu and Babayemi, 2009; Fasae *et al.*, 2010; Arigbede *et al.*, 2012). Brenda *et al.* (1997) reported a lower value of 30.5 ml/g DM for *G. sepium*. Makkar and Becker (1996) reported a lower value of 49.5ml/mg DM for *Moringa oleifera* leaves. The in vitro gas production revealed that *L. leucocephala*, *M. oleifera*, *A. lebbeck*, *O. basilicum*, *V. amygdalina* and *G. sepium* could be that they contain high amounts of soluble fermentable nutrients apart from high crude protein (Babayemi *et al.*, 2009), as judged by their relatively high gas production (Anele *et al.*, 2009; Arigbede *et al.*, 2012). The in vitro fermentation gas reported by Arigbede *et al.*, (2012) gave lower values of 43ml/g DM (*P. santalinoides*) and 57ml/g DM (*E. cyclocarpus*) and higher values of 44.0 ml/g DM, 45.0ml/g DM, and 65.0ml/g DM for *M. oleifera*, *L. leucocephala* and *M. griffoniana* respectively. *G. sepium* gas was lower than 41.6ml/g DM reported by Anele *et al.*, (2009). *F. thonnigii* gas production corroborate with the findings of Ogunbosoye and Babayemi (2010).

The variation in gas production and potential of gas production between the browse species may be attributed to compositional differences of the browse forages especially CP, fibre and other anti-nutritional components

as reported by (Babayemi *et al.*, 2004). Also high crude protein in feed enhances microbial multiplication in the rumen, which in turn determines the extent of fermentation (Aderinola and Binuomote, 2014).

Highest gas volume production was observed in *L. leucocephala*, whereas the methane gas production was highest in *G. sepium*. This is supported by earlier reports that tannin content of *G. sepium* leaves do not appear to interfere with its protein availability (Norton, 1994) and this could be that drying removes virtually all extractable tannins from the leaf (Ahn, 1990). Sunil (2014), observed the presence of saponin and tannin content in *E. cyclocarpum* could account for antimicrobial and subsequently reason for low gas production, hence low methane production (Goel *et al.*, 2008b). The saponin content of *E. cyclocarpum*, *V. amygdalina*, *G. sepium*, *P. santalinoides*, and *F. thonningii* were considerable higher than other species understudied and this probably account for their methane mitigation activity and this is in line with the findings of El hassan *et al.*, 1995; Bonsi *et al.*, 1995). Cieslak *et al.* (2013) reported that saponins mitigate methanogenesis mainly by reducing the number of protozoa. Bhatta *et al.* (2009) and Goel and Makkar, (2012) revealed that tannin suppresses methanogenesis by reducing the methanogenic populations directly or by reducing the protozoal population, thereby reducing methanogens symbiotically associated with the protozoal population. Newbold *et al.* (1995) and Delgado (2014) revealed that the process of methanogenesis, number of methanogens in the rumen has been directly correlated with the protozoal population. Condense tannin (CT) extracted from *L. leucocephala* caused a linear reduction in total methanogens (up to 99%) and total protozoa (up to 83%) with increasing levels (from 20 to 60 mg/g DM of substrate) of CT in an in vitro study (Tan *et al.*, 2011). However, the reduction in protozoal population was not always proportionally related to the decrease in methanogen population Cieslak *et al.* (2013), probably because some tannins have a direct effect on methanogens, which are not associated with protozoa (Bhatta *et al.*, 2012).

Tannins have an ability to inactivate microbial adhesions, enzymes and cell envelope proteins and may complex with polysaccharides (Ya *et al.*, 1988; Cowan, 1999). Lowest methane production was obtained in *A. repens* probably as a result of impaired protein digestion (Guillamón *et al.*, 2008) due to trypsin inhibitors. The phytochemicals of plant medicines seems to depend on the efficacy of the extraction, the solvent used (Cowan, 1999) amount in plant. Alkaloids have also been reported to intercalate with DNA and thus are potential anti-microbial agents (Raaman, 2006) and this could account for low methane production observed in *P. santalinoides*.

The variation in different groups of Methnogenic archaea due to a change of diet in rumen is difficult to study by way of the conventional methods of isolation and characterisation of culturable bacteria. Hence, variations in the number of rumen bacteria can be studied easily by using different oligonucleotide DNA probes, homologous to some regions of bacterial 16S rRNA. The variation in percent similarity implies divergence in Methnogenic archaea. Also, some lineages have modified the gene sequence substantially more than others, and thus have accumulated longer total branch lengths. Sample from *Ficus thonningii* was most divergent from the other plants species.

Conclusion

The proximate composition shows that the browse plants can contribute useful amounts of protein for livestock diets in the form of leaf meals. The MPTs, aromatic and medicinal plants contain secondary metabolites. Rumen microbial populations were affected by the secondary metabolites present in the MPTs, aromatic and medicinal plants in the in vitro study. Methane gas was produced during incubation of the forage plants, the highest was obtained from *G. sepium* and the lowest from *A. repens*. Methane production was inhibited by the secondary metabolites present in the MPTs, aromatic and medicinal plants in the in vitro study. Methanogens were found present in the incubated rumen liquor except for

the test sample containing *P. santalinoides*. *Methanobrevibacter* spp is the predominant methanogen in incubated rumen liquor in this study.

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THE HISTOLOGICAL ANALYSIS OF THE PROVENTRICULUS AND GIZZARD OF BROILERS FED WHEAT BRAN BASED DIET SUPPLEMENTED WITH NATUZYME AND MAXIGRAIN

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Abstract

This experiment was carried out to evaluate the effect of wheat bran based diet with or without commercial enzyme supplementation on the proventriculus and gizzard of Arbor acres broilers. The design of the experiment was a completely randomized design (CRD) with four treatments: T1- wheat bran based diet with no enzyme, T2- wheat bran based diet with Natuzyme, T3- wheat bran based diet with Maxigrain and T4- wheat bran based diet with Natuzyme and Maxigrain. One hundred and forty-four (144) one- day old unsexed Arbor Acre chicks were used in the trial. Performance record, carcass record and histological analysis were carried out. The dietary treatment had significant difference ($p < 0.05$) on the average feed intake of the birds. The slaughtered weight was higher in birds fed T4. Visceral weight, weight of filled gizzard and weight of empty gizzard were affected. Histological analysis showed inflammation and degeneration of the structure of the proventriculus and gizzard of those birds fed with enzyme supplement.

Keywords: Wheat bran, Enzyme, Proventriculus, Gizzard, Histological Analysis

L'ANALYSE HISTOLOGIQUE DU PROVENTRICULE ET DU GESIER DES POULETS DE CHAIR SOUMISA UN REGIME A BASE DE SON DE BLE COMPLETE AVEC NATUZYME ET MAXIGRAIN

Résumé

Cette expérience a été réalisée dans le but d'évaluer l'effet d'une alimentation à base de son de blé avec ou sans supplémentation en enzyme commerciale sur le proventricule et le gésier des poulets de chair Arbor. L'expérience a utilisé un dispositif complètement aléatoire (CRD : completely randomized design) avec quatre traitements : un régime T1 à base de son de blé sans enzyme ; un régime T2 à base de son de blé avec Natuzyme ; un régime T3 à base de son de blé avec Maxigrain ; et un régime T4 à base de son de blé avec Natuzyme et Maxigrain. Cent quarante-quatre (144) poussins Arbor Acre des deux sexes, âgés d'un jour, ont été utilisés dans cette expérience. Une analyse des données sur la performance et sur les carcasses ainsi qu'une analyse histologique ont été effectuées. Le traitement alimentaire a engendré une différence significative ($p < 0,05$) sur la consommation alimentaire moyenne des oiseaux. Le poids à l'abattage était plus élevé chez les oiseaux soumis au régime T4. Le poids viscéral, le poids du gésier rempli et le poids du gésier vide ont été affectés. L'analyse histologique a montré une inflammation et une dégénérescence de la structure du proventricule et du gésier des oiseaux recevant un supplément d'enzymes.

Mots-clés : son de blé, enzyme, proventricule, gésier, analyse histologique

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Introduction

Most unconventional feed resources which have emerged as potential feed resources are very high in fiber and non – starch polysaccharides. These fibrous and non-starch polysaccharides have placed a constraint on the use of these feed resources because of their high fibre, low energy, ether extract, protein and total digestible nutrient (Okorie *et al.*, 2013). There is need to find ways and means for improvement in the utilization of these fibrous materials so as to incorporate these materials in the poultry feed without any adverse effect on their health and production (Swain, 2008). The digestive system of any animal is important in converting the food the animal eats into the nutrients its body needs for growth, maintenance, and production. An animal's body breaks down food through both mechanical and chemical means. In many animals, mechanical action involves chewing; however, because birds do not have teeth, their bodies use other mechanical action such as grinding of feed particles by the gizzard. Chemical action includes the release of digestive enzymes and fluids from various parts of the digestive system as is the case of the proventriculus. In the gizzard the food is ground and mixed with the gastric juice. The muscular development of the gizzard is influenced primarily by diet. The level of fibre in broiler rations has an effect on the size of the gizzard (Gordon, 2015). This experiment was aimed at investigating the effect of high fibrous wheat bran fed with or without commercial enzyme supplementation on the proventriculus and gizzard of broilers.

Materials and Methods

This research was carried out at the Teaching and Research Farm, Landmark University, Omu-Aran, Kwara State, Nigeria which is located at 80, 8' 0" North, 50 6' 0" East and the ambient temperatures during the period of study were 25.6°C (morning), 31.1°C (afternoon) and 28.7°C (evening) with corresponding relative humidity of 69%, 40% and 51% respectively. The design of the

experiment was a completely randomized design (CRD). One hundred and forty four (144) Arbor Acre one- day old broilers were used for the experiment. The birds were divided into 4 dietary treatments and replicated thrice. 12 birds were allocated into each replicate the birds were fed the experimental diets for 49 days. Fresh water and feed were available to the birds *ad libitum*. The experimental feed was analyzed for proximate composition using A.O. A. C. (1996). The data collected were subjected to one-way analysis using SAS (2000) package and the means were separated using Duncan's multiple range test of the same software at 5% level of significance. The birds were weighed weekly for their weight gain and their feed intake was also recorded the feed conversion ratio was calculated to determine the efficiency of feed utilization which as measure for the performance record. At day 49, 2 birds per replicate were randomly selected and fasted for about 18 hours to empty their gastro intestinal tract, individual live weight were taken after which they were been slaughtered by slitting the jugular vein. Slaughtered weight, de-feathered weight, carcass and visceral weight were taken and recorded. The proventriculus and gizzards were harvested from the birds visceral and preserved in samples plates with 10% formalin. Slide preparation and microscopy were carried out at the Department of Clinical Pathology, University of Ibadan, Nigeria.

Results and Discussion

The effect of enzyme supplementation on the growth performance of broiler chickens is presented in Table 2. Feed intake was statistically similar across the treatments. No significant difference was observed in weight gain and feed conversion ratio across the treatments.

Some authors (Emiola *et al.*, 2007; Robin, 2012; Oyewole *et al.*, 2015; Makinde *et al.*, 2013; Makinde *et al.*, 2014) earlier reported decrease in feed intake with addition of fibrous diet. Fibre affects feed intake and energy utilization of birds. The primary factor in the voluntary feed intake of chicks appears to be the need

Table 1: Experimental Diet Composition

Ingredient	T1	T2	T3	T4
Maize	45.00	45.00	45.00	45.00
Wheat bran	15.00	15.00	15.00	15.00
Soy bean meal	30.00	30.00	30.00	30.00
Fish meal (65%)	5.00	5.00	5.00	5.00
Bone meal	2.25	2.25	2.25	2.25
Lime stone	1.00	1.00	1.00	1.00
Premix	0.50	0.50	0.50	0.50
Salt	0.25	0.25	0.25	0.25
Methionine	0.50	0.50	0.50	0.50
Lysine	0.50	0.50	0.50	0.50
Natuzyme		*	-	-
Maxigrain	-	-	**	-
Natuzyme + Maxigrain	-	-	-	***
Total	100.00	100.00	100.00	100.00
Calculated feed composition				
Crude protein (%)	22.80	22.80	22.80	22.80
Metabolizable energy (kcal/kg)	2602.90	2602.90	2602.90	2602.90
Crude fiber (%)	4.74	4.74	4.74	4.74
Ether extract (%)	3.52	3.52	3.52	3.52
Calcium (%)	1.41	1.41	1.41	1.41
Phosphorus (%)	0.97	0.97	0.97	0.97
Lysine (%)	1.64	1.64	1.64	1.64
Methionine (%)	0.919	0.919	0.919	0.919

*=presence of natuzyme, **=presence of maxigrain, ***=combination of both enzymes (35g Natuzyme and 10g Maxigrain) Premix 0.5% providing per kg Vitamin A 12,000,000IU, Vitamin D3 2,000,000IU, Vitamin E 7000IU, Vitamin B2 4000mg, Nicotinic acid 15,000mg, Calcium d-pentothenate 800mg, Biotin 40mg, Vitamin b12 10mg, Manganese 20,000mg, Iron 50,000mg, Zinc 100,000mg, Copper 10,000mg, Iodine 750mg, Cobalt 3000mg.

for energy. Birds will ordinarily eat to satisfy their energy requirement. As fibre content of diets increases, density of the diets decreases. The inclusion of fibre in feed dilutes energy concentration of diets. Hence, for birds to keep a constant energy level they have to change their feed intake as the energy density of the feed changes. Carcass characteristics of broiler chickens fed diets supplemented with enzymes are shown in Table 3. No significant differences were observed across the treatments except for slaughtered weight of those fed T4 (2.33kg) which was significantly heavier than those fed the control diet (2.13kg). The result obtained from the organ is similar to the findings of

lyayi et al. (2007) who reported no significant difference in the gizzard of broilers fed mucuna bean based diet and Heydar Zarghi et al., (2010) who also reported that there were no significant differences in relative weights of digestive organs of birds fed different dietary treatments. The result obtained in the weight of the organs also correlate with that of Zhu et al., (2014) relative sizes of proventriculus, gizzard and intestine were not affected by dietary metabolizable energy level or enzyme supplementation, but on the contrary it differs from that obtained by Alabi et al. (2014) who reported a significant difference in the weight of the proventriculus and the gizzard of

broilers fed enzyme supplemented rice husk, it also disagree with that of higher gizzard weights of laying hens which could be achieved when feeding 40 % of whole wheat grain to laying birds. This could be as result of the variation in the fibre content of the both feed ingredients used by the previous researchers compared to wheat bran used in this research. The insignificance in the weight is also not in line with that of Tossaporn (2013) who also reported that birds fed diets containing rice hull meal had heavier gizzards than birds fed the control diet.

Histological analysis of the Proventriculus

The result obtained in the histological analysis of the proventriculus is in line with the previous result as obtained by Fatimah *et al.*, (2013) who reported changes in the structure of the proventriculus of African ostriches fed vitamin A furnished feed, as well as the findings of Mostafa *et al.*, (2012) who reported an alteration in the structure of the proventriculus of quails in adaptation to their food habitats. The changes in the structure of the proventriculus of the birds with enzyme supplementation could not be traceable to the fiber content of the feed as treatment one remained normal, this findings is in accordance with the previous result as obtained by Alabi *et al.*, (2014) who reported that since the proventriculus is the main site of endogenous enzyme secretion such as the hydrochloric acid and the pepsin there could be an alteration in the concentration due to the interaction

between the endogenous and the exogenous enzyme resulting into the heterophils noticed in treatment two and the sloughing off of the cells in treatment three, although the visible lesions is moderate.

Histological analysis of the Gizzard

Result shows degeneration and inflammation of the gizzard and this finding is in agreement with that of Alabi *et al.*, (2014) who reported an increase in the gizzard size due to the need for more grinding activities resulting in increased musculature consequent on increased fibre content of the rice husk based diet. It is also in correlation with the past result of Sundu *et al.*, (2008) who reported that the birds fed enzyme supplemented diets had heavier and larger duodenums and larger overall size and weight of their small intestines than those of birds fed enzyme un-supplemented diets. This was possibly due to the fact that the enzymes accelerated the process of digestion in the digestive tract, particularly in the gizzard. The result obtained is in line with the findings of Emiola *et al.* (2007) who reported a distortion in the histological structures of the organs such as the liver, the kidney, the pancreas, the lungs and the heart of broilers fed kidney beans. The changes observed in the proventriculus and the gizzard also align with the findings of Mabelebele *et al.*, (2014) who concluded that, differences existed in the body weight, GIT and pH values of the indigenous Venda and broiler chickens when compared together under normal conditions.

Table 2: Performance result of broiler chickens fed diets supplemented with enzymes

Parameters	T1	T2	T3	T4	SEM
Average feed intake	65.41 ^{ab}	65.81 ^b	67.19 ^{ab}	70.37 ^a	0.84
Total feed intake/kg/bird	5.70	5.54	5.60	5.87	0.06
Total weight gain/kg/bird	2.09	2.09	2.15	2.31	0.04
Feed conversion ratio	2.73	2.65	2.61	2.55	0.03

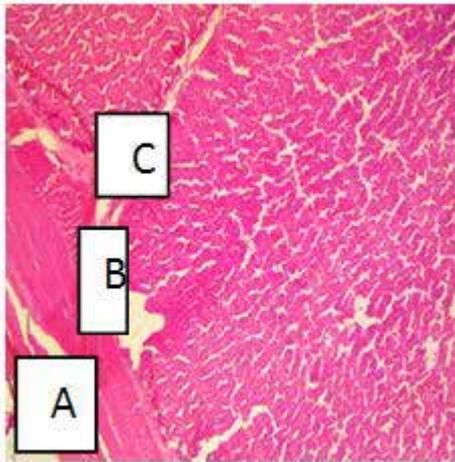
Means within the same row with different superscripts are significantly ($p < 0.05$) different.

Table 3: Carcass characteristics of broiler chickens fed diets supplemented with enzymes

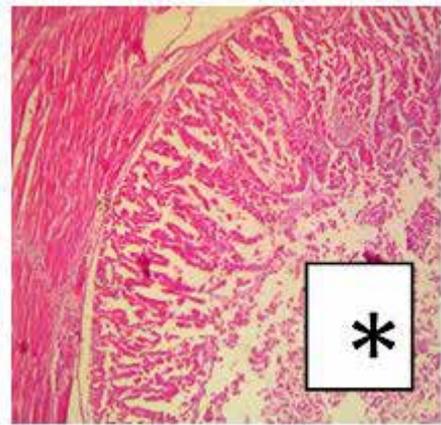
Parameters	T1	T2	T3	T4	SEM
Live_w	2.39	2.3	2.33	2.43	0.02
Sl_w	2.13 ^b	2.21 ^{ab}	2.23 ^{ab}	2.33 ^a	0.04
Df_w	2.07	2.07	2.11	2.24	0.04
Dr_w	1.64	1.59	1.66	1.70	0.02
Vis_w	0.28	0.32	0.30	0.28	0.01
Wfg	65.70	66.12	61.75	61.59	1.06
Weg	46.20	47.28	44.44	43.38	0.76

Means within a row with no common superscript differ significantly ($p < 0.05$). Live_w=live weight, Sl_w=slaughtered weight, Df_w=de-feathered weight, Dr_w=dressed weight, Vis_w=visceral weight, Wfg=weight of filled gizzard, Weg=weight of empty gizzard.

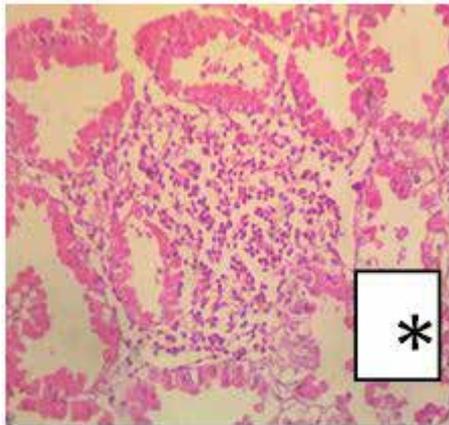
Plates of histological analysis for proventriculus and gizzard



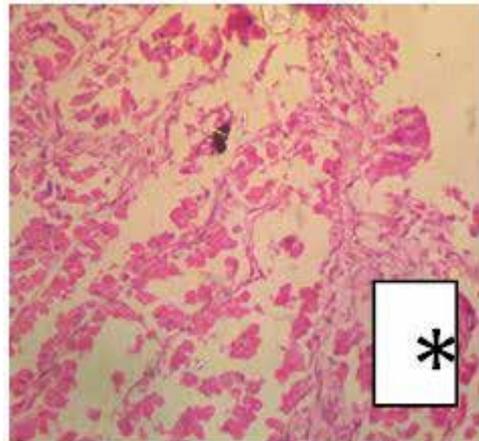
T1: Normal proventricular glands lying on the external muscular layer with no visible lesion.



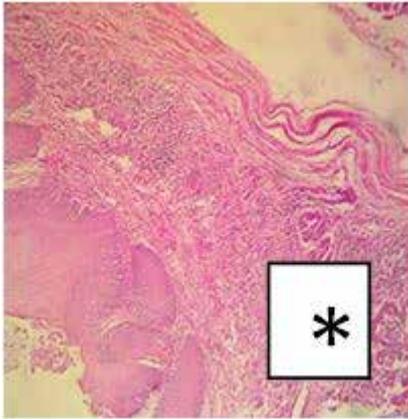
T3: Moderate sloughing off of tips of proventricular tubular glands.



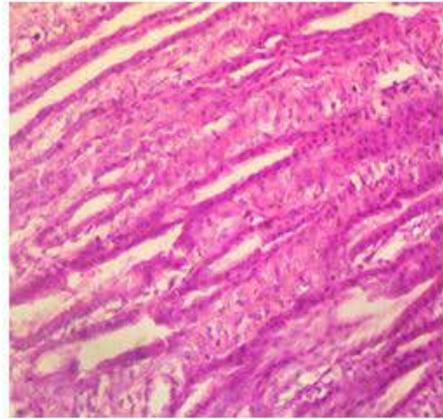
T2: Moderate aggregates of inflammatory cells (heterophils) between the glands



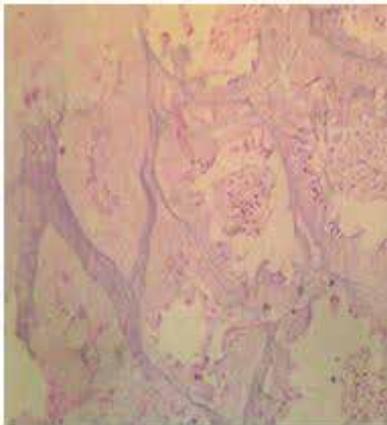
T4: Mild sloughing off/necrosis of proventricular glands



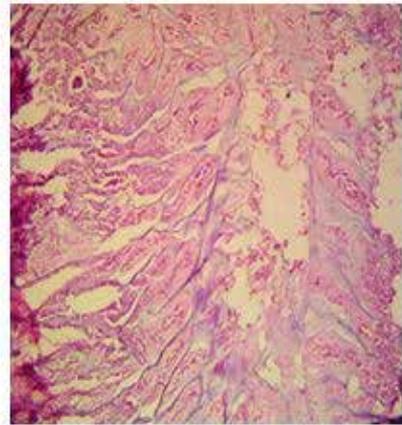
T1: Locally extensive necrosis of subcuticular glands and associated marked aggregates of numerous inflammatory heterophils and *lymphocytes*.



T2: No visible lesion: thick cuticle.



T3: Marked degeneration of sub-cuticular region



T4: Poor section; degenerate sub-cuticular region

Conclusion

The result of this study shows that birds fed enzyme supplemented fibrous feed had lesions both in their proventriculus and their gizzard. The supplementation with enzymes could not hamper or militate against this changes resulting from the rigorous activities of this organs in the digestion process of the feed. From the result obtained from this study, feeding of high level of wheat bran with enzyme supplementation showed distortion and inflammation of the proventriculus and the gizzard of the birds which increases the susceptibility of the birds to infections on the long run.

Recommendation

Based on the findings of this study caution should be taken while feeding bird with wheat bran supplemented with enzyme based feed as this could make the birds susceptible to various infections especially in the organs investigated in this experiment.

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ASSESSMENT OF THE NUTRITIONAL QUALITY OF VARIOUSLY-PROCESSED RUBBER SEED MEALS AS DIETARY INGREDIENTS USING THE LABORATORY RAT AS MODEL FOR PIGS

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Abstract

The study determined the growth performance and physiological parameters of laboratory rats fed variously processed rubber seed meals (RSM). Thirty six growing rats (18 males and 18 females) were randomly allotted into 6 groups and were fed a control diet with no RSM and 5 other diets containing 100 g of the raw RSM and 4 processed rubber seed meals (soaked, SRSM; sundried, SDRSM; boiled, BRSM; and roasted, RoRSM). The feeding of diets incorporating 100 g of the various types of rubber seed meals had no significant ($P > 0.05$) effect on feed intake, body weight gain and feed conversion efficiency, as compared to rubber seed meal-free (control) diet. However, water consumption by rats was significantly ($P < 0.05$) influenced by the dietary treatments. No deaths or health-related problems were recorded during the course of the study. Dietary treatments had significant ($P < 0.05$) impact on relative weights of the liver, heart and lungs but not on the kidney, spleen and intestinal weights. Treatment differences in blood cellular elements and biochemical indices were not significant ($P > 0.05$), except the WBC count, MCV value and blood sugar levels. With the exception of the RoRSM diet, cost per gram feed and feed cost per gram live weight gain were slightly reduced when the rubber seed meals were used, Seasonal variations in the prices of feedstuffs such as maize and soyabean meal would make the use of alternative feedstuffs such as rubber seed meal in animal diets more attractive.

ÉVALUATION DE LA QUALITÉ NUTRITIONNELLE DE TOURTEAUX DE GRAINES DE CAOUTCHOUC TRAITÉS DIFFÉREMMENT EN TANT QU'INGRÉDIENTS ALIMENTAIRES UTILISANT LE RAT DE LABORATOIRE COMME MODÈLE POUR LES PORCS

Résumé

L'étude a déterminé la performance de croissance et les paramètres physiologiques des rats de laboratoire nourris aux tourteaux de graines de caoutchouc (RSM : rubber seed meal) traités différemment. Trente-six rats en croissance (18 mâles et 18 femelles) ont été répartis de manière aléatoire à 6 groupes et ont reçu un régime témoin sans RSM et 5 autres régimes contenant 100 g de RSM brut et 4 repas de tourteaux de graines de caoutchouc traités (trempés – SRSM ; séchés au soleil – SDRSM ; bouillis – BRSM ; et grillés - RoRSM). L'alimentation des rats avec des régimes intégrant 100 g des différents types de tourteaux de caoutchouc n'a eu aucun effet significatif ($P > 0,05$) sur la consommation alimentaire, le gain de poids corporel et l'efficacité de la conversion alimentaire par rapport au régime (témoin) ne contenant pas de tourteaux de graines de caoutchouc. Cependant, la consommation d'eau par les rats a été significativement ($P < 0,05$) influencée par les traitements alimentaires. Aucune mortalité ou problème sanitaire n'a été enregistré au cours de l'étude. Les traitements diététiques ont eu un impact significatif ($P < 0,05$) sur les poids relatifs du foie, du cœur et des poumons, mais pas sur le poids des reins, de la rate et des intestins. Les différences de traitement au niveau des éléments cellulaires du sang et des indices biochimiques n'étaient pas significatives ($P > 0,05$), sauf le nombre de leucocytes, la valeur MCV et les

niveaux de sucre dans le sang. A l'exception du régime RoRSM, le coût par gramme d'alimentation et le coût de l'alimentation par gramme de gain de poids vif ont légèrement baissé lors de l'utilisation des tourteaux de caoutchouc. Les variations saisonnières des prix des aliments tels que le maïs et le tourteau de soja rendrait plus attrayant l'usage d'aliments animaux alternatifs tels que les tourteaux de graines de caoutchouc dans les régimes alimentaires pour animaux.

Introduction

The use of rubber seed meal in animal feeding systems has been limited due to the presence of toxic cyanogenic glycosides (George *et al.*, 2000; Ukpebor *et al.*, 2007; Eka *et al.*, 2010; Daulay *et al.*, 2014; Sharma *et al.*, 2014). In the presence of the enzyme, limarinase or in a slightly acid medium, the cyanogenic glycoside is converted to hydrogen cyanide which is poisonous. Small quantities of hydrogen cyanide do not result in death but may adversely affect the health of the animal (Stosic and Kaykay, 1981). Chronic cyanide toxicity on animals can also affect the growth phase of development of animals (Tewe and Maner, 1981; Tewe, 1983; Tewe and Kasali, 1986).

There are a wide variety of different methods of processing the rubber seeds to reduce their content of cyanogenic glycosides and hence their toxicity. These methods comprise of different combinations of drying, soaking, boiling and fermentation of whole seeds. All of these reduce the total hydrogen cyanide content of the seeds. In a recent study, Farr *et al.*, (2015) evaluated four simple methods of processing (soaking in water, sun-drying, boiling in water, and roasting) in terms of chemical compositions and energy values of the resultant rubber seed meals. The results indicated that the method of processing significantly affected the proximate compositions, the mineral contents, amino acid profiles, metabolizable energy and hydrogen cyanide concentrations of the resultant rubber seed meals.

In most areas where rubber seeds are produced, there is dearth of information on the effects of different types of processed rubber seed meals on different production indices of farm animals. Following on from the study of Farr *et al.*, (2015), this study was conducted to assess the effects of including the variously processed rubber seed meals in

diets on growth performance, physiological parameters and economy of gain using the laboratory as a model for pigs. The high cost associated with pig experimentation, coupled with the long delay in growth response, limits its use in the routine evaluation of feedstuffs. The growing laboratory rat, however, can be housed and reared relatively cheaper, consume only small amounts of food, and lends itself to ease of handling. Various studies (Moughan *et al.*, 1984, 1987; Smith *et al.*, 1990; Donkoh *et al.*, 1993; Donkoh *et al.*, 2012) indicate that overall the growing laboratory rat is a suitable and satisfactory model for the pig for nutritional studies.

Dietary Treatments

A control diet and five treatment diets (produced by adding 10% of one of the raw and 4 processed RSMs (soaked, SRSM; sundried, SDRSM; boiled, BRSM; and roasted, RoRSM) to the control to replace fishmeal and soyabean meal) were formulated (Table 1). The experimental diets were formulated to be isoproteic and isoenergetic. The source of the rubber seeds used in this study and the different processing methods are as described by Farr *et al.*, (2015)

Experimental Animals and Management

Thirty six Sprague-Dawley growing rats (18 males and 18 females) were kept individually in raised stainless steel cages with wire mesh floors, in a room with a 12 h light/dark cycle. The rats were randomly allocated to the six experimental diets such there were 6 rats per diet. The rats were dewormed before the start of the trial. Each rat had access to its respective diet for a 28-day period. Water was available *ad libitum*.

Table 1: Ingredient composition of the experimental diets fed to rats

Ingredients (g kg ⁻¹)	Dietary treatments					
	Control	RRSM	SRSM	SDRSM	BRSM	RoRSM
Maize	550	500	500	500	500	500
Fishmeal	120	120	120	120	120	120
Soyabean meal	160	110	110	110	110	110
RSM	0	100	100	100	100	100
Wheat bran	150	150	150	150	150	150
Vitamin/mineral premix	5	5	5	5	5	5
Salt (NaCl)	5	5	5	5	5	5
Dicalcium phosphate	5	5	5	5	5	5
Oyster shell	5	5	5	5	5	5
Chemical analysis (g kg⁻¹ DM)						
Crude protein	216.8	206.9	208.8	208.9	209.2	209.7
Crude fibre	37.83	47.21	42.12	46.26	42.43	43.63
Ether extract	39.35	53.15	56.65	50.15	54.15	53.65
Calcium	8.75	8.82	8.77	8.81	8.80	8.76
Phosphorus	8.34	8.13	8.16	8.30	8.30	8.27
ME (MJ kg ⁻¹) b	12.78	12.58	12.70	12.58	12.66	12.69

^aVitamin/mineral premix specified to provide the following kg⁻¹ diet: vitamin A 10,000 IU; D 2000 IU; K 3 mg; riboflavin 2.5 mg; niacin 12.5 mg; cobalamin 0.05 mg; pantothenic acid 5 mg; choline 175 mg; folic acid 0.5 mg; zinc 25 mg; iron 0.5 mg; copper 50 mg; cobalt 62.5mg; iodine 0.5 mg; selenium 0.3 mg; chlorine 1.6 g; sodium 1.3 g; magnesium 2mg; sulphur 0.4 g; potassium 3.0 g.

^bCalculated from data of NRC (1998) and the estimated metabolizable energy value of RSM.

Parameters Measured

Growth parameters

Rat growth performance were assessed weekly by measuring feed intake, body weight gain, water consumption, and feed conversion efficiency. The economy of gain was computed as the cost of feed per unit gain after feed cost per kg had been calculated from the market price of the individual ingredients.

Physiological Parameters

At the end of the feeding trial, 2 rats (1 male and 1 female) in each of the six (6) dietary treatment groups were randomly selected for blood collection and organ weight determination. The rats from each treatment were anesthetized by chloroform asphyxiation followed by decapitation and 10 ml of blood was collected into two sample tubes. Blood collection was done in the morning before feeding. The blood samples for the haematological studies were collected in sample bottles with EDTA before being

analyzed. Haematological attributes were estimated in whole blood just after bleeding, using the KX21N Sysmex Haematology Analyzer (Sysmex, Corporation, 2006) for its haemoglobin (Hb), red blood cells (RBC), packed cell volume (PCV), and white blood cells (WBC) contents. The blood samples for serum analysis were allowed to clot before centrifuging to obtain serum. The separated sera were decanted into bijoux bottles and stored at -20°C until analyzed. Blood glucose levels were determined using the One Touch Select Glucometer System of LifeScan Inc., USA. A test strip was fitted into the glucometer and a drop of blood sample was applied at the appropriate test area. The results were read on the screen of the meter after 5 seconds. The other serum metabolites (total protein, albumin, globulins, and cholesterol) were estimated using the Flexor Junior Chemistry Auto-Analyzer (Vital Scientific Dierer, the Netherlands).

For organ weights and histological studies, the abdomen was opened by an incision along the mid-ventral line and the skin and musculature folded back to expose the internal organs. The heart, liver, kidney, spleen, lung and intestines were excised, weighed immediately and expressed as g g-1 liveweight to ensure uniformity in comparison. The heart, liver, kidney, spleen, lung and intestines were examined to determine whether the diets had resulted in gross pathological changes.

Statistical Analysis

Data collected were subjected to Analysis of Variance (ANOVA) using the GenStat Statistical Software to identify significance of main effects. Where significant differences were found among treatments, specific effects

were tested by the least significant difference procedure. All tests for significance were based on the 5% probability.

Results and Discussion

The summary of the growth performance characteristics and organ weights of growing laboratory rats is presented in Table 2. Feed intake by rats for the 4-week period was not significantly ($P > 0.05$) affected by the various dietary treatments. The daily feed intake varied from 10.9 g (RoRSM diet) to 12.83 g (control and BRSM diets). The non-significant effect of the various rubber seed meals inclusion in diets on feed intake suggest that rats will consume containing processed rubber seed meals. A major factor affecting

Table 2: Effect of variously-processed RSM on rat growth performance and organ weights

Parameter	Control	RRSM	SRSM	SDRSM	BRSM	RoRSM	P-value and significance level
Total feed intake, g	359.1 ^a	340.0 ^a	353.7 ^a	332.4 ^a	359.5 ^a	305.3 ^a	0.178NS
Daily feed Intake, g	12.83 ^a	12.14 ^a	12.63 ^a	11.87 ^a	12.84 ^a	10.90 ^a	0.178NS
Initial body weight, g	61.84 ^a	59.17 ^a	65.00 ^a	61.67 ^a	62.50 ^a	62.50 ^a	0.106NS
Final body weight, g	162.0 ^a	152.0 ^a	166.16 ^a	142.0 ^a	166.82 ^a	134.16 ^a	0.120NS
Total weight gain, g	100.2 ^a	92.8 ^a	101.20 ^a	80.30 ^a	104.30 ^a	71.70 ^a	0.164NS
Daily weight gain, g	3.58 ^a	3.32 ^a	3.61 ^a	2.87 ^a	3.73 ^a	2.56 ^a	0.164NS
Feed:gain	3.58 ^a	3.67 ^a	3.50 ^a	4.14 ^a	3.45 ^a	4.26 ^a	0.124NS
Total water intake, ml	454.7 ^a	414.9 ^a	427.32 ^a	340.7 ^b	446.8 ^a	325.0 ^b	0.007*
Daily water intake, ml	16.24 ^a	14.82 ^a	15.26 ^a	12.17 ^b	15.96 ^a	1161 ^b	0.007*
Mortality, %	0	0	0	0	0	0	-
Organ weights, g g-1LBW							
Liver	0.496 ^c	0.506 ^{bc}	0.604 ^a	0.588 ^a	0.567 ^{ab}	0.490 ^c	0.009*
Heart	0.041 ^b	0.061 ^a	0.047 ^a	0.044 ^b	0.040 ^b	0.036 ^b	0.017*
Kidney	0.089 ^a	0.109 ^a	0.093 ^a	0.104 ^a	0.100 ^a	0.093 ^a	0.165NS
Lung	0.079 ^c	0.113 ^{ab}	0.099 ^{abc}	0.123 ^a	0.093 ^{bc}	0.111 ^{ab}	0.030*
Spleen	0.072 ^a	0.087 ^a	0.098 ^a	0.083 ^a	0.100 ^a	0.074 ^a	0.205NS
Intestines	0.506 ^a	0.487 ^a	0.470 ^a	0.534 ^a	0.457 ^a	0.453 ^a	0.194NS
Cost/g feed, GH¢	0.020	0.0185	0.0188	0.0187	0.0189	0.0188	-
Feed cost/g weight gain, GH¢	0.072 ^a	0.0679 ^a	0.0658 ^a	0.0774 ^a	0.0652 ^a	0.0801 ^a	0.016NS

^{abc}Means within a row with the same superscripts are not significantly different NS, non-significant ($P \geq 0.05$); * $P \leq 0.05$

feed consumption in animals is the dietary energy content because animals eat to satisfy their inner metabolic need for energy. In this study, the experimental diets were formulated to be isocaloric.

There was not much difference in average rat weight prior to the conduct of the feeding trial. Final body weight was also unaffected ($P>0.05$) by the dietary treatments. Consequently, there was no influence ($P>0.05$) of the dietary treatments on the mean daily live weight gain. Nevertheless, compared with rats on the BRSM diet and the control and the other rubber seed meal containing diets, the BRSM diet registered slightly faster growth. Similarly, the dietary treatments had no impact ($P>0.05$) on efficiency of feed utilization (feed: gain) of the rats. However, feed:gain ratio was slightly better for the BRSM diet than the control diet and other the rubber seed-containing diets.

There were no indications of ill health and no mortalities were recorded attributable

to the experimental treatments during the conduct of the trial. The structural and size of organs such as the liver, heart and gastrointestinal tract are often indication of the physiological state of the body. In this study, dietary treatments had significant ($P<0.05$) influence on the relative weights of the heart, liver and lung, however, kidney, spleen and empty intestinal weights were unaffected. Rats on the rubber seed meals, which on chemical analysis were found to contain certain amounts of HCN, in general, registered significantly higher liver weights in comparison with the control diet. Palmer and Olson (1979) reported an increase in liver weight in animals exposed to 4 mg cyanide /kg body weight/day. At the termination of the 4-week trial, examination of several organs (heart, liver, kidney, spleen, lung and intestines) from all the rats, however, revealed no macroscopic deviation from the normal in terms of gross tissue changes.

Table 3: Haematological and blood biochemical indices of rats fed diets containing variously-processed RSMs

Parameter	Control	RRSM	SRSM	SDRSM	BRSM	RoRSM	P-value and level of significance
Haemoglobin, g/dl	12.45 ^a	15.00 ^a	14.65 ^a	15.25 ^a	14.20 ^a	14.75 ^a	0.263NS
RBC X 106/ μ l	7.00 ^a	8.34 ^a	8.22 ^a	8.53 ^a	8.10 ^a	8.22 ^a	0.481NS
WBC X 103/ μ l	14.50	11.40	7.95	11.30	6.30	14.20	0.001***
Haematocrit, %	39.30 ^a	47.00 ^a	46.30 ^a	46.50 ^a	43.30 ^a	45.20 ^a	0.482NS
MCHC, g/dl	31.85 ^a	32.00 ^a	31.65 ^a	32.05 ^a	32.85 ^a	32.65 ^a	0.588NS
MCH, pg	17.95 ^a	18.00 ^a	17.85 ^a	17.90 ^a	17.60 ^a	18.00 ^a	0.977NS
MCV, fL	56.30	56.35	53.85	56.00	53.50	55.05	0.001*
Lymphocytes, X 103/ μ l	9.25 ^{ab}	9.15 ^{ab}	6.35 ^b	13.00 ^a	5.20 ^b	11.90 ^a	0.009*
Neutrophils, X 103/ μ l	1.05 ^a	0.45 ^a	0.30 ^a	0.90 ^a	0.20 ^a	0.30 ^a	0.075NS
PLT, X 103/ μ l	422.0 ^a	713.0 ^a	890.0 ^a	654.0 ^a	934.0 ^a	802.0 ^a	0.055NS
Blood sugar, mmol/l	4.50 ^a	3.35 ^{bc}	2.85 ^c	2.80 ^c	3.60 ^b	3.30 ^{bc}	0.001*
Globulins, g/l	46.0 ^a	43.0 ^a	46.50 ^a	49.0 ^a	47.0 ^a	56.0 ^a	1.130NS
Albumin, g/l	36.05 ^a	40.65 ^a	38.50 ^a	39.50 ^a	41.30 ^a	41.80 ^a	0.585NS
Total protein, g/l	82.15 ^a	83.35 ^a	84.80 ^a	88.35 ^a	88.00 ^a	97.85 ^a	0.935NS
Total bilirubin, μ mol/l	2.50 ^a	2.00 ^a	2.50 ^a	3.00 ^a	3.50 ^a	5.00 ^a	0.637NS
Cholesterol, mmol/l	1.25 ^a	1.50 ^a	1.40 ^a	1.70 ^a	1.55 ^a	2.05 ^a	2.219NS

Hb- haemoglobin; RBC- Red blood cells; WBC- white blood cells; HCT- haematocrit; MCHC- mean cell haemoglobin concentration; MCH- mean cell haemoglobin ; MCV- mean cell volume; PLT- platelet

^{abc}Means within a row with different superscripts are significantly different. NS, non-significant ($P\geq 0.05$); * $P\leq 0.05$; ** $P\leq 0.01$; *** $P\leq 0.001$

The results of the haematological and blood biochemical indices of rats fed diets which contained the raw and the variously-processed rubber seed meals are shown in Table 3.

Haematology and blood biochemistry are routinely used to evaluate the health status of animal (Mafuvadze and Erlwager, 2007). The degree to which blood constituents changes occur in apparently healthy animals indicate to what extent they can make physiological adjustments to stresses due to pathological, environmental, hormonal and nutritional factors, as well as the actions of drugs and toxic substances. Nutrition, particularly dietary protein intake, reportedly affects the liveweight and haematological indices of animals (Makinde, 1991). The effects of diets on haematological parameters have also been reported by other researchers (Ologhobo *et al.*, 1993; Otesile *et al.*, 1991). In the present study, the dietary treatment had no influence ($P>0.05$) on haematological and blood biochemical indices of rats, with the exception of the white blood cell count, MCV and lymphocyte values as well as the blood sugar levels.

The serum total protein, albumin and globulins of the rats in this study were not affected by the different types of rubber seed meals inclusion in the diets. This is an indication that the protein levels of the experimental diets were able to support the protein reserves of the rats. The insignificant differences in the total protein and albumin values between the control group and rubber seed meal fed groups suggests that there was not much depression in hepatic synthesis and or degradation of protein (Akanya *et al.*, 2015). In general, rats on the rubber seed meal diets, registered lower ($P<0.05$) blood sugar levels in comparison with those on the control diet devoid of rubber seed meal. This observation may be attributed to the high fibre contents of the rubber seed meal diets and supported by the assertion that high fibre diets have been associated with the reduction in blood sugar contents of animals (Dodson *et al.*, 1981).

The absence of variations in the serum metabolites, but for blood sugar levels, could

also be attributed to the comparable protein and feed intakes among the rats on the various diets. This is in support of the study conducted by Adesehinwa *et al.*, (2008). Jain (1986) reported that protein deficiency reduces most haematological and serum biochemical indices through reduction or impairment of the synthesis of blood cells which are proteinaceous in nature. The levels of haematological and blood biochemical indices observed in this study were within the normal ranges reported by Research Animal Resources (2015).

The cost per g of feed and the feed cost per g weight gain are presented in Table 2. Although the rubber seeds were obtained free of charge, the variously-processed rubber seed meals were assigned values of GH¢1.00; GH¢1.15; GH¢1.25; and GH¢1.10 for SDRSM, SRSM, BRSSM and RoRSM, respectively being the cost of picking the seeds, transportation and processing. The cost per kg maize and soyabean meal, which the variously-processed rubber seed meals replaced in the experimental diets were GH¢1.04 and GH¢3.00, respectively. Consequently, the cost per g of the control diet was higher (GH¢ 0.020) than all the various rubber seed meal-containing diets. This was due solely to the price disparities between soyabean meal and the different types of the rubber seed meals. Feed cost per gram live weight was lowest for rats on the BRSM-containing diet and highest for the RoRSM diet. The lowest cost of feed conversion registered by rats on the BRSM diet may be attributed to the slightly better efficiency of feed utilization and vice versa for rats on the RoRSM diet.

Based on the results of this study, processed rubber seed meal could be harnessed as a supplement for formulating animal or poultry feed. Seasonal variations in the prices of feedstuffs such as maize and soyabean meal would make the use of alternative feedstuffs such as rubber seed meal in animal diets more attractive.

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MULTIDRUG RESISTANT ENTEROHAEMORRHAGIC ESCHERICHIA COLI O157:H7 IN PIGEONS IN IBADAN, NIGERIA

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Abstract

Pigeons are commonly seen around human dwellings and in city centres. The movement of these birds from place to place makes them a veritable vehicle for environmental dissemination of pathogens. *Enterohaemorrhagic E. coli* (EHEC) O157:H7 can cause severe and sometimes fatal gastroenteritis in humans. This study investigated the occurrence and antimicrobial susceptibility of EHEC O157:H7 in the faeces. One hundred and twenty five cloacae samples (82 adult pigeons and 43 squabs) were collected from three different locations in Ibadan metropolis. *Enterohaemorrhagic E. coli* O157:H7 was isolated from samples on BCIG-SMAC following a selective pre-enrichment culture in modified Tryptic Soy Broth supplemented with novobiocin. Suspected non-sorbitol fermenting *E. coli* isolates were serologically identified as serotype O157:H7 using latex agglutination method. *Enterohaemorrhagic E. coli* isolates were tested for susceptibility to antimicrobial agents by the Kirby Bauer disk diffusion method. Out of all 125 samples examined, 23 (18.4%) were confirmed as EHEC O157:H7 serotype. Isolates showed resistance to nitrofurantoin (100%), ceftriazone (100%), amoxicillin (91.3%), augmentin (73.9%), gentamycin (60.9%), cotrimoxazole (60.9%), pefloxacin (47.8%), tetracycline (34.8%), ciprofloxacin (30.4%) and ofloxacin (17.4%). This study showed that pigeons harbour multidrug resistant EHEC O157:H7 and may contribute to environmental contamination through faecal shedding.

Keywords: EHEC O157:H7, Environmental contamination, Faeces, Multidrug resistance, Pigeon,

RESISTANCE DE L'ESCHERICHIA COLI ENTEROHEMORRHAGIQUE O157:H7 A PLUSIEURS MEDICAMENTS CHEZ LES PIGEONS A IBADAN (NIGERIA)

Résumé

Les pigeons sont communément vus autour des habitations humaines et dans les centres urbains. Le déplacement de ces oiseaux d'un endroit à un autre en fait de véritables facteurs de dissémination des agents pathogènes dans l'environnement. L'*E. coli* entérohémorragique (EHEC) O157:H7 peut causer une gastro-entérite grave et parfois mortelle chez les humains. La présente étude a examiné la présence et la sensibilité antimicrobienne de l'EHEC O157:H7 dans les matières fécales. Cent vingt-cinq échantillons cloacaux (82 pigeons adultes et 43 pigeonneaux) ont été collectés dans trois endroits différents dans la métropole d'Ibadan. L'*E. coli* entérohémorragique O157:H7 a été isolé dans des échantillons sur BCIG-SMAC à la suite d'une culture de pré-enrichissement sélective dans du bouillon trypticase soja (Tryptic Soy Broth) modifié additionné de novobiocine. Des isolats suspects d'*E. Coli* ne fermentant pas le sorbitol ont été identifiés sérologiquement comme sérotype O157:H7 en utilisant une méthode d'agglutination au latex. Des isolats d'*E. Coli* entérohémorragiques ont été testés pour détecter la sensibilité aux agents antimicrobiens par la méthode de diffusion sur disque de Kirby Bauer. Des 125 échantillons examinés, 23 (18,4%) ont été confirmés comme étant du sérotype EHEC O157:H7. Les isolats ont montré une résistance au nitrofurantoin (100%), à la ceftriazone (100%), à l'amoxicilline (91,3%), à l'augmentine (73,9%),

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à la gentamycine (60,9%), au cotrimozazole (60,9%), à la péfloxacine (47,8%), à la ciprofloxacine (30,4%) et à l'ofloxacine (17,4%). Cette étude a montré que les pigeons hébergent des EHEC O157:H7 résistant à plusieurs médicaments et qu'ils peuvent contribuer à la contamination de l'environnement par l'excrétion de matières fécales.

Mots-clés : EHEC O157:H7, contamination de l'environnement, matières fécales, résistance à plusieurs médicaments, pigeon,

Introduction

The presence of pigeon faeces in urban environments may contribute to the spread of infectious agents as they could harbour various microorganisms (Tanaka *et al.*, 2005). Thus, pigeons may play significant role in the dissemination and transmission of zoonotic pathogens including pathogenic *Escherichia coli*. Pathogenic *E. coli* strains are known to cause gastrointestinal and extraintestinal illnesses in humans and animals (Aranda *et al.*, 2004). In particular, *enterohaemorrhagic E. coli* (EHEC) strains are known to cause fatal gastroenteritis characterized with crampy abdominal pain, bloody diarrhoea and sometimes kidney failure in paediatric and geriatric patients (Nguyen and Sperandio, 2012). *Escherichia coli* serotype O157:H7 is the most commonly encountered serotype in sporadic cases and outbreaks of EHEC infection haemorrhagic colitis in humans (Karmali *et al.*, 2010). Human infections with EHEC O157:H7 are often zoonotic following direct or indirect contact with carrier animals or consumption of contaminated foods (Karmali *et al.*, 2010).

The increasing reports of antimicrobial resistance in pathogenic and commensal bacteria are a disturbing phenomenon of global dimension (CDDEP, 2015). Antimicrobial substances have been used for both treatment and prevention of bacterial diseases for decades. Nowadays, it appears the world is nearing the end of the antimicrobial age with the increasing rates of emergence of resistance (CDDEP, 2015). While antimicrobial usage has been identified as a major contributor to the development of antimicrobial resistance, there are reports supporting the presence of resistant bacteria in animals without prior exposure to antimicrobial (Zhang *et al.*, 2011).

Escherichia coli is often used as an indicator of the occurrence of antimicrobial resistance in both human and animal populations (Navarro-Gonzalez *et al.*, 2013).

Pigeons (*Columba palumbus*) could be potential reservoirs of resistant bacteria (Kimpe *et al.*, 2002). These birds are commonly around buildings and in public places in villages and in large cities. Various buildings provide suitable places for their nesting. The possibility of pigeons as sources of dissemination of potentially pathogenic and antimicrobial resistant bacteria transmissible to humans due to close proximity calls for attention (Cano-Terriza *et al.*, 2015). The present study evaluates the potential roles of pigeons as reservoirs and disseminator of antimicrobial resistant and pathogenic *E. coli* strains. It described the occurrence of *E. coli* including EHEC O157:H7 strains in fresh faeces from pigeons and squabs in Ibadan, Nigeria; as well as the antimicrobial drug susceptibility of the bacterial isolates.

Materials and Methods

Sampling

A total of 125 cloacae samples (82 adult pigeons and 43 squabs) were collected from three locations (Bode, Sasa and Molete) where pigeons were commonly seen in Ibadan, Nigeria. Samples were properly labelled, preserved in ice-pack and transported to the laboratory for immediate microbiological analysis.

Isolation and Identification of *E. coli*

Each cloacal sample was inoculated into nine milliliters of sterile tryptic soy broth (TSB) (Oxoid, Basingstoke, UK) in universal bottles. The broth cultures were incubated at 37 °C for 18 to 24h. After incubation, a loopful of the TSB

culture was inoculated onto MacConkey agar (Oxoid, Basingstoke, UK). Isolation of EHEC O157:H7 was as previously described (Ojo et al., 2014). Briefly, 1ml of the pre-enrichment culture was inoculated into 9 ml of modified Tryptone Soya Broth (mTSB) (CM0989, Oxoid®, Basingstoke, UK) supplemented with vancomycin (8 µg/mL), cefsulodin (10 µg/mL) and cefixime (0.05 µg/mL) (VCC selective supplement SR0190, Oxoid®, Basingstoke, UK). After 18h of incubation at 37OC, a loopful of the selective enrichment culture was transferred onto sorbitol MacConkey agar (SMAC) containing 5-bromo-4-chloro-3-indolyl-β-D-glucuronide (BCIG) (CM0981, Oxoid®) and cefixime tellurite supplement (SR0172, Oxoid®, Basingstoke, UK).

Identification of *E. coli*

Rose pink colonies on MacConkey agar (putative *E. coli*) and pale colonies on SMAC were selected for microscopy (following Gram staining) and biochemical test. Biochemical identification of Gram-negative rod-shape bacteria was achieved by using commercially available biochemical test kit for the identification of Gram-negative, oxidase negative bacilli (Microbact GNB 24E, OxoidR, Basingstoke, UK). The result of biochemical

reactions were interpreted according to manufacturer's instruction using accompanying computer software package (Oxoid Microbact® 2000 version 2.03). Furthermore, all non-sorbitol fermenting *E. coli* on SMAC-BCIG strains were tested using latex agglutination kit for *E. coli* O157:H7 (Oxoid DRO 120M, UK) according to the manufacturer's instruction.

Antimicrobial Susceptibility Test

The susceptibility of identified *E. coli* isolates to antimicrobial agents was determined by the Kirby-Bauer disk diffusion method. A single colony of fresh culture of the isolate under test was emulsified in normal saline. The turbidity of the bacterial suspension was adjusted to 0.5 McFarland standards. A sterile swab was dipped into the adjusted bacterial suspension and inoculated onto Mueller-Hinton agar (MHA) (Oxoid, Basingstoke, UK) plate by swabbing the entire surface of the MHA. The antimicrobial disks were individually placed firm on the inoculated MHA plate. The plates were incubated at 37 OC for 18h. After incubation, the diameter of the clear zone of inhibition around each antimicrobial disk was measured (in millimeters) and the result was interpreted in accordance with the recommendation of Clinical and Laboratory

Table 1: Rates of *E. coli* isolation from pigeons in location within Ibadan, Nigeria.

Samples by location (sample size)	Number (%) of <i>Escherichia coli</i>		
	EHEC O157	Non-EHEC	Total <i>E. coli</i>
Bode			
Adults (39)	8 (20.5)	28 (71.8)	36 (92.3)
Squabs (21)	5 (23.8)	15 (71.4)	20 (95.2)
Sub-total (60)	13 (21.7)	43 (71.7)	56 (93.3)
Sasa			
Adults (20)	3 (15.0)	11 (55.0)	14 (70.0)
Squabs (12)	2 (16.7)	8 (66.7)	10 (83.3)
Sub-total(32)	5 (15.6)	19 (59.4)	24 (75.0)
Molete			
Adults (23)	3 (13.0)	17 (73.9)	20 (87.0)
Squabs (10)	2 (20.0)	5 (50.0)	7 (70.0)
Sub-total (33)	5 (15.2)	22 (66.7)	27 (81.8)
Total (125)	23 (18.4)	84 (67.2)	107 (85.6)

Table 2: Antimicrobial Resistance Rates in EHEC O157 and non-EHEC isolates from pigeons in Ibadan, Nigeria

Antimicrobial agents	Number (%) of resistant isolates		Total (number tested = 107)
	EHEC O157:H7 (number tested = 23)	Non-EHEC (number tested = 84)	
Augmentin	17 (73.9)	72 (85.7)	89 (83.2)
Amoxicillin	21 (91.3)	83 (98.8)	104 (97.2)
Ceftriazone	23 (100)	84 (100)	107 (100.0)
Ciprofloxacin	7 (30.4)	38 (45.2)	45 (42.1)
Cotrimoxazole	14 (60.9)	53 (63.1)	67 (62.6)
Gentamycin	14 (60.9)	67 (79.8)	81 (75.7)
Nitrofurantoin	23 (100)	84 (100)	107 (100.0)
Ofloxacin	4 (17.4)	34 (40.5)	38 (35.5)
Pefloxacin	11 (47.8)	64 (76.2)	75 (70.1)
Tetracycline	8 (34.8)	64 (76.2)	72 (67.3)

Standards Institute (CLSI), (2013). Susceptibility to the following antimicrobials was determined: amoxicillin (AMX, 25µg), amoxicillin/clavulanic acid (AUG, 20 µg/10µg), ceftriazone (CRO, 30µg) ciprofloxacin (CPX, 5µg), cotrimoxazole (COT, 1.25/23.75µg), gentamicin (GEN, 10µg), nitrofurantoin (NIT, 300µg), ofloxacin (OFL, 5µg), tetracycline (TET, 30µg), pefloxacin (PFX, 5µg).

Results

A total of 107 (85.6%) *Escherichia coli* isolates were obtained from 125 cloacae samples of pigeons from three different locations where pigeons were being raised in Ibadan (Table 1). Thirty-eight of the 107 were non-sorbitol fermenters of which 23 were serologically identified as *Escherichia coli* O157:H7. The EHEC O157:H7 isolates showed resistance to tested antimicrobials as follows: nitrofurantoin (100%), ceftriazone (100%), amoxicillin (91.3%), augmentin (73.9%), gentamycin (60.9%), cotrimoxazole (60.9%), pefloxacin (47.8%), tetracycline (34.8%), ciprofloxacin (30.4%), ofloxacin (17.4%) (Table 2). Antimicrobial susceptibility profile showed that all the isolates were resistant to at least two antimicrobial agents resulting in 39 different resistance patterns (Table 3).

Discussion

As expected, findings from the present study indicated the presence of *E. coli* in the intestine of pigeons. In addition, enterohaemorrhagic *E. coli* O157:H7 serotype was detected in squabs and adult pigeons. The presence of EHEC O157:H7 in feral pigeons has significant public health implications. Enterohaemorrhagic *E. coli* O157:H7 is commonly associated with fatal cases of haemorrhagic colitis and haemolytic uraemic syndrome. The infection is mostly linked with the consumption of contaminated food substances. In the past three decades, outbreaks of severe gastrointestinal illness have occurred due to food-borne enterohaemorrhagic *E. coli* strains especially EHEC O157:H7 (Armstrong et al, 1996). Epidemiological studies have revealed the preponderance of EHEC O157 in the gastrointestinal tracts of farm animals especially ruminants (Ojo et al., 2010). The present study further showed that pigeons might serve as carriers of EHEC O157:H7, which can be transmitted to humans through faecal contamination of the environment. Pigeons are common sight around human dwellings and so may constitute a threat to public health with increased risk of exposure to EHEC O157:H7. People visiting public parks and other open spaces where feral pigeons are

Table 3: Antimicrobial resistance profiles of EHEC O157 and non-EHEC isolates from pigeons in Ibadan, Nigeria

Resistance group	Resistant Pattern	Number of isolates	
		EHEC O157:H7	Non-EHEC
R1	CRO, NIT	1	1
R2	AMX, NIT, CRO	1	0
R3	AMX, CRO, GEN, NIT	1	1
R4	AMX, CPX, CRO, NIT	1	1
R5	AMX, CRO, NIT, PFX	0	2
R6	AMX, CPX, CRO, GEN, NIT	0	3
R7	AMX, COT, CRO, GEN, NIT	0	1
R8	AMX, AUG, CRO, NIT, TET	0	1
R9	AMX, , CRO, NIT, GEN, TET	0	1
R10	AUG, COT, CRO, GEN, NIT	1	0
R11	AMX, AUG, COT, CRO, NIT	1	2
R12	AMX, AUG, CRO, GEN, NIT	1	2
R13	AMX, AUG, CRO, NIT, PFX, TET	0	2
R14	AMX, AUG, CPX, CRO, NIT, PFX	0	2
R15	AMX, AUG, COT, CRO, NIT, TET	0	1
R16	AMX, AUG, COT, CRO, PFX, NIT	2	3
R17	AMX, AUG, COT, CRO, GEN, NIT	2	0
R18	AMX, CPX, CRO, NIT, PFX, TET	2	0
R19	AMX, AUG, COT, CRO GEN NIT PFX	1	0
R20	AMX, AUG, CRO, GEN, NIT, PFX, TET	1	2
R21	AMX, AUG, CPX, CRO, NIT, OFL, TET	0	1
R22	AMX, AUG, CPX, CRO, GEN, NIT, TET	0	1
R23	AMX, AUG, COT, CRO, GEN, NIT, PFX	0	1
R24	AMX, CPX, CRO, GEN, NIT, OFL, TET	0	1
R25	AMX, AUG, CPX, CRO, GEN, NIT, PFX	1	0
R26	AMX, AUG, COT, CRO, GEN, NIT, TET	1	3
R27	AMX, AUG, COT, CRO, NIT, PFX, TET	0	1
R28	AMX, TET, COT, CRO, GEN, NIT, PFX	0	1
R29	AMX, AUG, CPX, CRO, GEN, NIT, PFX	0	1
R30	AMX, AUG, CRO, GEN, NIT, PFX, TET	0	1
R31	AMX, AUG, COT, CRO, GEN, NIT, PFX, TET	2	9
R32	AMX, AUG, CRO, GEN, NIT, OFL, PFX, TET	0	1
R33	AMX, AUG, CPX, CRO, GEN, NIT, PFX, TET	0	3
R34	AMX, AUG, COT, CPX, CRO, NIT, OFL, PFX	1	0
R35	AMX, AUG, COT, CRO, GEN, NIT, OFL, PFX	1	0
R36	AMX, AUG, COT, CRO, GEN, NIT, OFL, PFX, TET	0	10
R37	AMX, AUG, CPX, CRO, GEN, NIT, OFL, PFX, TET	0	4

Resistance group	Resistant Pattern	Number of isolates	
		EHEC O157:H7	Non-EHEC
R38	AMX, AUG, COT, CPX, CRO, GEN, NIT, PFX, TET	0	4
R39	AMX, AUG, COT, CPX, CRO, GEN, NIT, OFL, PFX, TET	2	17
Total	23	84	

commonly found are also at risk of infection with EHEC O157:H7 shed in the faeces of pigeons. In the present study, the prevalence of EHEC O157:H7 is higher in adult pigeons than in squabs. This is similar with the findings of Morabito et al. (2001) where a higher prevalence of EHEC O157:H7 was detected in adult pigeons than in squabs. The presence of vertically transferred passive immunity in the young could prevent the establishment of infection. The higher prevalence of both non-EHEC and EHEC O157:H7 in pigeons from Bode area than in those from Sasa and Molete areas suggests a better level of hygiene and environmental sanitation in the areas with lower prevalence. Thus, people living in Bode are at a higher risk of exposure to EHEC O157:H7 than those living in Sasa and Molete.

The high antimicrobial resistance rate in EHEC O157:H7 isolates from this study is very worrisome. The continued increase in antimicrobial resistance in pathogenic and commensal *E. coli* and other bacterial species is of global interest. The possible involvement of multidrug resistant EHEC O157:H7 in human infection could precipitate refractory illness making treatment problematic. Clinicians are confronted with the challenge of limited choice of antimicrobials available for treatment of refractory infection. This could lead to protracted illness, enhanced spread of infection, increased fatality and other socioeconomic implications. Among other causes, usage of antimicrobial agents in humans and animals has been identified as a major contributing factor to the emergence and widespread dissemination of antimicrobial resistant bacterial strains. However, there is no evidence of direct exposure of pigeons investigated in this study to antimicrobial agents. Notably, the pigeons were scavengers and could have

indirect exposure to antimicrobials residues in the environment. Improper disposal of antimicrobial leftovers and packages can provide residues in the environment. Scavenging pigeons can pick antimicrobial residues in water and feed items. Pigeons are also exposed to the existing burdens of antimicrobial resistant bacteria present in the environment due to contamination from various sources including humans and free-range animals.

Antibiotic resistance is an increasing worldwide phenomenon and there is urgent need for an improve surveillance. Indiscriminate antibiotics usage in livestock may have serious public health implications. Regulation and control of antibiotics usage in livestock production, environmental and meat hygiene as well as increased surveillance of antimicrobial resistance in bacteria of food animal origins are recommended for the protection of public health.

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BEHAVIOURAL RESPONSE OF WEST AFRICAN DWARF KIDS TO REPEATED SEPARATION FROM THEIR DAMS DURING THE FIRST WEEK OF LACTATION.

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Abstract

Eighty West African Dwarf kids were tested to investigate their behavioural responses to a 9 hr daily separation from their respective dams on 3 consecutive days, and scored 1-5. A kid was scored 5 when it was very active and emitted high-pitched bleats, while a least score of 1 was given when the kid was inactive and emitted no bleat. The effects of sex, birth weight, type of birth, day of separation and time interval from separation were measured using the chi-square procedure. No differences ($P > 0.05$) were found in the behaviour of kids among all the 3 days of separation. A higher proportion ($P < 0.05$) of kids, however, showed a higher level of disturbance on each of the 3 days of separation than control (when they were with their respective mothers). A higher proportion (92.5%; $P < 0.05$) of the kids were less disturbed immediately after separation than mid-way through separation (47.5%) and just before arrival of their mothers. Similarly, large proportion (47.5%) were less disturbed mid-way through separation than just before the arrival of their mothers. The proportion of undisturbed kids immediately after separation was similar ($P > 0.05$) to control, but higher ($P < 0.05$) than all separation intervals. A high proportion ($P < 0.05$) of kids had scores 4 and 5 midway through the separation period and just before arrival of their mothers than immediately after the separation period. Kids were generally stressed by the separation process, and the level of disturbance increased with increasing period of separation within the day, and must be avoided.

Keywords: kids, behaviour, vocalization, disturbance, isolation

REACTION COMPORTEMENTALE DE CHEVREUX NAINS D'AFRIQUE DE L'OUEST A LA SEPARATION REPETEE DE LEURS MERES PENDANT LA PREMIERE SEMAINE DE LACTATION

Résumé

Quatre-vingt chevreaux nains d'Afrique de l'Ouest ont été testés pour examiner leurs réponses comportementales à une séparation quotidienne de 9 heures de leurs mères respectives pendant 3 jours consécutifs, et ont obtenu un score de 1 à 5. Un chevreau a reçu la note 5 s'il était très actif et émettait des bêlements aigus, tandis qu'un score de 1 a été donné au chevreau inactif n'émettant aucun bêlement. Les effets du sexe, du poids à la naissance, du type de naissance, du jour de la séparation et de l'intervalle de temps écoulé depuis la séparation ont été mesurés en utilisant la procédure du chi-carré. Aucune différence ($P > 0,05$) n'a été observée dans le comportement des chevreaux au cours de trois jours de séparation. Cependant, une proportion plus élevée ($P < 0,05$) de chevreaux présentait un niveau de perturbation plus élevé au cours de chacun des trois jours de séparation par rapport aux témoins (lorsqu'ils étaient avec leurs mères respectives). Une proportion plus élevée (92,5%; $P < 0,05$) des chevreaux étaient moins perturbés immédiatement après la séparation qu'à mi-chemin de la séparation (47,5%) et juste avant l'arrivée de leur mère. De même, une grande proportion (47,5%) était moins perturbée à mi-chemin de la séparation que juste avant l'arrivée de leur mère. La proportion de chevreaux non perturbés immédiatement après la séparation était similaire ($P > 0,05$) aux témoins, mais plus élevée ($P < 0,05$) que tous les intervalles de séparation. Une proportion élevée ($P < 0,05$) des chevreaux avaient des scores de 4 et 5 au milieu de la période de séparation et juste avant l'arrivée de leur mère que juste après la période de séparation. Les chevreaux étaient généralement stressés par le processus de séparation, et le niveau de perturbation augmentait parallèlement au prolongement de la période de séparation dans la journée et devait être évité. Mots-clés : chevreaux, comportement, vocalisation, perturbation, isolement

Introduction

In Ghana the most common goat found and reared is the West African Dwarf goat. These animals are fertile, able to cope with climatic change, stress, scanty and irregular supply of feed and water (Davendra and Burns, 1983). There are also few crosses between the Sahelian and West African Dwarf breed of goats. In Northern Ghana, the sale of goat and sheep is second in line for meeting immediate cash needs (Ministry of Food and Agriculture, MoFA, 2004). Goats are considered to play a key role in ensuring food security for the people of Ghana. Goats also have high reproductive rate in the short term compared to cattle. Importantly, there are no religious or cultural barriers to rearing goats and consumption of chevron (Gatenby, 1991).

Several problems confront the small ruminant industry. Nwafor (2004) categorized these problems into biological, economic and cultural constraints. Biological constraints include high disease prevalence, poor feed conversion efficiency, inadequate quality feeds and high incidence of neonatal mortality (Opoku, 1982). The inability to commit greater resources to production and the incidence of holding livestock as a store of wealth are the economic and cultural constraints, respectively. After examining the main and secondary factors responsible for kid and lamb neonatal deaths in Ghana, Tuah and Baah (1985) concluded that starvation was the most important cause followed by pneumonia which results from poor husbandry practices.

Poor husbandry practices may impose some stress on farm animals. Any stress imposed on the farm animal reduces its ability to fight infections (Peacock, 1996). Stress also causes metabolic changes that can in turn adversely affect productivity in small ruminants (Apple *et al.*, 1995). Studies on stress response in farm animals are often conducted on the basis of single physiological alterations or irregular behavioural phenomenon that might be difficult to interpret. In the past, most physiological studies solely based on the analysis of catecholamine or glucocorticoids

without considering that multiple physiological system are altered during stress (Dantzer and Mormed, 1983). These measurement of single variable (i.e. cortisol) are of little value when considering the context in which substance is released and not knowing the consequence it has for animal well-being (Rushen, 1991). Animals can be stressed by either psychological stressor such as hunger, thirst, novelty and fatigue or physical stressor such as restraint, handling and early weaning or separation from the dam (Grandin, 1997).

The commonest way of feeding dams immediately after parturition is to temporarily separate mother and young, while the dam goes for grazing. This husbandry practice may have stressful effects on both mother and young within the first week of lactation. It is common that both the dam and offspring spend less time feeding and lying down, respectively, after separation is imposed (Stockey *et al.*, 1997). This has been shown to result in reduced feed intake in dam, reduced weight gain and even weight loss in both dam and offspring in various species (Haupt *et al.*, 1984). However, Temple (1988) reported that animal handling in early life is likely to influence how well it copes with weaning shock. Repeated separation of the young may therefore be beneficial in the long run. That notwithstanding, there is still the need to identify such psychological stressors in order to reduce them in animal husbandry systems, thereby avoiding the possible weight loss associated with the phenomenon (Haupt *et al.*, 1984). Abdul-Rahman *et al.* (2012a; 2012b) reported both the behavioural and physiological effects of this phenomenon in sheep. There is, however, not a single record on goats. This study, therefore, sought to investigate the adaptive response of West African Dwarf kids to repeated separation during the 1st week of lactation.

Materials and methods

Experimental site

The study was undertaken at the National Goats Breeding Station of the Animal Production Directorate, Ministry of Food and

Agriculture, Kintampo (Ghana). The station lies within latitude 7° 45' and 8° 45' N and longitude 1° 05' and 2° 05' W. It has a single rainy season from May to October, followed by the dry season from November to April. The annual rainfall of the area is between 1000 mm to 1200 mm. The temperature of the area ranges from 20 °C to 36 °C. (Metrological Service Department, Kintampo, Ghana). The vegetation of the area is the semi deciduous forest type. It is woody savanna characterized by scattered shrubs and trees such as *Adansonia digitata*, *Parkia biglobosa* and *Butyrospermum parkii*. Usually the dominant grass vegetation belongs to the Graminae (Innes, 1977).

Management of the Animals

The station has 11 paddocks in sole and mixed pastures. The animals are fed on pastures on rotational basis. They are allowed to graze ad-libitum in the dry season, while controlled grazing is practiced during the wet season. The animals are also given supplementary feed (such as maize, soya beans, cassava peels) during the dry season. The nannies are also flushed with soya beans, corn chaff, concentrates, rice bran and pito mash three weeks before crossing. Breeding is done seasonally from September to October and March each year. Controlled mating is practiced where the bucks are allowed to join the nannies during the mating season at a mating ratio of 1:25, for best breeding results (Luginbuhl, 2015). The animals are dewormed every two months in the rainy season and twice in the dry season. The kids are identified with indelible ink within a few hours post-partum and then ear tagged when about one month old.

Pregnant does are infrequently handled and are usually separated from the general flock towards the end of gestation period. Does are transferred to kidding pens 4-5 days prior to parturition and bedding material, consisting mostly of corn husk, provided for maximum comfort. From the day of expected parturition, does are closely monitored for possible intervention if required. After delivery, does, particularly heavy milkers, are milked soon if not sucked by the kid in order to relieve her of

udder pressure. Does and their kids are usually left undisturbed following kidding, in order to ensure establishment mother-young bond.

Soon after kidding, kids and their mothers are sent to the kid shed. The dam's udder is then checked for milk supply and potential problems. Kids are monitored closely to ensure that they nurse. Kids that fail to access the teat within the first 30 min postpartum are assisted to suck, and this usually include small, weak and deserted kids. In the case of deserted kids, they are dried with jute bags and further wrapped in fresh jute bags to keep warm. In such kids, tube feeding is done using colostrum obtained from the mother. Feed and water troughs are kept out of reach of kids. Navel cords more than 2 inches long are clipped. The navel area is sprayed with gentle iodine (1%) to prevent infection.

The goat pens are mainly stalls with concrete floors, with the walls being one meter high. The goats are housed in groups according to their ages. The kids and dams are housed in the kid shed up to weaning. The adult bucks and nannies are separated in general flock shed.

Animals

Eighty West African Dwarf kids were used for the study. Thirty-seven of them were born to primiparous dams while 43 were born to multiparous dams. Forty-one of the kids were males while the remaining 39 were females. Based on the average birth weight of West African Dwarf kids which is about 1.2 kg (Addae, 1998) the kids were grouped into low weight (< 1.2 kg) and high birth weight (\geq 1.2 kg) classes. Thirty-eight of the kids fell in to the low weight class while 42 fell into the high weight class.

Experimental procedure

Response of West African Dwarf kids to repeated separation from their dam during the first week of lactation: During the 1st week of lactation, mothers were allowed to go out for grazing with their flock mates while their kids were left behind. The flock spent 9 hours at grazing. Behavioural measurements were then made at 4 different intervals following

the separation period. These intervals were immediately after the dam had left for grazing (when the flock was visible to the kid while leaving for grazing), mid-way through the separation period (when the flock had spent about 4 hours 30 minutes at grazing), just before the arrival of the flock (when the flock was visible to the kid while returning from grazing) and 5 min after the arrival of the mother (control). For each day of separation, an average of the 3 intervals for each of the behavioural parameters was taken and considered as the reading for the particular day per animal. Similarly, for each interval from separation, an average of the 3 days was estimated and considered as the reading for the particular interval from separation per animal.

Behavioural measurement

The kid was observed at the various intervals from separation and its behaviour score recorded on a 5 - point scale similar to that used by Oppnng-Anane (1991) and shown in Table 1

Table 1: Key to score used for the behavioural response of kids to separation from the dam

Score	Lamb Behaviour
5	Very restless, continuous high-pitched bleats
4	Restless, continuous high pitched bleats
3	Restless, few bleats
2	Inactive, low bleats
1	Inactive, no bleats

Other parameters recorded

Parity of the dam, sex and birth weight of the kids: Dams were classified into 2 parity groups, namely, primiparous (one gestation and/or parturition) and multiparous (2 or more gestation and/or parturitions). Sex of kids was determined at birth and kids grouped into male and females. Birth weights were also recorded and were classified as in above.

Statistical Analysis

The effects of day of separation, time interval from separation, sex, birth weight and parity on the behavioural response of the young to separation from the mother was determined

using the chi-square procedure. All comparisons were done at 5% level of significance.

Results

Effects of day of separation on the behavioural response of West African Dwarf kids to repeated separation from its mother during the 1st week of lactation: The behavioural response of West African Dwarf kids to separation from their mothers is shown in Table 2. There were no differences ($P > 0.05$) in the behaviour of kids during all the 3 days of separation. Significant differences ($P < 0.05$) were, however, observed in the behaviour of the kids between each day of separation and their behaviour when their mothers had returned from grazing (control). All the kids that were together with their mothers showed significantly less signs of disturbance (scores 2 and 3) than when they were separated from their mothers on days 1 (67.5%), 2 (55%) and 3 (42.5%). Over 30%, 45% and 57.5% of the kids separated on days 1, 2 and 3, respectively, had scores 4 and 5, while none of them behaved in this manner when their mothers returned from grazing and stayed with them for about 5 minutes. The average kid behaviour score for the 3 days of separation and during the period when they were with their mothers were 3.5 and 2.4, respectively.

Effects of time interval from separation, parity of the dam, sex and birth weight of the kid on its behavioural response to repeated separation during the 1st week of lactation: The time period that elapsed after separation of the mother from the young significantly influenced ($P < 0.05$) kid behaviour (Table 3). A significantly higher ($P < 0.05$) proportion (92.5%) of the kids were less disturbed (scores 1, 2 and 3) immediately after separation than mid-way through the separation (47.5%) and just before the arrival of their mothers (27.5%). Similarly, more kids were less disturbed (scores 2 and 3) mid way through the separation period than just before the arrival of their mothers. All the kids that were together with their mothers showed significantly less signs of disturbance than when they were separated from their

Table 2: The effect of day of separation on the behavioural response of kids during the first week of lactation

Day of separation	No. (%) of kids that exhibited the various responses to separation from their mother					X ² (5)	No. of kids
	Kid behaviour score						
	1	2	3	4	5		
Day 1	0 (0)	8 (10.0)	46 (57.5)	18 (22.5)	8 (10.0)	*	80
Day 2	0 (0)	0 (0)	44 (55.0)	34 (42.5)	2 (2.5)	*	80
Day 3	0 (0)	2 (2.5)	32 (40.0)	34 (42.5)	12 (15.0)	*	80
Mother-young reunion (control)	0 (0)	52 (65.0)	28 (35.0)	0 (0)	0 (0)	*	80

*X² between day 1 and day 2 = 8.3, X² between day 1 and day 3 = 5.9, X² between day 2 and day 3 = 5.5, X² between day 1 and control = 31.3, X² between day 2 and control = 45.8, X² between day 3 and control = 46.3

Table 3: The effect of time interval from separation on the behavioural response of kids during the first week of lactation

Time interval from separation	No. (%) of kids that exhibited the various responses to separation from their mother					X ² (5)	No. of kids
	Kid behaviour score						
	1	2	3	4	5		
Immediately after sep	2 (2.5)	34 (42.5)	38 (47.5)	6 (7.5)	0 (0)	*	80
Midway through sep	0 (0)	2 (2.5)	36 (45.0)	38 (47.5)	4 (5.0)	*	80
Just before arrival of the dam	0 (0)	20 (25.0)	2 (2.5)	54 (67.5)	4 (5.0)	*	80
Mother-young reunion (control)	0 (0)	54 (67.5)	26 (32.5)	0 (0)	0 (0)	*	80

*X² between Immediately after and midway through separation = 45.9, X² between Immediately after sep and just before arrival of the flock = 65.4, X² between midway through Sep and just before arrival = 24.75, X² between immediately after sep and control = 7.4, X² between midway through sep and control = 45.9, X² between just before arrival of the flock and control = 76.3, Sep: Separation

Table 4: The effects of sex, birth weight and parity on the behavioural response of kids to separation during the first week of lactation

Groups of kids	No. (%) of kids that exhibited the various responses to separation from their mother					X ² (5)	No. of kids
	Kid behaviour score						
	1	2	3	4	5		
Sex							
Male	0 (0)	0 (0)	17 (68.0)	8 (32.0)	0 (0)	5.2	41
Female	0 (0)	0 (0)	6 (24.0)	7 (28.0)	2 (8.0)		39
Birth weight							
Low (<1.2 kg)	0 (0)	0 (0)	3 (19.0)	12 (75.0)	1 (6.0)	8.3	38
High (≥1.2 kg)	0 (0)	0 (0)	20 (83.0)	4 (17.0)	0 (0)		42
Parity							
Primiparous	0 (0)	0 (0)	9 (60.0)	6 (40.0)	0 (0)	2.0	37
Multiparous	0 (0)	0 (0)	14 (58.0)	8 (32.0)	3 (12.0)		43

*X² between day 1 and day 2 = 8.3, X² between day 1 and day 3 = 5.9, X² between day 2 and day 3 = 5.5, X² between day 1 and control = 31.3, X² between day 2 and control = 45.8, X² between day 3 and control = 46.3

mothers at all intervals, except the period immediately following separation, when kids were equally ($P>0.05$) less disturbed.

Sex, birth weight and type of birth of the kid did not influence ($P >0.05$) the behavioural response of West African dwarf kids to separation from their mothers during the first week of lactation (Table 4).

Discussion

The insignificant influence of day of separation on the behavioural response of the young to separation from its mother in the present study is indicative of the fact that the response of West African dwarf kids to a psychological stressor such as separation from its mother does not change with day. A gradual adaptation to the separation process was not observed but rather a trend towards increasing disturbance. These increases were, however, marginal. Similar observations were made by Abdul-Rahman *et al.* (2012a; 2012b) in sheep. These observations, however, contradict that of Price *et al.* (2003) who reported a decrease in the behavioural response of calves at few days past separation; they observed a decline in vocalization in calves on the 3rd and 5th days of separation and suggested that the animals were gradually adapting to the separation process. This may, therefore, reflect behavioural differences between large and small ruminants. While large ruminants respond to separation by gradually adopting to the psychological stressor, small ruminants on the other hand remain constantly disturbed throughout the period of repeated separation.

The fact that kids were highly disturbed during the separation period than when they were with their respective mothers is an indication that separation generally is a stressful experience to the young. This is in agreement with the reports of Abdul-Rahman *et al.* (2012a; 2012b) in sheep and Price *et al.* (2003) in cattle. Price and associates (2003) noted that beef calves separated on 3 days vocalized 2000-4000 times greater than unseparated controls. They also observed that separated calves spent 28.1%

of their times walking, while unseparated calves spent only 8.6% of their time doing so. Stockey *et al.* (1997) indicated that during separation both dam and offspring spent less time feeding and lying down, respectively, besides increased vocalization, leading to reduced feed intake in the dam, reduced weight gain, and net weight losses in both the dam and offspring in various species (Haupt *et al.* 1984). In contrast, however, Haupt *et al.* (1983) reported that young calves remained relatively inactive when separated from their mothers during the 1st week of life.

The result of the present study showed that the longer the young is separated from its mother, the more disturbed, and therefore stressed it becomes, indicating that even on the same day of separation, the young did not adjust to the separation process. This trend is similar to that observed by Abdul-Rahman *et al.* (2012a) in sheep. Even though a similar trend was reported in a physiological study by Abdul-Rahman *et al.* (2012b), the authors noted that the differences in levels of disturbance between time intervals from separation were minor. Turner and co-workers (2010) showed that female sheep had a greater cortisol response than their male counterparts in isolation/restraint stress. In the present study, however, no difference was found between males and females, even though males tended to be more tolerant to stress than females.

Conclusion and Recommendation

Kids were generally more disturbed on each day of separation, and the level of disturbance increased with increasing period of continuous separation within the day, indicating that separation during the 1st week of lactation is a stressful process and must be avoided. It is advised that zero grazing be practiced until the kids are old enough to go out for grazing with their mothers. In such a case, possible weight losses associated with the practice may be avoided.

Conflict of interest

The author declares that there is no conflict of interest that could be perceived as prejudicing the impartiality of the article.

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EFFECT OF TAMARINDUS INDICA (LINN, 1753) PULP AND LEAF- FORTIFIED DIETS ON EXPERIMENTAL AEROMONAS HYDROPHILA INFECTION IN CLARIAS GARIEPINUS (BURCHELL, 1822)

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Abstract

Intensification of aquaculture is associated with infectious diseases with consequent increase in the use of synthetic antibiotics. The rise in antibiotic-resistant bacteria is of global criticism. The role of *Tamarindus indica* as antimicrobial agents has been reported. This study evaluated the utilization of tamarind pulp and Leaves in the diets of *Clarias gariepinus* as antimicrobial agent against *Aeromonas hydrophila* infection. Ten experimental diets consisting of 0.5, 1.0, 1.5, 2.0% of basal diets each of tamarind pulp or leaf meal and 0.0% (untreated control) and 0.2% oxytetracycline (treated control) were fed to *C. gariepinus* for 12 weeks. After the feeding trial, fish were subjected to bath challenge with *Aeromonas hydrophila* and observed for 28 days to determine mortality and relative percentage survival. The surviving fish after 28 days post-challenge were sampled for haematological studies. Data were analyzed using one-way analysis of variance at $P = 0.05$. All experimental groups of the fish on tamarind-treated diets exhibited significantly lower ($P \leq 0.05$) mortality compared to the groups fed with the control diets. 100% relative percentage survival was exhibited by the groups of fish on 1.5-2.0% pulp and 2.0% leaf diets. Higher white blood cell counts were obtained from tamarind-treated groups compared to the untreated control diets. The heterophil of the challenged fish fed diet with oxytetracycline was significantly lower ($P \leq 0.05$) than fish fed 2.0% tamarind leaf diet. Inclusion of tamarind in the diets of the challenged *C. gariepinus* did not alter the monocytes, eosinophil and basophil significantly ($P > 0.05$) compared to those on the control diets. Fortifying the diets of *Clarias gariepinus* with 2.0% tamarind pulp and leaf meal significantly enhanced protection against *Aeromonas hydrophila* infection, hence these inclusion levels are recommended as alternatives to oxytetracycline in the control of motile aeromonas septicemia.

Keywords: *Clarias gariepinus*, tamarind, synthetic antibiotics, *Aeromonas hydrophila* infection, relative percentage survival

EFFET DES REGIMES ENRICHIS DE PULPES ET FEUILLES DE TAMARINDUS INDICA (LINN, 1753) SUR L'INFECTION EXPERIMENTALE A AEROMONAS HYDROPHILA CHEZ LES CLARIAS GARIEPINUS (BURCHELL, 1822)

Résumé

L'intensification de l'aquaculture est associée à des maladies infectieuses qui ont pour conséquence une augmentation de l'utilisation d'antibiotiques de synthèse. L'augmentation des bactéries résistantes aux antibiotiques fait l'objet de critiques au niveau mondial. Le rôle de *Tamarindus indica* en tant qu'agent antimicrobien a été rapporté. La présente étude a évalué l'utilisation de pulpes et feuilles de tamarin dans les régimes de *Clarias gariepinus* comme agent antimicrobien contre l'infection à *Aeromonas hydrophila*. On a administré à *C. gariepinus* pendant 12 semaines dix régimes expérimentaux constitués de 0,5, 1,0, 1,5 et 2,0% de régimes basaux de farine de pulpes ou de feuilles de tamarin et 0,0% (témoin non traité) et 0,2% d'oxytétracycline (témoin traité). Après l'essai alimentaire, les poissons ont été soumis à une infection en bain à *Aeromonas hydrophila* et observés pendant 28 jours pour déterminer la mortalité et le pourcentage

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de survie relatif. Les poissons ayant survécu après 28 jours post-infection ont été échantillonnés pour des études hématologiques. Les données ont été analysées en utilisant une analyse de variance à un facteur à $P = 0,05$. Tous les groupes expérimentaux de poissons soumis aux régimes traités avec le tamarin ont présenté une mortalité significativement plus faible ($P < 0,05$) par rapport aux groupes nourris aux régimes témoins. Un pourcentage de survie relatif de 100% a été observé pour les groupes de poissons recevant des régimes de 1,5-2,0% de pulpe et 2,0% de feuilles. Un nombre plus élevé de globules blancs a été obtenu chez les groupes traités au tamarin par rapport aux régimes témoins non traités. L'hétérophile du poisson infecté soumis à un régime alimentaire contenant de l'oxytétracycline était significativement plus faible ($P < 0,05$) que celui du poisson soumis au régime à 2,0% de feuilles de tamarin. L'inclusion du tamarin dans les régimes de *C. gariepinus* infectés n'a pas modifié significativement les monocytes, les éosinophiles et les basophiles ($P > 0,05$) par rapport à ceux soumis aux régimes témoins. L'enrichissement des régimes alimentaires de *Clarias gariepinus* avec 2,0% de farine de pulpes et de feuilles de tamarin a significativement amélioré la protection contre l'infection à *Aeromonas hydrophila* et, par conséquent, ces niveaux d'inclusion sont recommandés comme solution de rechange à l'oxytétracycline dans le contrôle de la septicémie à *aeromonas* mobile.

Mots-clés : *Clarias gariepinus*, tamarin, antibiotiques de synthèse, infection à *Aeromonas hydrophila*, pourcentage de survie relatif

Introduction

Intensification of aquaculture has led to remarkable improvement in productivity (FAO (Food and Agriculture Organization), 2014, 2016), but it is also associated with diseases, considered to be the major source of mortality in aquaculture, and consequently increase in the use of antimicrobials (Defoirdt *et al.*, 2011). Bacterial diseases (including aeromonad septicemia) are reported to be an important economic limiting factor in intensive aquaculture, responsible for mass losses (Claudia and Jettrey, 2009; Hossain *et al.*, 2011; Pridgeon and Klesius, 2012; Oladosu *et al.*, 2013) leading to therapeutic use of synthetic antibiotics. The use of synthetic antibiotics and other drugs in aquaculture is complicated because of the need to administer them directly into the water where the fish are reared. Hence, safety of the fish products for consumption and environmental impacts are crucial factors for consideration (FAO, 2005).

Aeromonas hydrophila is a bacterial pathogen with a wide geographical distribution in both the terrestrial and aquatic environments. It has been isolated from a variety of sources including soil, lakes, rivers, water reservoirs, ground water, drinking water leaving treatment plant, wound infections and human stools (United State Environmental Protection Agency, 2005; Subashkumar *et al.*, 2006; Newaj-

Fyzul *et al.*, 2008; Rogo *et al.*, 2009; Anuradha *et al.*, 2010; Shayo *et al.*, 2012; Saidi *et al.*, 2013). *A. hydrophila* is the aetiological agents of motile aeromonas septicemia which have infected a wide variety of fish species such as carp, tilapia, catfish, perch, salmon and gold fish (Janda and Abbott, 2010; Parker and Shaw, 2011). Motile aeromonas septicemia is a devastating disease of cultured freshwater fish with acute to chronic clinical signs and causing significant mortality (Plumb and Hanson, 2011; Edward, 2012). The incidence of motile aeromonas septicemia resulting to mass fish mortality that threatened livelihood of people has been reported (Shayo *et al.*, 2012) and great economic losses globally (Pridgeon *et al.*, 2012, Oladosu *et al.*, 2013).

To control infectious diseases in aquaculture, the same antibiotics used in other areas of animal production are employed. The intensive use of antibiotics for prevention and control of disease has contributed to increased antibiotic-resistant bacteria, immune suppression and environmental pollution (FAO, 2011; Sajid *et al.*, 2011; Gent *et al.*, 2012). Limited number of the antibiotics was approved for aquaculture use. The three antibiotics authorized for use in aquaculture by Food and Drug Administration in United States of America are oxytetracycline, sulfadimethoxine /or metoprim and aquaflor / florfenicol (Pridgeon *et al.*, 2011; Romero

et al., 2012). Motile aeromonas septicaemia is usually treated with oxytetracycline in the food of the fish at the rate of 55 mg per kg fish/day for 10 days (George, 1987; Edward, 2012, Ferrandez et al., 2014). Oxytetracycline is one of the most commonly used antibiotics in fish farming, both in the hatcheries and grow-out ponds. It is however poorly absorbed through the intestinal tracts of fish, hence it has to be administered at a high rate of 100-150mg/kg fish/day for 10-15 days (Romero et al., 2012). The role of herbal and medicinal plants as antimicrobial agents for fish pathogens and as immunostimulants in fish has been reported (Aly and Mohamed, 2010; Pakravan et al., 2011; Sajid et al., 2011, Amirkhani and Firouzbakhsh, 2013). The efficacy of any prophylactic and therapeutic agents for aquaculture use is usually tested through in vivo experimental challenge tests. Some researchers have reported higher level of protection of experimentally challenged fish and shrimp fed herbal products (Logambal et al., 2000; Immanuel et al., 2004; Praseetha, 2005; Aly and Mohamed, 2010; Pakravan et al., 2011; Bello et al., 2012; Yilmaz et al., 2013; Pujarini et al., 2014; Manaf et al., 2016). Tamarind, *Tamarindus indica* (Linn, 1753), is a medicinal tree species which grows widely in tropical and sub- tropical regions of the world (El-Siddig et al., 2006; Kumar and Bhattacharya, 2008). In vitro antimicrobial activity of tamarind has been reported (Dhoughari, 2006; Daniyan and Muhammad, 2008; Adeniyi et al., 2017). Our previous study demonstrated the in vitro antimicrobial activity of tamarind pulp and leaf on *Aeromonas hydrophila* (Adeniyi et al., 2017). There is paucity of information on the effects of dietary tamarind on disease resistance in fish. Therefore, the aim of this study was to evaluate the effect of dietary tamarind pulp and leaf meal as feed additives on protection of *Clarias gariiepinus* against experimental *Aeromonas hydrophila* infection.

Materials and methods

Experimental diets

Experimental diets (Table 1) consisted of ten groups (eight tamarind-treated and

two control diets). Air-dried tamarind pulp and leaves were processed into meals and included singly at 0.5, 1.0, 1.5 and 2.0% each to fortify the basal diets while the controls were included at 0.0% (untreated control) and 0.2% oxytetracycline (treated control).

Pathogen

Pathogenic *Aeromonas hydrophila* from a stock isolated from moribund *Clarias gariiepinus* was used for the challenge test. The isolate from the frozen ampules was sub-cultured for 24 hour at 37°C on nutrient agar. The 24-hour bacteria were suspended in physiological saline solution and standardized to 0.5 McFarland standard (1×10^8 CFU/ml) (CLSI, 2012). The inoculum was mixed with the water in the challenge tank at 0.2ml/litre of water.

Challenge protocols

Clarias gariiepinus (30 – 45g) previously fed the experimental diets (Table 1) for 12 weeks were transferred from the culture tanks to the challenge tanks. A bath challenge was conducted with twenty *C. gariiepinus* in each tank in triplicate per treatment. The fish were immersed in the culture suspensions for 1 hour (Adelmann et al., 2008; Emmenegger et al., 2013) and then returned to the culture tanks. The challenged fish were offered the experimental diets (Table 1) and observed for 28 days for external signs, erratic swimming, behavioural and clinical signs, response to feed and mortality rates were recorded. The Cumulative Mortality Index (CMI) and Relative Percentage Survival (RPS) were also calculated using these formulae (Amend, 1981);

- i. $CMI = Dt_1 + Dt_2 + Dt_3 + \dots + Dtn$
(D is the number of dead fish at the given time (days), t1, t2, t3, tn)
- ii. $RPS = 1 - \frac{\% \text{ mortality in treatment}}{\% \text{ mortality in untreated control}} \times 100$
(Amend, 1981; Thorborn et al., 1987)

Haematological studies

The survived fish after 28 days post-challenge were sampled for haematological studies. Blood samples were collected from

Table 1: Gross composition (g/100g dry matter) of experimental diets at varying inclusion levels of tamarind pulp and leaves fed to *Clarias gariepinus* for 16 weeks

Ingredients	Diets (% inclusion levels)									
	+ C	- C	0.5P	1.0P	1.5P	2.0P	0.5L	1.0L	1.5L	2.0L
Fish meal	24.11	24.11	24.11	24.11	24.11	24.11	24.11	24.11	24.11	24.11
SBM	30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00
GNC	25.50	25.50	25.50	25.50	25.50	25.50	25.50	25.50	25.50	25.50
Maize	10.89	10.89	10.89	10.89	10.89	10.89	10.89	10.89	10.89	10.89
Soy oil	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Salt	0.50	0.50	0.50	0.50	0.50	0.50	1.50	0.50	0.50	0.50
Bone meal	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
O. shell	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Premix*	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Cr ₂ O ₃	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Starch	3.50	3.30	3.00	2.50	2.00	1.50	3.00	2.50	2.00	1.50
Tamarind	-	-	0.50	1.00	1.50	2.00	0.50	1.00	1.50	2.00
OTC	-	0.2	-	-	-	-	-	-	-	-
Total	100	100	100	100	100	100	100	100	100	100

*Vitamin A = 20,500.00IU, Vitamin B1 = 20,000.00mg, Vitamin B2 = 15,000.00, Vitamin B3 = 90,000mg, Vitamin B4 = 4,000.00mcg, Vitamin B5 = 40.00mg, Vitamin B6 = 20,000.00, Vitamin B7 = 500.00mcg, Vitamin B12 = 15.00mcg, Vitamin C = 350,000.00mg, Vitamin D3 = 4,250,000.00IU, Vitamin E = 250,000.00IU, Vitamin K = 8,000.00mg, Copper sulphate = 4,000.00mg, Potassium Iodine = 2,000.00mg, Inositol = 50,000.00mg, Methionine = 50,000.00mg, Choline chloride = 600,000.00mg, Ferrous sulphate = 40,000.00mg, Manganese oxide = 30,000.00mg, Magnesium = 60,000.00mcg, Molybdenum = 100.00mg, Antioxidant = 125,000.00mg, Lysine = 50,000.00mg, Cobalt = 750.00mg, Sodium Selenite 200.00 mcg, Zinc oxide = 40,000.00 mg.
 + C = untreated Control - C = OTC - treated Control T = Pulp L= Leaves SBM = Soybean Meal GNC= Groundnut Cake O. = Oyster OTC = Oxytetracycline

the caudal vein of fish in each treatment from the caudal vein hypodermic syringe (Ejraei et al., 2015; Erfan et al., 2015) into heparinised tubes. Haemoglobin (Hb: g/dl) was determined spectrophotometrically (SM23A, England) by measuring the formation of cyanomethaemoglobin using Drabkin's solution. The absorbance of the solution was measured at wave length 540 nm against Drabkin's solution as a blank. Packed Cell Volume (PCV: %) was determined by placing fresh blood in glass capillary tubes and centrifuging at 10,500 rpm in a microhaematocrit centrifuge for five minutes and the height of the tubes measured using haematocrit reader (Hawksley, SH120). Red Blood Cell (RBC), White Blood Cell (WBC) and platelets were determined (Dacie and Lewis, 1991) using a light microscope (Olympus, Olympus Corporation, China) with improved Neubauer bright-line haemocytometer (Marienfeld, Agoda Company

Pte. Ltd., Germany).

Leucocytes differential cells were analyzed from blood smears were made on slides and left to air-dry. The slides were stained with Giemsa stain (Giemsa Laboratories Ltd. Molbase, Shanghai) using differential stains. Slides were examined using a light microscope (Olympus, Olympus Corporation, USA) with 1000x magnification. Each slide was moved in one direction while the number of neutrophil, heterophil, basophil, monocytes and lymphocytes were counted using blood cell differential counter (Durga, Miniscience, Inc., USA) until a total of 100 leucocytes were counted. The differential cells were expressed in percentage (Harikrishnan et al., 2010). Sera liver enzymes (Alanine aminotransferase, Aspartate aminotransferase and Alkaline phosphatase) were determined by using commercial (Randox) kits, following the procedure described by the manufacturer.

Statistical analysis

Data were analyzed using One-way Analysis of Variance (ANOVA). Duncan multiple range tests was used to compare differences among means at 5% probability level using statistical software SAS (Statistical Analysis System, 2010).

Results

Fish survival and nutrient utilization

After 28 days post-challenge, all experimental groups of the *C. gariepinus* on tamarind-treated diets exhibited significantly lower ($P \leq 0.05$) cumulative mortality index compared to the groups fed with the control diets (Figure 1). Fish on the diet containing oxytetracycline had significantly lower ($P \leq 0.05$) percentage mortality compared to the fish on the untreated control diet. 100% relative percentage survival was exhibited by the groups of fish on 1.5-2.0% TP and 2.0% TL diets. Also groups of fish fed tamarind-treated diets exhibited better ($P \leq 0.05$) level of protection against *Aeromonas hydrophila* than oxytetracycline-treated groups. The feed utilization (Figure 2) was significantly enhanced ($P \leq 0.05$) in tamarind-treated groups than in the control groups.

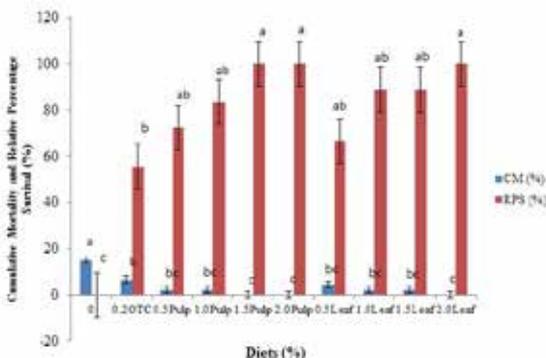


Figure 1: Effect of tamarind-fortified diets on cumulative mortality and nutrient utilization of *Clarias gariepinus* challenged with *Aeromonas hydrophila* after 28 days post-challenge

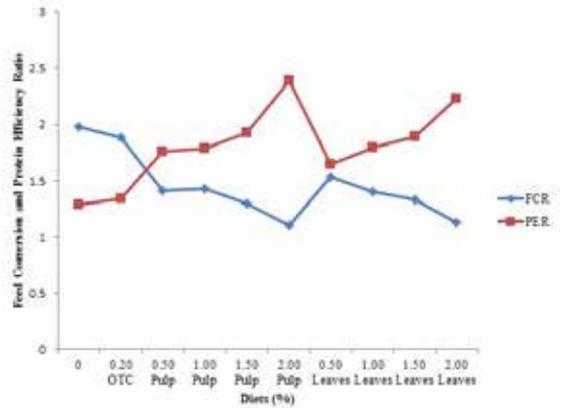


Figure 2: Effect of tamarind-fortified diets on feed utilization of *C. gariepinus* challenged with *Aeromonas hydrophila* after 28 days post-challenge

Haematology

Haematological profiles of the groups of *C. gariepinus* challenged with *Aeromonas hydrophila* are shown in Table 2. The pack cell volume and haemoglobin of the group of fish fed with diets containing TP did not differ significantly ($P > 0.05$) from those fed the control diets. The RBC counts of *C. gariepinus* on 1.5% TP and 1.0% TL were significantly higher than in the control groups. The platelet counts of the fish on diet containing 1.0% TP and TL were significantly lower ($P \leq 0.05$) than the values obtained from those on other diets. Treating the diets with tamarind did not alter the WBC counts of the challenged fish significantly ($P > 0.05$). However, higher values of WBC counts were obtained from tamarind-treated groups compared to the untreated control diets. At 2.0% TP and TL inclusion levels, WBC counts were higher than in the control diets. The lymphocyte count of the fish fed 1.5% TP diet was significantly higher ($P \leq 0.05$) than those fed other TP groups, 1.0 and 2.0% TL and untreated control diets. The neutrophil of the challenged fish fed with OTC-treated diet was significantly lower ($P \leq 0.05$) than fish fed 2.0% TL diet. Inclusion of tamarind in the diets of the challenged *C. gariepinus* did not alter the monocytes, eosinophil and basophil significantly ($P > 0.05$) compared to those on the control diets. Sera aspartate aminotransferase and alkaline phosphatase were significantly ($P \leq 0.05$) altered among the treatment groups with

Table 2: Effect of tamarind-fortified diets on haematological profiles of *Clarias gariepinus* challenged with *Aeromonas hydrophila* after 28 days post-challenge

Diets (%)	PCV (%)	Hb (g/dl)	RBC (x10 ⁶ /µl)	PLA (x10 ⁶ /µl)	WBC (x10 ⁶ /µl)	LYM (%)	HET (%)	MON (%)	EO (%)	BA (%)
0.00	25.50±1.29 ^{abc}	8.68±0.09 ^{abc}	2.18±0.45 ^{cd}	242.50±92.40 ^{ab}	14.19±4.42	52.75±3.61 ^{bc}	41.00±5.57 ^{ab}	3.25±0.97	3.00±0.82	0.00±0.00
0.20OTC	25.75±1.71 ^{abc}	8.60±0.22 ^{abc}	2.11±0.51 ^{cd}	195.75±15.00 ^{abc}	16.83±1.45	59.25±1.71 ^{ab}	33.50±1.29 ^{bc}	4.25±0.50	2.75±0.50	0.25±0.50
0.50P	30.25±4.73 ^{ab}	10.28±3.46 ^a	2.74±1.34 ^{abc}	250.00±71.02 ^a	15.41±1.55	56.75±2.99 ^{bc}	33.75±3.11 ^{ab}	2.50±0.58	2.00±1.15	0.25±0.50
1.00P	27.75±1.26 ^{abc}	8.78±0.09 ^{abc}	2.46±0.95 ^{bcd}	158.75±34.36 ^c	15.56±0.25	59.25±2.36 ^{ab}	37.50±2.22 ^{bc}	3.50±0.58	4.00±2.00	0.5±0.58
1.50P	31.25±1.26 ^a	10.65±0.39 ^a	3.51±0.08 ^a	179.00±48.51 ^{abc}	15.41±2.99	57.00±4.73 ^{bc}	37.50±4.73 ^{ab}	2.75±1.50	2.50±1.29	0.25±0.50
2.00P	24.50±4.20 ^{bc}	8.05±1.45 ^{bc}	2.45±1.04 ^{bcd}	200.50±64.66 ^{bc}	18.54±4.63	54.50±2.64 ^{ab}	39.75±2.09 ^{ab}	2.50±1.00	3.25±0.50	0.00±0.00
0.50L	30.25±1.71 ^{ab}	9.73±0.63 ^{ab}	3.32±0.07 ^{ab}	194.00±40.43 ^{abc}	14.96±5.33	61.50±1.91 ^{ab}	32.25±2.63 ^{bc}	3.00±1.82	3.25±1.26	0.00±0.00
1.00L	30.25±1.71 ^{ab}	9.90±0.65 ^{ab}	3.49±0.29 ^a	154.25±50.56 ^c	14.89±1.29	56.00±4.58 ^{bc}	37.00±5.03 ^{ab}	2.75±1.71	4.25±0.96	0.00±0.00
1.50L	23.75±1.71 ^c	7.53±0.66 ^c	1.72±0.03 ^d	171.00±35.81 ^{bc}	17.14±1.05	66.25±4.99 ^a	27.00±4.97 ^c	2.75±0.96	3.75±1.50	0.25±0.50
2.00L	29.75±4.99 ^{abc}	9.80±1.27 ^{ab}	2.95±0.52 ^{abc}	211.50±34.11 ^{abc}	18.30±1.39	50.00±3.63 ^c	43.50±5.97 ^a	3.75±0.96	2.75±0.50	0.00±0.00

Means with the same letter on the same column are not significantly different at P < 0.05

Packed Cell Volume Hb = Haemoglobin RBC = Red Blood Cell PLA = Platelet WBC = White Blood Cell LYM = Lymphocytes HET = Heterophil MON = Monocyte EO = Eosinophil BA = Basophil

OTC = Oxytetracycline P= Pulp L = Leaves PCV =

the highest in the untreated control group (Figure 3).

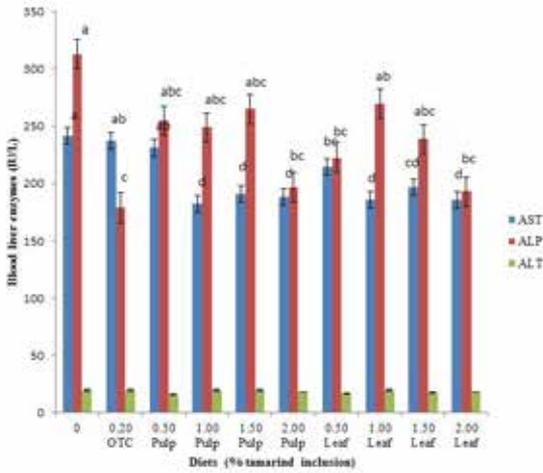


Figure 3: Effects of dietary tamarind pulp and leaf on Blood liver enzymes of *Clarias gariepinus* challenged with *Aeromonas hydrophila* ALT=Alanine aminotransferase AST = Aspartate aminotransferase ALP = Alkaline phosphatase

Discussion

Higher level of protection was exhibited by the groups of fish fed tamarind-treated diet and OTC-treated diet compared to the untreated control group. Significantly higher relative percentage survival demonstrated by *C. gariepinus* on dietary tamarind pulp and leaf against *Aeromonas* infection at 1.5-2.0% inclusion levels than the treated-control group shows the great antimicrobial potentials of these phytochemicals as natural alternative to oxytetracycline. Relative percentage survival above 70 is good potency index of aquaculture vaccine (European Pharmacopoeia, 2002). The RPS of fish fed diets containing 1.0-2.0% TP and TL were above 80%. This might be as a result of the antimicrobial activity of TP, TL and OTC (Adeniyi *et al.*, 2017) and enhanced feed utilization. This observation coincided with the reports of other researchers on the effects of phytobiotics on disease resistance of fish. Higher level of protection was obtained in *Oreochromis niloticus* fed diets containing Echinacea and garlic (Aly and Mohamed, 2010) and green tea (Abdel-Tawwad *et al.*,

2010) against *A. hydrophila*. Goldfish fed diets supplemented with mixed herbal extracts (Harikrishnan *et al.*, 2010) also exhibited higher protection against *A. hydrophila*. Also, feeding carps on diets treated with herbal mixture (Sudagar and Hajibeglou, 2010), willow herb (Pakravan *et al.*, 2011), basil (Amirkhani and Fibarouzkhsh, 2013) and *Euphorbia hirta* leaves extracts (Pratheepa and Sukumaran, 2014) resulted in higher level of protection against *A. hydrophila*. Furthermore, *Mystus montanus* fed powdered *Ocimum tenniflorum*, *Zingiber officinale* and *Allium cepa* (Kumar *et al.* 2014) and rohu fed garlic-based adjuvant (Pujarini *et al.*, 2014) were significantly protected against *A. hydrophila* infection.

Similarly, shrimps fed diets treated with sea weed (Immanuel *et al.*, 2004) and *Withania somnifera* (Praseetha, 2005) as well as *O. mossambicus* fed with herbal additives (Immanuel *et al.*, 2009) against *Vibrio* species exhibited higher survival. Higher percentage survival was also exhibited by African catfish fed diets containing walnut leaf and onion bulb against *Pseudomonas aeruginosa* (Bello *et al.*, 2012b). *O. mossambicus* fed diets treated with cumin (Yilmaz *et al.*, 2013), *Vitex trifolia*, *Strobilanthes crispus* and *Aloe vera* mixtures (Manaf *et al.*, 2016) against *Streptococcus* species also exhibited higher protection against the infection. The observed higher protection of the fish against the pathogens enhanced by the herbal additives compared to synthetic antibiotics further establish their potential as suitable alternative to synthetic antibiotics.

Haematological parameters are indicators of the health status of fish (Fagbenro *et al.*, 2013). WBCs are the blood cells mediating the innate and adaptive responses in animals, providing protection against infection caused by chemical and microbial agents (Mak and Saunders, 2006; Aly and Mohammed, 2010; Pakravan *et al.*, 2011). The improved erythrocytes and leukocytes counts of the groups of challenged fish fed with the tamarind-treated diets, although without significant difference, is an indication of the ability of the phytochemical additives to enhance non-specific immune system of the experimental fish. Also,

the higher *lymphocytes* count in the fish fed tamarind-treated diets and the complementary higher values of heterophil in the groups of fish fed diet containing 2.0% tamarind pulp and leaves might have contributed to the significantly higher protection of these groups against Motile *Aeromonas Septicaemia* (MAS) in the experimental fish compared to the group fed OTC-treated and negative control diets.

Varying haematological observations on the effects of phyto-genic additives on experimentally challenged fish have been reported. WBC counts increased while RBC and Hb reduced significantly when infected goldfish were fed diets containing 0.1-0.2g/kg of mixture of some herbal extracts (Harikrishnan *et al.*, 2010). Improvements in haematological parameters of *A. hydrophila* infected *O. niloticus* fed diets containing green tea (Abdel-Tawwab *et al.*, 2010) and carp fed varying inclusion levels of herbal extract mixture (Sudagar and Hajibeglou, 2010) were also reported. Pakravan *et al.* (2011) also reported lower RBC and PCV of *A. hydrophila* infected carp fed diets containing willow herb extracts while the authors also observed higher WBC counts and insignificant differences in Hb, *lymphocytes*, monocytes and neutrophils. Treating the diets of carp with basil leaves extract also resulted in significant increase in Hb, RBC and WBC counts when challenged with *A. hydrophila* (Amirkhani and Firouzbakhsh, 2013). Increase in activities of blood liver enzymes (aspartate aminotransferase and alkaline phosphatase) in the blood is an indication of reduction in the functional integrity of cell membrane of the liver, resulting to hepatic necrosis (Modesto *et al.*, 2013). The lower activities of these enzymes in fish on tamarind-fortified diets might be because the level of haemorrhage caused by *Aeromonas hydrophila* was significantly reduced and consequently enhanced their survival.

In conclusion, feeding *Clarias gariepinus* with diets fortified with 2.0% tamarind pulp and leaf meal improved some immunity characteristics, reduced blood liver enzymes and consequently gave highest protection against *Aeromonas hydrophila* infection.

Hence, this inclusion level is recommended as alternatives to oxytetracycline in the control of motile *aeromonas septicaemia* in African catfish.

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SERO-PREVALENCE AND ASSOCIATED RISK FACTORS OF CONTAGIOUS CAPRINE PLEUROPNEUMONIA IN GOATS IN SELECTED DISTRICTS OF BALE ZONE PASTORAL AREA, SOUTH EASTERN ETHIOPIA

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Abstract

A cross-sectional study was undertaken from March 2017 - June 2017 to estimate the seroprevalence of CCPP and to assess its putative risk factors in goats in three selected districts of Bale Zone pastoral area, Oromia regional state, South Eastern Ethiopia. A total of 423 sera samples collected from goats were examined for Mccp specific antibodies using Competitive Enzyme-Linked Immuno Sorbent Assay (c-ELISA) test. Out of the total examined sera, 147 samples were positive for CCPP, giving an overall seroprevalence of 34.75% in the study areas. Seroprevalence rates of 41.79%, 33.64% and 28.82% were recorded in Sawena, Dawe Kachan and Madda Walabu districts, respectively. However, there was no statistical significant difference ($\chi^2=1.743$; $P=0.418$) in CCPP antibodies seropositivity among the three districts. Moreover, no significant difference ($p>0.05$) was observed between seropositivity and sex and body condition scores. Multivariable logistic regression statistical analysis revealed that age category, flock size, newly introduced goats and accessibility to veterinary service delivery were significantly associated ($p<0.05$) with the CCPP seropositivity. Consequently, adult age groups (OR=2.496), large flock (OR=3.416), inaccessibility to veterinary service (OR=2.508) were at higher risk of infection with contagious caprine pleuropneumonia than their counter groups ($p<0.05$). All of the pastoralists responded as their goats frequently contacted with other flocks, uncontrolled movement of goats and congregation at watering point that implied potential risk factors which played a role for the existence and transmission of the disease in the study districts. The present seroepidemiological investigation indicated that CCPP is the major goat health problem in the area which warrants implementation of appropriate and integrated control and prevention programs to mitigate the disease impact through multidirectional approaches.

Key words: Bale zone, c-ELISA, CCPP, Goats, pastoral area, Risk Factors, sero-prevalence

SEROPREVALENCE ET FACTEURS DE RISQUE ASSOCIES DE LA PLEUROPNEUMONIE CONTAGIEUSE CAPRINE DANS CERTAINS DISTRICTS DE LA ZONE PASTORALE DE BALE DANS LE SUD-EST DE L'ÉTHIOPIE

Resume

Une étude transversale a été réalisée de mars 2017 à juin 2017 en vue d'estimer la séroprévalence de la PPCC et d'évaluer ses facteurs de risque putatifs chez les chèvres de trois districts sélectionnés de la zone pastorale de Bale dans la région d'Oromia (sud-est de l'Éthiopie). Un total de 423 échantillons de sérums prélevés sur des chèvres a été examiné pour déterminer les anticorps spécifiques à Mccp en utilisant un test de dosage immuno-enzymatique de compétition (c-ELISA). De l'ensemble des sérums examinés, 147 échantillons étaient positifs pour la PPCC, soit une séroprévalence globale de 34,75% dans les zones étudiées. Des taux de séroprévalence de 41,79%, 33,64% et 28,82% ont été enregistrés respectivement dans les districts de Sawena, Dawe Kachan et Madda Walabu. Cependant, on n'a pas noté de différence statistique significative ($\chi^2 = 1,743$; $P = 0,418$) au niveau de la séropositivité des anticorps anti-PPCC entre les trois districts. De plus, aucune différence significative ($p > 0,05$) n'a été observée entre la séropositivité et le sexe

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et l'état corporel. L'analyse statistique multivariable de la régression logistique a révélé que la catégorie d'âge, la taille des troupeaux, les chèvres nouvellement introduites et l'accessibilité des services vétérinaires étaient significativement associés ($p < 0,05$) à la séropositivité pour la PPCC. En conséquence, les groupes d'âge adulte (OR = 2,496), les grands troupeaux (OR = 3,416), l'inaccessibilité du service vétérinaire (OR = 2,508) sont des facteurs de risque plus élevés d'infection par la pleuropneumonie contagieuse caprine par rapport aux autres groupes ($p < 0,05$). Tous les éleveurs ont répondu que leurs chèvres étaient souvent en contact avec d'autres troupeaux, que des mouvements incontrôlés de chèvres et des rassemblements aux points d'abreuvement étaient des facteurs de risque potentiels qui ont joué un rôle dans l'existence et la transmission de la maladie dans les districts étudiés. L'enquête séro-épidémiologique actuelle a indiqué que la PPCC est le principal problème sanitaire des chèvres dans la région, ce qui justifie la mise en œuvre de programmes de contrôle et de prévention appropriés et intégrés pour atténuer l'impact de la maladie par des approches pluridirectionnelles.

Mots-clés : zone de Bale, c-ELISA, PPCC, chèvres, zone pastorale, facteurs de risque, séroprévalence

Introduction

CCPP is one of the most important infectious diseases of goats that pose a significant threat to production capacities of this animal. Contagious caprine pleuropneumonia (CCPP) is a highly contagious, infectious fibrinous pleuropneumonia of goats caused by *Mycoplasma capricolum* subspecies *capripneumoniae* (Mccp), characterized by fever, respiratory distress with coughing, nasal discharge, high morbidity and mortality rates (Radostitis *et al.*, 2007; Rurangirwa and McGuire, 2012). It is highly contagious severe respiratory disease. It is one of those rampant and highly contagious animal diseases with potential of rapid spread irrespective of national borders (Gelagay *et al.*, 2007).

CCPP is a major cause of economic losses in the goat industry globally as these intracellular bacteria can infect domestic as well as wild breeds of goat (Nicholas, 2002a; Arif *et al.* 2007; Ostrowski *et al.* 2011), with 100% morbidity and 60–80% mortality rates, reduced milk yield, cost of treatment and vaccination of the disease and indirect loss due to the imposition of trade restrictions (Rurangirwa and McGuire, 2012; OIE, 2009). It is a devastating disease of goats included in the list of notifiable diseases of the Office International des Epizooties (OIE) (MansoSilvan *et al.* 2011). It is a major threat to the goat farming industry in developing countries (Lorenzon *et al.*, 2002) and is pandemic in Africa, the Middle East and Asia (Manso-Silvan *et al.*, 2011; Nicholas and

Churchward, 2012).

The presence of CCPP in Ethiopia had been suspected since 1983 and was confirmed later in 1990 by isolation and identification of Mccp (F-38) following an outbreak of CCPP in Ogaden, Eastern Ethiopia (Thiaucourt *et al.*, 1992). Since then the disease has been known to be endemic in different regions of the country (Sharew *et al.*, 2005). Outbreaks of CCPP have been reported from almost all regions of the country, especially from the low lands areas, which are known goat rearing regions (APHRD, 2010). It is more prevalent in the arid and semi-arid lowland area of the Rift Valley, Borena range lands, South Omo, Afar and other pastoral areas and imposes severe losses in goat population (Gezahegn, 2006). The frequently reported outbreaks of CCPP in Ethiopia almost certainly represent an underestimate as this disease is having a major socio-economic impact in the country (Nicholas and Churchward, 2012). Every year an outbreak of respiratory diseases occurs in the goat population with an alarming rate of morbidity and mortality but the lack of advanced techniques is a big hindrance in its proper diagnosis.

In present day of Ethiopia, there is a national drive to alleviate the existing food deficit by devising different agricultural strategies including improvements of the productivity of livestock sector by controlling some of the major diseases through regular monitoring to achieve transformation plan. Knowledge and understanding of the epidemiological profile of animal diseases, is critical for evaluating and

addressing the veterinary health needs of the livestock population of a local area (Khan, 2010). Hence, the disease deserves special attention as it diminishes the capacity of goats to achieve their inherent potential level of production for any given feeding and management regimen.

Dawe Kachan, Sawena and Mada Walabu districts are among the known pastoral areas of Bale Zone and possess huge live stock resource. Goats being an important component of live stock play a significant role in supporting the pastoralists' livelihood in the area. Despite the high population density of goats in the region, little attention has been given to the health problems of goats. The exact picture, dynamics and distribution of CCPP in the areas is not well documented. Hence, there is scarcity of well documented information on the current status and distribution of the disease in the area. In addition, most livestock disease outbreaks, particularly in more remote parts of the country, remain undiagnosed and therefore, information on the sero-epidemiology and significance of CCPP can only readily be obtained through serological studies in order to apply control measures by application of prevention measures and veterinary extension.

Hence, this study was conducted to determine the seroprevalence of CCPP that potentially affect goat production system and to assess potential risk factors that played role for the existence of the disease in goat population in Dawe Kachan, Sawena and Mada Walabu districts of Bale Zone pastoral area, Oromia regional state, south eastern Ethiopia. Therefore, our study complemented the paucity of information about seroprevalence of CCPP in goat production system of the area.

Materials and Methods

Description of the Study Area

The study was conducted in three selected districts of Bale Zone pastoral areas, namely: Dawe Kachan, Sawena and Mada Walabu districts of Bale Zone, Oromia Regional State, south east of Ethiopia. The three study districts were selected purposely to represent the goat rearing districts of the zone based on

number of goats they possess and ecological conditions.

Dawe Kachan district is located at a distance of 565km south east of Addis Ababa, the capital city of Ethiopia. The mean annual temperature of the district is 17.5 oc. The maximum and minimum temperatures are 25oc and 10oc, respectively. The annual rainfall of the area ranges from 400-1200mm with mean annual rainfall of 800mm. As majority of the population of the district are pastoralists, their livelihoods are mainly depend on livestock rearing. There are about 89,184 bovines, 35,563 sheep, 100,725 goats, 5,951 equines, 20,289 camels and 10,472 chickens (DKDPDO, 2016).

Sawena district is located at a distance of 623 kms south east of Addis Ababa. The mean annual rainfall is 375 mm where as the lowest and highest rainfall is 250mm and 500mm, respectively. The mean annual temperature of the district is 35oc. The lowest temperature is 30oc and highest is 40oc, respectively. Livestock rearing is the main stay as livelihood in the district. There are about 52,850 bovines, 29,500 sheep, 61,990 goats, 10,630 equines, 19,540 camels and 18,044 poultry (SDPDO, 2016).

Mada Walabu district is located at 430 km southeast of Addis Ababa. The mean annual temperature of the district is 29.5oc. The lowest temperature is 21oc and highest is 38oc, respectively. The mean annual rainfall is 701.5 mm where as the lowest and highest rainfall is 628mm and 775mm, respectively. From early days, livestock rearing has played an important role in the life of district population. In the rural and lowland areas of the district, rearing and breeding is the main stay of the people. There are about 213,962 bovines, 11,901 sheep, 233,020 goats, 14,179 equines, 66,644 camels and 133,249 poultry (MDPDO, 2016).

Study animals

The study animals were local breeds of goats managed under extensive pastoral production system by district pastoralists. Randomly selected goats with no history of vaccination for CCPP was used as a source of

serum samples regardless of age, sex or status of health. Goats of both sex and various age groups were sampled. Age of the animals was determined based on owners' information and dental eruption (Bekele *et al.*, 2011). The study animals consisted of 423 goats selected by simple random sampling method from smallholder pastoralists.

Study design

A cross sectional type of study supported by questionnaire survey and serological tests was carried out to determine the sero-prevalence of contagious caprine pleuropneumonia in goats and to assess potential risk factors that played role for the existence of the disease in goat population in Dawe Kachan, Sawena and Madda Walabu districts of Bale Zone pastoral area, Oromia regional state, south eastern Ethiopia. Blood samples were collected once from jugular vein of individual goats. Sera samples were tested using competitive enzyme linked immunosorbent assay (c-ELISA) according to the standard test procedure at National Veterinary Institute (NVI) of Ethiopia.

Sample size and sampling method

The sample size for goats in the study area was calculated using a method recommended by Thrusfield (2007):

$$n = \frac{1.96^2 \times P_{exp} (1 - P_{exp})}{d^2}$$

Where:

n = required sample size,
P_{exp} = expected prevalence, and
d = desired absolute precision

Since there is no reasonable research done in these areas so far; the sample size was calculated using a method recommended by Thrusfield (2007), with 95% confidence interval, at 5% desired absolute precision and expected prevalence of 50%. Accordingly; the total numbers of sample required for this study was 384 goats, but to increase the precision level a total of 423 goats were sampled for

investigation.

The three districts from Bale zone pastoral areas was selected purposively based on concentration of goats and ecological conditions. A total of 9 kebeles (3 from Dawe Kachan, 2 from Sawena and 4 from Madda Walabu district) were selected purposely based on accessibility to road, feasibility to sample collection and number of goats they possess (higher concentration of goats). Prior to commencement of the study, list of all households of those kebeles (sampling frame) were obtained from districts' pastoral development office. Then, list of households possessing goats were sorted with the help of agricultural development agents (sampling frame). Identification numbers were given for the sorted goats. Then, simple random sampling method was applied to select 423 goats. In addition, proportionality of incorporating goats in the sample was applied as per the population size of each district and kebeles (Table 1).

The sample size of households was determined by the formula given by Arsham (2007) for survey studies.

$$N = 0.25 / (SE)^2$$

Where: N= sample size and SE= standard error of the proportion. Assuming the standard error of 3.5% at a precision level of 5%, and the confidence interval of 95%, 204 households possessing goats were selected by a simple random sampling method for interview. Proportionality of households in the sample was applied as per the population size of each district and kebeles.

Inclusion criteria:

Goats of all ages with no history of vaccination for CCPP were included.

Exclusion criteria:

Apparently healthy goats with history of CCPP vaccination were excluded.

Questionnaire survey

Semi-structured questionnaires was prepared, pre-tested and adjusted by translating

Table 1: Proportional allocation and number of animals sampled from each districts.

Districts	Total No. of goat population	Total No. of households	No. of kebeles sampled	Total No. of goats sampled
Dawe Kachan	100,725	11,884	3	107
Sawena	61,990	6,177	2	67
Madda Walabu	233,020	16,753	4	249
Total	395,735	34,814	9	423

in to Afan Oromo and administered by the interviewer who speaks the same language (Afan Oromo) with the participant pastoralists. Likely sampling techniques were followed the same procedures using proportionality of goats that were sampled. Thus, 204 pastoralists (goat owners) were selected for detail interviews using semi-structured questionnaires. It was focused on the potential risk factors that played a role for the existence and transmission of the disease in the study areas. It was conducted after carefully explaining the purpose of the work to the interviewees.

Sample Collection and Laboratory Tests

Sera collection: Animals were restrained by animal handlers and 5-7 ml of blood was collected directly from jugular vein of each animal using sterile plain vacutainer tubes with disposable 18-20 gauge hypodermic needles. The samples were allowed to stand in slant position for 2-6 hours at room temperature until sufficient amount of clot is formed. Then the samples were placed in the refrigerator at +4oc till serum extracted. The serum was separated in to cryovials. All the samples were labeled with animal identification and stored temporarily at -20°C. Transportation to the National Veterinary Institute (NVI) was done by placing packed sample using an ice box containing preformed blocks of ice.

Serological tests: collected sera samples were examined for the presence of specific antibodies against Mccp by using c-ELISA in serology laboratory of National Veterinary Institute (NVI). The c-ELISA test was employed using Mccp antibody test kit. It is used to determine sero-prevalence of the disease. Those samples with percentages of

inhibition greater than or equal to 50% were considered positive for the presence of Mccp antibodies.

Data analysis

The data collected form field were entered into computer using Microsoft Excel and transferred to STATA version 11.0 for Windows (Stata Corp. College Station, TX, USA) for statistical analyses. All categorical variables were expressed in percentages. Individual animal prevalence was defined as the number of c-ELISA positive animals per total number of animals tested. The association sero-positivity with potential risk factors (origin, age, sex, flock/ herd size, contact with other herds, introduction of new animals and body condition score and etc.) were computed using chi-square (χ^2) and logistic regression. A p-value <0.05 was considered statistically significant. Those risk factors which showed associations with the prevalence of contagious caprine pleuropneumonia were further analyzed by logistic regression. The independent or explanatory variables considered in the model were those that showed statistical significance (< 0.2). Odds ratio was calculated to see the degree of association of the risk factors with the occurrence of contagious caprine pleuropneumonia.

Results

Pastoralists’ perception and knowledge on CCPP in the study districts

A 100% response was found when selected households were visited and the pastoralists were interviewed. Questionnaires were administered to 204 individual

pastoralists possessing goats to investigate the risk factors that played role in transmission of CCPP from goat to goat in the study areas as depicted in Table 2. Common goat diseases in the study area in order of importance as listed by the pastoralists included: PPR, CCPP, tick infestations, mange, pox, orf and endoparasites were highlighted as the major health challenges in goats in the study districts. Of the 204 pastoralists who responded to the questionnaire, 113 (55.39%) were aware of CCPP and gave the local name of CCPP as “sombessa”. It was observed that 89.22%

of the goat owners responded that source of replacement stock was raised from own flock. The present questionnaire survey revealed that 100% of the households interviewed were responded as their goats frequently contacted with other flocks, uncontrolled movement of goats and congregation at watering point. Moreover, all of the goat owners responded as there was uncontrolled movement of goats in the area. Furthermore, all of the respondents (pastoralists) interviewed disclosed that there was no CCPP test and provision of vaccine for their goats.

Table 2: Knowledge, attitudes and practices (KAP) of goat owners about CCPP (N=150).

Factors	Category	No. of respondents	Percentage of respondents (%)
Level of education	Illiterate	111	54.41
	Literate	93	45.59
CCPP awareness	No	91	44.61
	Yes	113	55.39
CCPP vaccine	No	204	100.00
	Yes	-	-
CCPP test	No	204	100.00
	Yes	-	-
Culling of sick goats	No	198	97.06
	Yes	6	2.94
Contact with other flock	No	-	-
	Yes	204	100.00
Replacement flock	Raised in own herd	182	89.22
	Buy in	15	7.35
	Mixed	7	3.43
Uncontrolled movement of goats in the area	No	-	-
	Yes	204	100.00
Congregation at watering point	No	-	-
	Yes	204	100.00
Overcrowding	No	117	57.35
	Yes	87	42.65

Overall seroprevalence of CCPP in the study districts
 Out of 423 goat sera samples examined, 147 were found to be positive for Mccp specific antibodies using c-ELISA, giving an overall sero-prevalence of 34.75% in the study areas (Table 3). The sero-prevalence was highest in Sawena district (41.79%) followed by Dawe Kachan (33.64%) and the lowest was recorded in Madda Walabu district (28.82%). There was no significance difference ($P > 0.05$) in CCPP seroprevalence among three districts as depicted in Table 3.

Seropositivity of CCPP within selected kebeles of the study districts

The number of goats tested within each 9 study kebeles and proportions found to be positive for CCPP are depicted in Table 4. Of the six 9 kebeles selected, Micha was with the highest CCPP seroprevalence (51.61%) while Barisa was the least (23.08%). There was no significant association between the selected kebeles of the studied districts and Mccp seropositivity.

Table 3: Seroprevalence of CCPP in the three districts of Bale zone pastoral area, Ethiopia.

District	Sample tested	Sample positive	Seroprevalence (%)
Sawena	67	28	41.79
Dawe Kachan	107	36	33.64
Madda Walabu	249	83	28.82
Overall	423	147	34.75

Pearson $\chi^2 (2) = 1.743; Pr = 0.418; Pr = Precision value$

Table 4: Seroprevalence of CCPP within the selected kebeles of the districts.

District	Sampled kebeles	No. of sera tested	Samples positive	Prevalence (%)
Sawena	Micha	31	16	51.61
	Biliso	36	12	33.33
Dawe Kachan	Hantulle Basaka	42	17	40.48
	Meo	31	8	25.81
	Arda Orru	34	11	32.35
Madda Walabu	Madda	72	27	37.50
	Hora Kore	59	19	32.20
	Oda Boji	66	25	37.88
	Barisa	52	12	23.08
Total		423	147	34.75

Table 5: Chi-square analysis of association of the putative risk factors with CCPP seropositivity.

Variables	Category	No tested	No positive (%)	$\chi^2 (p\text{-value})$
Origin	Sawena	67	28 (41.79)	1.743 (0.418)
	Dawe Kachan	107	36 (33.64)	
	Madda Walabu	249	83 (28.82)	
Age	Young	92	18 (19.57)	11.958 (0.001)
	Adult	331	129 (38.97)	
Sex	Male	154	51 (33.12)	0.905 (0.342)
	Female	269	96 (35.69)	
Flock size	Small	48	8 (16.67)	

Variables	Category	No tested	No positive (%)	χ^2 (p-value)
	Medium	196	62 (31.63)	11.157 (0.001)
	Large	179	77 (43.02)	
New introduction	Present	67	31 (46.27)	4.657 (0.031)
	Absent	356	116 (32.58)	
Body condition scores	Poor	113	47 (41.59)	3.416 (0.181)
	Medium	208	69 (33.17)	
	Good	102	31 (30.39)	
Accessibility to veterinary service	Accessible	319	94 (29.47)	15.981 (0.000)
	Inaccessible	104	53 (50.96)	

Table 6: Multivariable logistic regression analysis of risk factors with CCPV sero-positivity.

Factor	Mccp specific antibodies test result		Odds ratio		P value
	Total No. tested	Prevalence (%)	COR (95%CI)	AOR (95% CI)	
Age					
Young	92	18 (19.57)	1	1	
Adult	331	129 (38.97)	2.625 (1.499, 4.598)	2.496 (1.399, 4.454)	0.002
Flock size					
small	48	8 (16.67)	1	1	
Medium	196	62 (31.63)	2.313 (1.022, 5.235)	2.073 (0.892, 4.818)	0.090
Large	179	77 (43.02)	3.775 (1.671, 8.525)	3.416 (1.472, 7.927)	0.004
New introduction					
Absent	356	116 (32.58)	1	1	
Present	67	31 (46.27)	1.782 (1.050, 3.024)	1.644 (0.942, 2.871)	0.080
Accessibility to veterinary service					
Accessible	319	94 (29.47)	1	1	
Inaccessible	104	53 (50.96)	2.487 (1.580, 3.915)	2.508 (1.563, 4.025)	0.000

COR, Crude Odds Ratio; AOR, Adjusted Odds Ratio; CI, Confidence Interval; 1, Reference.

Chi-square analysis of association of the putative risk factors with CCPV

A Chi-square analysis revealed that seroprevalence of CCPV was significantly associated with the age groups, flock size and

accessibility to veterinary service ($P < 0.001$) and new introduction ($P < 0.05$), however its association with origin, sex and body condition scores was not statistically significant ($P > 0.05$) (Table 5).

Multivariable logistic regression analysis of putative risk factors associated with CCPP

The results of logistic regression analysis of the association of different risk factors with the seroprevalence of CCPP are depicted in Table 6. Analysis of the association of associated risk factors with the seroprevalence using multivariable logistic regression showed that older goats (OR = 2.496, 95%CI: 1.399, 4.454), large flock size (OR = 3.416, 95%CI: 1.472, 7.927) and inaccessibility to veterinary infrastructure (OR = 2.508, 95%CI: 1.563, 4.025) were at higher risk of infection with CCPP as compared to younger goats, small flock size and accessibility to veterinary infrastructure, respectively.

Discussion

The ranking of CCPP by pastoralists in the study area as second among the health challenges of their goats concurs with the report of Mekuria et al. (2008) from Southern Ethiopia participatory investigation of CCPP where farmers ranked the disease as major constraint in goat production. The study discovered the local name of CCPP to be “sombessa” in the study areas. Communities in this study had a higher level (55.32%) of awareness of CCPP and comparable with the work of Tambuwal et al. (2011) and Billy et al. (2015) who reported a high level in the two transboundary states of north western Nigeria (65.0%) and in Kaduna State of Nigeria (88.9%), respectively. Uncontrolled movement of goats in the area, congregation at watering point, overcrowding and frequent contact with other flock observed in the present questionnaire survey were previously reported to favor spread of CCPP when animals meet at watering points and grazing areas because of increased contact rates between infected goats and naive ones essential for effective transmission of Mccp (Thiaucourt et al., 1996; Mekuria and Asmare, 2009).

The overall seroprevalence of CCPP in the study areas was 34.75%. The current finding was in close agreement with the reports of Lakew et al. (2014), 31.6% in Borana pastoral area,

Southern Ethiopia; Birhanu et al. (2009), 32.68% in Afar and Tigray regions of Ethiopia; Awati and Chavhan (2013), 35% from Karnataka, India. The present recorded seroprevalence rate was higher than the results of Mekuria and Asmare (2009), 18.61% in south Omo and Arbaminch of southern Ethiopia; Yousuf et al. (2012), 4.92% in and around Dire Dawa, Eastern Ethiopia. However, the current report was lower than the seroprevalence rate of 50% (Mebratu, 1986), 51.5% (Gezahegn, 1993), 53% (Roger and Zekaries, 1996) in east Shoa, Melkasedi (Hararge) and Gewane (Afar), respectively. The variation in seroprevalence rate of CCPP reported by various investigators might be due to temporal and spatial factors associated with sampling, the situation of the disease during the time of sampling, management practices, stocking density, level of accessibility to veterinary services, agro-ecological conditions, differences in study methods and materials and the variation in the specificity and sensitivity of the different serological tests employed.

The current study revealed that origin of goats was not significantly associated with CCPP seropositivity ($P > 0.05$), however, the results showed highest seroprevalence rates in Sawena (41.79%) compared to Dawe Kachan (33.64%) and Madda Walabu (28.82%). The possible reasons for the variations in CCPP seroprevalence among the study areas might be related to the difference in management practices, agro-ecological factors and presence of carrier animals in the districts.

Higher seropositivity of Mccp specific antibodies in older goats (38.97%) compared to younger ones (19.57%). The differences in CCPP seroprevalence among age categories were significantly associated ($p < 0.001$). Odds ratio indicated that older age group were 2.496 times more likely to be exposed to CCPP than younger age category. This is in agreement with the previous report of Regassa et al. (2010) and Bekele et al. (2011) and Yousuf et al. (2012) who revealed statistical significant association among age groups. The higher prevalence of the disease in older age goats as compared to the younger ages might be explained by the fact that humoral immunity

to CCPP is influenced by age. Furthermore, as age increases, the goats are often repeatedly exposed to different stress conditions (due to malnutrition, movement over long distances, adverse weather conditions and the likes) which can predispose the animal to the disease. Movements of goats especially adults away from homestead for search of water and pasture probably exposes them to infection, hence, chances of being infected increases due mixed goats from different households during these long distance movements. Moreover, they also tend to be infected repeatedly. Therefore, the probability to be seropositive in older ages for CCPP would be high as compared to young goats. Seropositivity may be high in adult but mortality is higher in young animals than in adults (Radostits *et al.*, 2007).

The present study disclosed that sex of the animal was not significantly associated with Mccp specific antibodies seropositivity. This result was comparable with the previous reports of Mekuria and Asmare (2009), Bekele *et al.* (2011) and Lakew *et al.* (2014). It has also been reported that CCPP is highly contagious and fatal disease affecting susceptible goats of both sexes (OIE, 2008).

Higher seroprevalence of CCPP was recorded in large flock size (43.02%) compared to medium (31.63%) and small (16.67%) ones with statistically significant association ($p < 0.001$) between them. The values of odds ratio indicated that large flock size was about 3.4 times more likely to be seropositive than small herd size. Likewise, Solomon (2005) and Bekele *et al.* (2011) reported that large flocks were more affected with CCPP than smaller ones. This might be attributed to the fact that larger stocking density (increasing number of susceptible population) and overcrowding as a factors which favor spread and occurrence of the disease within the flock. Increased contact rates between infected goats and naive ones are essential for effective transmission of Mccp. The disease is readily contagious and a short period of contact is enough for successful transmission through coughing (Thiaucourt and Bolske, 1996; OIE, 2009).

It was observed that higher CCPP seropositivity in newly introduced goats with statistically significant association ($p < 0.05$). OIE (2009) reported that the outbreak of the disease follows the introduction of an infected animal into a group of susceptible goats. Moreover, Disease outbreak may occur after animal transportation over a long distance (OIE, 2009; Thiaucourt and Bolske, 1996).

In this study the seroprevalence observed among the distance categories was significant and the prevalence of CCPP was higher in distant communities. Previous study by Sori (1999) and Bekele *et al.* (2011) in the area has indicated that an impact of insufficient veterinary service in mobile livestock production system increases the spread of diseases. This finding could be attributed to the inaccessibility of such areas to veterinary services targeted to the disease. In pastoral areas, the users of the static clinical service and veterinary extension system are mostly those communities residing within the distance of 10kms to 15kms from veterinary service centers. Therefore, most areas beyond this radius do not have access to the service. Therefore, the extension of veterinary service to such and inaccessible corners is very limited (MoARD, 2003).

Conclusion

The present study revealed that seroprevalence of CCPP is high in the study areas. The present finding indicates endemicity of the infection in the study areas. It was observed that age, flock size, newly introduced goats and distance from veterinary service centre were the major risk factors associated with the occurrence of CCPP. Determination of seroprevalence of the disease and potential risk factors could pave the way in devising control and prevention strategies. Moreover, all of the pastoralists responded as their goats frequently contacted with other flocks, uncontrolled movement of goats and congregation at watering point that implied potential risk factors which played a role for the existence and transmission of the disease in the study

districts. The present seroepidemiological investigation indicated that CCPP is the major goat health problem in the area which warrants appropriate measures to be in place towards the prevention and control of the disease. Advanced molecular methods should be conducted to isolate and type the pathogenic causative agent of CCPP in study districts.

Conflict of Interests

The authors have not declared any conflict of interests.

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CHEMICAL COMPOSITION AND IN VITRO DRY MATTER DIGESTIBILITY OF MORINGA OLEIFERA, ASPILIA AFRICANA AND AZADIRACHTA INDICA LEAVES USING RABBIT INOCULUM

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Abstract

The effect of caecal inoculum of rabbit on in vitro gas production and dry matter digestibility of *Moringa oleifera*, *Azadirachta indica* and *Aspilia africana* leaf meals at different levels of 0%, 15% and 30%. Leave samples were analyzed for crude protein (CP), lignin (ADL), acid (ADF) and neutral (NDF) detergent fibres. The gas production was continuously measured by incubating samples in buffered solution for 96 hr. Cumulative gas production was recorded from 2 - 96 hrs of incubation periods using non-linear regression equation. Concentrations of CP (13.66-26.26 %DM), NDF (48.00-60.00 %DM) and ADF (36-54 %DM), ADL (48.00 - 60.00 %DM) differed among species. Mineral results showed that K (417.52 ppm), Fe (10.01 ppm), Cu (0.22 ppm) and Zn (0.88 ppm) were highest in *A. Africana*. Highest ($p < 0.05$) metabolizable energy (3.94 MJ/kg-1), organic matter digestibility (28.06%) and short chain fatty acids (0.05 μ mol) estimated from the in vitro gas production parameters were observed at 30% MOM. The present experiment has shown that the leaves of the plants improve the efficiency of protein digestion in the caecum and are sufficient to meet the requirements of rabbits.

Keywords: *Moringa oleifera*, *Azadirachta indica*, *Aspilia africana*, in vitro gas production and Rabbits

COMPOSITION CHIMIQUE ET DIGESTIBILITE INVITRO DE LA MATIERE SECHE DE FEUILLES DE MORINGA OLEIFERA, D'ASPILIA AFRICANA ET D'AZADIRACHTA INDICA UTILISANT L'INOCULUM DE LAPIN

Resume

L'effet de l'inoculum caecal du lapin sur la production de gaz in vitro et la digestibilité de la matière sèche de feuilles de *Moringa oleifera*, *Azadirachta indica* et *Aspilia africana* à différents niveaux de 0%, 15% et 30% a été étudié. Les échantillons de feuilles ont été analysés pour déterminer les teneurs en protéine brute (CP), lignine (ADL), fibres à détergent acide (ADF) et à détergent neutre (NDF). La production de gaz a été mesurée en continu en incubant des échantillons dans une solution tamponnée pendant 96 heures. La production cumulative de gaz a été enregistrée entre 2 et 96 heures de périodes d'incubation en utilisant une équation de régression non linéaire. Les teneurs en CP (13,66-26,26% DM), NDF (48,00-60,00% DM) et ADF (36-54% DM), ADL (48,00-60,00% DM) étaient différentes entre les espèces. Les résultats minéraux ont montré que les teneurs en K (417,52 ppm), Fe (10,01 ppm), Cu (0,22 ppm) et Zn (0,88 ppm) étaient les plus élevées chez *A. Africana*. L'énergie métabolisable la plus élevée ($p < 0,05$) (3,94 MJ/kg-1), la digestibilité de la matière organique (28,06%) et les acides gras à courte chaîne (0,05 μ mol) estimées à partir des paramètres de production de gaz in vitro ont été notées à 30% de MOM. L'expérience a montré que les feuilles des plantes améliorent l'efficacité de la digestion des protéines dans le caecum et sont suffisantes pour répondre aux besoins des lapins.

Mots-clés: *Moringa oleifera*, *Azadirachta indica*, *Aspilia africana*, production de gaz in vitro et lapins

Introduction

Livestock production depends on the availability and quality of feed provided to the animal. For ruminant animals, forages such as grasses and legumes are the main source of feeds to satisfy their nutritional requirements, either for maintenance, production or reproduction (Sarnklong *et al.*, 2010; Kim *et al.*, 2012). Leaves from some tropical legumes and plants notably *Moringa (Moringa oleifera)*, Bush marigold (*Aspilia africana*) and neem leaf (*Azadirachta indica*) have attracted attention for their ability to provide large quantity of high quality forages all year round as well as their ability to maintain a sustainable environment and soil fertility through nitrogen fixation (Omoikhoje *et al.*, 2006; Ogbuewu, 2008). In rabbits, Calabro *et al.*, (1999) and Stanco *et al.* (2003) proposed the GPT to predict the nutritive value of rabbit diets, using fresh rabbit caecal content as inoculum. Rabbit has a low utilization of fibrous fraction due to the rapid passage of feed through the gastro intestinal tract. However, through the caecotrophic activity, the digestibility of nutrients, especially protein, is incremented (Machado *et al.*, 2012). Determination of in vitro gas production method is an accurate and reliable tool for the estimation the nutritive quality of different classes of forages (Njidda and Nasiru, 2010) and evaluation of fermentation potentials of forages or feeds microbial nitrogen supply and animal performance (Blummel and Orskov, 1993). The in vitro gas production technique (IVGPT) is based on the fact that the anaerobic digestion of carbohydrates by rumen or caecal micro-organisms produces gas (CO₂, CH₄ and traces of H₂) and volatile fatty acids (acetate, propionate, butyrate) gas production can therefore, be measured to estimate the rate and extent of feed degradation.

This study was designed to assess the nutritive values of *Moringa oleifera*, *Aspilia africana* and *Azadirachta indica* based on their: chemical analysis, mineral composition, in vitro gas production and dry matter degradation using rabbit inoculum.

Materials and methods

Collection and preparation of the plant materials

Azadirachta indica, *Moringa oleifera* and *Aspilia africana* were used for the study. Leaf samples from each of the plants were harvested during dry season within the arboretum of Ogun State, Nigeria. The leaves were air - dried and milled to 2 mm particle size to obtain a leaf meal product for determination of chemical analysis of the plants according to AOAC (2005). Non-fibre carbohydrate (NFC) was calculated as (NFC= 100- CP- EE- NDF- ASH). Neutral detergent fibre (NDF), Acid detergent fibre (ADF) and Acid detergent lignin (ADL) were determined according to Van Soest *et al.* (1991). Concentration of Ca, K, Mg, Cu, Zn, and Fe were determined with atomic absorption spectrophotometry (Fritz and Schenk, 1979).

Experimental design

Seven samples were formulated in a 2 x 3 + 1 factorial arrangement. The samples constitute of 0% (Concentrate), 15% and 30% of *Moringa oleifera* (MOM) *Azadirachta indica* (AIM) and *Aspilia africana* (AAM) leaf meal with concentrate.

Inoculum preparation

Selection of animals and preparation of inoculum were carried out according to the method used by Calabro *et al.* (1999). Freshly collected caecum contents of rabbits were used to prepare the inoculum. Five 90-day old New Zealand Red rabbits showing normal weight gain were randomly selected prior to slaughtering. The animals were slaughtered at about 11.00 am and the caecum was isolated by tying off the two ends to prevent movement of the digesta. The buffer solution was mixed together at ratio 1:50w/v with the caecum contents and with the aid of a dispenser. The suspension was then squeezed through six layers of sieve gauze to constitute the inoculum and the filling of the syringes were not more than 45 min after slaughtering.

In vitro gas production was determined according to the procedure described by Menke and Steingass (1988). All manipulation (mixing,

weighing, diluting, filtering through gauze and constitution of inoculum) and the filling of syringes were done under the constant flushing of oxygen free CO₂ gas to assured anaerobic conditions.

Syringes were filled with 30 ml of inoculum consisting of 10 ml of fluid caecum and 20 ml of buffer solution. Two blanks containing 30 ml of inoculum only. The syringes were placed in an incubator with temperature regulated at 39° C.

Gas measurements and analysis at end of incubation

At the end of the fermentation period OMD and pH was determined. The volume of gas produced in each syringe was recorded at 2, 4, 6, 8, 12, 18, 24, 36, 48, 60, 72, 84 and 96 h of incubation.

Curve fitting and statistical analysis

Readings were converted to volume by using a pre- established linear regression between pressures (Mota *et al.*, 2005). Gas volume at each incubation time was expressed per unit of incubated dry matter (DM).

The data obtained were fitted to the non-linear regression equation:

$$V \text{ (ML/200 mg DM)} = b (1 - e^{-ct})$$

Where V= potential gas production at time t, b= the volume of gas that evolved with time, and c = the fractional rate of gas production.

Statistical analysis

Data obtained were analyzed separately to determine the interaction effect of the browse plant and inclusion levels using the general linear model of SPSS (Release 20.0) statistical package (SPSS, 2011). Model sums of squares were partitioned to linear, quadratic and cubic trends (Gomez and Gomez, 1983). A probability of P<0.05 was considered to be statistically significant.

Results

The chemical composition and fibre fraction of dried leaves of the browse plants is presented in Table 1. The results showed that all the parameters were significant (P<0.05). The CP contents of the leaves were significantly (P<0.05) different with values ranging from 13.66% DM for *A. africana* leaf to 26.66% DM for *M. oleifera* leaf. Values for ether extract and ash contents ranged from 2.00% DM for *A. indica* leaf to 5.85% DM for *M. oleifera* leaf and 11.00% DM for *A. indica* leaf to 20.05% DM for *A. africana* leaf respectively. In addition, non-fibre carbohydrate (NFC) values of 2.89 to 9.84% DM respectively were significantly (P<0.05) different among the dietary treatment. There were significant (P<0.05) differences for neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) values. Values for NDF ranged from 48.00 to 60.00% DM. ADL values ranges from 18.00% to 30.00% DM. Similar values of 18.00% DM were obtained for both *M. oleifera* and *A. africana* leaves.

The mineral concentration of the leaves was ppm (mg/lit) on dry weight basis were significantly (P< 0.05) different for all the plants in Table 2. Concentration of Ca and Mg ranges from 113.54 to 200.51 ppm and 59.02 to 67.58 ppm respectively. The K concentration significantly (P<0.05) ranged from 193.23 ppm in *M. oleifera* leaves to 417.52 ppm. Concentration of Cu (0.22 ppm) was significantly (P< 0.05) higher in *A. africana* leaves. The range of Fe in the studied plant varies from 4.86 ppm to 10.01 ppm in a significant (P< 0.05) difference and *A. africana* significantly (P< 0.05) had higher Zn content (0.88 ppm).

Table 3 shows the in vitro gas (ml/200mg) production of the experimental diets. The volume of gas production steadily (P< 0.05) increases from 12 hours to 96 hours. Gas was not produced from 2 hours to 8 hours of incubation. At 12 hours of incubation 15% *A. indica* leaf meal had significant (P< 0.05) highest volume of gas produced (1.00 ml/200mg) followed by 30% *M. oleifera* leaf meal (0.83 ml/200mg) resulting in (L: Q: P< 0.05) linear

Table 1: Chemical composition and fibre fraction (% DM) of dried leaves of the browse plants

Parameters	Dried leaves of the browse plants			SEM
	<i>M. oleifera</i>	<i>A. africana</i>	<i>A. indica</i>	
Crude protein	26.26 ^a	13.66 ^c	17.16 ^b	1.88
Ether extract	5.85 ^a	2.85 ^b	2.00 ^c	0.58
Ash	17.00 ^b	20.05 ^a	11.00 ^c	1.33
NFC	2.89 ^c	7.44 ^b	9.84 ^a	1.02
Fibre fraction				
NDF	48.00 ^b	56.00 ^a	60.00 ^a	1.53
ADF	36.00 ^c	50.00 ^b	54.00 ^a	1.53
A ADL	18.00 ^b	18.00 ^b	30.00 ^a	1.53

^{abc} Means along the same row with different superscripts are significantly different ($P < 0.05$)

SEM- Standard Error of Mean

NFC- Non-Fibre Carbohydrate

ADF-Acid Detergent Fibre

ADL-Acid Detergent Lignin

Table 2: Mineral composition of dried leaves of the browse plants

Element	Dried leaves of the browse plants			SEM
	<i>M. oleifera</i>	<i>A. africana</i>	<i>A. indica</i>	
Concentration ppm (mg/lit)				
Macro element				
Ca	200.51 ^a	165.21 ^b	113.54 ^c	33.50
Mg	67.58 ^a	59.02 ^b	61.52 ^b	5.78
K	193.23 ^b	417.52 ^a	310.56 ^{ab}	47.26
Micro element				
Fe	4.86 ^c	10.01 ^a	7.03 ^b	0.58
Cu	0.08 ^b	0.22 ^a	0.07 ^b	0.03
Zn	0.59 ^b	0.88 ^a	0.72 ^{ab}	0.58

^{abc} Means along the same row with different superscripts are significantly different

($P < 0.05$) SEM- Standard Error of Mean

and quadratic trend whereas, no gas was obtained in control 0% and 15% *M. oleifera* leaf meal. The least volume of gas produced was recorded in 30% *A. africana* (0.30 ml/200mg) which was not significantly ($P > 0.05$) different from 30% *A. africana* leaf meal (0.30 ml/200mg). Gas produced at 84 hours and 96 hours of incubation significantly ($P < 0.05$) differed as 30% *M. oleifera* leaf meal produced the highest volume of gas production ($P < 0.05$) (9.67; 10.10 ml/200mg) followed closely by 30% *A. africana* (9.30; 9.63 ml/200mg). The least amount of gas produced was obtained in 15% *M. oleifera* leaf meal (6.60; 6.63 ml/200mg) at 84 hours and 96

hours when compared with the gas produced by the other diets. However, the gas produced by 30% *M. oleifera* leaf meal consistently increased ($P < 0.05$) throughout the incubation period from 18 hours to 96 hours.

The control 0%, 15% *M. oleifera* leaf meal and 15% *A. africana* leaf meal produced the least gas volume at 24 hours of incubation with similar value ranging from 3.00 to 3.33 ml/200mg. At 48 and 60 hours of incubation 30% *M. oleifera* leaf meal and 30% *A. indica* leaf meal, had the similar value (7.83 ml/200mg) of gas produced followed by the control 0% (7.67 ml/200mg).

Table 3: In vitro gas production (ml/200mg) of the experimental diets up to 96hrs of incubation

Forage	Levels (%)	Period of Incubation													
		2hrs	4hrs	8hrs	12hrs	18hrs	24hrs	36hrs	48hrs	60hrs	72hrs	84hrs	96hrs		
Control	0	0.00	0.00	0.00	0.00 ^c	0.47 ^c	3.00 ^c	5.17 ^e	7.67 ^b	7.67 ^b	8.32 ^b	9.00 ^{ab}	9.33 ^b		
<i>Moringa oleifera</i>	15	0.00	0.00	0.00	0.00 ^c	0.30 ^c	3.33 ^{bc}	3.83 ^g	4.80 ^f	5.33 ^f	6.17 ^c	6.00 ^c	6.63 ^c		
	30	0.00	0.00	0.00	0.83 ^{ab}	2.83 ^a	5.00 ^a	6.83 ^a	7.83 ^a	7.83 ^a	9.00 ^a	9.67 ^a	10.10 ^a		
<i>Azadirachta indica</i>	15	0.00	0.00	0.00	1.00 ^a	3.00 ^a	4.33 ^{ab}	6.17 ^c	7.50 ^c	7.50 ^c	8.83 ^{ab}	9.13 ^{ab}	9.83 ^{ab}		
	30	0.00	0.00	0.00	0.43 ^{abc}	1.33 ^b	4.33 ^{ab}	6.33 ^b	7.83 ^a	7.83 ^a	8.67 ^{ab}	9.00 ^{ab}	9.50 ^{ab}		
<i>Aspilia Africana</i>	15	0.00	0.00	0.00	0.33 ^{bc}	1.30 ^b	3.33 ^{bc}	5.13 ^f	5.67 ^e	5.83 ^e	6.50 ^c	6.77 ^c	7.00 ^c		
	30	0.00	0.00	0.00	0.36 ^{bc}	0.67 ^c	3.69 ^{bc}	5.33 ^d	6.67 ^d	7.17 ^d	8.67 ^{ab}	9.30 ^a	9.63 ^{ab}		
SEM		0.00	0.00	0.00	0.19	0.194	0.35	0.01	0.03	0.02	0.19	0.38	0.19		
Forage effect		0.000	0.000	0.000	0.009	0.000	0.008	0.000	0.000	0.000	0.000	0.000	0.000		
Level effect		0.000	0.000	0.000	0.027	0.025	0.004	0.004	0.000	0.000	0.001	0.001	0.001		
Linear		0.000	0.000	0.000	0.048	0.991	0.933	0.539	0.751	0.871	0.776	0.978	0.871		
Quadratic		0.000	0.000	0.000	0.029	0.059	0.178	0.058	0.143	0.278	0.445	0.797	0.485		
Cubic		0.000	0.000	0.000	0.255	0.082	0.081	0.129	0.177	0.411	0.622	0.772	0.656		

^{abcdefg} Means along the same column with different superscripts are significantly different ($P < 0.05$).

$P < 0.05$ Probability for Linear, Quadratic and Cubic

SEM- Standard Error of Mean

Table 4: Parameters estimated by the model and characteristics of in vitro fermentation kinetics at 96 hours

Forage	Level (%)	B (h)	C	LAG	PH	OMD (%)	IVDMD (%)	ME (MJkg ⁻¹)	SCFA (μmol)
Control	0	13.7 ^{ab}	0.02 ^{ab}	6.42 ^a	7.64 ^{ab}	24.51 ^d	70.00 ^{ab}	3.47 ^e	0.10 ^d
<i>Moringa oleifera</i>	15	9.19 ^b	0.01 ^b	6.20 ^b	7.67 ^a	25.79	53.33 ^e	3.63 ^d	0.20 ^{cd}
	30	10.57 ^b	0.03 ^a	5.13 ^e	7.53 ^c	28.06 ^a	60.00 ^c	3.94 ^a	0.50 ^a
<i>Azadirachta indica</i>	15	8.30 ^b	0.02 ^{ab}	5.79 ^d	7.68 ^a	25.75 ^c	43.33 ^f	3.63 ^d	0.20 ^{cd}
	30	17.66 ^a	0.01 ^b	6.10 ^c	7.66 ^a	26.32 ^{bc}	56.67 ^d	3.69 ^c	0.30 ^{bc}
<i>Aspilia Africana</i>	15	11.69 ^b	0.02 ^{ab}	4.90 ^f	7.59 ^b	26.15 ^{bc}	23.33 ^g	3.71 ^c	0.40 ^{ab}
	30	11.27 ^b	0.02 ^{ab}	5.77 ^d	7.66 ^a	26.77 ^b	23.33 ^g	3.77 ^b	0.40 ^{ab}
SEM		0.01	0.00	0.01	0.00	0.01	0.12	0.00	0.00
Forage effect		0.000	0.136	0.000	0.000	0.000	0.000	0.000	0.000
Level effect		0.001	0.781	0.001	0.355	0.000	0.000	0.000	0.000
Linear		0.529	0.560	0.080	0.826	0.037	0.000	0.026	0.010
Quadratic		0.705	0.830	0.221	0.969	0.050	0.000	0.048	0.020
Cubic		0.050	0.307	0.174	0.941	0.027	0.000	0.023	0.046

^{ab,cd,de} Means along the same column with different superscripts are significantly different ($P < 0.05$).

P * < 0.05 Probability for Linear, Quadratic and Cubic

SEM- Standard Error of Mean

OMD -Organic matter digestibility,

ME-Metabolizable Energy,

IVDMD-In vitro dry matter digestibility,

B-Asymptotic amount of the produced gas Maximum amount of produced gas or total potential gas production.

C- Specific gas production rate as affected by t.

The result of Metabolizable energy (ME MJKg⁻¹), Organic matter digestibility (OMD %), Short chain fatty acids SCFA (μ mol) and in vitro dry matter Digestibility (IVDMD %) of the samples are shown in Table 4. The linear, quadratic and cubic relationships were significant ($P < 0.05$) for the parameters mentioned above. The values of ME, Organic Matter digestibility (OMD) and Short chain fatty acid (SCFA) ranged as follows in control diet to 30% *M. oleifera* 3.47 - 3.94%; 24.51 - 28.06% and 0.01 - 0.05 mmol respectively. *M. oleifera* at 30% level of inclusion had higher value than the other samples in ME; OMD and SCFA and the least ($P < 0.05$) value was obtained in control at 0% level of inclusion. IVDMD ranged from 23.33% to 70.00%. It increased as the inclusion levels increases across the treatments. Statistically, similar value of (23.33%) was achieved for *A. indica* leaf meal at 15% and 30% level of inclusion. OMD ranged from 24.51 to 28.06% and similar value of SCFA (0.04 μ mol) was obtained for 15% and 30% *A. indica* leaf meal. In addition, 15% *M. oleifera* leaf meal and *A. indica* leaf meal had similar value for SCFA (0.02 μ mol).

The in vitro fermentation kinetics at 96 hrs showed that the volume of gas produced was significant ($P < 0.05$) across the treatments and ranged from 8.30 h to 17.66 h leading to a cubic trend (C: $P < 0.05$). The values for specific gas production rate (C) ranged from 0.01 to 0.03. *M. oleifera* leaf meal at 30% level had ($P < 0.05$) highest rate of gas production with a value of 0.03. Similar value of 0.02 was recorded for 0%, 15% *A. africana* leaf meal, 15% and 30% *A. indica* leaf meal. The least Lag value was obtained at 15% *A. indica* leaf meal 4.90 while the highest value of 6.42 was obtained at 0% Control. pH values ranged from 7.53 to 7.68 and similar pH value of 7.66 was achieved for 30% *A. africana* and *A. indica* leaf meal.

Discussion

Chemical composition varied among plant species. The CP content of *M. oleifera* leaf used in this study was higher than the two other leaves. This confirmed the potential of

the leaf as a good alternative source of protein when incorporated into animal feed (Nouala *et al.*, 2006). The CP content of *A. africana* leaf observed in this study was higher than the value 8.45% reported by (Ukanwoko and Igwe, 2012). However, the crude protein value recorded for *A. indica* leaf was substantially above the value of 8 - 10% required to satisfy the maintenance requirement of ruminants (Norton, 2003). Variations in CP content could possibly be due to differences in ratio of leaves and twigs in forages as similarly reported by Larbi *et al.* (2011).

The ash content reflects the mineral composition of the forages and is an indication of the intrinsic ability of the plants to supply minerals to farm animals. The observed variation in chemical composition of forage agrees with the report of (Aseigbu and Anugwa, 1988; Topps, 1992). NFC is a crude estimate of the carbohydrate pool that differs in digestibility from NDF. It has a positive relationship with ammonia nitrogen (NH₃-N) utilization in the rumen (Tylutki *et al.*, 2008). The Non-fibre carbohydrate (NFC) recorded in the current study fell below the range 12.7 - 25.9% reported by Anele *et al.* (2009). The differences observed here could be attributed to the variation in the genetic constitution of *A. indica* plant and various processing method used. NRC (1996) also reported that the problem of utilizing the *A. indica* leaf remains. This is because neem tree scattered around the world are generally distinct and its nutritional potentials are affected by climatic condition, method of processing and to a lesser extent, the genetic make-up of the animal.

According to Onwuka and Akinsoyinu (1988), the presence of mineral elements in animal feed is vital for the metabolic processes of the animals. Macro nutrients like K, Ca, and Mg play key roles in building tissues and balancing the physiological, metabolic, and biochemical processes of livestock (NRC, 1996).

The mineral composition of the leaves of the selected plants (*M. oleifera*, *A. africana* and *A. indica* leaves were higher than those reported for some tested leaf meals. The concentrations of Ca, K and Mg were higher in the present leaf

meals than the values reported by Anjorin et al. (2010) and Asaolu et al. (2011) for some leaf meals. The abundance of these elements was in agreement with findings that these three elements represent the most abundant metal constituents of many plants (Chizzola and Franz, 1996; Lavilla et al., 1999). The variation in mineral composition of the leaf meals used in this study and those reported in literature shows that the mineral content of leaf meal varies with location as reported by Anjorin et al. (2010). The leaves of *M. oleifera*, *A. africana* and *A. indica* had adequate calcium content that is within the recommended level for livestock (NRC, 1984). The potential of *A. africana* to stop bleeding and heal wound could be because of high calcium content and zinc content.

The Mg concentrations of the leaves are within the tolerable level reported by (NRC, 1984) and the values were high enough to meet the Mg requirement for rabbit. The potassium deficiency in ruminants leads to muscular weakness, decreased feed intake, reduced weight gain, and / or decreased milk production.

In this study, values obtained for Zn and Cu were lower when compared with the values of 21.70 and 5.73 parts per million (ppm) for Zn and Cu, respectively reported by Mutayoba et al. (2011). The presence of Zinc in high amounts is of special interest in view of the importance of the inclusion of Zinc in the diet of animals.

The occurrence of anaemia in Fe deficiency may probably be related to its role in facilitating iron absorption and in the incorporation of iron into haemoglobin (FAO/WHO 1984).

Gas production parameters suggested differences in nutritional values that were generally closely related to chemical composition (Cerrillo and Juarez, 2004; Kamalak et al., 2005). Values obtained for the fractional rate of gas production (c) might indicate a better nutrient availability for rumen micro organism (Getachew et al., 2004). The values were similar to the values of 0.02 - 0.04 ml/h and 0.03 - 0.04 ml/h reported by (Anele et al., 2008 and Sodeinde et al., 2009) for

multipurpose trees species. The higher values obtained in this study might be attributed to higher digestible at the rate at which a feed or thier chemical constituent are digested in the caecum. It might also be attributed to higher nutrient composition of the forage as influenced by the different factors to which they were subjected to Khazaal et al. (1996) who suggested that the intake of a feed is mostly explainable by the fractional rate of gas production (c) which affect the rate of passage of the feed through the rumen.

The lag time observed in this study was much higher than 1.09-1.13 h; 1.41-1.95 h than those reported by (Ojo, 2011 and Dele, 2012) respectively. According to Murrillo et al. (2011) gas production is a nutritional wasteful product but provides useful basis from which metabolizable energy (ME), organic matter digestibility (OMD) and short chain fatty acid (SCFA) might be predicted (Babayemi, 2006).

Chemical composition in combination with in vitro digestibility and ME values could be considered as useful indicator for preliminary evaluation of the potential nutritive value of previous uninvestigated shrubs and tree leaves (Ammar et al., 2005). Menke and Steingass (1988) reported a strong correlation between metabolizable energy (ME) values, in vivo, in vitro and chemical composition of feed. The values obtained for ME in this study fell below 6.00-8.70 MJ/KG-I reported by Torbatinejad et al. (2009). Variation in ME content is comparable with the report of Getachew et al. (2002) who reported 3.00 MJ/KG-IDM variation for tropical browses but was slightly higher than 2.29-2.61 MJ/KG-I reported by Kamalak et al. (2005). Lower ME observed could be ascribed to presence or absence some certain secondary metabolites in the selected plants.

Aganga and Mosace (2001) observed a mutual relationship between total gas production, metabolizable energy, organic matter digestibility and short chain fatty acid. The highest short chain fatty acid reported at 30% MOM was similar to the report value 0.05mmol by Njidda and Ikhimioya (2010) for *Ficus thonningii*. Short chain fatty acid (SCFA) value is an indication that the forage could be

readily utilized after digestion for maintenance and production. The mean in vitro dry matter digestibility (IVDM) values reported in this study for MOM compared favourably with 50 - 60% reported by Sandoval and Mendoza (2000) for tropical grasses, as well as 50.56% and 51.14% for leguminous and non leguminous browse plants reported by Ahamefule et al. (2006). The results obtained for *A. africana* leaf meal (AAM) was close to 32.72 - 52.44% reported by Ibeawuchi et al. (2002) but were higher than the result for *A. indica* leaf meal (AIM).

The organic matter digestibility (OMD) values obtained in this study fell below the findings of Omoniyi et al. (2013) and were similar to the report of 29.15% by Ajayi and Babayemi (2008). Reduction in OMD values could be attributed to higher concentrations of secondary metabolites. Yusuf et al. (2013) reported that the presence of tannin inhibits degradability.

Sallam (2005) and Sommart et al. (2000) suggested that gas volume is a good parameter for predicting digestibility fermentation end product and microbial protein synthesis of the substrate by rumen microbes in the in vitro microbial protein synthesis of the substrate by rumen microbes in the in vitro system. There was steady increase in the volume of gas production for over a period of 96h as significant differences occurred among the dietary treatments. Highest volume of gas produced in 30% MOM (5.00 ml/200mgDM) at 24h of incubation fell below the range of gas obtained by Anele et al. (2008) for *Moringa oleifera*, *Millittia griffoniana* and *Pterocarpus santalinoides* (35.8, 32.2 and 27.8 ml/200mgDM) respectively. At 48h of incubation, the volume of gas production at 15% and 30% AIM were lower than the findings by Ali et al. (2012) and Isah et al. (2012) for *A. indica* (15.00 mg/200mgDM). Many factors determine the amount of gas produced during fermentation which also depend on the nature and level of fibre, presence of secondary metabolites (Babayemi et al., 2004) and potency of the rumen or caecum liquor for incubation (Babayemi, 2006).

However, a correlation occurred between the crude protein and the cumulative

gas production as observed in 30% *M. oleifera* at 96h of incubation. This indicate that high level of crude protein content contribute to high gas production as well as high crude protein in feed which also enhances microbial load and determines the extent of fermentation in which the gas produced is related to microbial protein synthesis. It is possible to attain the potential gas production of feedstuffs if the donor animal from which rumen liquor for incubation was collected meets its nutrient requirement (Babayemi et al., 2004).

Conclusion and Recommendation

The present experiment has shown that the examined browse plants *Moringa oleifera*, *Aspilia africana* and *Azadirachta indica* are potential good source of protein and contained adequate amounts of minerals which are sufficient to meet the requirements of rabbits. The leaves of the plants improve the efficiency of protein digestion in the caecum and nutritional status when successfully incorporated into the feeding regime of animals.

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COMPARISON OF INFRARED, ELECTRONIC DIGITAL AND MERCURY-IN-GLASS THERMOMETERS: I. WEST AFRICAN DWARF GOATS

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Abstract

Monitoring body temperature accurately is essential in livestock production. Data of body temperature measurements taken concurrently with electronic digital (ED), mercury-in-glass (MG) and infrared (IR) thermometers in 107 (male=38 and female=69) West African Dwarf (WAD) goats aged between 6 months and 3 years were compared to assess the accuracy. ED and MG thermometry was taken via the rectum (TEMPd and TEMPa respectively) while the IR thermometer was used on the opening of the rectum (TEMPiR) and forehead (TEMPiH). The data were subjected to analysis of variance and Pearson correlation analysis. Scatter diagrams were plotted to generate R-squared for the relationships between the readings of the thermometers. Thermometer type had significant ($P < 0.001$) effect on body temperature (BT) in WAD goats. The BT recorded was in the order: TEMPd > TEMPa > TEMPiR > TEMPiH. There was strong positive significant ($P < 0.001$) correlation between TEMPd and TEMPa ($r = 0.896$). The correlations between TEMPd and TEMPiR ($r = 0.237$) and TEMPa and TEMPiR ($r = 0.222$), though significant ($P < 0.05$), were weak. TEMPiH and TEMPiR had positive and significant ($P < 0.001$) correlation ($r = 0.503$). TEMPiH had no significant ($P > 0.05$) correlation with TEMPd and TEMPa. Linear regression of TEMPd with TEMPa, TEMPiR and TEMPiH yielded R^2 of 0.802, 0.056 and 0.00006 respectively. Deviations from TEMPd obtained were 0.34, 0.92 and 2.54°C for TEMPa, TEMPiR and TEMPiH respectively. Taking body temperature of WAD goats with IR thermometer on the forehead may not give correct measurement as with the traditional MG and ED thermometers. Temperature measurement with IR thermometer on the opening of the rectum of WAD goats seems to be more accurate than on the forehead.

Keywords: rectal temperature, heat stress, health, welfare, diagnosis

COMPARAISON DES THERMOMETRES INFRAROUGE, ELECTRONIQUE NUMERIQUE ET A MERCURE EN VERRE : I. LES CHEVRES NAINES D'AFRIQUE DE L'OUEST

Résumé

La surveillance correcte de la température corporelle est essentielle dans la production animale. Les données sur la température corporelle prises simultanément avec des thermomètres électronique numérique (ED), à mercure en verre (MG) et infrarouge (IR) chez 107 chèvres (mâles=38 et femelles=69) naines d'Afrique de l'Ouest (WAD) âgées de 6 mois et 3 ans ont été comparés dans le but d'évaluer leur précision. Les thermomètres ED et MG ont été utilisés dans le rectum (respectivement TEMPd et TEMPa) tandis que le thermomètre IR a été utilisé à l'ouverture du rectum (TEMPiR) et sur le front (TEMPiH). Les données ont été soumises à une analyse de variance et à une analyse de corrélation de Pearson. Les diagrammes de dispersion ont été tracés pour générer R-squared pour les relations entre les relevés des thermomètres. Le type de thermomètre utilisé a eu un effet significatif ($P < 0,001$) sur la température corporelle (BT) des chèvres WAD. La température corporelle (BT) enregistrée était dans l'ordre TEMPd > TEMPa > TEMPiR > TEMPiH. On a noté une forte corrélation positive significative ($P < 0,001$) entre TEMPd et TEMPa ($r = 0,896$). Les corrélations entre TEMPd et TEMPiR ($r = 0,237$) et TEMPa et TEMPiR ($r = 0,222$), bien que significatives ($P < 0,05$), étaient faibles. TEMPiH et TEMPiR avaient une corrélation ($r = 0,503$) positive et significative ($P < 0,001$). TEMPiH n'avait pas de corrélation significative ($P > 0,05$) avec

TEMPd et TEMPa. La régression linéaire de TEMPd avec TEMPa, TEMPiR et TEMPiH a respectivement donné R^2 de 0,802, 0,056 et 0,00006. Les écarts par rapport à TEMPd obtenus étaient de 0,34, 0,92 et 2,54°C respectivement pour TEMPa, TEMPiR et TEMPiH. L'utilisation du thermomètre IR pour prendre la température corporelle des chèvres WAD sur le front peut ne pas donner un résultat correct, tout comme les thermomètres traditionnels MG et ED. La mesure de la température avec un thermomètre IR à l'ouverture du rectum des chèvres WAD semble plus précise par rapport à la température prise sur le front de l'animal.

Mots-clés : température rectale, stress thermique, santé, bien-être, diagnostic

Introduction

Monitoring of vital signs in livestock species especially body temperature is important to assessing health status and ensuring proper diagnosis of disease conditions (Stephens Devalle, 2005; Fayomi *et al.*, 2007; Brunnel, 2012). Deviation in body temperature beyond normal range for particular species under thermoneutral zone may be an indication of sickness in animals (Lee *et al.*, 2016). In the tropics, the challenge of heat stress stemming from high environmental temperature, which may exceed 30°C (Abioja *et al.*, 2012), has been a perennial problem to livestock production. One of its indicators is elevation in body temperature which must be monitored. These underscore the imperativeness of accurate measurement of body temperature of farm animals both in research and production scenarios.

Physiologists hypothetically divide the body of an animal into two: the core and the shell (Smith *et al.*, 1988). The core comprises of the brain and other internal organs where the metabolic heat production takes place while the extremes and outer coverings are termed the shell. Obtaining the core body temperature require invasive techniques involving implantation of temperature transponder microchips or probes. This may be difficult to achieve and expensive to maintain. Therefore, non-invasive methods are explored on farms, in clinics and in research. These include temperature taken via armpit (axillary), ear (tympanic), anus (rectal) and mouth (oral). These have varying degrees of accuracy because they only mimic the thermal situations in the core of the subject. Axillary temperature is usually a degree lower than oral (Singh *et al.*,

2000). Several authors had compared accuracy of different thermometer types in human beings (Gasim *et al.*, 2013). Prendiville *et al.* (2002) had made several attempts to measure body temperature from different parts of the body in cattle.

In farm animals, rectal temperature is the commonest way of obtaining body temperature. These involve the use of traditional clinical (mercury-in-glass (MG) and electronic digital (ED) thermometers via the rectum. Rectal temperature measurement in West African Dwarf (WAD) goats with the aid of MG thermometer (Shelton, 2000; Abioja *et al.*, 2007; Adediji, 2012; Daramola *et al.*, 2015; Kubkomawa *et al.*, 2015) and ED thermometer (Gaughan *et al.*, 1999; Alamer, 2006; Otoikhian *et al.*, 2009; Okoruwa, 2014) had been reported in literatures. Many researchers in biometeorology and animal welfare claimed that the ED thermometers are easier to use than the MG thermometers.

Introduction of pyrometers into body temperature measurement has proved to be easier than the previous two. Devices that are able to determine surface temperature of objects without needing to touch them are called pyrometers. An infrared (IR) thermometer is one particular type of pyrometers for measuring temperature of objects without making direct contact because they specifically measure the energy being radiated from an object. Its use in humans has really gained ground. However in livestock, non-contact IR thermometer is being adopted gradually by some farmers and researchers. Infrared (IR; non-contact) thermometers became known and used in livestock species after the scourge of Ebola in coastal West Africa. This has raised the concern for its accuracy and the

point at which it should be used. Tympanic IR thermometer has been developed and used in goats, sheep, horses, cats, dogs (Goodwin, 1998; Rexroat *et al.*, 1999; Stephens Devalle, 2005; Brunnel, 2012). In humans, one the common ways of using IR thermometer is to beam the laser on the forehead. Osio and Carnelli (2007) had earlier compared the accuracy of body temperature measurement with IR thermometer on forehead, navel and axilla with measurement with other conventional thermometers. It was reported by the authors that the readings from different devices used were correlated. The question is whether this is the same in goats. Therefore, this study aimed at comparing the data of thermometry taken with clinical (mercury-in-glass and electronic digital) thermometers with the noncontact infrared thermometer on the forehead and at the opening of the rectum.

Materials and methods

Location

The research was carried out at the Small Ruminant Unit of the University Teaching and Research Farm, Federal University of Agriculture, Alabata Road, Abeokuta, Nigeria (latitude 7° 13' 49.46"N; longitude 3° 26' 11.98"E (Google Earth, 2013) and altitude 76 m above sea level).

Meteorological observations

Ambient temperature and relative humidity in the pens were monitored immediately at the point of body temperature measurements with digital thermal hygrometer.

Experimental animals and management

One hundred and seven (male=38 and female=69) West African Dwarf goats kept in open-sided, slatted-floor pens were used for this experiment. There were two age groups; <1 year (n=63) and >1 year (n=44). The animals were fed with fresh elephant grass and concentrate 5% body weight basis. Fresh water was made available *ad libitum* daily. Vaccination programme and recommended medications were adequately adhered to.

Data collection

Body temperature measurement on all the goats was carried out using three different (electronic digital, mercury-in-glass and infrared) thermometers. The first two thermometers (TEMPd and TEMPa respectively) were used via rectum of the animals. However, body temperature measurement with the latter was done on the forehead (TEMPiH) and at the opening of the rectum (TEMPiR). The four readings were taken at the same time on individual animals.

Mercury-in-glass thermometer

Rectal temperature of goats was measured with a mercury-in-glass (MG) thermometer (0.1°C accuracy) inserted into the rectum and held for 1 minute.

Electronic digital thermometer

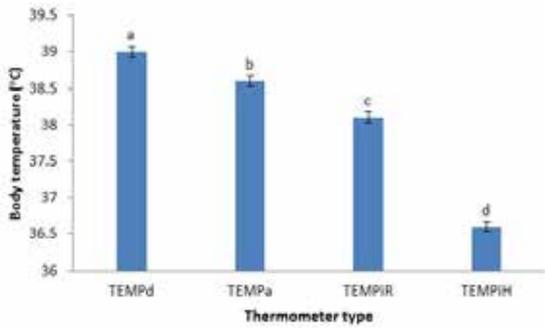
Electronic digital (ED) thermometer (0.1°C accuracy) was inserted into the rectum of goats and held in contact with the epithelial lining until it beeped as earlier described by Abioja *et al.*, (2012).

Infra-red non-contact thermometer

Body temperatures of goats were taken by beaming the laser from the Infrared (IR) thermometer (0.1°C accuracy) on the forehead and at the opening of the rectum. It was ensured that the distance between the animal and the thermometer did not exceed the value recommended by the manufacturer.

Data analyses

Data collected were subjected to analysis of variance using SYSTAT analytical statistical package Version 5.0 (SYSTAT, 1992). Ambient temperature and relative humidity were included in the model as covariates. Means found to be statistically different were separated with Duncan Multiple Range Test. The data on TEMPd, TEMPa, TEMPiR and TEMPiH were further subjected to Pearson correlation analysis. Taken TEMPd as the dependent variable, scatter diagrams were plotted to generate R squared for the relationships between the readings of the thermometers.



^{a,b,c,d}Means with different letters in different section of the chart differ significantly ($P < 0.05$)

Figure 1: Body temperature (°C) of WAD goats as affected by thermometer type

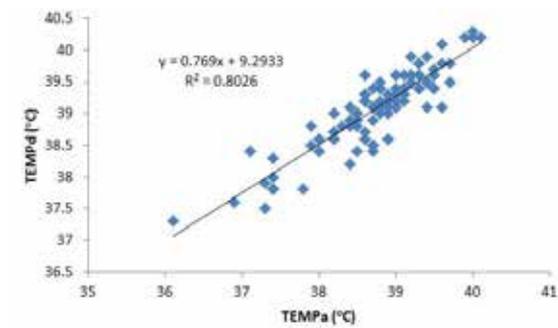


Figure 2: Scatter diagram of temperature readings of ED against MG

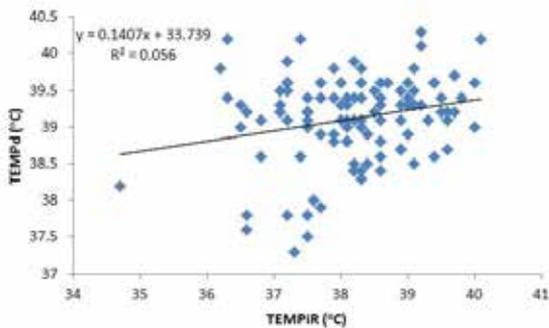


Figure 3: Scatter diagram of temperature readings of ED against IR at the opening of the rectum

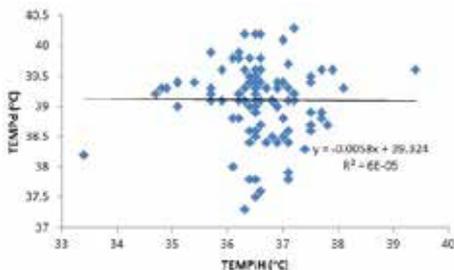


Figure 4: Scatter diagram of temperature readings of ED against IR on the forehead

Results

Effect of thermometer type on body temperature of WAD goats is presented in Figure 1. There were significant ($P < 0.001$) differences in the temperatures obtained by various thermometers. The body temperature recorded was in the order: $TEMPd > TEMPa > TEMPiR > TEMPiH$. Table 1 shows the correlation matrix between the values obtained with different thermometers in West African Dwarf goats. There was strong positive significant ($P < 0.001$) correlation between $TEMPd$ and $TEMPa$ ($r = 0.896$). The correlations between $TEMPiR$ and $TEMPd$ ($r = 0.237$) and $TEMPiR$ and $TEMPa$ ($r = 0.222$), though significant ($P < 0.05$), were weak. $TEMPiH$ and $TEMPiR$ had positive and significant ($P < 0.001$) correlation ($r = 0.503$). Scatter diagrams showing the relationships between readings of $TEMPd$ against $TEMPa$, $TEMPiR$ and $TEMPiH$ are presented in Figures 2-4 respectively. $TEMPd$ had regression coefficients R^2 of 0.802, 0.056 and 6×10^{-5} with $TEMPa$, $TEMPiR$ and $TEMPiH$ respectively. Table 2 shows the deviations of the readings of other thermometer types from the electronic digital thermometer. Deviations from $TEMPd$ obtained were 0.34, 0.92 and 2.54°C for $TEMPa$, $TEMPiR$ and $TEMPiH$ respectively.

Discussion

Accurate measurement of body temperature is important to accurate diagnosis of disease conditions in farm animals. The ease and short span of time with which infrared thermometer is used gives it an advantage as a ready tool of choice instead of usual electronic digital and mercury-in-glass thermometers (Rexroat *et al.*, 1999). It also avoids the need to touch or handle animals before temperature readings can be taken as it is non-contact. However, in consonance with the present findings, Rubia-Rubia *et al.* (2011) had reported that the body temperature depends on the type of thermometer and the area of the body at which the temperature is taking. Infra-red thermometers recorded lower measurements for the forehead and rectum opening than

Table 1: Correlation matrix of the body temperature of WAD goats measured with electronic digital (ED), mercury-in-glass (MG) and infrared (rectum opening and forehead) thermometers

	TEMPd	TEMPa	TEMPiR	TEMPiH
TEMPd	1.000			
TEMPa	0.896***	1.000		
TEMPiR	0.237*	0.222*	1.000	
TEMPiH	-0.007	-0.017	0.503***	1.000

Table 2: Deviations from readings of thermometer types from electronic digital thermometer

Temperature readings	Bias (°C)	Standard deviation (°C)	Maximum (°C)	Minimum (°C)
ED-MG	0.34	0.66	1.3	-0.5
ED-IR rectum	0.92	0.94	3.9	-1.0
ED-IR forehead	2.54	1.44	4.8	0.2
MG- IR rectum	0.59	0.90	3.8	-1.5
MG- IR forehead	2.20	1.32	5.0	-0.2
IR rectum - IR forehead	1.62	1.20	3.5	-2.2

the mercury-in-glass and electronic digital thermometers. The latter were actually placed in the rectum which is closer to the body core of the animal. The measurement on the forehead tends to take skin temperature (shell), which is always lower than the core body temperature. Moreover, lower temperature might have resulted from barrier of the skull limiting the extension of brain temperature to the outside. Contrary to the finding of Rexroat and his colleagues with infrared (Vet-Temp™ VT100 instant ear) thermometers, whose readings were close (within 0.1oF) to traditional rectal thermometers in cats and dogs, the reading of infrared thermometer on the forehead of WAD goats were far below the reading from ED and MG clinical thermometers. The reason may be adduced to the fact that tympanic temperature is a core temperature in human (Brinnel and Cabanac, 1989). However, Goodwin (1998) reported a higher temperature taken with digital thermometer than infrared thermometer taken via tympanic membrane in goats. Electronic digital thermometer seems to be more sensitive than mercury-in-glass. It beeps showing readiness at shorter span of time than the analogue. Analogue thermometer is left in the rectum for at least 60 seconds before

the reading is taken. Yet, consistently lower readings are obtained than in electronic digital thermometer. It becomes important to allow the mercury-in-glass thermometers stay longer than 60 seconds if accurate measurement will be ensure.

In the present study, temperatures obtained by using mercury-in-glass thermometer recorded higher correlation and agreed more closely with temperature readings with electronic digital thermometer than with non-contact infrared thermometer. Though, infrared thermometer had been used on the forehead to take body temperature in humans, it may not be reliable in WAD goats. Skin temperatures are most likely lower than core body temperature. Skin temperature may be affected easily by environmental temperature. Brunnel (2012) stated that a time lag between changes in core and subcutaneous temperatures could account for some of disparity obtained in temperature readings. A cursory look at the differences in the readings of infrared thermometer at forehead and opening of rectum signals that the accuracy of measurement will depend on the location. It suggests that taking reading at other parts of the body may yield closer readings to rectal

temperatures. Gasim et al. (2013) had reported that thermometry infrared tympanic membrane thermometer is reliable and as accurate as axillary mercury-in-glass thermometer in humans, yet Yaron et al. (1995) reported that infrared tympanic thermometry did not agree with rectal temperature measurements. Both Chue et al. (2012) and Rabbani et al. (2010) recorded agreement in readings with infrared tympanic thermometer and oral mercury-in-glass thermometers.

Conclusion

IR thermometer measures the skin temperature rather than the core body temperature, unlike the ED and MG thermometers that takes the core body temperature. Taking body temperature of WAD goats with IR thermometer on the forehead may not give correct measurement as with the traditional MG and ED thermometers. Temperature measurement with IR thermometer on the opening of the rectum of WAD goats seems to be more accurate and nearer the core body temperature than on the forehead.

Impact

Traditionally, body temperature in ruminants is taken with either mercury-in-glass or digital thermometers via the rectum. Infrared (IR; non-contact) thermometers became known and used in livestock species after the scourge of Ebola in coastal West Africa. Its use in livestock raises two concerns: Will it give similar readings; and the actual point of taking the measurement on the animals. IR thermometer recorded lower temperature with little relationship with the traditional thermometers especially when used on the forehead of WAD goats. IR readings at the opening of the rectum showed a closer relationship.

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PHENOTYPIC DIVERSITY OF THE DOMESTIC DONKEY (*EQUUS AFRICANUS ASINUS*) IN THE SUDANO-SAHELIAN ZONE OF CAMEROON

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Abstract

This study was carried out in the Sudano Sahelian Zone of Cameroon to contribute to a better understanding of the biodiversity of donkeys of the area. 205 adult donkeys (111 males and 94 females) were randomly sampled in 3 localities (Bogo, Gazawa and Kaélé). The results show that the grey color (80.02%) is dominant while white (4.85%) is least represented. Majority of the donkeys display the St. Andrew cross (84.50%) and not zebra lines (76.20%). The mean body measurements assessed (cm) were as follows: thoracic: 108.31 ± 6.82 , height at withers: 98.05 ± 6.88 , trunk length: 101.37 ± 13.07 , neck length: 40.47 ± 8.79 , right ear length: 25.09 ± 1.24 , length of the left ear: 25.43 ± 1.07 and adult live weight: 122.11 ± 1.24 kg. The effect of sex on biometric measurements and indices was not significant ($P \leq 0.05$) however females were heavier (124.72 ± 1.65 kg) than males (120.44 ± 1.79 kg). The average height at withers of Gazawa's animals was significantly higher ($P > 0.05$) than those from the other localities. Compactness, body profile and mass indexes were significantly high ($P \leq 0.05$) in Bogo and Kaélé. The body and profile indexes were significantly higher in Gazawa compared to the other two localities. The correlations were significant ($P \leq 0.01$), positive and perfect between live weight and thoracic circumference ($r = 1.00$) both in males and females. The linear type equation ($LW = -155,50 + 2,565PT$) better predicts the body weight ($R^2 = 1,00$). The corporal profile index (CPI: 0.98 ± 0.01) and the relative corporal index (RCI: 0.93 ± 0.01) made it possible to classify the donkeys of the Sudano-Sahelian zone as of longiline profiles ($RCI \geq 0,90$) for Gazawa and medians ($0.84 \leq RCI \leq 0.90$) for animals of the other localities (Bogo, kaélé). The genetic diversity of the donkey obtained by the principal component analysis (PCA) revealed that live weight, trunk length and thoracic circumference contribute to 62.17% of the genetic variability in the population. The discriminant factorial analysis (DFA) shows that the population consists of 3 genetic types as indicated by the phylogenetic tree. The observed diversity leads to the conclusion that there are opportunities for genetic improvement of donkey population from the Sudano Sahelian zone of Cameroon.

Keywords: Biodiversity, morphobiometry, donkey, Sudano-Sahelian zone, Cameroon.

LA DIVERSITE PHENOTYPIQUE DE L'ANE DOMESTIQUE (*EQUUS AFRICANUS ASINUS*) DANS LA ZONE SOUDANO-SAHELIENNE DU CAMEROUN

Resume

Cette étude s'est déroulée dans la Zone Soudano Sahélienne du Cameroun afin de contribuer à une meilleure connaissance de la biodiversité des ânes de la région. 205 ânes adultes (111 mâles et 94 femelles) ont été échantillonnés au hasard dans 3 localités (Bogo, Gazawa et Kaélé). Les résultats ont montré que la robe grise (80,02%) est dominante alors que la blanche (4,85%) est moins représentée. La majorité des ânes présentent la croix saint André (84,50%) et ne présentent pas de zébrures (76,20%). Les moyennes des principales mensurations corporelles évaluées (cm) ont été les suivantes : le pourtour thoracique : $108,31 \pm 6,82$; la hauteur au garrot : $98,05 \pm 6,88$; la longueur du tronc : $101,37 \pm 13,07$; la

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longueur du cou : $40,47 \pm 8,79$; la longueur de l'oreille droite : $25,09 \pm 1,24$; la longueur de l'oreille gauche : $25,43 \pm 1,07$ et le poids vif adulte : $122,11 \pm 1,24$ kg. L'effet du sexe sur les mensurations et les indices biométriques n'a pas été significatif ($P > 0,05$) cependant les femelles ont été plus lourdes ($124,72 \pm 1,65$ kg) que les mâles ($120,44 \pm 1,79$ kg). Dans la localité de Gazawa comparée aux deux autres la hauteur au garrot, la longueur corporelle et la longueur du cou ont été significativement plus élevées ($P < 0,05$). Les indices de compacité, corporelle de profil et de massivité ont été significativement plus élevées ($P > 0,05$) à Bogu et Kaélé. Les indices corporels de profil et de format quant à eux ont été significativement plus élevés dans la localité de Gazawa. Les corrélations sont significatives ($P > 0,01$), positives et parfaites entre le poids vif et le pourtour thoracique. L'équation de type linéaire $LWV = -155,50 + 2,565PT$ semble mieux prédire le poids vif ($R^2 = 1,00$). L'indice corporel relatif a permis de classer les ânes de la zone soudano sahélienne comme longilignes ($ICR \geq 0,90$) à Gazawa et médiolignes ($0,84 \leq ICR \leq 0,90$) à Bogu et kaélé. La diversité génétique des ânes obtenue par l'analyse en composante principale (ACP) a révélé que le poids vif, la longueur du tronc et le pourtour thoracique contribuent à 62,17% de la variabilité génétique au sein de la population. L'analyse factorielle discriminante (AFD) montre que la population est constituée de 3 types génétiques ainsi que le précise l'arbre phylogénétique. La diversité observée permet de conclure qu'il existe des possibilités d'amélioration génétique de la population asine de la zone soudano sahélienne du Cameroun.

Keywords: Biodiversité, morpho-biométrie, âne, zone Sudano-Sahélienne, Cameroun.

Introduction

The donkey (*Equus asinus*) appears as an untapped wealth thanks to its numerous advantages. It is used both for breeding, milking and meat; easy to maintain and inexpensive it can be assigned to any role in the farm. The donkey population of Cameroon estimated at over 50 thousand heads (INS, 2013) is spread in almost all regions. Although some work has been done on the socioeconomics of donkeys (Ebangi and Vall, 1998; Vall *et al.*, 1996; Vall *et al.*, 2001; Nguekeng, 2015) and the morphology of donkeys in the Northwest region of Cameroon (Defeu, 2015), very little information is available on the genetic diversity of donkeys in Cameroon and its production is neglected. Accordingly, FAO (2007) recognised the understanding of the genetic characteristics of livestock as one of the strategic priorities of the global plan of action for animal genetic resources, and this is according to Melesse *et al.*, (2013) a prerequisite for improving the productivity, utilization and sustainable conservation of local genetic resources. It is in this perspective that this work has been initiated in order to contribute to a better understanding of the biodiversity of donkeys in the Sudano-Sahelian zone of Cameroon. More specifically, the aim was to evaluate morphobiometric variability and to deduce the structure and phylogenetic relationships of donkeys in the region.

Materials and methods

Location and geo-climatic characteristics of the study area

The Sudano Sahelian zone of Cameroon (Figure 1) extends between latitudes 9° and 13° North and longitudes 13° and 16° East.

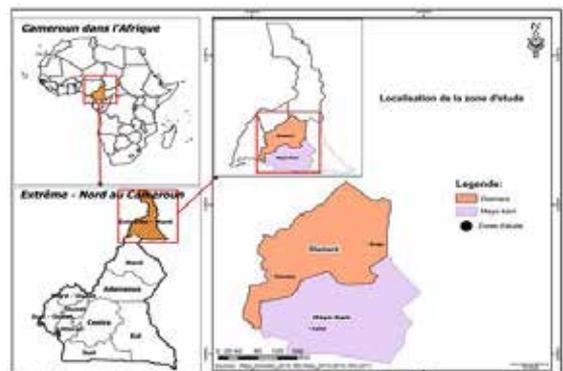


Figure 1: Location of the study area

It is characterized by a climate punctuated by an annual unimodal rainy season with a long dry season (October to May) and a short rainy season (June to September). Temperatures vary in the similarly, with average minima of 28° C (December to February) and maxima reaching 45° C in April. The annual solar radiation varies from 2500 to 3300 hours (MNAGRI-FASA, 2000). Rainfall varies from 700 to 800 mm per year (Cardinale *et al.*, 1997).

The vegetation is composed of several species: *Pennisetum* spp., *Schoenefeldia gracilis* in Yaeres (floodplain), woody (sandy soils) (*Combretum glutinosum*, *Annona senegalensis*, *Strychnos spinosa* etc.).

Animal material

A sample of 205 adult donkeys was examined according to the sex and area on origin (Table 1). The selection criteria concerned the existence of donkeys and the availability of the farmers in these localities.

Data collection

The collection method is derived from the approach developed by Lauvergne (2006) and adapted by FAO (2013) for quantitative characteristics. The age of the animal given by the breeders was complemented by observation of the dentition (Rabier, 2012).

The body measurements (figure 2) were: body length (BL in cm), chest circumference (CC in cm), height at withers (HW in cm), neck length (NL in cm) left ear length (LEL in cm), right ear length (REL in cm).

Table 1: Distribution of donkey sampled in the Sudano-Sahelian zone of Cameroon.

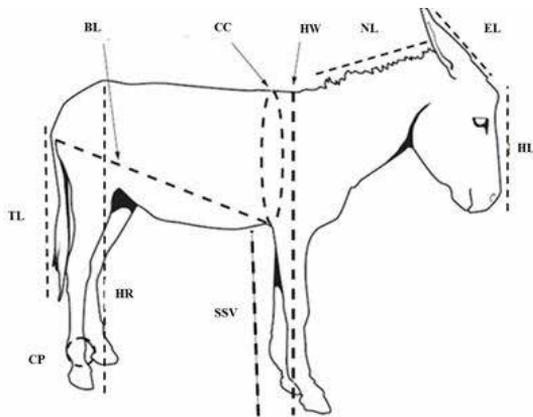
Sex	Localities			Total N	Percentage (%)
	Bogo	Gazawa	Kaélé		
Females	44	23	27	94	45.85
Males	35	49	27	111	54.15
Total	89	72	54	205	100.00

They were obtained using a measuring tape for length and circumference parameters and a height gauge (0.1 mm accuracy).

The coat colour was identified by direct observation under natural daylight, as well as the sex of the animal, the presence or not of the Saint Andrew's cross; the presence or absence of eye encirclement. The weight of the animal was determined by the Salifou's formula (2014): $LW (Kg) = 2.565 \times PT - 155.528$

From the body measurements, five body biometric indices were calculated according to formulas described by Khatouf (2005) Nicks *et al.*, (2006) and Boujenane *et al.* (2008):

- Format Index (FI): $IF = BL / HW$.
- Compactness index (CI) : $CI = CC / BL$
- Massiveness index (MI) : $MI = CC / HW$
- Body profile index (BPI) : $BPI = HW / BL$
- Relative body index (RBI) : $ICR = BL / CC$



LW = live weight; HW = height at withers; HR: height at the rump; BL: body length; CC= chest circumference; SSV= Sub-sternal vacuum; CP=cannon perimeter; NL=neck length; HL = head length; TL = tail length; EL= ear length

Figure 2: Body measurements in donkey

Statistical Analysis

The descriptive statistics was used to characterize phenotypic traits and body measurements. The Analysis of variance (ANOVA) was used to test the influence of factors on biometric measurements and indices.

When the effects of the variation factors were significant, the Duncan test was used for mean separation. The type and degree of association between measurements and biometric indices were assessed using Pearson correlation coefficients, the principal component analysis (PCA) was performed on the basis of biometric features. Discriminant factorial analysis (DFA) based on body measurements made it possible to identify the

genetic types of the population studied. The genetic relationship between the three genetic types (Carpentier, 2007) was established by constructing the phylogenetic tree according to the hierarchical ascending classification (HAC) protocol. The barometric equations were established by the regression of the live weight on the different measurements and the indices. All these statistical analyses were carried out using SPSS 21.0 and XLSTAT 2014 software.

Results

Morphological characteristics of donkeys in the Sudano-Sahelian zone Cameroon

Phaneroptic characters

Zebra, St. Andrew's cross, eye encirclement and coat colour

Table 2 summarizes the phaneroptic characteristics of the donkey populations of the Sudano-Sahelian Zone of Cameroon.

The frequencies of animal not bearing stripes (76%) and having the St Andrew cross (84%) are highly significant ($p > 0.05$); the proportion of eye encirclement are comparable between the localities. The coat colour of the animals according to the localities is diversified: the gray coat and its variants is dominant (80%), followed by the black (19%), bay (12%) and the white coat (10%).

Figure 3 illustrates the different coat colors observed in donkeys in the Sudano-Sahelian zone of Cameroon.

Body measurements of the donkey populations of the Sudano-Sahelian zone Cameroon

Body measurements of donkeys' populations by location

Of the six measurements considered, only the thoracic circumference and the length of the left ear were not significantly influenced by the locality ($P \leq 0.05$).

The body measurements of the populations of donkeys as well as their

Table 2: Modalities, numbers and frequencies of the phaneroptic characters of donkeys in the Sudano-Sahelian zone of Cameroon

Phanéroptic traits	Modalities	Localities			Total	Frequencies (%)	P*
		Bogo	Gazawa	Kaélé			
Sex	Females	44	23	27	94	45.85	0.24
	Males	35	49	27	111	54.15	
Coat colour	Dark gray	36	49	20	105	51.20a	0.00
	Light gray	23	13	24	60	29.30b	
	Black	7	7	4	18	8.80c	
	Bay	7	1	4	12	5.90c	
	White	6	2	2	10	4.90c	
Zebra Stripes	Presence	9	29	11	49	23.90b	
	Absence	70	43	43	156	76.10a	
St. Andrew's Cross	Presence	60	69	44	173	84.40a	
	Absence	19	3	10	32	15.60b	
Eye encirclement	Presence	38	34	31	103	50.20	
	Absence	41	38	23	102	49.80	

* $p < 0.05$



Figure 3: Different coat colours in the donkey of the Sudano-Sahelian zone Cameroon

Table 3: Body measurements of donkey populations according to the locality of the Sudano-Sahelian zone of Cameroon.

Parameters		Localities			Total (n=205)	P Value
		Bogo (n=79)	Gazawa (n=72)	Kaélé (n=54)		
Chest circumference	X ± E.S	108.35±0.77	108.98±0.80	107.52±0.93	108.36±0.48	0.49
	CV%	6.76	4.82	7.25	6.28	
Height at withers	X ± E.S	97.41±0.76 ^b	100.10±0.79 ^a	96.39±0.92 ^b	98.09±0.48	0.01
	CV%	8.56	3.27	7.77	7.01	
Body length	X ± E.S	96.35±1.23 ^b	111.21±1.29 ^a	95.74±1.49 ^b	101.09±0.78	0.00
	CV%	12.62	5.38	14.48	12.91	
Neck length	X ± E.S	38.32±0.93 ^b	44.88±0.97 ^a	37.72±1.12 ^b	40.32±0.58	0.00
	CV%	24.53	7.40	28.21	21.77	
Wright ear length	X ± E.S	25.47±0.13 ^a	24.43±0.14 ^b	25.43±0.16 ^a	25.09±0.08	0.00
	CV%	5.03	4.22	4.17	4.94	
Left ear length	X ± E.S	25.46±0.12	25.28±0.13	25.63±0.15	25.44±0.08	0.18
	CV%	4.40	3.52	4.56	4.17	
Life weight	X ± E.S	122.40±1.96	124.02±2.06	120.26±2.38	122.40±1.24	0.49
	CV (%)	15.34	10.86	16.61	14.24	

^{a,b} in the row, means with the same superscripts in the same row are not significantly different ($P \leq 0.05$); X ± E.S: mean ± standard error; CV: coefficient of variation; n: sample size.

coefficients of variation according to the locality of the Sudano-Sahelian zone of Cameroon are presented in Table 3.

The mean chest circumference and left ear length in the donkey's populations of the Sudano-Sahelian zone Cameroon are statistically ($p \leq 0.05$) similar between localities, and most variable in Kaélé's donkeys. The height at withers, body length, neck length and the right ear length are however showing significant differences ($p > 0.05$) among localities, and displaying the greatest variability in Bogo's donkeys.

Regardless of the locality, the average live weight of donkeys in the Sudano Sahelian zone of Cameroon is 122.40 kg with a CV = 14.24%. This parameter is comparable ($p \leq 0.05$) between the localities and Kaélé's donkeys are the most variable (16.61%). The heaviest donkeys are those from Gazawa with 124.02 \pm 2.06 kg.

Body measurements of donkeys' populations according to sex in the Sudano-Sahelian zone Cameroon.

Table 4 presents the body measurements according to the sex of donkeys' populations in the Sudano-Sahelian zone of Cameroon as well as their coefficients of variation.

There is no significant differences among sexes for the body measurements of donkeys from the sudano-sahelian zone of Cameroon..

Correlation between body measurements in the donkeys' populations of the Sudano-Sahelian zone of Cameroon.

Table 5 presents the general matrix of coefficients of correlations between body measurements in the donkey populations of the Sudano-Sahelian zone of Cameroon.

The correlations between body measurements can be positive or negative, and are weakly or strongly correlated with one another. The live weight is positively correlated ($P < 0.01$) with all measurements, except for the length of the right and left ears. However, the highest positive correlation is observed between the chest and live weight ($r = 1.00$).

Table 4. Body measurements according to the sex of donkeys' populations in the Sudano-Sahelian zone of Cameroon as well as their coefficients of variation

Parameters		Sex		Total (n=205)	P value
		Male (n=111)	Female n=94)		
Chest circumference	X \pm E.S	107.59 \pm 0.64	109.26 \pm 0.70	108.36 \pm 0.47	0.08
	CV%	6.35	6.11	6.28	
Height at withers	X \pm E.S	98.17 \pm 0.65	97.99 \pm 0.71	98.09 \pm 0.48	0.86
	CV%	7.26	6.74	7.01	
Body length	X \pm E.S	101.09 \pm 1.25	101.79 \pm 1.35	101.41 \pm 0.92	0.71
	CV%	13.41	12.35	12.91	
Neck length		40.05 \pm 0.91	40.96 \pm 0.84	40.46 \pm 0.62	0.46
	CV%	22.55	20.95	21.77	
Wright ear length	X \pm E.S	25.11 \pm 0.13	25.07 \pm 0.12	25.09 \pm 0.09	0.85
	CV%	4.82	5.07	4.94	
Left ear length	X \pm E.S	25.55 \pm 0.11	25.31 \pm 0.10	25.44 \pm 0.07	0.11
	CV%	3.95	4.43	4.17	
Poids vif (kg)	X \pm E.S	120.44 \pm 1.79	124.72 \pm 1.65	122.40 \pm 1.22	0.08
	CV (%)	14.54	13.74	14.24	

^{a, b} in the row, means with the same superscripts in the same row are not significantly different ($P \leq 0.05$); X \pm E.S: mean \pm standard error; CV: coefficient of variation; n: sample size.

Table 5: General matrix of coefficients of correlations between body measurements in the donkey populations of the Sudano-Sahelian zone of Cameroon

Variables	CC	HW	BL	NL	WEL	LEL	LW
CC	1						
HW	0.65**	1					
BL	0.60**	0.61**	1				
NL	0.53**	0.52**	0.85**	1			
WEL	0.03	-0.08	-0.35**	-0.39**	1		
LEL	-0.04	0.07	-0.17*	-0.19**	0.66**	1	
LW	1.00**	0.65**	0.60**	0.53**	0.03	-0.04	1

** $P < 0.01$ * $P < 0.05$ (bilatéral), CC : chest circumference ; HW : height at withers ; BL : body length ; NL : neck length ; WEL : wright ear length ; LEL : Left ear length ; LW : poids vif.

Table 6. Matrix of coefficients of correlations between body measurements in males (above diagonal) and females (below diagonal) in the donkey populations of the Sudano-Sahelian zone of Cameroon

Variables	CC	HW	BL	NL	WEL	LEL	LW
CC	1	0.48**	0.61**	0.58**	-0.03	-0.14	1.00**
HW	0.80**	1	0.50**	0.37**	-0.10	0.01	0.48**
BL	0.60**	0.69**	1	0.81**	-0.43**	-0.27**	0.61**
NL	0.50**	0.64**	0.85**	1	-0.44**	-0.35**	0.58**
WEL	0.09	-0.06	-0.28**	-0.34**	1	0.65**	-0.03
LEL	0.08	0.12	-0.07	-0.04	0.68**	1	-0.14
LW	1.00**	0.80**	0.60**	0.50**	0.09	0.08	1

The matrix of coefficients of correlations between body measurements by sex in the donkey population of the Sudano-Sahelian Zone of Cameroon is presented in Table 6.

Table 6 shows that both females and males have significant correlations, positive or negative. All the negative and/or non-significant correlations are those between the ears and the other measurements. The thoracic circumference and body length, live weight and chest circumference are positive and highly significantly correlated measurements in both sexes.

Barymetric equations in the donkey populations of the Sudano-Sahelian zone of Cameroon

Table 7 presents the different forms of prediction equation of the live weight of donkeys from their body measurements in the Sudano-Sahelian zone of Cameroon.

It is found that the chest circumference is a good predictor of live weight for donkeys in the Sudano-Sahelian zone of Cameroon (R^2 between 0.99 and 1). The linear equation with the thoracic circumference seems to be better suited for the prediction of live weight regardless of sex and gender, thanks to its simplicity and its high coefficient of determination.

Biometric indices of donkeys in the Sudano-Sahelian zone Cameroon

Biometric indices of donkeys according to the locality of the Sudano-Sahelian zone Cameroon

Table 8 present the body indices of donkeys in the Sudano-Sahelian zone of Cameroon and their coefficients of variation according to the locality. All biometric indices are significantly influenced ($p > 0.05$) by the locality. The compactness index of donkeys is lowest ($P < 0.05$) in Gazawa (0.98) with less variability.

n=94) Table 7: Types of weight prediction equations from to the body measurements of donkeys in the Sudano-Sahelian zone Cameroon

Types of equations	Measurements	Sex	Equations	R ²
Linéaire	HW	Male	$LW = -71.24 + 1.952HW$	0.63
		Female	$LW = 2.08 + 1.25HW$	0.23
		Male & Female	$LW = -40.95 + 1.664HW$	0.43
	CC	Male	$LW = -155.50 + 2.565HW$	1.00
		Female	$LW = -155.50 + 2.565HW$	1.00
		Male & Female	$LW = -155.5 + 2.565PT$	1.00
Exponential	HW	Male	$LW = 20.12e^{0.018HW}$	0.67
		Female	$LW = 124.50e^{-2E-0HW}$	0.001
		Male & Female	$LW = 26.50e^{0.015HW}$	0.46
	CC	Male	$LW = 10.17e^{0.022PT}$	0.99
		Female	$LW = 124.5e^{-2E-0PT}$	0.001
		Male & Female	$LW = 10.27e^{0.022PT}$	0.99
Logarithmic	HW	Male	$LW = 179.8LnHW - 704$	0.63
		Female	$LW = 121.3LnHW - 431.2$	0.26
		Male & Female	$LW = 155.9LnHW - 592.4$	0.44
	CC	Male	$LW = -1137.0 + 268.9LnPT$	0.99
		Female	$LW = -1141.0 + 269.9LnPT$	0.99
		Male & Female	$LW = -1139.0 + 269.5LnPT$	0.99
Power	HW	Male	$LW = 0.054HW^{1.677}$	0.68
		Female	$LW = 0.654HW^{1.143}$	0.29
		Male & Female	$LW = 0.151HW^{1.458}$	0.48
	CC	Male	$LW = 0.001PT^{2.410}$	0.99
		Female	$LW = 0.001PT^{2.404}$	0.99
		Male & Female	$LW = 0.001PT^{2.406}$	1.00
Polynomial	HW	Male	$LW = -68.38 + 1.890HW + 0.000HW^2$	0.63
		Female	$LW = -772.9 + 18.01HW - 0.089HW^2$	0.40
		Male & Female	$LW = -425.7 + 9.992HW - 0.044HW^2$	0.47
	CC	Male	$LW = -155.5 + 2.565PT + 0PT^2$	1.00
		Female	$LW = -155.5 + 2.565CC + 0CC^2$	1.00
		Male & Female	$LW = -155.5 + 2.565CC + 0CC^2$	1.00

CC: chest circumference; HW: height at withers; LW: live weight; R²: Coefficient of determination.

The only index statistically influenced by sex ($p > 0.05$) is the massiveness index 1.11 ± 0.00 with a coefficient of variation of 5.41%. The body indexes of profile, massiveness and format are variable in females.

Correlation between the biometric indices of donkeys in the Sudano-Sahelian zone of Cameroon

Table 10 summarizes the type and degree of association between biometric indices in the donkeys from the Sudano-Sahelian zone of Cameroon.

Table 8: Body indices of donkeys in the Sudano-Sahelian zone Cameroon and their coefficients of variation according to the locality

Parameters		Localities			Total (n=205)	P values
		Bogo (n=79)	Gazawa (n=72)	Kaélé (n=54)		
Compactness index	X ± E.S	1.14±0.01 ^a	0.98±0.01 ^b	1.14±0.01 ^a	1.08±0.01	0.00
	CV%	9.65	4.08	10.53	11.11	
Body profile index	X ± E.S	1.02±0.01 ^a	0.90±0.01 ^b	1.02±0.01 ^a	0.98±0.01	0.00
	CV%	10.78	4.44	10.78	11.22	
Relative body index	X ± E.S	0.89±0.01 ^b	1.02±0.01 ^a	0.89±0.01 ^b	0.93±0.10	0.00
	CV%	8.99	4.90	11.24	10.75	
Massiveness index	X ± E.S	1.12±0.01 ^a	1.09±0.01 ^b	1.12±0.01 ^a	1.11±0.00	0.01
	CV%	7.14	3.67	5.36	5.41	
Format Index	X ± E.S	0.99±0.01 ^b	1.11±0.01 ^a	0.99±0.01 ^b	1.03±0.01	0.00
	CV%	10.10	4.50	10.10	9.71	

^{a, b} in the row, means with the same superscripts in the same row are not significantly different ($P \leq 0.05$); X \bar{x} ± E.S: mean ± standard error; CV: coefficient of variation; n: sample size.

Correlation coefficients independently of locality and sex are highly variable. They may be negative or positive according to index pairs. A positive and highly significant correlation coefficient ($p > 0.01$) is observed between the format index (FI) and the relative body index (RBI), the compactness index (CI) and the body profile index (BPI). The highest negative correlation ($p > 0.01$) was obtained between the compactness index (CI) and the relative body index (RBI).

The matrix of correlations between the biometric indices by sex of donkeys in the Sudano-Sahelian zone of Cameroon is presented in Table 11.

The positive and highly significant correlation in females (0.91) and males (0.81) is the difference between the compactness index and the body profile index. The correlation between the format index and the relative body index is highly significant in females (0.91) and males (-0.41) but of opposite sign

Genetic variability, structure and genetic relationships between the donkey populations of the Sudano-Sahelian zone Cameroon.

Genetic variability of the donkey population

Table 12 and Figure 4 show the contribution of the 6 main components to the genetic variability observed in the donkey population of the Sudano-sahelian zone of Cameroon.

The first 2 components PC1 (F1) and PC2 (F2) would explain to 78.22% the variability observed in our population.

Specifically for the PC1 component, the measurements are live weight (LW), body length (BL) and chest circumference (CC). As for the PC2 component, it is the right ear (WEL) and left (LEL) length. The factorial discriminant analysis (FDA) revealed that the study population consisted of 3 genetic types (1, 2, 3), illustrated by figure 5 and 6.

Figure 5 and 6 show that the population of donkeys in the Sudano-Sahelian zone of Cameroon is made up of 3 genetic types 1, 2 and 3, and the genetic relationships between the three genetic types of the Sudano-Sahelian zone of Cameroon are represented by the distances between the barycenters of the classes (table 13). The genetic type 3 has higher body characteristics, followed by type 1 and type 2 respectively, although type 2 seems to be remote from the other.

Table 9: Body indices of donkeys and their coefficients of variation according to sex in the Sudano-Sahelian zone of Cameroon.

Parameters		Sex		Total (n=205)	P value
		Male (n=111)	Female n=94)		
Compactness index	X ± E.S	1.08±0.01	1.09±0.01	1.08±0.01	0.70
	CV%	11.11	10.09	11.11	
Body profile index	X ± E.S	0.98±0.01	0.97±0.01	0.98±0.01	0.57
	CV%	10.20	11.34	11.22	
Relative body index	X ± E.S	0.94±0.01	0.93±0.01	0.93±0.01	0.58
	CV%	10.64	9.68	10.75	
Massiveness index	X ± E.S	1.10±0.01 ^b	1.12±0.01 ^a	1.11±0.00	0.02
	CV%	4.55	6.25	5.41	
Format Index	X ± E.S	1.03±0.01	1.04±0.01	1.03±0.01	0.46
	CV%	9.71	10.58	9.71	

a, b in the row, means with the same superscripts in the same row are not significantly different ($P \leq 0.05$); X \bar{x} ± E.S: mean ± standard error; CV: coefficient of variation; n: sample size.

Table 10: Matrix of correlations between the biometric indices of donkeys in the Sudano-Sahelian zone of Cameroon.

	MI	RBI	BPI	FI	CI
MI	1				
RBI	-0.34**	1			
BPI	-0.21**	-0.85**	1		
FI	0.19**	0.86**	-0.99**	1	
CI	0.32**	-0.99**	0.86**	-0.87**	1

** $P < 0.01$ **. La corrélation est significative au niveau 0.01 (bilatéral). IF = Indice de compacité. BPI = body profile index. RBI = relative body index. MI = massiveness index. FI = format index, CI=compactness index

Table 11: Matrix of correlations between biometric indices in males (above the diagonal) and females (below the diagonal) of donkeys in the Sudano-Sahelian zone Cameroon.

	MI	RBI	BPI	FI	CI
MI	1	0.80**	-0.99**	0.23*	-0.82**
RBI	-0.45**	1	-0.79**	-0.41**	-0.99**
BPI	0.04	-0.91**	1	-0.24*	0.81**
FI	-0.06	0.91**	-1.00**	1	0.37**
CI	0.44**	-0.99**	0.91**	-0.92**	1

** $P < 0.01$ **. La corrélation est significative au niveau 0.01 (bilatéral). IF= Indice de compacité. BPI = body profile index. RBI = relative body index. MI = massiveness index. FI = format index, CI=compactness index

Table 12: Eigenvalue and cumulative variance of the various main components in the analysis of the variability within the population of donkeys in the Sudano-Sahelian zone of Cameroon.

Principales Components (PC)	Eigen value	Variability (%)	Cumulative %
PC1	3.69	52.54	52.54
PC2	1.80	25.68	78.22
PC3	0.71	10.14	88.36
PC4	0.43	6.09	94.45
PC5	0.23	3.31	97.76
PC6	0.16	2.24	100.00

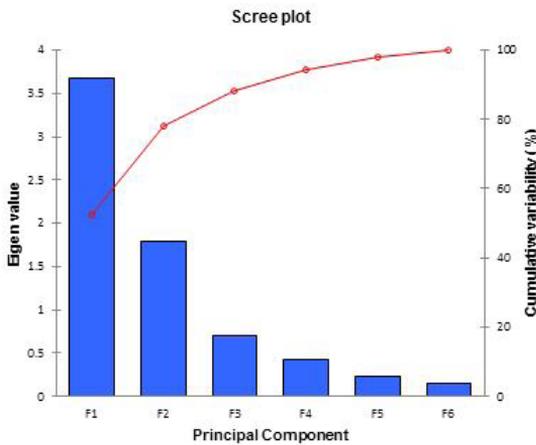


Figure 4: Contribution of the main components to the genetic variability of the donkey population of the Sudano-Sahelian zone Cameroon

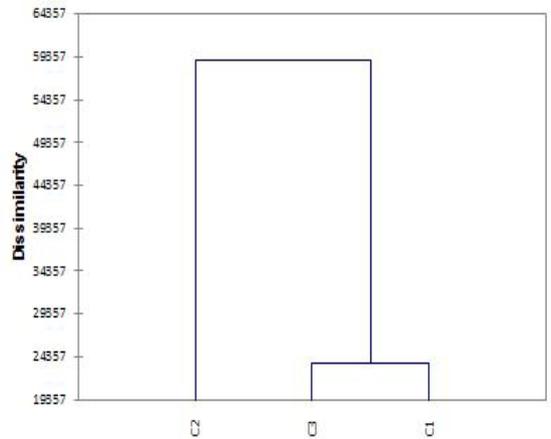


Figure 6: Phylogenetic tree of the genetic types of donkeys of the Sudano-Sahelian zone of Cameroon. C1: Genetic Type 1, C2: Genetic Type 2, C3: Genetic Type 3

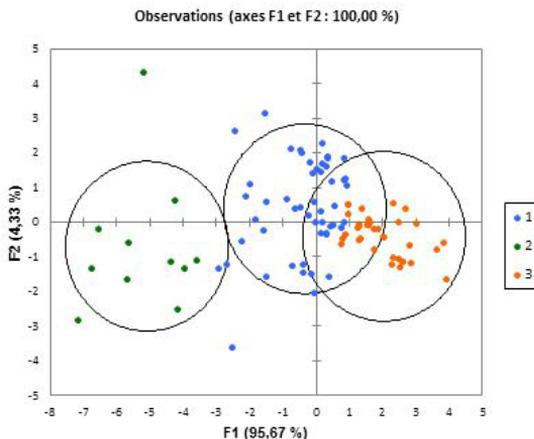


Figure 5: Disposition of the three genetic types making the donkey populations of the Sudano-Sahelian zone of Cameroon.

Table 13: Distances between the barycenters of classes

	1	2	3
1	0		
2	47.147	0	
3	23.239	69.978	0

The differences observed may be due to the different endogenous (species, race, strain...) and/or exogenous factors (climate, livestock management, feeding ...).

The phylogenetic tree, while confirming the structure of the population, has established the relationships between them. Thus the genetic types C3 and C1 are closer together whereas the genetic type 2 is far apart.

Discussion

This study revealed a varied range of coat colours (gray, black, bay, white), within the same group of animals, revealing that this population has not yet been purified by selection. The dominant coat colour is gray (80%), having variants from light to dark, with a black band on the shoulder and shades of white colour around the eyes, nose, abdominal region and the inner part of the legs. This is similar to the description made by Raveneau and Daveze (1996) corresponding to *Equus asinus nubicus* or *Equus asinus africanus*, which originates from the Horn of Africa in northern Sudan. Similar observations on the coat colour were also made by Roamba (2014), Salifou (2014) and Defeu (2015). Nevertheless, our results are not in line with those of Labbaci (2016) for Algerian donkeys with 4.9% grey and 65.6% brown. The diversity of robes is due to the migration of donkeys across the continent and to expression of expressive mutants given the ecological environment (Kefena *et al.*, 2011). The effect of natural selection or the intervention of the breeder through the crosses.

Wilson (1981) reports that there is little physical variation in donkeys across Africa and they rarely exceeds 105 cm in height at the withers. This is verified with our results on the mean height of the donkeys studied at 98.09 cm. La hauteur au garrot a été plus élevée chez les mâles que les femelles. Ces résultats corroborent ceux de Roamba (2014) sur les ânes du Sénégal (98.29 et 97.5 cm), bien que la hauteur de nos ânes soit relativement faible, elle ne s'éloigne pas de Salifou (2014) sur les ânes du Burkina Faso (97.4 cm) et Defeu (2015) au Nord-Ouest Cameroun 99.72 cm. However, our results differ from those found by Folch and Jordana (1997) who obtained a height at the withers of 139.2 cm and Labbaci (2016) in donkeys of Algeria 142.00 cm.

The chest circumference (108.24 cm) obtained on the donkeys of the Sudano-Sahelian zone of Cameroon is similar to the results of Ebangi and Vall (2005) on other donkey populations in Northern Cameroon (107.53 cm), Roamba (2014) in Senegal (108.9

cm) and by Salifou (2014) in Burkina Faso (105.1 cm). Moreover, our results are not far from those of by Defeu (2015) on the donkeys from the Northwest Cameroon (110.07 cm), while Labbachi (2016) revealed 124.26 cm of chest circumference in Algerian donkeys, value higher to our by 16 cm. The average length of the donkey neck of the Sudano Sahelian zone of Cameroon is (40.47 cm) and does not depart from that obtained by Defeu. (2015) on donkeys in the western highlands of Cameroon (44.41 cm). Our results are higher than those found by Salifou (2014) 28.7 cm on the donkeys of Burkina Faso and Roamba. (2014) 30.7 cm in Senegal but weak compared to Omo (47.21 cm) and Sinnar (47.46 cm) donkeys in Ethiopia (Kefena *et al.*, 2011).

In terms of length of ears (25.26 cm) our results are similar to those obtained by Roamba (2014) in Senegal (26.5 cm), Salifou (2014) in Burkina Faso (24.6 cm), Tebourdanne (2015) and Daloum (2015) cm in Chad. However, the results obtained with the Catalan ass (33.13 cm) in Spain (Folch and Jordana, 1997) and Labbachi (2016) 30.15 cm in Algeria are low. Yilmaz and Eturgrul (2012) also in their study on the donkeys of Turkey reported a length of ears (21.9 cm) less than ours. The length of the body recorded in donkeys in the Sudano-Sahelian zone of Cameroon is relatively close to that of Roamba (2014) for donkeys in Senegal (105.5 cm), Salifou (2014) in Burkina Faso (103.9 cm) and Daloum. (2015) for donkeys in Chad respectively. On the other hand, the results found by Labbachi (2016) for Algerian donkeys 157.62 cm have higher values than those of Cameroon in general, including that of Defeu (2015).

Salifou (2014) found values of live weight (114.02 kg) low in Burkina Faso than those of our results (122.11 kg). Our values however corroborate those found by Roamba (2014) in Senegal and Ebangi and Vall (2005) in Northern Cameroon (123.8 and 123.45 kg respectively). Labbachi (2016) on the other hand reported an average adult live weight of about 196.45 kg less than the European donkeys estimated at 350 kg by Audiot (1977) for donkeys in Algeria. The genetic make-

up, farming system and the type of use may explain those differences. The large variability recorded in height at the withers and length of the body, neck length and right ear may be of great interest in the future when genetic improvement targets are considered. This large observed variability can be the consequence of the different systems of breeding that exist between the populations since some animals are in free grazing while others are claustration. The effect of environmental factors may also be responsible for these different degrees of variability observed within the population.

Our results corroborate those of Pearson and Ouassat (1996); Nengomasha *et al.*, (1997); Roamba (2014); Kabore (2014); Defeu (2015) who found a high correlation coefficient between live weight and thoracic circumference.

The chest circumference was the best predictor of body weight. R^2 between 0.99 and 1.00 in both males and females. This is consistent with the results of several authors (Pearson and Ouassat, 1996, Pearson and Ouassat, 2001, Nengomasha *et al.*, 1997, Roamba, 2014, Kabore, 2014, Defeu, 2015). In addition, Pearson and Ouassat (1996) suggested the use of the chest circumference as a more reliable variable than the length of the trunk to determine the live weight of the donkeys, as some may have a distended belly.

As for the compactness index of the ass studied (1.09), this value is lower than that found by Daloum (2015, 1.66), Defeu (2014, 1.29). The principal component analysis showed that of the 6 components, the first 2 components, explain the variability observed in the population at 78.22%. However, it should be noted that the variables (live weight, thoracic trunk length) contribute respectively to 20.76%; 20.76%; 20.64% of the variance observed in our sample. Defeu (2015) found that the nine main components account for 90.24% of the total variability within the North-West Cameroun's donkey populations and of the 16 variables collected, only 2 made it possible to differentiate the population as well as possible. However, live weight alone contributes to 49.48% of the total variability.

The DFA shows us that there are three genetic types of donkeys in the Sudano-Sahelian zone of Cameroon, which corroborates those found by Defeu (2015) in the Northwest. Roamba (2014) found that the Senegalese donkey population is homogeneous as that of Salifou (2014) in Burkina Faso. However, Papa and Kume (2012) obtained two genetic types in Albania, Daloum (2015) and Labbachi (2016) mentioned five genetic types in Chad and Algeria respectively. The phylogenetic tree made from the Hierarchical Ascending Classification (HAL) protocol and the Discriminant Factorial Analysis (AFD) both revealed the existence of three genetic types with 2 close genetic types (type 1 and 3) one farther away from the other two (type 2).

Conclusion

The morphological characteristics were influenced by the localities and there is a variability of the colour of the dress in the donkey of the Sudano-Sahelian zone of Cameroon with a predominance of gray and its variants (light gray, dark gray). The localities did not influence the thoracic circumference. The body measurements of donkeys in the Sudano-Sahelian zone were comparable between sexes.

Correlations between measurements are highly variable in males and females. The linear, polynomial and power regression equations show that the thoracic circumference is a good predictor of the weight in the donkey of our study area. The highly variable biometric indices were influenced by the localities and not by the sex, these indices reveal longiline and medioline shaped animals. Principal component analysis revealed a high genetic variability within the donkey population of the study area and discriminant analysis showed that the population is composed of three genetic types: type 1 with higher characteristics, followed by type 3 and type 2 respectively. The dendrogram confirmed the existence of these three genetic types grouped into two subgroups: subgroup 1 consisting of genetic types I and III which are very similar, and subgroup 2 consisting of the genetic type II which is more distant. There is

a high phenotypic variability in donkeys in Far North Cameroon for all traits studied. This diversity suggests the possibility of a genetic improvement.

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CHARACTERISTICS OF CATTLE SLAUGHTERED IN THE MUNICIPAL ABATTOIR OF BAFOUSSAM AND PREVALENCE OF TUBERCULOSIS

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Abstract

The study was carried out in June 2016 using a semi-structured questionnaire, interviews and direct observations at the municipal slaughterhouse of Bafoussam in the Western Region of Cameroon (NL 5°27'58" and EL 10°24'32"). The objective was to determine the characteristics of cattle slaughtered and the prevalence of *tuberculosis* in that region. A total of 773 cattle were observed before slaughter and their carcasses subjected to a veterinary health ante mortem inspection. The animals (48.4 %) are mostly crossbred, of male sex (61.2%) and aged 5-8 years (50.1%). The overall prevalence of *tuberculosis* was 6.9%. That prevalence was higher among white Fulani (9.0%), cows (10.3%), and animals older than 8 years (12.8 %). The general average carcass weight was 151.76 ± 23.03 kg. Crossbreeds animals recorded an average carcass weight of 154.43±20.92 kg which is not significantly different from those of other breeds. The bulls have the highest average carcass weight (157.64 ± 22.84 kg), which is significantly different from that of cows. The study therefore confirms that bovine *tuberculosis* is endemic in the Bafoussam slaughterhouse and suggests that systematic knowledge on epidemiology and control of the disease as well as the interrelationship between animal and human *tuberculosis* should be updated.

Keywords: Bafoussam, Carcasses, Characteristics, Cattle, Prevalence, Slaughterhouse, *Tuberculosis*

CARACTERISTIQUES DES BOVINS ABATTUS DANS L'ABATTOIR MUNICIPAL DE BAFOUSSAM ET PREVALENCE DE LA TUBERCULOSE

Resume

Cette étude a été réalisée en juin 2016 à l'aide d'un questionnaire semi-structuré, d'entretiens et d'observations directes à l'abattoir municipal de Bafoussam dans la Région Ouest du Cameroun (NL 5°27'58" et EL 10°24'32"). L'objectif de l'étude était de déterminer les caractéristiques des bovins abattus et la prévalence de la tuberculose dans cette région. Un total de 773 bovins a été observé avant l'abattage, et les carcasses ont été soumises à une inspection vétérinaire ante mortem. Les animaux (48,4%) étaient majoritairement croisés, ils étaient mâles (61,2%) et âgés de 5 à 8 ans (50,1%). La prévalence globale de la tuberculose était de 6,9%. Cette prévalence était plus élevée chez les races Fulani blanc (9,0%), les vaches (10,3%) et les animaux âgés de plus de 8 ans (12,8%). Le poids moyen général de la carcasse était de 151,76 ± 23,03 kg. Les animaux croisés avaient un poids de carcasse moyen de 154,43 ± 20,92 kg, qui n'est pas significativement différent de ceux des autres races. Les taureaux ont le poids carcasse moyen le plus élevé (157,64 ± 22,84 kg), qui est significativement différent de celui des vaches. L'étude confirme donc que la tuberculose bovine est endémique à l'abattoir de Bafoussam, et propose une mise à jour des connaissances

systématiques sur l'épidémiologie et le contrôle de la maladie ainsi que sur les liens entre la tuberculose animale et la tuberculose humaine.

Mots-clés : Bafoussam, carcasses, caractéristiques, bovins, prévalence, abattoir, tuberculose

Introduction

Animal proteins supply in tropical Africa is not only insufficient, but also challenged by many livestock production constraints. Among issues faced by livestock development in tropical Africa, diseases account among the major ones, hindering the productivity of animals, supply of their products and by-products, hence affecting farmers' incomes.

Tuberculosis is of paramount importance to livestock officials and farmers, both because of its impact on animal productivity and as a zoonotic disease, jointly affecting almost all domestic and feral animals, and human beings, there considered a legally notifiable contagious disease. *Tuberculosis* is endemic in Cameroon (Awah-Ndukum *et al.*, 2005). The prevalence of the disease could also be attributed to the interaction between wildlife, some domestic animals and human beings. Measures that could help to eradicate the disease include: good knowledge of the disease and its epidemiology, application of appropriate screening measures in farms and slaughter houses for disease epidemio-surveillance and control. In Africa, *tuberculosis* is one of the major diseases leading to huge economic losses estimated at many million US dollars yearly (Ly, 2007). It affects different farming systems (Thys *et al.*, 2006) and human development as well (Cosivi *et al.*, 1998). The lack of biosecurity measures and contacts between infested animals and human beings or vice versa are among risk factors that promote the spread of this disease. The studies of *tuberculosis* in the Democratic Republic of Congo, Cameroon and Chad indicate prevalence of 7.6, 2.7 and 10.3 % respectively (Awah-Ndukum *et al.*, 2005).

In Cameroon, cattle account for 54% of all meat products locally produced and consumed by population. The cattle annually provides 110 000 tons of meat and 174 000 tons of milk which is entirely consumed by the local

population. It contributes around 950 billion CFA francs in capital GDP (MINEPIA, 2009). Due to its many advantages as source of protein, income generation, power and employment, cattle breeding is an important asset that can help to quickly increase meat production (Boukar *et al.*, 2015). However, increase production needs to take into consideration the prevalence of *tuberculosis*. Despite its potential in the economy of the country, information related to the epidemiology of this zoonotic disease is limited, (Awah-Ndukum *et al.*, 2005; Tiega, 2012; Meka, 2014). Bafoussam is the regional head quarter of the Western Region of Cameroon with the one of the highest population density in Cameroon (BUCREP, 2010). In the municipal slaughterhouse of Bafoussam, post-mortem information on the health of these animals is lacking seriously. Hence the need to conduct this study to take stock and contribute to improving the productivity and performance, biosecurity and biosafety of the beef sector in Cameroon is imperative. The objective was to contribute in improving the knowledge on the prevalence of *tuberculosis* in Cameroon. More specifically, to analyse the characteristics of cattle slaughtered and assess the effect of cattle origin, breed, sex and age on the prevalence of bovine *tuberculosis* at the municipal slaughterhouse of Bafoussam.

Material and Methods

Location of the study area

The study was conducted in June 2016 at the Bafoussam municipal abattoir (5°27'58"N, 10°24'32"E) (Figure 1). The area is dominated by highlands and valleys. The zone has no major rivers; the populations get most water from streams. The soil is red (feralitic). The prevailing climate is Cameroonian type with two seasons: the dry season running from mid-November to mid-March and a rainy season from mid-March to mid-November.

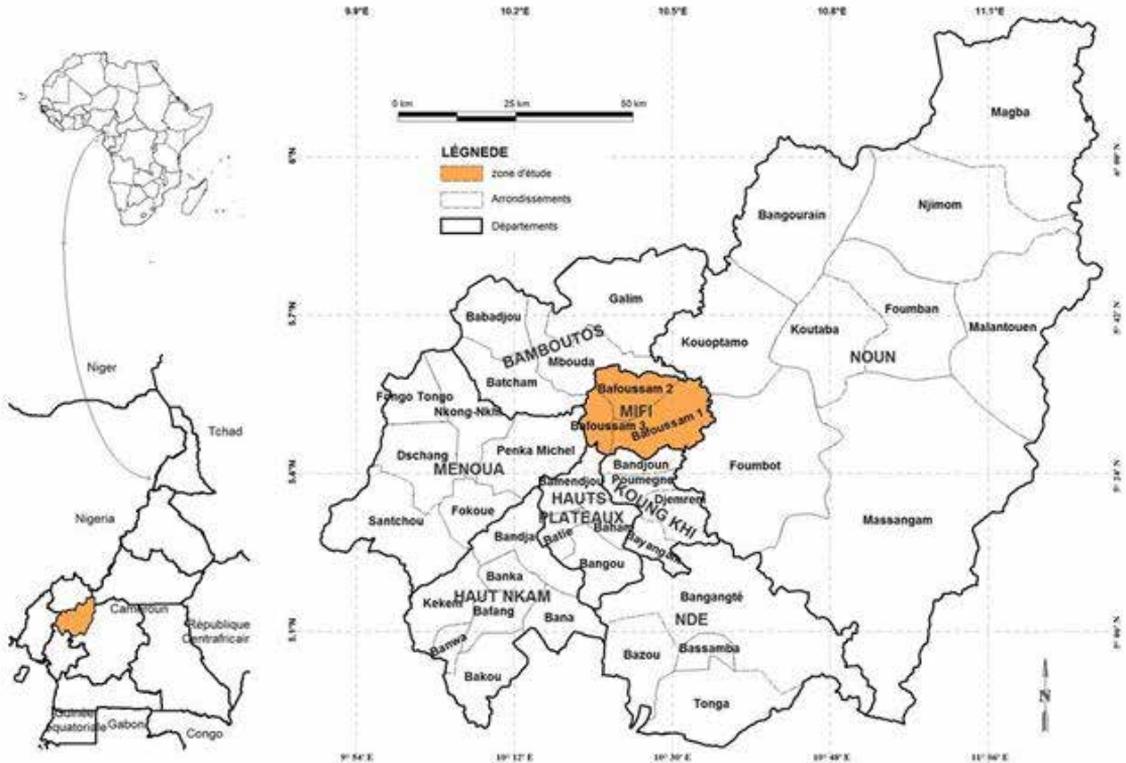


Figure 1: Location of the study area

The temperatures range from 18 to 23°C and the average annual rainfall is 1832.2mm corresponding to 153 days of rain, with the pic rainfalls registered during June, July, August and September. The months of December and January are marked with severe drought. The area of study (Bafoussam I) is a melting pot of many ethnicities, religions and social strata. Livestock activities here are dominated by poultry, swine and ruminants (mostly goat and sheep) production (DREPIA/O, 2013) most often associated with non-conventional species.

Animal material

The animals concerned for this study were cattle. After their arrival at the slaughterhouse they are housed in the holding pen (380 m2) for a variable period of time before slaughter. During this period the animals are registered and subjected to the ante mortem inspection before starting the slaughtering process.

Data collection

A structured questionnaire was used directly to livestock owners at the level of slaughter house to collect data on the origin of animals, the breed was identified as described by Lhotse (1969). Sex determination was made by visual observation of the animals, seexual dimorphism being marked and supported by the presence of testis in males and the udders in females. The age of the cattle was estimated using observations on dental table (Poivey et al., 1981). The physiological status (pathological situation, pregnancy or not in females on carcass, etc.) was determined. In this study we used the hot carcass weight, which is the weight of the carcass after removing leg, viscera, head, skin and genitals before the packaging method in the cold room. The diseases diagnosis involved examination of the state of the carcass and the control of hazardous diseases to human health. Determining the prevalence of diseases was based on post-mortem examination of carcasses, which was done by visual inspection,

palpation and incision of different organs (lungs, liver and kidneys). Lymph nodes of the head, chest, mesenteric and other skin diseases that can present lesions were also inspected.

Parameters studied

The parameter investigated was the prevalence of bovine *tuberculosis*, calculated based on the total population, the incidence of the disease at a specific time.

$$\text{Prevalence \%} = \frac{\text{Number of infected animals}}{\text{Number of total animals}} \times 100$$

Data analysis

Descriptive statistics were performed using Excel software version 2007. The influence of breed, sex, age on prevalence of *tuberculosis* and carcass weight of animals was subjected to analysis of variance, to one way ANOVA. In case of significant difference, Duncan multiple range test was used for the separation of means at 5% with SPSS version 21.

Results

Characteristics of cattle slaughtered at the municipal abattoir of Bafoussam

Characteristics of cattle slaughtered at the abattoir of Bafoussam are presented in table I.

The cattle slaughtered come exclusively from the cattle market of Foumban, upon investigations, it was revealed that most of the animal sold on that market also come from Banyo (Adamawa region), Bansa (North West region) and the various sub-divisions of the Noun Division (Western region).

The crossbreed represent up to 48.4% of the animals slaughtered, followed by the white Fulani (40.2%) and Red Fulani (11.4%). Gudali cattle are absent. Among the crossbreeds, mostly males are slaughtered (66.3%), the same situation is observed in white Fulani (59.5%), while in red Fulani, mostly females are slaughtered (54.5%).

Table I: Distribution of cattle slaughtered at the abattoir of Bafoussam according to the breed, age and sex.

Origin Breed	Sex	Foumban (100%) Age in years, (%)				Total
		< 3	3-5	5 -8	>8	
White fulani	Male	0	111	68	6	185 (59.5)
	Female	7	12	70	37	126 (40.5)
	Total	7 (0.9)	123 (15.9)	138 (17.8)	43 (5.6)	311 (40.2)
Red fulani	Male	0	20	20	0	40 (45.5)
	Female	0	7	28	13	48 (54.5)
	Total	0 (0.0)	27 (3.5)	48 (6.2)	13 (1.7)	88 (11.4)
Crossbreeds	Male	7	122	101	18	248 (66.3)
	Female	0	14	100	12	126 (33.7)
	Total	7 (0.9)	136 (17.6)	201 (26.0)	30 (3.9)	374 (48.4)
Total	Male	7	253	189	24	473 (61.2)
	Female	7	33	198	62	300 (38.8)
	Total	14 (1.8)	286 (37.0)	387 (50.1)	86 (11.1)	773 (100.0)



Figure 2: Distomatosis (a), lung tuberculosis (b), liver tuberculosis (c) and spleen tuberculosis (d) observed at the municipal abattoir of Bafoussam

The cattle slaughtered are predominantly males (61.2%), mostly of the age group of 5 to 8 years (50.1%), followed by those cattle aged between 3 to 5 years (37.0%) and lastly those less than 3 years (1.8%).

Pathologies observed on cattle slaughtered at the municipal abattoir of Bafoussam

From the post-mortem inspection of 773 carcasses, two main pathologies were identified namely tuberculosis and distomatosis (figure 2). Despite that distomatosis was predominant (9.1%), our attention for this study focused on tuberculosis which was found in 6.9% of the animals slaughtered. Splenomegaly was also noticed at 1.0%.

Influence of breed, sex and age on the prevalence of tuberculosis of cattle slaughtered at the municipal abattoir of Bafoussam

The prevalence of tuberculosis according to breed, sex and age of cattle is presented by table 2

The white Fulani and the crossbreeds recorded the significantly ($P>0.05$) highest prevalences (9.0 and 8.0 % respectively) compared to the red Fulani (4.8%).

The males presented the significantly ($P>0.05$) low prevalence (4.7%) than females (10.3%).

Animals aged five years and above were significantly ($P>0.05$) most affected, the highest prevalence been observed in > 8 years group (12.8%) followed by animals aged between 5 to 8 years (10.1%), though the prevalence of tuberculosis was statistically comparable ($P>0.05$) between cattle of these two age groups. The most affected organs were the lungs, liver and spleen (figure 2).

Carcass weight of cattle slaughtered at the municipal abattoir of Bafoussam

The carcass weights based on breed, sex and age of cattle slaughtered at the municipal abattoir of Bafoussam are presented in table 3.

The crossbred animals have the highest average carcass weight (154.43kg), though not significantly different ($P\leq 0.05$) from that of red and white Fulani breed.

Table 2: Prevalence of *tuberculosis* according to breed, sex and age of cattle slaughtered at the municipal abattoir of Bafoussam

Characteristics		Number inspected	Number infected	Percentage (%)
Breed	White fulani	311	28	9.0 ^a
	Red fulani	88	7	4.8 ^b
	Crossbreeds	374	18	8.0 ^a
	Total	773	53	6.9
Sex	Male	473	22	4.7 ^a
	Female	300	31	10.3 ^b
	Total	773	53	6.9
Age (years)	< 3	14	0	0.0 ^a
	3-5	286	3	1.0 ^a
	5-8	387	39	10.1 ^b
	> 8	86	11	12.8 ^b
	Total	773	53	6.9

Table 3: Carcass weight according to breed, sex and age of cattle slaughtered at the municipal abattoir of Bafoussam

Characteristics		Sample size	Average weight carcass (kg)
Breed	Red fulani	88	148.60±24.21 ^a
	White fulani	311	152.26±23.96 ^a
	Crossbreed	374	154.43±20.92 ^a
Sex	Female	300	139.80±21.68 ^a
	Male	473	157.64±22.84 ^b
Age (years)	< 3	14	126.08±22.35 ^a
	3-5	286	147.13±19.72 ^b
	5-8	387	155.59±25.04 ^{bc}
	> 8	86	162.26±24.47 ^c

^{a, b, c} : on the same line, the means with the same letter are not significantly different.

**Figure 3:** Calf of nearly 8 months age from a slaughter cow at the municipal abattoir of Bafoussam

Male animals exhibited the highest ($P>0.05$) average carcasses weight (157.64kg), while animals aged 8 years and above had significantly ($P<0.05$) the highest carcass weight (162.26 ± 24.47 kg) compared other age groups.

Losses of calves by slaughtering of pregnant cows at the municipal abattoir of Bafoussam

This study noticed that there were serious calves losses (figure 3) registered upon slaughtering of pregnant cows. 24 cows on 300 after post mortem examined were pregnant (8, 0%). When combined to the carcass

condemnation due to infections, this represent a great loss of incomes for farmers and poses the problem of pregnancy diagnosis test on cow before slaughter.

Discussions

All the cattle slaughtered at the municipal abattoir of Bafoussam come from the cattle market of Fouban. This revealed the deficiency in local cattle production to satisfy the consumer needs in the headquarter of the Western region of Cameroon. This could be resultant form the high demographic growth occupying all range lands, and the local crop farming tradition encouraging small livestock which could be easily integrated to agriculture. The result obtained here is contrasting with those reported in the slaughterhouse of Dschang by Meka (2014) where the animals came from Bamenda, Fouban and Dschang. The explanation could be from the fact that the cattle market of Fouban is closer in distance to the city of Bafoussam compared to Bamenda, and the status of the road from other regions by trucks is a highly limiting factor for farmers to bring their animals to the market.

The crossbred animals (48.4%) and the white Fulani (40.2%) are the most slaughtered animals. A survey among farmers regarding crossbreeding revealed that this action is mostly meant to improve growth rate and carcass characteristics, hence intended for better gain in meat production. The higher presence of white Fulani cattle could be due to the fact that being reared mostly by Nomadic Mbororo communities, they are more adapted to distant walking than Gudali and are well adapted to the extensive farming system practiced in the hilly areas of the western highland of Cameroon.

Males are the most slaughtered cattle. This result is similar to that reported by Dilla (2013) and Meka (2014) and. This is explained by the fact that the male cattle are generally culled for sale at early age by farmers to control the sex ration and stabilize the herd size. Only best bulls are maintained for longer period in the herd, while younger ones are the

main target for the market in case of any family financial issue, while females are more likely to be retained in the herd for reproduction. Only females believed to be underproductive are sent to the market. However, this is most of the time done without careful evaluation, as the proportion of pregnant cows accidentally slaughtered is high.

The prevalence of *tuberculosis* (6.9%) among cattle slaughtered at the municipal abattoir of Bafoussam should be a serious concern to be addressed to the livestock and public health decision makers. The presence of these diseases can be explained by the fact that the cattle are raised in extensive, nomadism and transhumance system where they graze in the same pasture and drink from the same water points. This proximity creates conditions favorable to the propagation of these diseases, while proper biosecurity issues in livestock farming in general in Cameroon still to be addressed. This actual results on the prevalence of *tuberculosis* in Bafoussam metropolitan town collaborates with the findings of Awah-Ndukum et al. (2005) at the SODEPA Douala abattoir thus confirming the disease being endemic in Cameroon. The prevalence of *tuberculosis* in Cameroon cattle production has increased from 2.7 ± 2.4 % in 2010 to about 6.9 % 2016 as reported by (Awah- Ndukum et al., 2010). This rate is eight times higher than that recorded by Tiéga (2012) in Yaoundé and similar to those of Ebolowa (Meka, 2016). This may be explained by the fact that the probability that the carcasses infected with bovine *tuberculosis* escaping the vigilance of inspectors is higher in the slaughterhouses of Yaoundé. This is due to the larger number of animals slaughtered per day (188-250) in Yaoundé with fewer inspectors (3-5). While in the slaughterhouse of Bafoussam, we have just 25 animals slaughtered per day, for 4-5 veterinarians involved, it is thus easier to detect cases of diseases in smaller abattoirs than in larger once. But in general, these prevalence in any region of Cameroon are most likely to be higher if proper diagnosis methods were applied. The females being most affected in this study confirmed the records (Dilla, 2013). This could be explained by the fact

that, the slaughtered females cattle generally are very old, bovine *tuberculosis* is a disease that evolves with age, females have more chances to be disease carriers (Cleaveland *et al.*, 2007). This also explains why animals of more than 8 years are the most affected. Blancou *et al.* (1971) in Madagascar, Sidibe *et al.* (2003) in Mali, Cleaveland *et al.* (2007) in Tanzania reported a comparable result. Then *tuberculosis* prevalence increases with age of the animals, especially in areas where the infection is endemic. In the same line, *tuberculosis* is a slow killer disease, young infected animals will manifest the disease at an older age (Thorel, 2003).

Regarding carcass weight, the male are heavier. This could be explained by the fact that male cattle are sold in responds to speculation or for investment purposes and are marketed in better body condition than cows, which are mainly sold under culling. The highest carcass weight of males also holds in that this category of animals is the most used for fattening, to take advantage of the sexual dimorphism in growth performances. It is for this reason that the largest gap of carcass weight is observed in males. Animals over 8 years have the highest carcasses weight but still no significant difference compared to carcass weight of animals between 5 to 8 years. This is mainly due to the fact that animals usually reach their adult weight between 6 and 8 years.

Conclusion

Base on this study on characteristics of cattle slaughtered in the municipal abattoir of Bafoussam and prevalence of *tuberculosis*, it is established that the animals come from the cattle market of Foumban, they are mainly crossbreed and of bulls and animals between 5 to 8 years are most slaughtered. Two main pathologies were detected: *tuberculosis* and distomatosis, though the present study mostly focused on *tuberculosis*, which prevalence is 6.9%. cows and animals over 8 years old are the most affected. The rate of calve loss by the slaughtering of pregnant cows is about 8.0%, while the average carcass yield is still quite low as compared to that of known improved

breeds in the region.

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EFFECTS OF VARYING FEEDING TIMES ON FERTILITY AND HATCHABILITY OF BROILER CHICKEN BREEDERS IN A TROPICAL ENVIRONMENT

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Abstract

This study investigated the effects of feeding times on total egg production, fertility and hatchability of broiler chicken breeders in a tropical environment. The experiment was conducted using 240 Marshal Broiler breeder flocks for eight weeks between 40 to 48 weeks of age. The birds were randomly assigned to 3 treatment groups of feeding times (3, 5 and 7 am), with four replicates per treatments. Each feeding time consisted of 80 birds replicated in quadruplets of 20 birds each in a Completely Randomized Design. Prior to the eighth week data collection, the birds were allowed to get accustomed to the new feeding pattern for the first two weeks. The first four weeks (1 – 4 weeks) of the experiment was scheduled for the feeding time and eggs were collected and taken to the hatchery on regular basis. The second four weeks (5 – 8 weeks) was for monitoring of the eggs in the hatchery and the stages of embryonic development. Significant ($p < 0.05$) differences were obtained only in the hen-day egg production, average number of chicks hatched and percentage hatchability. Broiler chicken breeders on 3 and 5 am feeding times recorded similar hen-day egg production. Survivability was best ($p < 0.05$) at 3 am (85.16%) feeding time and poorest in broiler chicken breeders on 5 am feeding time. Feeding did not impact negatively on the embryonic development. It was then concluded that feeding broiler breeders at 3 am or 5 am in tropical environment enhanced better hen-day egg production, and hatchability.

Keywords: Broiler breeders, egg production, feeding time, embryonic development

EFFETS DES DIFFERENTS TEMPS D'ALIMENTATION SUR LA FERTILITE ET LE TAUX D'ECLOSION DES POULETS DE CHAIR REPRODUCTEURS DANS UN ENVIRONNEMENT TROPICAL

Resume

La présente étude a analysé les effets des temps d'alimentation sur la production totale d'œufs, la fertilité et le taux d'éclosion des poulets de chair reproducteurs dans un environnement tropical. L'expérience a été menée en utilisant 240 troupeaux de poulets de chair reproducteurs âgés de 40 - 48 semaines pendant huit semaines. Les oiseaux ont été répartis de manière aléatoire à 3 groupes de traitement de temps d'alimentation (3, 5 et 7 heures du matin), avec quatre répétitions par traitement. Chaque temps d'alimentation comportait 80 oiseaux répliqués en quadruplets de 20 oiseaux chacun dans un dispositif complètement randomisé. Avant la collecte des données de la huitième semaine, les oiseaux ont été autorisés à s'habituer au nouveau mode d'alimentation pendant les deux premières semaines. Les quatre premières semaines (1 à 4 semaines) de l'expérience ont été programmées pour la période d'alimentation et les œufs ont été régulièrement recueillis et amenés à l'écloserie. Les deux autres quatre semaines (5 à 8 semaines) visaient la surveillance des œufs dans l'écloserie et les stades de développement embryonnaire. Des différences significatives ($p < 0,05$) n'ont été obtenues qu'au niveau de la production journalière d'œufs, du nombre moyen de poussins éclos et du pourcentage d'éclosion. Les poulets de chair reproducteurs dont les temps d'alimentation étaient fixés à 3 et 5 heures du matin ont enregistré une

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production journalière similaire d'œufs. Le taux de survie était meilleure ($p < 0,05$) au temps d'alimentation de 3 h a.m. (85,16%) et le plus faible chez les poulets de chair reproducteurs soumis au temps d'alimentation de 5 heures du matin. L'alimentation n'a pas eu d'impact négatif sur le développement embryonnaire. On a alors conclu que l'alimentation des poulets de chair reproducteurs à 3 heures du matin ou à 5 heures du matin dans un environnement tropical améliorerait la production d'œufs et le potentiel d'éclosion.

Mots-clés : poulets de chair reproducteurs, production d'œufs, temps d'alimentation, développement embryonnaire

Introduction

The efficiency of hatcheries is often measured in terms of hatchability which greatly influences the production and distribution of day-old chicks and eventually initiates the poultry production chain (King'ori, 2011). Although, genetic factors could influence fertility and hatchability of breeder birds, but with proper management and handling of these birds, some genetical traits can be suppressed. According to Zuidhof *et al.*, (2014), there have also been unintended consequences, and a prime example is the challenge of allocating feed to parent stocks, which is faced daily by hatching egg producers. Overweight hens produce excess follicles in their ovaries and their reproductive output is seriously compromised in terms of settable egg numbers, fertility and chick quality.

Changing time of feeding (from morning to afternoon) was among many experimental alternatives used to improve egg shell quality of broiler breeder hens maintained under optimal environmental conditions (Bootwalla *et al.*, 1983; Wilson and Keeling, 1991). It was reported that afternoon feeding resulted in a higher rate of egg production (Balnave, 1977; Bootwalla *et al.*, 1983). However, a decline in egg production was observed by Harms (1991) when feeding time was changed from morning to afternoon. Wilson and Keeling (1991) also speculated that a change in time of lay or increased transit time of the egg through the oviduct, could be responsible for the delay in time of oviposition observed when broiler breeder hens were fed in the afternoon. A study by Spradley *et al.*, (2008) reported that broiler breeders fed twice a day laid more and heavier eggs through 42 weeks of age and had better overall body weight uniformity than those fed

once a day. Chen *et al.* (2006) reported that high glucose availability due to hyperphagia in broiler breeders could result in lipotoxicity and ovarian dysfunction. This thereby hinders fertility and hatchability which are major parameters of reproductive performance and are most sensitive to environmental and genetic influence.

Several feeding regimes systems are applied to improve the performance of fertile egg production in poultry. The usual procedure is to feed broiler breeder hens once a day particularly in the morning. Cave (1981) evaluated different feeding schemes for female broiler breeders from 24 to 63 weeks of age, and reported no difference in egg production. The lowest body weight gain, together with the heaviest egg mass, indicated that more frequent feeding allowed better partition of nutrients for egg formation instead of body tissue.

Feeding time seems to have the potential to influence the performance of adult broiler breeder flocks and it is thus of great importance (Backhouse and Gous, 2006). Formerly, feeding breeders in the late afternoon was a standard procedure, but latter study (Leeson and Summers, 2000) showed that choice of feeding time for adult breeders could influence the production of settable eggs, egg shell quality, fertility and hatchability. The authors further stated that feeding in the late afternoon resulted in probable reduction of mating activities and the increase of the amount of broken eggs because the hens are more interested in feeding than in mating activity and there are more aggression between males. Additionally, the later the hens are fed the higher the chances for the production of eggs with abnormal shells resulting in a reduction in hatchability (Clunies *et al.*, 1993). Hence, choosing an adequate feeding system as

well as the best time in the day for providing the feed could be considered among the most important practices, which help small poultry producers to achieve the best possible production performance especially under hot and humid climatic conditions in tropical countries. The adequate time in the day for poultry feeding, especially in hot regions, could be considered as one of the most important factors that play an important role in the body thermo-regulation of birds (De Avila *et al.*, 2003; Ashour *et al.*, 2004). This study therefore aimed at determining the effects of changing the time of poultry feeding in the dry season and at cooler hours in a tropical environment in a broiler breeder farms on the productive and reproductive performance of the birds.

Materials and methods

Experimental site

The research was conducted at Obasanjo Farms Nigeria Ltd., Igbo-Ora breeder's Farm, Oyo State, Nigeria. The site is situated in the rain forest zone of Nigeria, on latitude 7°26'1.79" N and longitude 3°17'16.37" E with an elevation of 140m above sea level (Google earth, 2016).

Experimental birds and management

The experiment was conducted using 240 Marshal Broiler breeder flocks for eight weeks between 40 to 48 weeks of age. These birds were randomly assigned to 3 treatment groups, with four replicates per treatments. The birds were housed in a fenced hall, with cages equipped with nipple drinker system. Room's cleaning and disinfection programs were carried out in accordance with standards employed by the Farm. Prior to the eighth week data collection, the birds were allowed to get accustomed to the new feeding pattern for the first two weeks. The first four weeks of the experiment was scheduled for the feeding time and eggs were collected from the birds and taken to the hatchery on regular basis (not more than 5 days storage period). The second four weeks was then used for the monitoring of the eggs in the hatchery and the stages of

the embryonic development.

Feeding Times

Three feeding times were used, 3.00 am (as control; the practice on the farm), 5.00 am and 7.00 am i.e. 3:00 hours, 5:00 hours and 7:00 hours. All birds were fed once a day at the chosen varying feeding times on 135 g of feed per bird per day. All the treatments were replicated four times to contain 20 birds per replicate. The lighting schedules for the birds were for 12 hours of day light, 4 hours of artificial lighting and 8 hours of darkness.

Data Collection

Laying performance of breeders

Daily records of feeds consumed, mortality from the start of the experiment till the end of the experiment was recorded and used for this study.

1. Feed Conversion Ratio (FCR): This was calculated as the ratio of feed consumed (g) to weight of hen-day egg laid (g):

$$FCR = \frac{\text{Total feed intake (g)}}{\text{Total egg produced (g)}}$$
2. Hen-day production (HDP): This was measured by the ratio of the egg laid in a day divided by the number of birds alive multiplied by 100.

$$HDP = \frac{\text{Egg laid per day}}{\text{No of birds alive}} * 100 \%$$
3. Breeder house mortality: This was a measure of birds that are alive, which gave an indication of the survivability of the breeder stocked. It was calculated by the formula:

$$\text{Breeder house survivability} = \frac{\text{No of broiler breeders that are alive}}{\text{Total No of broiler breeders in stocked}} * 100 \%$$

Percentage Egg production

This was calculated by the ratio of the total number of eggs produced to the total number of birds stocked per pen multiplied by 100. That is:

$$\text{Percentage egg production} = \frac{\text{Total No of eggs produced}}{\text{Total No of birds stocked per pen}} * 100$$

Fertility Test

This was determined on the 5th day of incubation. A total of 390 eggs set were moved into the candling room and candling was done to check for fertility. Clear eggs (eggs with no living shrimp-like structure) were separated.

Percentage Fertility

This was calculated as the number of fertile eggs divided by the number of eggs set and multiplied by 100% Fertility = (No of fertile eggs/No of eggs set) *100

The percentage infertile (% infertile) eggs was determined by subtracting the percentage fertile eggs from 100.

Percentage Hatchability

This was determined by the ratio of number of chicks hatched to the number of fertile eggs multiplied by 100% Hatchability = (No of chicks hatched/No of fertile eggs) *100

Determination of Embryonic Development

A total number of three hundred and thirty-six (336) hatching eggs (4 eggs from each replicate of 3 treatments) at days 1, 3, 5, 7, 10, 15 and 18 of incubation were broken into petri dishes for the determination of embryonic development. Also, 4 eggs (an egg per replicate) from each treatment were gently broken to determine the weight of the growing embryo, the amount of remaining yolk, albumen and moisture loss. Pictures of the more pronounced development of the embryo were taken.

Chick Quality Assessments

Ten chicks were picked at random from each treatment to assess chick quality using PASGAR® score for parameters observed (Meijerhof, 2009). For each chick, the average score was calculated. The parameters evaluated include the following:

1. **Chick Vitality:** if when the chick is made to lie on its back and it sits up immediately (score=0) but if it takes more than 3 seconds to sit up (score=1).
2. **Navel:** the navel is normal when it is completely closed and all yolk is absorbed (score=0). If navel is open and/or a dried cord can still be seen (score=1).

3. **Hock Joint:** if the hock joint is not enflamed and have a normal colour (score=0). But if the hock joint is enflamed and/or red (score=1).
4. **Beak:** if the beak is clean and the nostrils are closed (score=0), but if the beak is dirty and/or has a red dot (score=1).
5. **Abdomen:** the size of the abdomen depends on the size of the yolk sac and is essentially linked to temperature and humidity in during incubation. If the chick has a soft abdomen (score=0), but if the abdomen is hard and the skin is stretched (score=1).

Statistical Analysis

Data obtained were subjected to completely randomized design. Significantly ($p < 0.05$) different means among variables were separated using Duncan's multiple range test as contained in SAS (2004).

Results

Effects of feeding times on egg production of broiler chicken breeders

Table 1 shows the effects of feeding time on the total egg production of broiler chicken breeders. Significant ($p < 0.05$) difference was obtained only in the hen-day egg production among the treatments. Comparable values of 69.19% and 69.83% were observed in broiler chicken breeders on 3 and 5 am feeding times, respectively and these were significantly ($p < 0.05$) higher than 60.55% recorded in birds on 7 am feeding time.

Effects of feeding times on egg fertility and hatchability of broiler chicken breeders

The effects of feeding time on egg fertility and hatchability of broiler chicken breeders in tropical environment are shown in Table 2. Feeding times significantly ($P < 0.05$) affected the average number of chicks hatched and the percentage hatchability. Broiler chicken breeders on feeding times 3 am and 5 am recorded similar values in the average number of chicks hatched and percentage hatchability. Birds on 7 am feeding time recorded the least (25.34) average number of chicks hatched and

the poorest percentage hatchability (90.53 %). The other parameters measured were not significantly ($p>0.05$) affected by the feeding times. Broiler chicken breeders on 3 am and 5 am feeding times recorded numerically higher values (90.31 and 84.84 eggs, respectively) in egg laid compared to the birds on 7 am (79.69 eggs) feeding time. The same observation was made for the egg set across the treatments. However, birds on 5 am feeding time recorded numerically highest percentage fertile eggs (91.27 %) and percentage infertile eggs (9.57 %) than birds on 3 am and 7 am feeding times. Effects of Feeding Time on survivability of Broiler Chicken Breeders

Table 3 shows the effects of feeding time on the survivability of broiler chicken breeders in a tropical environment. The best ($P<0.05$) survivability was obtained in broiler chicken breeders on 3 am feeding time (85.16 %) followed by birds fed at 7 am (74.69 %) while the poorest survivability was observed in broiler chicken breeders on 5 am feeding time.

The other parameters measured were not significantly ($P>0.05$) influenced by the feeding times.

Effects of Feeding Time on the quality of chicks of Broiler Chicken Breeders

Table 4 shows the effects of feeding time on the quality of chicks of broiler chicken breeders in a tropical environment. Feeding times did not significantly ($p>0.05$) influence the average chick weight, vitality of the chicks, navel, hock joint, beak and abdomen.

Effects of feeding time on embryonic development of broiler chicken breeder eggs

Plates 1 to 7 show the embryonic development of broiler chicken breeder eggs as affected by the feeding times. It was observed that feeding did not impact negatively on the embryonic development hence at every stage of measurement, a plate describes the development irrespective of the feeding times.

Table 1: Effect of feeding time on total egg production of broiler chicken breeder birds

Parameter	Feeding time		
	3 am	5 am	7 am
Feed intake (g/bird/day)	126.02±5.59	117.64±1.20	126.23±1.09
Average egg mass (g)	45.27±3.20	47.81±4.49	38.60±1.31
Average egg laid	90.31±3.89	84.84±4.78	76.69±2.71
Feed conversion ratio	1.95±0.15	1.73±0.05	1.98±0.01
Hen-day egg production (%)	69.19±1.10 ^a	69.83±4.05 ^a	60.55±1.65 ^b

^{a,b}: Means on the same row having different superscripts are significantly ($p<0.05$) different

Table 2: Effects of feeding time on egg fertility and hatchability of Broiler chicken breeders

Parameter	Feeding time		
	3 am	5 am	7 am
Average number of eggs laid	90.31± 3.89	84.84± 4.76	79.69± 2.71
Average number of eggs set	32.75±2.68	31.50±2.56	30.75±2.56
Average number of fertile egg	29.75±2.72	28.75±1.38	28.00±1.47
% Fertile eggs	90.84±2.91	91.27±2.90	91.06±3.53
Average number of infertile eggs	3.00±0.41	2.75±0.48	2.75±0.25
% Infertile eggs	9.16±1.43	9.57±1.33	8.94±1.36
Average number of chicks hatched	28.67±2.72 ^a	27.69±1.75 ^a	25.34±0.75 ^b
% Hatchability	96.39±0.37 ^a	96.32±1.57 ^a	90.53±2.52 ^b

^{a,b}: Means on the same row having different superscripts are significantly ($p<0.05$) different

Table 3: Effects of feeding time on survivability of broiler chicken breeders

Parameter	Feeding time		
	3 am	5 am	7 am
Feed intake (g/bird/day)	126.02± 5.59	117.64± 1.20	126.23± 1.09
Average number of eggs laid	90.31±3.89	84.84±4.76	79.69±2.71
Feed conversion ratio	1.95±0.15	1.74±0.05	1.98±0.01
Breeder house survivability (%)	85.16±9.82 ^a	63.72±3.96 ^b	74.69±2.02 ^b

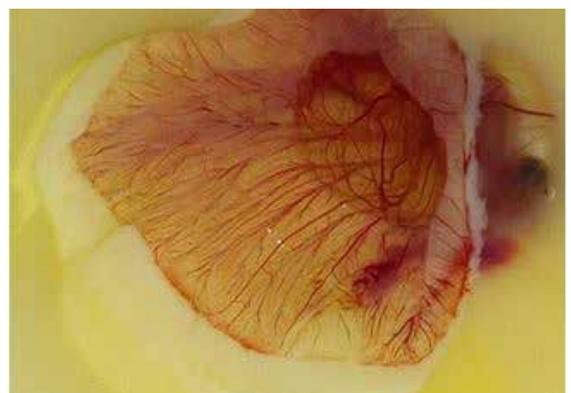
^{a,b}: Means on the same row having different superscripts are significantly ($p<0.05$) different

Table 4: Effects of Feeding Time on the Quality of chicks of Broiler Chicken Breeders

Parameter	Feeding time		
	3 am	5 am	7 am
Average chick weight (g)	41.73±0.91	42.08±0.71	39.93±1.35
Vitality	2.00±0.41	2.50±0.50	1.75±1.18
Navel	0.75±0.48	0.25±0.25	0.75±0.25
Hock joint	0.00±0.00	0.00±0.00	0.00±0.00
Beak	0.00±0.00	0.00±0.00	0.00±0.00
Abdomen	0.00±0.00	0.00±0.00	0.00±0.00

^{a,b}: Means on the same row having different superscripts are significantly ($p<0.05$) different

Plate 1 shows the picture of the first day of incubation. No development of embryo could be observed at this stage. The development of blood vessel and formation of appendages could be seen in Plate 2 which depicts the pictorial representation of an incubated chicken egg at day 3. Plate 3 shows the development of a growing chicken embryo on day 5 of incubation. The blood vessels were more obvious at this stage and formation of eyes could be observed. Day 7 of the embryonic development is shown in Plate 4; the eyes are

**Plate 2.** Embryonic development at day 3 (x 10)**Plate 1:** Embryonic Development at day 1 (x10)**Plate 3.** Embryonic development at day 5 (x 10)

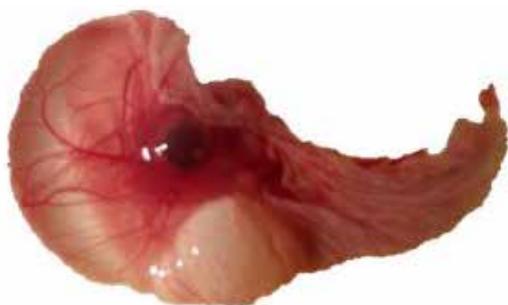


Plate 4: Embryonic development day 7 (x 10)



Plate 5. Embryonic development at day 10 (x 10)



Plate 6: Embryonic development day 15 (x 10)



Plate 7: Embryonic development at day 18 (x 10)

more conspicuous at this stage of incubation, also feather development could be observed. Plate 5 shows the embryonic development at day 10 of incubation; the head of the embryo is formed and the feathers are more obvious. The chick is well-developed, the eyes are more visible, beak, legs and feathers are well-developed on day 15 of incubation which is depicted in Plate 6. Plate 7 shows the stage of incubation of chicken egg at day 18. All parts of the chick are well-formed and conspicuously well-developed at this stage.

Discussion

In this study, significant differences were observed in the total hen-day egg production, birds fed at 3 and 5 am recorded higher hen-day egg production than flocks fed at 7 am. This result contrasted the findings of Londero et al. (2016) who reported that egg production of broiler breeders was not affected by the time of feeding. The result obtained by Londero et al. (2016) could be attributed to the difference in the time of feeding of the experiment which was on single feeding of 8 am, twice feeding times of 8 am and 3 pm, and single feeding of 3 pm.

Eggs laid across the treatments were not significantly affected by the varying times of feeding. This is however in agreement with the findings of Samara et al. (1996); De Avila et al. (2003); Backhouse and Gous (2006) who found out that the number of eggs produced per hen were not affected by feeding time. This study shows that average egg mass was not significantly influenced by different feeding times contrary to the findings of Backhouse and Gous (2006), who observed that feeding times and frequency affected egg mass. This could be due to difference in strains of breeders used in the experiments.

Feeding time has no effect on the feed intake of broiler breeders. This is in contrast with the findings of Ukachukwu and Akpan (2007) who reported a significant difference in the feeding regime of laying birds on restricted feeding. The difference between the present study and the findings by Ukachukwu

and Akpan (2007) could be as a result of the different management systems practiced.

In poultry reproductive flocks, it is essential to achieve a large number of eggs with normal structure, optimal morphological composition and interior quality (Majid *et al.*, 2013). Egg laid were not affected by time of feed allocation. This confirmed the findings of Samara *et al.* (1996), that different feeding schedules had no effect on the egg production. Fertility among the treatments were not affected by feeding time as reported by Bootwella *et al.* (1983) who found out that feeding time had no effect on breeders' fertility. Gibson (2006) also reported that different feeding schedules had no significant effect on fertility of broiler chicken breeders. However, the observed insignificance in the treatments could be as a result of timing and artificial insemination done for the breeders thereby confirming the reports by Penfold *et al.* (2000) in the endangered Northern Pintail duck.

Significant differences were observed in the breeder house mortality; birds fed at 5 am and 7 am recorded a higher mortality rate than those fed at 3 am. This could be as a result of increase in temperature as the sun rises compared to when the birds were fed earlier at 3 am thereby causing heat stress for the birds. This result however is in agreement with Wilson *et al.* (1989), who observed that the time of feeding is a factor that may result in heat stress, due to the heat increment from exothermic reactions that occur during feed metabolism. The authors observed that there is an increased interior temperature 5 hours after feeding time which causes a remarkable increase in body temperature of the birds.

Results from this study showed significant differences in the percentage hatchability at different times of feeding. This result corroborates the finding of Petek (2006) who reported that birds fed during the late hours of the morning and afternoon records a higher hatchability performance. Feeding time has no effect on the embryonic development of broiler eggs. The development followed the normal embryonic development similar to the finding of Tona *et al.* (2005). Moreover, feeding

time has no effect on the chick weight of the broiler chicks across the treatments. This could be as a result of similarity in egg weight during incubation. Chick vitality, navel, hock, beak and abdomen were also not affected by the feeding time of the broiler breeders. The chicks observed recorded a healed navel, clean beak and of no deformities thereby confirming the findings of Tona *et al.* (2005).

Conclusion

From the results of the experiment it could be concluded that:

1. Feeding broiler chicken breeders at 3 am and 5 am had better hen-day egg production and hatchability
2. Survivability of broiler chicken breeders fed at 3 am was better than those fed at 5 am and 7 am.
3. Embryonic development was not influenced by time of feeding.

Impact

Based on this study, 3 am and 5 am feeding time could be recommended for a better productive performance of broiler chicken breeders in terms of hen-day egg production and higher percentage hatchability. However, for the best chicks' survivability, feeding time of 3 am could be retained by the Broiler Breeder Farm.

Ethical approval:

All applicable international, national and/or institutional guideline for the care and use of animals were followed.

Informed Consent:

Consent of every individual included in this study was obtained.

Conflict of interest:

The authors hereby declare that they have no conflict of interest.

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PREVALENCE AND PATHOLOGY OF INDIGESTIBLE FOREIGN BODIES IN RUMEN AND RETICULUM OF CATTLE SLAUGHTERED AT KOMBOLCHA ELFORA ABATTOIR, NORTH EAST ETHIOPIA.

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Abstract

A cross-sectional study was conducted from October, 2015 to April, 2016 at Kombolcha ELFORA Abattoir, South Wollo Zone, Amhara National Regional State, with the objectives of determining the prevalence of foreign bodies in forestomach of slaughtered cattle, gross pathological lesions and associated risk factors for the occurrences of foreign bodies. Postmortem examination was employed to examine the foreign body from rumen and reticulum after slaughter. The study animals were selected by using systematic random sampling method. From the total of 700 cattle examined, 219 (31.28%) were found positive for indigestible foreign bodies in rumen and reticulum. Sands (12.32%), clothes (10.50%), ropes (8.67%) and stones (8.67%), were the most common types of foreign bodies observed. The prevalence of foreign body in cattle was significantly associated with sex ($\chi^2 = 7.11$, $P < 0.05$), breed ($\chi^2 = 8.87$, $P < 0.05$) and body condition ($\chi^2 = 8.03$; $P < 0.05$) of cattle. However there was no significant difference in the prevalence of foreign bodies in animals of different age groups ($\chi^2 = 3.08$, $P > 0.05$) and origins ($\chi^2 = 2.62$; $P > 0.05$). Abscess (60%) was the most common lesion encountered followed by hemorrhage (21.81%), ulcer (9.09%), ruminal atony (7.27%) and reticular fistula (1.81%). Most of the lesions (67%) were caused by metallic foreign bodies compared to non-metallic foreign bodies. The study demonstrated that ruminants in the area ingest various types of indigestible foreign materials, which can hamper their health and productivity. To avert the problem, collaborative intervention schemes need to be applied involving professionals, policy makers, livestock keepers, and environmental activists

Key words: Abattoir, Cattle, Foreign body, Ethiopia, Reticulum, Rumen

PREVALENCE ET PATHOLOGIE DE CORPS ÉTRANGERS INDIGESTES DANS LE RUMEN ET LE RETICULUM DES BOVINS ABATTUS A L'ABATTOIR DE KOMBOLCHA ELFORA, DANS LE NORD-EST DE L'ÉTHIOPIE

Resume

Une étude transversale a été menée d'octobre 2015 à avril 2016 à l'abattoir Kombolcha ELFORA, dans la zone sud de Wollo de l'Etat régional national Amhara en Éthiopie, avec pour objectif de déterminer la prévalence des corps étrangers dans le préestomac des bovins abattus, les lésions pathologiques flagrantes et les facteurs de risque associés à la présence de corps étrangers. Un examen post-mortem a été utilisé pour examiner les corps étrangers dans le rumen et le réticulum après l'abattage. Les animaux étudiés ont été sélectionnés en utilisant une méthode d'échantillonnage aléatoire systématique. Sur un total de 700 bovins examinés, 219 (31,28%) ont été trouvés positifs pour les corps étrangers indigestes dans le rumen et le réticulum. Les types de corps étrangers les plus fréquemment observés étaient le sable (12,32%), les vêtements (10,50%), les cordes (8,67%) et les pierres (8,67%). La prévalence des corps étrangers chez les bovins était significativement associée au sexe ($\chi^2 = 7,11$, $P < 0,05$), à la race ($\chi^2 = 8,87$, $P < 0,05$) et à l'état corporel ($\chi^2 = 8,03$; $P < 0,05$) des animaux. Cependant, on n'a pas noté de différence significative dans la prévalence des corps étrangers entre les différents groupes d'âge ($\chi^2 = 3,08$, $P > 0,05$) et les origines ($\chi^2 = 2,62$; $P > 0,05$). L'abcès (60%) était la lésion la plus fréquente, suivie de l'hémorragie (21,81%), de

l'ulcère (9,09%), de l'atonie ruminale (7,27%) et de la fistule réticulaire (1,81%). La plupart des lésions (67%) étaient plus causées par des corps étrangers métalliques par rapport aux corps étrangers non métalliques. L'étude a montré que les ruminants de la région ingèrent divers types de matières étrangères indigestes, susceptibles d'avoir des effets néfastes sur leur santé et productivité. Pour éviter le problème, il est nécessaire de mettre en oeuvre des programmes d'intervention concertés faisant participer les professionnels, les décideurs, les éleveurs et les militants écologistes.

Mots-clés : abattoir, bovins, corps étranger, Éthiopie, réticulum, Rumen

Introduction

Gastrointestinal foreign bodies are among the most common surgical emergency in veterinary medicine. Because of their indiscriminate feeding habits, cattle are known to ingest and, at times, choke because of ingestion of different types of foreign bodies (Baumont, 1996). Ingestion of indigestible foreign bodies is mainly associated with nutritional deficiencies, environmental pollution and poor feeding management, and causes various problems in the rumen and reticulum of ruminants (Jones *et al.*, 1997). In developed countries, industrialization and agriculture mechanization have further increased the occurrence of foreign bodies in ruminants, whilst in developing countries the high rate of occurrence is associated with poor farming management (Misk *et al.*, 2004; Semieka, 2010). Ingestion of foreign bodies in cattle is a pathological condition of economic importance which leads to severe economic losses as a result of high morbidity and mortality rates (Radostits *et al.*, 2007; Ramin *et al.*, 2008). In Jordan for example an estimated loss of \$25 million in ruminant productivity and health associated with plastic impaction was reported (Hailat *et al.*, 1996).

The problems that are caused vary with the duration that the foreign body has been present, the location of the foreign body, the degree of obstruction that is caused as well as problems associated with the material of the foreign body. Cattle with such foreign bodies are usually asymptomatic in nature and only diagnosed live animals rarely (in severe cases). The foreign bodies can be well studied in abattoirs. Ingestion of indigestible foreign bodies can have various harmful effects on the animals, which include reduced feed intake, failure to absorb volatile fatty acids, reduced rate of fattening, internal injury, and death following

the blockage of intestinal tract (Omid *et al.*, 2012). Also, it causes various health problems that include: ruminitis, impaction of the rumen, absence of defecation, traumatic pericarditis, and traumatic reticuloperitonitis (Radostits *et al.*, 2007).

Ethiopia being among the fastest developing country is undergoing drastic changes especially with construction of new roads and buildings in major town and cities. Kombolcha town is serving as the main industry zone for the Amhara region and the country as well. More than six heavy factories and many middle and small factories are operating in the town. However lack of proper disposal methods of wastes from construction sites predisposes dairy cattle to ingest these wastes. Keeping in view the importance of foreign bodies and paucity of information, present study was undertaken to assess the prevalence, pathology (gross) and associated risk factors of rumen and reticulum foreign bodies in cattle slaughtered at Kombolcha ELFORA Abattoir, North East Ethiopia.

Materials and Methods

Study Area

The study was conducted at Kombolcha ELFORA Abattoir, South Wollo Zone from October 2015 to April 2016. Kombolcha is located 375Km North East of Addis Ababa at an altitude of 1500-1840 meter above sea level (Figure 1). The annual average rainfall is 750-900 mm with a mean minimum and maximum temperature of 11.7 and 23.9°C, respectively. The area receives a bimodal rainfall where the short rainy seasons are between March and May while the long rain season extends from June to end of September. The relative humidity of the area varies from 23.9-79%.

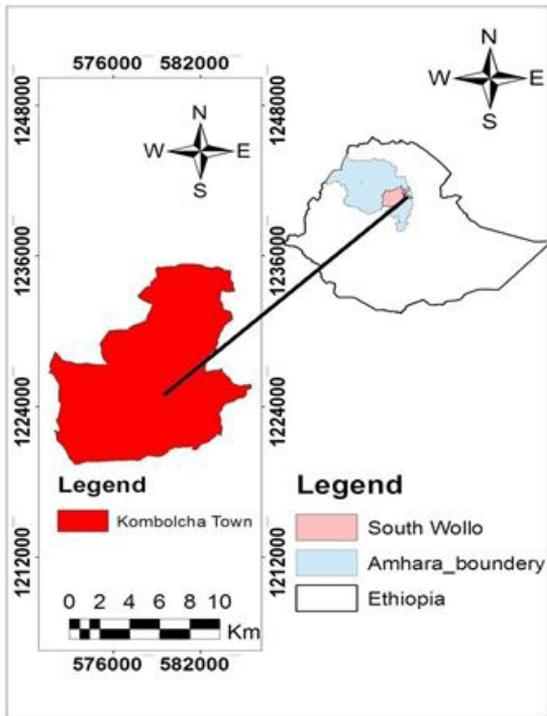


Figure 1: Location map of study area

Study Animals

Out of the total number of 3234 cattle brought for slaughter during the study period, 700 animals were selected and examined for presence of foreign bodies in rumen and reticulum. The figures included both indigenous Ethiopian zebu cattle (687) and cross-bred cattle (13). Of the 700 slaughtered animals, 688 were males and 12 were females (that were culled due to reproductive problems or old age). The major sources of cattle to this abattoir are Kombolcha, and adjoining areas. The farming system in these areas is mixed livestock and crops and animals are kept under extensive and semi intensive farming systems. Information regarding age, origin, sex, body condition and breed were collected to determine their association with the prevalence of foreign body in study animals. The animals were categorized into three age groups such as Young (≤ 5 year of age), adult (5-10 year) and old (≥ 10 year) and also grouped based on body score condition as poor, medium and good. Age of the animals was determined based on dental eruption as described by Pace and Wakeman (2003). Body

condition was recorded as poor, medium and good based on the appearance of the animal and manual palpation of the dorsal spines and transverse processes of the lumbar vertebrae.

Study Design

A cross-sectional study was conducted from October, 2015 to April, 2016 to assess the prevalence of the rumen and reticulum foreign bodies and to identify the type of indigestible foreign bodies and their associated risk factors for the occurrence of foreign bodies. Breed, age, body conditions, sex and origin of studied animals were considered as risk factor for occurrence of foreign bodies.

Sampling Technique and Sample Size Determination

The animals were selected by systematic random sampling procedure. during the study period The sample size for this study was calculated by considering 50% of the population with indigestible foreign body (IFB) since there were no earlier studies done in the study areas. Thus, the sample size was determined according to Thrusfield (2005) using 95% confidence interval and 0.05 absolute precision. This was calculated by using the following formula:

$$N = \frac{1.96^2 \times P_{exp}(1-P_{exp})}{d^2}$$

Where, N = required sample size; P_{exp} = Expected prevalence; d = Desired absolute precision (0.05).

Therefore, the desired sample size for the present study was 384. However 700 cattle have been included in the study to increase accuracy, representativeness and randomness in the study animals.

Postmortem examination

Post-mortem examination involved visual inspection, palpation and making incision of rumen and reticulum for the presences of indigestible foreign body in rumen and reticulum. All the contents were examined thoroughly for the presence of foreign bodies.

Foreign bodies obtained during inspection were washed with water to remove adhering feed material and to identify type of foreign bodies. The location, type of the foreign bodies and the associated lesions if any were recorded.

Data collection Analysis

The collected data were entered into Microsoft Excel data sheets and analyzed using SPSS version 20. The prevalence was calculated by dividing the total number of positive animals to the total number of animals inspected for foreign bodies multiplied by 100. The association between the effects of different risk factors and prevalence was analyzed using the Pearson chi-square (χ^2) test. A statistically significant association between variables was said to exist if the calculated P-value was <0.05 and the 95% confidence interval (CI).

Results

Prevalence of foreign body

From the total of 700 cattle's examined

for the presence of foreign bodies in their rumen and reticulum, 219 (31.28%) of animals had foreign bodies in their rumen and /or reticulum. Of these 219 animals, 127(57.99%) animals had foreign bodies in rumen while 61 (27.85%) animals' foreign bodies were present in reticulum. In only 31 (14.15%) animals foreign bodies were present in both rumen and reticulum. The types of foreign bodies observed during the study were wires, nails, needles, blades, stones, sands, clothes, plastics, sacks, ropes, leathers and hairball. Overall the sands were the most common accounting for 27 (12.32%), which was followed by clothes 23 (10.50%), ropes 19 (8.67%), stones 19 (8.67%), sands and stones 17 (7.76%), sacks 16 (7.30%), plastics 16 (7.30%), wires 11 (5.02%), hairball 11 (5.02%), clothes and sacks 10 (4.56%), blades 10 (4.56%), clothes and ropes 9 (4.10%), leathers 8 (3.65%), needles 7 (3.19%), leathers and plastics 7 (3.19%), nails 6 (2.73%), and ropes and plastics 3 (1.36%). The types of foreign bodies, their distribution in rumen and reticulum, and their proportions are shown in Table 2.

Table 1: Prevalence of foreign bodies in relation to sex, breed, age, origin and body conditions of cattle slaughtered at Kombolcha ELFORA abattoir.

Risk factors		N	n	Prevalence (%)	χ^2	P value
Sex	Male	688	211	30.67	7.11	0.02
	Female	12	8	66.66		
Breed	Local	687	210	30.56	8.87	0.01
	Cross	13	9	69.23		
Age	Young (≤ 5 years)	18	5	27.77	3.08	0.54
	Adult (5-10 years)	268	77	28.73		
	Old (≥ 10 years)	414	137	33.09		
Origin	Lowland	350	110	31.42	2.60	0.62
	Midland	278	91	32.72		
	Highland	72	18	25		
Body condition	Poor	297	106	35.69	8.03	0.009
	Medium	285	81	28.42		
	Good	118	32	27.11		
Total		700	219	31.28		

N= Number of animals examined

n= Number of animals with foreign bodies

Table 2: Frequency of occurrence of foreign body in cattle slaughtered at Kombolcha ELFORA abattoir in relation with their location.

Type of Foreign body	Location			Total
	Rumen	Reticulum	Both organs	
Wire	1(0.78%)	9(14.75%)	1(3.22%)	11
Nail	-	6(9.83%)	-	6
Blade	3(2.36%)	7(11.47%)	-	10
Needle	1(0.78%)	6(9.83%)	-	7
Stone	7(5.51%)	8(13.11%)	4(12.9%)	19
Sand	15(11.8%)	5(8.19%)	7(22.58%)	27
Cloth	19(14.96%)	-	4(12.9%)	23
Plastic	10(7.87%)	4(6.55%)	2(6.45%)	16
Sack	13(10.23%)	1(1.16%)	2(6.45%)	16
Rope	17(13.38%)	-	2(6.45%)	19
Leather	4(3.14%)	4(6.55%)	-	8
Hair	9(7.08%)	2(3.27%)	-	11
Sand and stone	10(7.87%)	4(6.55%)	3(9.67%)	17
Rope and plastic	2(1.57%)	-	1(3.22%)	3
Leather and Plastic	1(0.78%)	4(6.55%)	2(6.45%)	7
Cloth and Rope	6(4.72%)	-	3(9.67%)	9
Sack and cloth	9(7.08%)	1(1.63%)	-	10
Total	127(57.99%)	61(27.85%)	31(14.15%)	219(31.28%)

$\chi^2=6.58, P \text{ value}=0.000$

Table 3: Distribution and type of lesions due to presence of foreign bodies in rumen and reticulum in cattle slaughtered at Kombolcha ELFORA abattoir.

Lesion	Location			Foreign body type	Total
	Reticulum	Rumen	Reticulum and rumen		
Hemorrhage	12	-	-	Metallic	12 (21.81 %)
Abscess	17	16	-	Metallic 24 Non-metalic 9	33(60 %)
Ulcer	-	4	1	Non metallic	5(9.09 %)
Reticular fistula	1	-	-	Metallic	1(1.81 %)
Ruminal atony	-	4	-	Non metallic	4(7.27 %)
Total	30(54.54%)	24(43.63%)	1(1.81%)		55(25.11)

Association of prevalence of foreign body with risk factors

The prevalence was foreign body in cattle was significantly associated with sex ($\chi^2=7.11$; $P<0.05$) and breed ($\chi^2=8.87$; $P<0.05$) of cattle. The prevalence was significantly higher in females and crossbreeds when compared to males and local breeds respectively. Also prevalence was significantly ($\chi^2=8.03$; $P<0.05$) higher in animals with poor body condition compared to animals with medium or good body condition. However there was no significant difference in the prevalence of foreign bodies in animals of different age groups ($\chi^2=3.08$; $P>0.05$) and among animals of different origins ($\chi^2=2.60$; $P>0.05$) (Table 1).

Out of a total of 219 cattle with foreign bodies, 127 (57.99%) cattle were having foreign bodies in rumen only and 61 (27.85%) cattle in reticulum only. Only 31 (14.15%) cattle had foreign bodies in both in rumen as well as in reticulum. Also rumen harbored mostly non metallic materials while reticulum was the major site for the retention of metallic objects. The statically analysis also showed that there exist highly significant differences among different stomach compartment ($\chi^2=6.58$; $P<0.05$) in the occurrences of foreign bodies as shown in Table 2.

Pathological lesion in rumen and reticulum

From 219 animals with foreign bodies, 55 (25.11%) has lesions either in examined organs. Of the 55 abnormalities, majority of the lesions (54.54%) were observed in reticulum followed by rumen (43.63%) while only 1.81% pathological lesion was observed both in rumen and reticulum (1.81%) as shown in Table 3. Abscess (60%) was the most common lesion observed followed by hemorrhage (21.81%), ulcer (9.09%), ruminal atony (7.27%) and reticular fistula 1.81(%). Most of the lesions were caused by metallic foreign bodies when compared to non-metallic foreign bodies (Table 3).

Discussion

Ingestion of indigestible foreign materials by ruminants is a common worldwide problem including the sub-Saharan countries (Remi-Adewumi *et al.*, 2004; Ghurashi *et al.*, 2009; Bakhiet, 2008). The present study revealed an overall prevalence of foreign bodies as 31.28% in cattle slaughtered at Kombolcha ELFORA abattoir. The present prevalence rate of foreign bodies is relatively higher than the earlier reports in Ethiopia (17.07% in Hawassa Municipal Abattoir by Rahel, 2011: 23.9% at Hirna municipal abattoir by Dawit *et al.* 2012). However the present results were lower than the findings of Negash *et al.*, (2015) and Sheferaw *et al.*, (2014) who reported a prevalence of foreign bodies in forestomch of cattle as 43.4% in Harmaya and 41.8% in Bahir Dar respectively . These differences in the prevalence of foreign bodies between various areas may be attributed to differences in animal management systems and the extent of foreign body management both in the rural and/or urban areas and in the grazing areas. The higher prevalence of foreign bodies in the current study area is probably related to the unrestricted and increased use of construction materials and their improper disposal. Ingestion of foreign bodies is associated with a shortage of forage (Hailat *et al.*, 1996) as well as increased pollution of grazing lands with indigestible materials (Tesfaye *et al.*, 2012). If owners do not provide supplementary feed during feed shortages, their animals are likely to face a negative energy balance that will force them to ingest unusual materials including plastic, cloth, rope and even metallic objects (Hailat *et al.* 1996). Another reason for high prevalence of foreign bodies might be associated with scarcity of feed due to prevailing drought conditions in study area.

Overall, the prevalence of foreign bodies in study animals was significantly higher ($\chi^2 = 7.11$, $P<0.05$) in female (66.67 %) than male (30.66%) cattle. These results are in concur with the findings of Tiruneh and Yesuwork (2010). Higher prevalence in female cattle may be explained by the fact that females

generally have a longer lifespan than males, as livestock farmers normally do not sell females because they reproduce and increase the herd size. Similarly the prevalence of foreign bodies was significantly higher ($\chi^2 = 8.87$; $P < 0.05$) in cross breed cattle (69.23%) than local breeds (30.56%) which is in agreement with the findings of Desiye and Mersha (2012) and Rahel (2011). This might be associated with the level of body size which requires high demand of nutrition and hence increase exposure for foreign bodies. The prevalence of foreign bodies in study animals with poor body condition (35.69%) was significantly higher ($\chi^2 = 8.03$, $p < 0.05$) than in those with medium (28.42%) and good body condition (27.11%). This finding is in agreement with the reports of Abebe and Nuru (2011), Hailat *et al.*, (1996), Desiye and Mersha (2012), Rahel (2011) and Tesfaye *et al.*, (2012). Poor body condition could be due to the interference of foreign body with the absorption of volatile fatty acid (VFA) in forestomach and thus causing reduced weight gain (Rahel, 2011; Ismael *et al.*, 2007; Remi-Adewunmi *et al.*, 2004). Presence of hairballs in forestomach over a long period of time become large tight balls hinder the process of fermentation and mixing of contents leading to anorexia, decreased production and loss of body condition (Tyagi, and Singh, 1993).

This study indicated that most foreign bodies occurred in the rumen 127(57.99%) than reticulum 61(27.85%) which is in agreement with the findings of Desiye and Mersha (2012) and Remi-Adewunmi *et al.*, (2004). This study also indicated that Metallic foreign bodies were most frequently recovered from reticulum, while non-metallic foreign bodies were detected from rumen. The reason might be due to retention of these foreign bodies by the honey comb structure of the reticular mucosa and their heavy weight give chance to be attracted to the lumen of the reticulum due to gravitational attraction force of these heavy foreign bodies to the ventral part of the forestomach.

The foreign bodies that were observed during the study were wires, nails, needles, blades, stones, sands, clothes, plastics, sacks,

ropes, leathers and hairball. Similar types of foreign bodies were reported by Nugusu *et al.*, (2013), Dawit *et al.*, (2012), Reddy and Sasikala (2012). The presence of sand as major foreign body can be correlated the widespread construction going in Kombolcha and nearby areas. Moreover drought and scarcity of water in the area might have predisposed the animals to drink sandy or muddy water.

Abscess (60%) was the most common lesion observed followed by hemorrhage (21.81%), ulcer (9.09%), ruminal atony (7.27%) and reticular fistula (1.81%). Similar types of lesions were reported by Reddy *et al.* (2014). Abscess formation in forestomach is a common complication of perforation by metallic objects (Andrews *et al.*, 2003). Most of the lesions (67.27%) were caused by metallic foreign bodies when compared to non-metallic foreign bodies. Though non-metallic foreign bodies didn't produce any significant gross lesions however they (like ingested polythene) generally hinder the process of fermentation and mixing of contents leading to indigestion. In Ethiopia, stray cows are generally seen on the roadsides eating plastic bags and their contents in search of food items (Singh, 2005). These polythenes can also obstruct the orifice between reticulum and omasum and may become fatal. The plastic bags cannot be digested or passed as such through faces by an animal. Moreover, the toxic contents of plastic may enter in man through milk produced by such cows (Singh, 2005)

Conclusion

The prevalence of foreign bodies observed in the rumen and reticulum of cattle presented at the Kombolcha ELFORA abattoirs was high (31.28%). The study showed that improper disposal of and pollution of the environment with indigestible materials like sand, plastic, cloth, metal, rope and stone could pose serious health risks for free-grazing ruminants. Amongst the foreign bodies observed, sand packs constituted the major challenge. Ingestion of these foreign bodies in cattle may cause economic losses due to mortality, morbidity and decreased productivity.

Such losses are of particular importance in Ethiopia, which has low economic output with a per capita income of less than one US dollar per day. Further research should be conducted at national to highlight the problem and to estimate the associated economic losses.

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INFLUENCE OF MORINGA OLEIFERA LEAF MEAL-BASED CONCENTRATES AND STAGES OF LACTATION ON MILK YIELD AND UDDER PARAMETERS OF KALAHARI RED DOES

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Abstract

This study addressed the hypothesis that the influence of *Moringa oleifera* leaf meal-based concentrates and stages of lactation on milk yield and udder parameters of Kalahari Red does was null during twelve weeks of lactation. Twenty lactating Kalahari Red does were allotted to four *Moringa oleifera* leaf meal-based concentrates at 0, 5, 10 and 15 % in an on-farm experiment in a Complete Randomized Design. The highest milk offtake, milk secretion rate, daily milk yield, udder circumference, udder length and udder volume were recorded in Kalahari Red does fed 10% MOLM. 614.90±33.44 cm³ of Udder Volume yielded 637.74±28.56 g per day during the early stage of lactation while the milk off take and milk secretion were 60.85±9.46 ml and 25.79±1.23 ml at mid and early lactations respectively. The interaction of inclusion levels of MOLM in the concentrates and stages of lactation significantly influenced all the parameters. It can be concluded that MOLM levels, stages of lactation and their interaction influenced udder parameters and milk yield of Kalahari Red does.

Keywords : Moringa, Milk yield , Udder parameters , Kalahari Red

INFLUENCE DES CONCENTRES A BASE DE FARINE DE FEUILLES DE MORINGA OLEIFERA ET DES STADES DE LACTATION SUR LE RENDEMENT LAITIER ET LES PARAMETRES DE LA MAMELLE DES CHEVRES KALAHARI ROUGES

Résumé

La présente étude a examiné l'hypothèse selon laquelle l'influence des concentrés à base de farine de feuilles de *Moringa oleifera* (MOLM) et des stades de lactation sur le rendement laitier et les paramètres de la mamelle des chèvres Kalahari rouges était nulle pendant 12 semaines de lactation. Vingt-cinq chèvres kalahari rouges allaitantes ont été réparties à quatre concentrés à base de farine de feuilles de *Moringa oleifera* aux taux de 0, 5, 10 et 15% dans une expérience à la ferme, selon un dispositif complètement aléatoire. La plus grande quantité de lait trait, le taux de sécrétion de lait, le rendement journalier en lait, la circonférence de la mamelle, la longueur de la mamelle et le volume de la mamelle ont été enregistrés pour les chèvres Kalahari rouges ayant reçu 10% de MOLM. 614,90 ± 33,44 cm³ de volume de la mamelle ont donné 637,74 ± 28,56 g par jour pendant le stade initial de lactation, tandis que le lait trait et la sécrétion de lait étaient respectivement de 60,85 ± 9,46 ml et 25,79 ± 1,23 ml aux stades intermédiaire et initial de lactation. L'interaction des taux d'inclusion de MOLM dans les concentrés et les stades de lactation a influencé significativement tous les paramètres. On peut conclure que les niveaux de MOLM, les stades de lactation et leur interaction ont influencé les paramètres de la mamelle et le rendement laitier des chèvres Kalahari rouges.

Mots-clés : Moringa, rendement en lait, paramètres de la mamelle, Kalahari rouge

Introduction

Milk synthesis commences following kidding in the gland epithelium at a relatively high rate and the amount continues to increase for about 2-5 weeks (Egbowon *et al.*, 2007). Antunac *et al.* (2001) reported that in the first stage of lactation produce small amount of milk per day and milk yield increased progressively until the third lactation when kids are weaned. Milk production traits in goats are affected by different non-genetic factors and the knowledge of these factors is essential for efficient management and for accurate estimation of breeding values. Adjusting for the known non-genetic factors is necessary to increase efficiency of animal selection in dairy goats (Assan, 2015). Milk production is a function of the stage of lactation, usually measured in the number of days in milk (Swalve, 1995). Milk from the early stage of lactation normally contained high milk components. Milk yield and its composition are influenced by nutrition, health of the animals, environment, stage of lactation, etc. Among these factors, the stage of lactation is very significant (Pavić *et al.*, 2002, Oravcova *et al.*, 2006, 2007, Kuchtik *et al.*, 2008). A number of animal (species, breed) or environmental (lactation stage, feeding regime, animal health and management) factors affect milk composition (Wojtowski *et al.*, 2001; Chillard *et al.*, 2003; Gorecki *et al.*, 2004). In a trial with goats, supplementation with *Moringa* leaves at a level of 0.3 % of body weight resulted in a milk yield of 0.57 kg day⁻¹, and this was 13 % higher than grazing only (Sarwatt *et al.*, 2004). It is been reported that goat milk yield composition is affected by breed, stage of lactation and plane of nutrition (Malau-Aduli *et al.*, 2001). Level of nutrition, mainly referred to energy and protein levels of feed intake, is a main factor affecting milk yield and milk composition in dairy ruminants. While there have been some reports on the effect of *Moringa* leaf meal on milk, body and udder parameters on some breeds of goats, not much has been done to estimate the effect of nutrition on milk, body and udder parameter of Kalahari Red goats.

H0: *Moringa oleifera* leaf meal-based

concentrates and stages of lactation have no effects on milk yield and udder parameters of Kalahari Red does.

H1: *Moringa oleifera* leaf meal-based concentrates and stages of lactation have effects on milk yield and udder parameters of Kalahari Red does.

Materials and method

Experimental Site

The experiment was carried out at the Kalahari Unit of the Institute of Food Security, Environmental Resources and Agricultural Research, Federal University of Agriculture, Abeokuta, Ogun State, which is located in the tropical rainforest zone in Nigeria within 7°13'47.41"N, 3°23'43.48"E. The climate is humid and located in the forest zone of South-Western Nigeria. The mean precipitation and the temperature are 1,112.70 mm and 23.5 °C respectively. Relative humidity averaged 81.50 % throughout the year. Seasonal distribution of rain is approximately 110.90 mm (9.97 %) in the dry season (October – March) (OORBDA, 2012).

Brachiaria ruziziensis paddock

About two hectares of previously established *Brachiaria ruziziensis* grass were allowed to be grazed by lactating Kalahari Red does as basal diets at Kalahari Unit.

Harvesting and Processing of *Moringa oleifera* Leaf meal

Fresh leaves of *Moringa oleifera* (Nigerian ecotype) were collected from previously established plot at Kalahari Unit. The harvested *Moringa* leaves were air-dried by spreading on a tarpaulin and cemented floor in a roofed and well ventilated room. The leaves were frequently turned until they were crispy to touch while retaining their greenish colouration. The leaves were then hand-milled to obtain a product herein referred to as *Moringa oleifera* leaf meal (MOLM) which was stored in air-tight sacs until ready to use.

Experimental Animals and Management

Table 1.0: Composition (% Dry Matter) of *Moringa oleifera* leaf-based concentrates fed to lactating Kalahari Red does

Parameters	0 %	5 %	10 %	15 %
<i>Moringa oleifera</i> (air-dried)	0.00	5.00	10.00	15.00
Groundnut Cake	28.00	23.00	18.00	13.00
Corn Bran	65.00	65.00	65.00	65.00
Molasses	2.00	2.00	2.00	2.00
Bone meal	2.00	2.00	2.00	2.00
Salt	2.75	2.75	2.75	2.75
Livestock premix	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00

A total of twenty (20) lactating Kalahari Red does weighing 39.16 ± 0.56 were used in the experiment for a duration of 98 days (14 days of adjustment period, 84 days for data collection). The animals were managed under semi-intensive system. Udder parameters were measured using measuring tape.

Kids' Management during milk collection

Newly born twenty seven (27) kids were allowed to suckle their dams freely for the first 20 days post-partum in order to have access to colostrum and good quantity of milk before commencement of the experiment. Prior to each day's milking, kids were separated from their dams for three hours (8:00 – 11:00 hours). Within this period of separation, kids were fed part of the milk collected in a bowl and were reintroduced after milking.

Collection of Milk

The two halves of the udders were hand-milked weekly from week 3 to 14 (12 weeks). The milk off-take is here in described as the first hand milking (residual milk after suckling) early in the morning after which they were allowed to stay in the milking parlour and milked the second time after 3 hours interval as the milk yield. The animals were led to a milking parlour where individual doe was restrained in a milking cage. Prior to milk expression, hands were washed with soap and rinsed with water. Two clean soft towels were used, one to wipe the udders clean while the other was used

to dry up left over milk. On the days of yield determination, kids were separated from their dams for 3 hours. Hand milking commenced by calm massage of the udders, then the teats were placed in between the thumb and index finger, pressed gently against the palm and pushed upward with the aim of simulating natural jab by the kids during suckling and stripped out the milk by several downward strokes which forced the milk out into a small bowl placed directly under the udders. The teats were released at the end of downward strokes at regular interval. Aliquots (10 mls) of the milk were composited for analysis .

Milk Yield

The extractable 3-hour milk yield was measured using a measuring cylinder. Milk yield per day was determined by multiplying the 3 hour milk yield by 8. Milk secretion rate was obtained on hourly basis by dividing the 3-hour milk yield by 3.

Statistical Analysis

General linear model was used for the unbalanced data. Levels of significance was taken at 5 % probability, while the significant means were separated using Duncan's multiple range test (Duncan, 1955). Microsoft Excel 2000 was used for plotting graphs while Systat version 5.02 was used for regression analyses.

Statistical Model

$$Y_{ijkl} = \mu + M_j + S_k + (MS)_{jk} + \square_{ijkl}$$

Where:

Y_{ijkl} = Milk yield.

μ = Population mean.

M = Effect of the l th postpartum substitution of i air-dried *Moringa oleifera* leaf meal in the concentrate at 0, 5, 10 and 15 %.

S_k = Stage of lactation (early lactation: 3 – 6 weeks; mid- lactation: 7 – 10 weeks; and late lactation: 11 – 14 weeks).

$(MS)_{jk}$ = Interactive effects of i th postpartum substitution of air-dried *Moringa oleifera* leaf meal and stage of lactation.

\square_{ijkl} = Random Error.

Results

Table 2 shows main effect of *Moringa oleifera* leaf meal-based concentrates on milk yield and udder parameters of lactating Kalahari Red does. Milk yield measured in grammes was in the range of 476.53 to 570.33 g/day ($P > 0.05$). Milk secretion rate, milk yield per 3 hour,

daily milk yield (ml), udder circumference, udder length and udder volume were significantly influenced ($P < 0.05$) by the substitution levels of *Moringa oleifera* leaf meal in the diets.

Table 3 shows main effects of stage of lactation on milk yield and udder parameters of lactating Kalahari Red does fed *Moringa oleifera* leaf meal-based concentrates. All the parameters were significantly different ($P < 0.05$). Daily milk yield (g) decreased from early lactation (637.74 g) to mid lactation (525.97 g) while late lactation had 332.55 g.

Table 4a and b show interactive effect of *Moringa oleifera* leaf meal-based concentrates and stage of lactation on growth performance of lactating Kalahari Red does. Concentrate intakes ($p < 0.05$) of 0 % inclusion level of MOLM at early, mid and late lactations were 0.66 kg, 0.71 kg and 0.73 kg respectively while that of 5 % at early, mid and late lactations were 0.90 kg, 0.84 kg and 0.93 kg respectively. The feed conversion ratio was not significantly ($p > 0.05$) affected by the inclusion level and stage of lactation.

Table 2: Main effect of *Moringa oleifera* leaf meal-based concentrates on milk yield and udder parameters of lactating Kalahari Red does

Parameters	<i>Moringa oleifera</i> leaf meal inclusion level			
	0 %	5 %	10 %	15 %
Milk Offtake	46.56±8.52 ^{ab}	41.50±7.49 ^{ab}	63.47±9.50 ^a	38.11±7.47 ^b
Milk Secretion Rate(ml/Hour)	19.98±1.21 ^b	19.36±1.68 ^b	26.27±2.26 ^a	18.20±1.13 ^b
Milk Yield per 3Hour	59.95±3.65 ^b	58.09±5.06 ^b	78.81±6.78 ^a	54.60±3.41 ^b
Daily Milk Yield (ml)	479.66±29.25 ^b	464.78±40.50 ^b	630.48±54.27 ^a	436.85±27.28 ^b
Daily Milk Yield (g)	499.83±31.08	494.56±42.67	570.33±49.91	476.53±30.19
Udder Circumference (cm)	26.73±0.91 ^c	29.45±0.88 ^b	32.64±0.93 ^a	27.72±0.73 ^{bc}
Udder Length (cm)	18.53±0.36 ^c	19.66±0.43 ^b	21.70±0.69 ^a	17.32±0.40 ^d
Udder Volume (ml ³)	441.18±22.83 ^{bc}	512.64±38.75 ^b	643.14±44.32 ^a	417.05±36.68 ^c

^{a,b,c,d} means in the same row with different superscripts differ significantly ($p < 0.05$)

Table 3: Main effect of stage of lactation on milk yield and udder parameters of lactating Kalahari Red does fed *Moringa oleifera* leaf meal-based concentrates

Parameters	Stage of Lactation		
	Early lactation	Mid Lactation	Late Lactation
Milk Offtake	42.77±4.69 ^{ab}	60.85±9.46 ^a	34.42±5.90 ^b
Milk Secretion Rate(ml/Hour)	25.79±1.23 ^a	21.85±1.48 ^b	13.10±0.82 ^c
Milk Yield per 3 Hour	77.38±3.69 ^a	65.55±4.45 ^b	39.32±2.47 ^c
Daily Milk Yield (ml)	619.09±29.58 ^a	524.46±35.63 ^b	314.63±19.80 ^c
Daily Milk Yield (g)	637.74±28.56 ^a	525.97±35.44 ^b	332.55±20.71 ^c
Udder Circumference (cm)	32.32±0.58 ^a	28.48±0.61 ^b	25.17±0.93 ^c
Udder Length (cm)	21.37±0.39 ^a	18.31±0.37 ^b	16.83±0.28 ^c
Udder Volume (cm ³)	614.90±33.44 ^a	430.43±26.80 ^b	407.09±30.35 ^b

^{a,b,c} means in the same row with different superscripts differ significantly (p < 0.05)

Table 4a: Interactive effects of substitution levels of *Moringa oleifera* leaf meal-based concentrates and stages of lactation on milk yield and udder parameters of lactating Kalahari Red does

Sage of Lactation	<i>Moringa oleifera</i> leaf meal inclusion level						
	Early lactation	0 %			5 %		
		Mid Lactation	Late Lactation	Early lactation	Mid Lactation	Late Lactation	
Milk Offtake (ml)	32.33±4.94 ^{bc}	82.45±23.10 ^a	23.06±2.37 ^c	44.15±9.20 ^{abc}	53.00±19.66 ^{abc}	27.35±5.77 ^{bc}	
Milk Secretion rate (ml/Hr)	23.69±1.84 ^{bcd}	22.07±2.19 ^{bcd}	12.60±1.26 ^e	24.98±2.61 ^{bc}	20.06±3.50 ^{cde}	13.05±1.89 ^e	
Milk Yield per 3 Hour (ml)	71.07±5.53 ^{bcd}	66.23±6.59 ^{bcd}	37.80±3.80 ^e	74.94±7.85 ^{bc}	60.20±10.51 ^{cde}	39.15±5.68 ^e	
Daily Milk Yield (ml)	568.62±44.26 ^{bcd}	529.90±52.77 ^{bcd}	302.44±30.44 ^e	599.55±62.84 ^{bc}	481.60±84.15 ^{cde}	313.20±45.49 ^e	
Daily Milk Yield (g)	583.46±49.05 ^{abc}	562.20±55.24 ^{abc}	315.57±31.54 ^d	654.67±67.45 ^{ab}	502.82±85.92 ^{bcd}	326.19±46.78 ^d	
Udder Circumference (cm)	30.82±0.81 ^{bcd}	27.98±0.73 ^{cde}	19.03±2.02 ^f	32.62±1.39 ^{ab}	28.96±1.31 ^{bcde}	26.76±1.61 ^d	
Udder Length (cm)	20.54±0.51 ^{bc}	17.33±0.41 ^{ef}	16.70±0.38 ^f	21.98±0.70 ^{ab}	19.24±0.55 ^{cde}	17.15±0.45 ^f	
Udder Volume (cm ³)	517.10±33.34 ^{bcde}	384.27±28.99 ^{cde}	343.47±54.87 ^e	647.88±77.88 ^{ab}	458.15±57.04 ^{bcde}	422.90±52.82 ^{cde}	

Table 4b: Interactive effects of substitution levels of *Moringa oleifera* leaf meal-based concentrates and stages of lactation on milk yield and udder parameters of lactating Kalahari Red does (Cont'D)

Sage of Lactation	<i>Moringa oleifera</i> leaf meal inclusion level					
	0 %			5 %		
	Early lactation	Mid Lactation	Late Lactation	Early lactation	Mid Lactation	Late Lactation
Milk offtake (ml)	71.06±11.39 ^{ab}	54.12±10.09 ^{abc}	65.83±29.25 ^{abc}	31.30±10.78 ^{bc}	52.50±18.04 ^{abc}	30.15±7.01 ^{bc}
Milk secretion rate (ml/Hr)	32.56±3.50 ^a	29.54±3.61 ^{ab}	13.52±2.52 ^e	23.72±1.80 ^{bcd}	17.25±2.06 ^{de}	13.39±1.19 ^e
Milk yield per 3hour (ml)	97.68±10.50 ^a	88.62±10.84 ^{ab}	40.56±7.57 ^e	71.16±5.41 ^{bcd}	51.75±6.20 ^{ed}	40.18±3.58 ^e
Daily milk yield (ml)	781.46±84.07 ^a	709.00±86.76 ^{ab}	324.50±60.56 ^e	569.31±43.35 ^{bcd}	414.00±49.63 ^{de}	321.47±28.70 ^e
Daily milk yield (g)	730.56±54.87 ^a	634.50±96.27 ^{abc}	345.94±64.91 ^d	630.28±51.72 ^{abc}	445.98±50.89 ^{cd}	346.86±30.05 ^d
U d d e r circumference (cm)	35.55±0.90 ^a	31.07±1.76 ^{bc}	30.61±1.96 ^{bcd}	31.24±1.24 ^{bc}	26.69±1.09 ^{de}	25.22±1.08 ^e
Udder Length (cm)	23.80±1.04 ^a	21.77±0.85 ^b	17.86±1.23 ^{def}	19.73±0.72 ^{cd}	15.95±0.54 ^f	16.04±0.31 ^f
Udder volume (cm ³)	780.93±57.70 ^a	567.04±76.50 ^{bc}	548.19±88.17 ^{bcd}	566.44±82.88 ^{bc}	353.24±46.49 ^{ed}	321.97±32.69 ^e

^{a,b,c,d,e,f} means in the same row with different superscripts differ significantly ($p < 0.05$)

Discussion

The results obtained showed that udder characteristics (udder circumference, length and volume) were influenced by *Moringa oleifera* inclusion and stages of lactation. Udder traits were the most variable components in comparison with others such that it is unclear which is the most developed udder amongst them. Consequently, their mammary glands are better developed after parturition which could be attributed to the fact that the mammary glands are suckled more frequently by kids. Udder length and circumference increased to its peak at 5th week which is in line with Stanton et al. (1992) who observed the same effect in dairy cows and suggested that this pattern could be due to the fact that mammary gland are still developing during the first lactation. This becomes evident by increased number of mammary cells and their synthetic activity which Zamiri et al. (2001) confirmed to be due to hormones. It was further reported by Fernandez et al. (1995) that udder circumference increased as udder

length increased. The result revealed clearly that gradual decline in udder traits of Kalahari Red goats occurred from the 6th week of lactation till the 14th week of lactation. The relative decline in udder traits before milk removal measured as week of lactation progressing from the 1st week till the 12th week is normal because as week of lactation progressed there is probably a decrease in udder secretory tissue. Mephram (1987) reported that the involution of mammary secretory cells is triggered by the disruption of the tight junctions between adjacent cells and decrease in oxytocin (Zamiri et al., 2001). Udder volume is a function of amount of milk stored. It has earlier been shown that udder morphology and milk yields are positively correlated (Marnet and McKusick, 2001). Udder volume was calculated in this study using the volume of a sphere which is contrary to Izadifard and Zamiri (1997) who considered udder as a cylindrical object. The radius was calculated by dividing the circumference by the factors of 2 and pi which is in consonance with James and Osinowo (2004) and Amao (1999)

who both divided the addition of udder length and udder width by 4. This will not be adequate for udder volume of Kalahari Red goats with well-developed and asymmetric udders with supernumerary teats.

The stage of lactation had a significant effect on daily milk offtake, secretion rate, daily milk yield (DMY) and udder parameters, which is consistent with the results of Ochoa-Cordero et al. (2002) for Rambouillet ewes. The same conclusions were submitted by Bencini and Pulina (1997) and Sevi et al. (2004). In this study, daily milk yield of Kalahari Red does decreased from 637.74 g in the early lactation to 525.97 g during the mid lactation to 332.55 g in late lactation which is in contrast with Kraličkova et al. (2012) who submitted a relatively stable mean value between the early and mid lactation which is from 0.66 to 0.83 kg. In the same vein, Nuda et al. (2003) and Kuchtik et al. (2008) reported similar trend. It can be adduced that the low DMY till mid lactation was probably caused by the inadequate production of high nutrient-pasture as a result of low rainfall. Ploumi et al. (1998) also pointed to the fact that long term droughts and high temperatures lead to a drop in milk yield. Milk offtake and milk yield were 60.85 ml and 637.74 g at the mid and early lactation respectively. It has been reported that goat milk yield is affected by stage of lactation (Olumo, 1995 and Malau-Aduli et al., 2001). The observed trend of milk yield could have resulted from proliferation of myoepithelial cells of the mammary gland especially at the early stage of lactation but which were now available for milk synthesis at the mid lactation. Wilde and Knight (1990) reported that mammary cells multiplied during early lactation and declines after mid of lactation. There is a phase of rapid cellular activation, starting from the end of gestation and followed by cellular regression at varying rates, that ends with the cessation of lactation or dry-off owing to smaller udder circumference, length and volume (Hurley, 1989). Higher milk production during early lactation, in Kalahari Red does could be as a result of the processes of synthesis and secretion of nutrients and of active and passive blood filtration by specialized

epithelial cells of the mammary gland at this period (Mephram, 1987). A typical pattern of milk yield over time is often characterized by an initial phase of increasing production which reaches a maximum and then declines rapidly until dry-off. Based on the results obtained from this research, it can be concluded that there were significant Interactive effects of substitution levels of *Moringa oleifera* leaf meal and stages of lactation on milk yield and udder parameters of lactating Kalahari Red does.

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Conflict of Interest Statement:

The authors declare that they have no conflict of interest.

Statement of Animal Rights:

All applicable International, National, and Institutional guidelines for the care and use of animals were followed in the conduct of this research.

Informed Consent:

Informed consent was obtained from all individual participants included in this study.

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ASSESSMENT ON MAJOR LIVESTOCK HEALTH PROBLEMS IN SOUTHERN ZONE OF TIGRAY, NORTHERN ETHIOPIA

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Abstract

A cross sectional study was conducted to identify the major livestock health problems in southern zone of Tigray, northern Ethiopia from July 2014 to June 2016. Questionnaire survey and case observational study were employed for data collection. A total of 120 respondents were interviewed for the questionnaire survey. Moreover, a total of 1152 animal cases were tentatively diagnosed based on history and clinical signs. The most frequent diseases of cattle mentioned by the respondents include; blackleg (47.5%), anthrax (35%), pneumonic pasteurellosis (33.3%), ectoparasitism (11.7%) and foot and mouth disease (9.16%). In small ruminants the major health problems identified based on the respondents response were Peste des petits ruminants (27.5%), sheep and goat pox (18.3%), foot rot (14.1%), Orf (10.8%) and ectoparasites (7.5%). The most prevailing equine health problem was African horse sickness (7.5%). The most important poultry disease in the area was Newcastle disease (30%) and the most common camel disease was hemorrhagic septicemia (18.3%). From the case observational study the most encountered diseases of cattle were Black leg (7.9%), Lumpy skin disease (6.25%) and Mastitis (4.86%). Sheep pox (3.21%) and PPR (4.08%) were most commonly diagnosed diseases in sheep and goats respectively. Furthermore, strangles (5.3%), Newcastle disease (5%), and hemorrhagic septicemia (3.13%) were the major diseases diagnosed in equines, poultry and camel. In general the study indicated that various infectious, parasitic and miscellaneous diseases were the major livestock health problems in the area which results death and production loss. Hence, there is a need of developing proper animal health delivery system, disease prevention and control program.

Key words: Livestock, Health, Problem, Southern Tigray

ÉVALUATION DES PRINCIPAUX PROBLÈMES DE SANTÉ ANIMALE DANS LA ZONE SUD DE TIGRAY, DANS LE NORD DE L'ÉTHIOPIE

Résumé

Une étude transversale a été menée dans le but d'identifier les principaux problèmes de santé animale dans la zone sud du Tigré, dans le nord de l'Éthiopie, de juillet 2014 à juin 2016. Une enquête par questionnaire et une étude observationnelle ont été utilisées pour la collecte des données. Au total, 120 répondants ont été interrogés pour l'enquête par questionnaire. En outre, un total de 1152 cas d'animaux a été provisoirement diagnostiqué sur la base d'antécédents et de signes cliniques. Les maladies les plus fréquentes des bovins telles que mentionnées par les répondants comprennent : le charbon symptomatique (47,5%), le charbon bactérien (35%), la pasteurellose pneumonique (33,3%), l'ectoparasitisme (11,7%) et la fièvre aphteuse (9,16%). Chez les petits ruminants, les principaux problèmes de santé identifiés sur la base des réponses des répondants comprennent la peste des petits ruminants (27,5%), la clavelée du mouton et la variole caprine (18,3%), le piétin (14,1%), l'orf (10,8%) et les ectoparasites (7,5%). Le problème de santé le plus courant des équidés était la peste équine africaine (7,5%). La maladie la plus importante des volailles dans la région était la maladie de Newcastle (30%), tandis que la maladie du chameau la plus fréquente était la septicémie hémorragique (18,3%). D'après l'étude observationnelle des cas, les maladies les plus rencontrées chez les bovins étaient le charbon symptomatique (7,9%), la dermatose nodulaire contagieuse (6,25%) et la mastite (4,86%). La clavelée du mouton (3,21%) et la peste des petits ruminants (4,08%) étaient les maladies les plus fréquemment diagnostiquées respectivement chez les ovins et les caprins. En outre, les principales maladies diagnostiquées chez les équidés, les volailles et les chameaux étaient la gourme

(5,3%), la maladie de Newcastle (5%) et la septicémie hémorragique (3,13%). En général, l'étude a révélé que diverses maladies infectieuses, parasitaires et mixtes étaient les principaux problèmes de santé animale dans la région et sources de mortalités et de pertes de production. Par conséquent, il est nécessaire de mettre au point un système de prestation de soins de santé animale appropriés et un programme de prévention et de contrôle des maladies.

Mots-clés : animaux, santé, problème, sud du Tigré

Introduction

Livestock production in most African countries has been an integral part of agricultural system and it has been considered as the main component of agricultural development in most parts of sub-Saharan Africa (Sileshi *et al.*, 2016). Like in many developing countries, livestock play a crucial role in Ethiopia; Ethiopia is believed to have the largest livestock population, being the first in Africa and tenth in the world. An estimate indicates that the country is home for about 56.7 million cattle, 29.3 million sheep and 29.1 million goats, 1.2 million camel, 9.9 million equine and 56.9 million poultry (CSA, 2015). The livestock subsector has an enormous contribution to Ethiopia's national economy and livelihoods of many Ethiopians through meat, milk, egg, drought power and source of cash. The subsector contributes about 16.5% of the national GDP and 35.6% of the agricultural GDP (Metaferia *et al.*, 2011).

Despite the large number of livestock in Ethiopia, the livestock sub-sector's contribution to the economy is very low as per the country expectation and potential of the sector (Shitaye *et al.*, 2007; Sileshi *et al.*, 2016). The low productivity is attributed to the low genetic potential of indigenous cattle, poor nutrition and reproductive performance, inadequate management, high disease incidence and parasitic burden (Shitaye *et al.*, 2007; Leta and Mesele, 2014; Sileshi *et al.*, 2016).

Knowing the type and extent of the common or major health problems is very important for the development of herd health strategies and the selections of possible interventions (CSA, 2007). An organized research that can elucidate major animal health problems is a central issue for further study of epidemiological study on the diseases of

livestock. In southern zone of Tigray there was no detail previous study on the major diseases which hinder the productivity of livestock sector: hence, identifying the major livestock health problems was found to be a priority task for further animal health research in the area. Therefore, the study was carried out with the following objectives:

- To assess the major livestock diseases in southern zone of Tigray
- To enquire base line information on major livestock health problems in the study area.

Materials and methods

Study Area and Population

The study was conducted in Southern zone of Tigray Regional State, Northern Ethiopia. It is located at 660 km North of Addis Ababa, and 120 km South of Mekelle. Geographically it is located at 12o 15' and 13o 41' north latitude and 38o 59' and 39o 54' east longitude, constituting an area of 9,446 km² at an altitudinal range of 930 – 3925 masl. The total human population of the study area is 1,004,558 (12, 4813 from urban and 879,745 from rural) (CSA, 2007). The zone consists of five administrative districts; namely Raya Alamata, Alaje, Endamohoni, Ofla, and Raya azebo under different agro ecological zones. However, it is dominated by two major agro-ecologies (lowlands and highlands) and the study mainly focused on these two major agro-ecologies covering a large area of the zone. The livestock population consists of 404427 cattle, 322774 sheep, 161415 goats, 516 horses, 66910 donkeys, 381 mule, 27762 camel, 397512 poultry and 24129 beehives (CSA, 2015).

Study design

In this study a cross-sectional questionnaire survey and clinical case observation was employed to identify the major animal health problems in the study area.

Questionnaire survey

The study was conducted on purposely selected districts (Endamehoni, Raya azebo & Raya alamata). A structured questionnaire was developed, Four PAs were randomly selected from each district then from each PA 10 households were randomly selected. Therefore, a total of 120 total households were interviewed. The questionnaire addressed socioeconomic characteristics, major livestock diseases, traditional practices and access to veterinary services. Clinical symptoms perceived by the respondent was used for identification (diagnosis) of a particular disease.

Clinical case observation

Animals brought to three governmental veterinary clinics (namely: Maichew, Mehoni & Alamata clinics) were clinically examined and tentative diagnosis was set based on the history of the animal and clinical signs of the problem. A total of 1152 animals (510 cattle, 104 ovine, 102 caprine, 189 equine, 147 poultry and 100 Camel) were randomly examined based on systematic random selection.

Data analysis

The collected data from the study area was recorded in database based on Microsoft® Excel (Microsoft Corporation, USA) spread sheet and coded properly. The collected data was subjected to statistical analysis using SPSS soft ware, version 20. Descriptive statistics such as mean, frequency distribution and percentages was used for analysis. Ranking of the different diseases identified in the study area were done using the rank index formula as described by Musa et al. (2006). Rank index = sum of (3 X number of household ranked first + 2 X number of household ranked second + 1 X number of household ranked third) for an individual preference, reason or criteria divided by the sum of (3 X number of household ranked first

+ 2 X number of household ranked second + 1 X number of household ranked third) for overall reasons, criteria or preferences.

Result

Questionnaire survey

Demographic features of respondents

The majority of the respondents were male (88.3%) and the rest were female (11.7%). The maximum and minimum age of respondents was 83 and 22 years respectively. The average age of the respondents was about 44 years. The respondents educational status indicated that 37.5% were illiterate (can't read and write) and only 3.3% were preparatory school and above (higher educational level). Respondents' marital status also showed that 91.7% were married whereas 8.3% were single.

Table 1: Respondents access to veterinary clinic

Veterinary service	Frequency	Percentage
Governmental veterinary clinic	57	47.5
Private veterinary clinic	15	12.5
No access	48	40
Total	120	

Farming system

This study revealed that the majority of the respondents (96.7%) were engaged in mixed crop-livestock farming system while the rest (3.3%) were rearing only livestock.

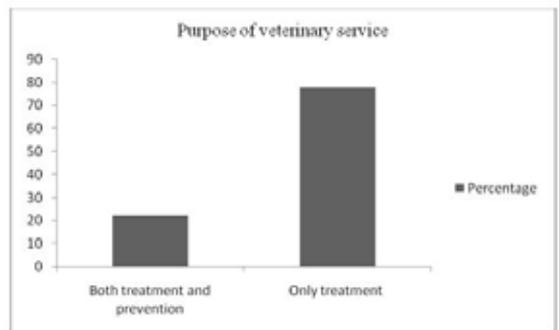


Figure 2: Purpose of veterinary service

Access to veterinary service

Majority of the respondents (60%) reported that they have access to either private (12.5%) or governmental veterinary service (47.5%) though the veterinary clinics are not well equipped (Table 1). The respondents indicated that the distance of veterinary clinics from home is mostly 1-4km (41.7%) where as 25.8% and 32.5% indicated <1km and >4km respectively.

Out of the respondents who had access to veterinary service only 22.2% get service for both treatment and prevention purposes while the rest majority (77.8%) only treat their animals when they got sick (Figure 2).

Traditional practices of disease treatment

Out of the total respondents 47.5% have the habit to treat their animals at home by buying drugs from veterinary pharmacy. The majority 64.2% traditionally treat their animals by branding, bleeding and using herbs/plants which have medicinal purpose (Table 2).

Major diseases identified

The respondents indicated that various infectious, parasitic and miscellaneous diseases are the major livestock health problems in the area which cause death and production loss. They also indicated that factors like feed shortage, inadequate veterinary service, season and agro ecology aggravate the disease dynamics. The most frequent diseases of cattle mentioned by the respondents include; blackleg (47.5%), anthrax (35%), pneumonic pasteurellosis (33.3%), ectoparasites (11.7%), foot and mouth disease (9.16%), bloat (8.3%), mastitis (5%), wound (4.16%), endoparasites (4.16%), enteritis (3.3%), lumpy skin disease (3.3%), abortion (2.5%), contagious bovine pleura pneumonia (1.7%), difficulty of urination (1.7%) and retained placenta (0.83%) (Table 3).

In small ruminants the major health problems identified based on the respondents response are Peste des petits ruminants (27.5%), sheep and goat pox (18.3%), foot rot (14.1%), Orf (10.8%), ectoparasites (7.5%), pneumonic pasteurellosis (5.83%), anthrax (5%), endoparasites (2.5%), and enteritis (1.66%)

(Table 4).

The most prevailing equine health problem was African horse sickness (7.5%) followed by GIT parasitism (6.66%), anthrax (5.8%), ectoparasites (4.1%), colic (2.5%), and difficulty of urination (0.83%) (Table 5).

The most important poultry disease in the area was Newcastle disease (30%) followed by fowl cholera (4.1%), ectoparasites (3%), coccidiosis (2.5%) and fowl pox (1.66%) (Table 6).

The respondents indicated that the most common camel diseases were hemorrhagic septicemia (18.3%), plant poisoning (6.6%), anthrax (5.8%), ectoparasites (5%), bloat (4.1%), difficulty of urination (2.5%), camel pox (1.6%) and saddle sore (0.83%).

Clinical case observation

A total of 1152 animals (510 cattle, 104 ovine, 102 caprine, 189 equine, 147 poultry and 100 camel) were tentatively diagnosed based on clinical features and history of the animals at three selected veterinary clinics (Maichew, Mehoni and Alamata clinics) in the study area. From each clinic a total of 384 animals were examined. Among the diagnosed diseases of cattle Black leg (7.9%), Lumpy skin disease (6.25%), Mastitis (4.86%), Pneumonic pasteurellosis (3.65%) and Anthrax (4%) were the most major ones. In sheep sheep pox (3.21%), pneumonic pasteurellosis (2.69%), GIT parasitism (1.74%), and ectoparasitism (1.39%) were the most common diseases while in goats PPR (4.08%), GIT parasitism (1.74%) and pneumonic pasteurellosis (1.34%) were most commonly encountered. The study revealed that strangles (5.3%), wound (4.86%), colic (2.69%) and African horse sickness (2.52%)

Table 2: Traditional practice of treating animals

Practice	Frequency	Percentage
Branding	22	18.3
Bleeding	20	16.7
Using herbs/plants	14	11.7
All	21	17.5
No	43	35.8
Total	120	

Table 3: Major cattle diseases identified based on respondents response and knowledge

Diseases	Degree of importance					Total	Percentage (%)	Overall rank
	1st	2nd	3rd	4th	5th			
Anthrax	25	14	2	1	-	42	35	2
Pasterellosis	26	10	1	3	-	40	33.3	3
Abortion	1	1	1	-	-	3	2.5	12
Mastitis	3	-	2	1	-	6	5	7
Bloat	6	2	-	2	-	10	8.3	6
Black leg	34	9	12	2	-	57	47.5	1
Ectoparasite	8	3	2	1	-	14	11.7	4
Retained placenta	-	1	-	-	-	1	0.83	15
Foot and mouth disease	3	6	2	-	-	11	9.16	5
Lumpy skin disease	1	1	2	-	-	4	3.3	11
Contagious bovine pleura pneumonia(CBPP)	1	1	-	-	-	2	1.7	13
Enteritis/diarrhea	2	1	1	-	-	4	3.3	10
Difficulty of urination	-	-	1	1	-	2	1.7	14
Endoparasite	2	3	-	-	-	5	4.16	9
Swelling(wound)	2	1	1	1	-	5	4.16	8

Table 4: Major small ruminant diseases identified based on respondents response

Diseases	Degree of importance						Total	Percentage (%)	Overall rank
	1st	2nd	3rd	4th	5th	6th			
Anthrax	5	-	1	-	-	-			
Peste des petits ruminants (PPR)	20	8	4	1	-	-	33	27.5	1
Orf(Contagious Ecthyma)	4	1	6	-	1	1	13	10.8	4
Endoparasitosis	1	1	-	1	-	-	3	2.5	8
Pastuerellosis	1	1	1	2	2	-	7	5.83	6
Sheep and goat pox	6	10	3	2	1	-	22	18.3	2
Ectoparasitism	3	4	1	1	-	-	9	7.5	5
Foot rot	8	6	3	-	-	-	17	14.1	3
Enteritis/diarrhea	1	1	-	-	-	-	2	1.66	9

Table 5: Major equine diseases identified based on respondents response

Diseases	Degree of importance				Total	Percentage (%)	Overall rank
	1st	2nd	3rd	4th			
Colic	2	1	-	-	3	2.5	5
GIT parasitism	7	-	1	-	8	6.66	2
African Horse Sickness	6	1	-	2	9	7.5	1
Anthrax	5	2	-	-	7	5.8	3
Ectoparasite	2	2	1	-	5	4.1	4
Difficulty of urination	-	1	-	-	1	0.83	6

Table 6: Major poultry disease identified based on respondents response

Diseases	Degree of importance			Total	Percentage	Overall rank
	1st	2nd	3rd			
Foul pox	2	-	-	2	1.66	5
Foul cholera	2	2	1	5	4.1	2
Newcastle disease	33	1	2	36	30	1
Ectoparasite	1	2	1	4	3.3	3
Coccidiosis	-	1	2	3	2.5	4

Table 7: Major camel disease identified based respondents response

Diseases	Degree of importance				Total	Percentage	Overall rank
	1st	2nd	3rd	4th			
Hemorrhagic septicemia	14	4	4	-	22	18.3	1
Camel pox	-	2	-	-	2	1.6	7
Saddle sore	-	1	-	-	1	0.83	8
Plant poisoning	2	2	2	2	8	6.6	2
Ectoparasite	3	-	1	2	6	5	4
Anthrax	5	1	1	-	7	5.8	3
Bloat	1	1	1	2	5	4.1	5
Difficulty of urination	1	1	1	-	3	2.5	6

Table 8: Major diseases tentatively diagnosed at the selected veterinary clinics

Diseases	Animal species						Total
	Bovine	Ovine	Caprine	Equine	Poultry	Camel	
Blackleg	91(7.9)	-	-	-	-	-	91(7.9)
Epizootic lymphangitis	-	-	-	7(0.6)	-	-	7(0.6)
Salmonellosis	19(1.65)	-	-	-	-	-	19(1.65)
Tetanus	5(0.43)	-	-	5(0.43)	-	-	10(0.87)
Actinomycosis	8(0.69)	-	-	-	-	-	8(0.69)
Brucellosis	18(1.56)	-	-	-	-	-	18(1.56)
Colibacillosis	9(0.78)	-	-	-	-	-	9(0.78)
Foul pox	-	-	-	-	39(3.39)	-	39(3.36)

Diseases	Animal species						Total
	Bovine	Ovine	Caprine	Equine	Poultry	Camel	
Anthrax	46(4)	-	-	-	-	-	46(4)
GIT parasitism	35(3)	20(1.74)	20(1.74)	-	-	17(1.46)	92(7.97)
Dermatophytosis	5(0.43)	-	-	-	-	-	5(0.43)
Pneumonic Pasteurellosis	42(3.65)	31(2.69)	16(1.34)	-	-	-	80(6.94)
Wound	-	-	-	56(4.86)	-	-	56(4.86)
Hemoparasites	13(1.13)	-	-	-	-	-	13(1.13)
Bloat	19(1.65)	-	-	-	-	-	19(1.65)
urinary tract infection	13(1.13)	-	-	-	-	-	13(1.13)
Ectoparasitism	7(0.6)	16(1.39)	-	-	-	24(2.08)	47(4.07)
AHS	-	-	-	29(2.52)	-	-	29(2.52)
LSD	72(6.25)	-	-	-	-	-	72(6.25)
camel pox	-	-	-	-	-	23(2)	23(2)
Coccidiosis	-	-	-	-	50(4.34)	-	50(4.34)
Colic	-	-	-	31(2.69)	-	-	31(2.69)
Actinobacillosis	23(2)	-	-	-	-	-	23(2)
Newcastle disease	-	-	-	-	58(5)	-	58(5)
Orf	-	-	6(0.52)	-	-	-	6(0.52)
Strangles	-	-	-	61(5.3)	-	-	61(5.3)
Mastitis	56(4.86)	-	-	-	-	-	56(4.86)
PPR	-	-	47(4.08)	-	-	-	47(4.08)
sheep and goat pox	-	37(3.21)	13(1.13)	-	-	-	50(4.34)
bovine farcy	8(0.69)	-	-	-	-	-	8(0.69)
hemorrhagic septicemia	10(0.87)	-	-	-	-	36(3.13)	46(4)
Lung worm	11(0.95)	-	-	-	-	-	11(0.95)
	510(44.3)	104(9)	102(8.85)	189(16.4)	147(12.76)	100(8.68)	1152

were the major health problems of equines. The study also indicated that Newcastle disease (5%) was the dominant poultry disease followed by coccidiosis (4.34%) and fowl pox (3.39%). Moreover, the diseases diagnosed in camel were hemorrhagic septicemia (3.13%), ectoparasitism (2.08%), camel pox (2%) and GIT parasitism (1.46%) (Table 8).

Discussion

Questionnaire survey

In this study the farming system of the farmers who participated was dominantly characterized by mixed crop-livestock type (96.7%). Hence, this showed that the study

area's main income comes from both crop and livestock. Both crop and livestock are dependable between each other. They use oxen as drought power for crop production; in turn they use crop-residue to feed their animals. Therefore, livestock is a key agricultural component in the area; it serves in terms of milk, meat, drought power and also source of cash. This was in line with the report of other studies (Moges and Bogale, 2012; Haftu *et al.*, 2014) who reported a similar crop-livestock farming system as dominant farming in Gantaafeshum district (eastern Tigray) and Lay- Armachiho district (northern Gondar) respectively. Though livestock was key component in southern zone of Tigray, its production and productivity

was low due to shortage of feed and disease prevalence. Disease accounts big to affect the livestock production as there was insufficient veterinary service delivery and lack of farmers' awareness in the area. This was in agreement with other reports (Moges and Bogale, 2012; Haftu *et al.*, 2014). The study revealed that the majority of the respondents (60%) had access to veterinary clinics though the service was not sufficient as the veterinary clinics were unequipped and lack of qualified professionals. This was in agreement with Moges and Bogale (2012). The veterinary clinics in the area were dominantly governmental (47.5%) while the rest 12.5% were private health posts, thus it indicated that the private veterinary sector coverage was small. The respondents also indicated they travel long distance to access those veterinary clinics. Though the veterinary service was insufficient those who get service mostly get service for disease treatment only (77.8%) unless there was campaign vaccination program, while the few respondents (22.2%) had the habit of treating and vaccinating their animals. This indicated the vaccination (prevention) approach of disease control in the area was inadequate.

Moreover there was also a report of 47.5% respondents that they had a habit of treating animal disease by self by buying drugs from veterinary pharmacies around. This can lead to inappropriate use of drugs; hence it can contribute to drug resistance which favors disease prevalence. The study also revealed that the majority of the contacted farmers (64.2%) traditionally treat animals by branding, bleeding and using herbs/plants in addition to modern treatment. This was in agreement with the report of Haftu *et al.* (2014) who reported 43.4% of respondents from Gantaafeshum district (eastern Tigray) use traditional treatment.

The respondents indicated that various infectious, parasitic and miscellaneous diseases were the major livestock health problems in the area which cause death and production loss. As per the farmers response the disease dynamics in the area was affected by factors like feed shortage, inadequate veterinary service, season

and agro-ecology.

The most frequent diseases of cattle mentioned by the respondents include; Blackleg, Anthrax, Pneumonic Pasteurellosis, Ectoparasite infestation, and foot and mouth disease. This was in line with the findings of other studies in different study areas (Belayneh, 2002; Gebremedhin, 2007; Haftu *et al.* (2014). Moreover it was in agreement with Moges and Bogale (2012) who reported Anthrax and Black leg as most important diseases of cattle in Lay-Armacheho district of northern Ethiopia. The study also indicated that Bloat, Mastitis, Wound (swelling), GIT-parasitism, Enteritis, Lumpy skin disease, Abortion, CBPP, Dysuria (difficulty of urination) and Retained placenta were the less frequently mentioned diseases of cattle. Similarly other report of Yohannes (2007) indicated that Lumpy skin disease was also reported as important disease in Alamata district of Tigray. Moreover, the finding of GIT-parasitism in this study was in agreement with other reports (Ameni *et al.*, 2001; Belete, 2006; Haftu *et al.*, 2014). The finding of Enteritis was also reported by Tariku (2000).

The major health problem mentioned in small ruminants was Peste des petits ruminants, followed by Sheep and goat pox, Foot rot, Orf, Ectoparasites, Pneumonic pasteurellosis, Anthrax, GIT-parasitism, and Enteritis. However, in the reports of Ayet *et al.* (2004), Moges and Bogale (2012) the first important disease of small ruminant was Pneumonic pastuerellosis. At the same time Gizachew (2007) reported that Orf was main problem of small ruminant. The difference might come due to location.

The most prevailing equine health problem was African horse sickness followed by GIT- parasitism, Anthrax, Ectoparasites, Colic, and Dysuria (difficulty of urination). Unlike the current study a report of Haftu *et al.* (2014) in Gantaafeshum district of northern Ethiopia indicated Colic was the first important equine disease.

The most important poultry disease mentioned by the respondents in the area was Newcastle disease which was in agreement with Haftu *et al.* (2014). Besides Newcastle disease Foul cholera, Ectoparasite, Coccidiosis,

and Foul pox were important poultry diseases mentioned. This was in line with other studies (Yohanes, 2007; Haftu *et al.*, 2014) who reported Foul pox, Coccidiosis as important diseases of poultry following Newcastle disease.

The respondents indicated that the most common camel diseases were Hemorrhagic septicemia (18.3%), Plant poisoning (6.6%), Anthrax (5.8%), Ectoparasites (5%), Bloat (4.1%), difficulty of urination (2.5%), Camel pox (1.6%) and Saddle sore (0.83%).

Case observational study

The case observational study revealed that among the diagnosed diseases of cattle Black leg, Lumpy skin disease, Mastitis, Pneumonic pasteurellosis, and Anthrax were the major important diseases. This was in agreement with Moges and Bogale (2012). The case observational study of Haftu *et al.* (2014) similarly indicated Pneumonic pasteurellosis, Mastitis and Anthrax as major important cattle diseases in Gantaafeshum district of Tigray.

In sheep Sheep pox, Pneumonic pasteurellosis, GIT parasitism, and Ectoparasitism were the most common diseases while in goats PPR, GIT parasitism and Pneumonic pasteurellosis were most commonly encountered. It agreed with other reports (Moges and Bogale, 2012; Haftu *et al.*, 2014).

In line with Moges and Bogale (2012) strangles was the first major problem of equines in the area. In agreement with the questionnaire survey African horse sickness, colic and wound were the other observed problems of equines.

The study also revealed that Newcastle disease was the dominant poultry disease followed by Coccidiosis and Foul pox. It agreed with the report of other studies in other parts of Ethiopia (Moges and Bogale, 2012; Haftu *et al.*, 2014). It was also consistent with the questionnaire survey of this study. It indicated that Newcastle disease was the main problem of poultry production in Tigray. In camel Hemorrhagic septicemia was the most frequently observed problem which was in line with the questionnaire survey study of this research.

Conclusion and recommendations

The study indicated that various infectious, parasitic and miscellaneous diseases were the major livestock health problems in the area which results death and production loss. Moreover, the veterinary service provision coverage and farmers awareness towards disease control was insufficient. With this conclusion the following points were recommended:

- Development of proper animal health delivery system and expansion of veterinary service in the area; the private veterinary service delivery should be encouraged
- Awareness should be created for farmers about proper animal health management and disease prevention
- Further animal disease investigation, research and monitoring of specific animal diseases
- Proper disease prevention and control program should be promoted

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LIVESTOCK MANAGEMENT PRACTICES AND MORTALITY PROFILE IN ANIMAL HUSBANDRY IN NORTH-EASTERN NIGERIA

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Abstract

Livestock development is one of the key economic indices of Nigeria and livestock management is aimed to enhance animal health and welfare. The present study investigated management practices and mortality profile in livestock husbandry in northeastern Nigeria. The survey instrument was a close-and open-ended Questionnaire designed to retrieve information from livestock farmers relative to farmer/ animal demographics, livestock management practices and livestock mortality. The questionnaire was used in face – to – face interviews carried out between March and December 2013. Data collected were entered into a personal computer and analyzed using an SPSS data package software version 16.0 for windows, for descriptive statistic. Results indicated that Total confinement (54.5%), housing more than one species of animals under same housing area (32%), use of water tank for multiple animals (52%), use of ground water as source of drinking water for animals (41%), hand-cleaning of animal manure (32%), and regular vaccination (51%) were the most common livestock management practices observed amongst farmers, while mortality was observed more often in female than male animals (58%). In conclusion, the findings in this study revealed that farmers in north-eastern Nigeria managed male animals very well than female animals, possibly for their economic benefits. The practice of housing more than one type of animal species in the same housing unit observed in this study may influence the use of antimicrobials, as well as promoting cross-transfer of diseases between different species.

Key words: Livestock, husbandry, management, mortality, northeast Nigeria

PRATIQUES DE GESTION ET PROFIL DE MORTALITE DES ANIMAUX D'ELEVAGE AU NORD-EST DU NIGERIA

Résumé

Le développement de l'élevage est l'un des principaux indices économiques du Nigeria, et la gestion du bétail vise à améliorer la santé et le bien-être des animaux. La présente étude a examiné les pratiques de gestion et le profil de mortalité des animaux d'élevage dans le nord-est du Nigeria. L'instrument d'enquête était un questionnaire comprenant des questions ouvertes et fermées, conçu pour extraire des informations des éleveurs de bétail en rapport avec la démographie des éleveurs / animaux, les pratiques de gestion et la mortalité des animaux d'élevage. Le questionnaire a été utilisé lors d'entretiens en face à face effectués entre mars et décembre 2013. Les données collectées ont été saisies dans un ordinateur personnel et analysées à l'aide d'un logiciel SPSS version 16.0 pour les statistiques descriptives. Les résultats indiquent que le confinement total (54,5%), le fait d'abriter plus d'une espèce animale dans la même zone d'hébergement (32%), l'utilisation de réservoirs d'eau pour plusieurs animaux (52%), l'utilisation des eaux souterraines comme source d'eau potable (41%) pour les animaux, l'enlèvement à la main du fumier animal (32%) et la vaccination régulière (51%) étaient les pratiques de gestion des animaux d'élevage les plus courantes observées chez les éleveurs, tandis que la mortalité a été observée plus souvent chez les femelles par rapport aux mâles (58%). En conclusion, les résultats de cette étude ont révélé que les éleveurs du nord-est du Nigéria gèrent mieux les animaux mâles par rapport aux femelles, probablement pour leurs avantages économiques. La pratique observée dans cette étude, consistant à abriter plus d'une

espèce animale dans la même unité de logement, peut influencer l'utilisation d'antimicrobiens et favoriser le transfert croisé de maladies entre différentes espèces.

Mots-clés : animaux, élevage, gestion, mortalité, nord-est du Nigeria

Introduction

In ancient times, humans were hunter-gatherers, moving over vast space and environments as nomadic rearers, and they started breeding wild, and domestic animals such as sheep, cattle, goats, pigs, poultry and horses for food and labour (Pantosti, 2012). Livestock development occupies one of the top economic indices of a country, and the goal of livestock production, whether organic or conventional is to enhance animal health and welfare (Vaarst *et al.*, 2005; Alrøe *et al.*, 2001). Health and welfare were advocated as the most distinctive features of a more nature-friendly production, as a way to achieve a price premium for livestock products (Tybirk *et al.*, 2004). Adoption of animal husbandry as a main occupation depends on the availability of feed and fodder, adequate knowledge of preservation and management practices, good roads and transport facilities (Meena *et al.*, 2007).

Livestock management practices comprises strategies that when implemented correctly, address, reduce, or control a potential animal health problems and increase the productivities and reproduction of such animals (Pennington *et al.*, 2015; Chaudhary *et al.*, 2013). Animal health problems are closely linked to nutrition and breed, and shortage of quality feed has been found to affect animal health adversely, as nutritional stress significantly contributes to animal's susceptibility to disease agents or infections (Meena *et al.*, 2007). Underfeeding results in late maturity, high mortality, poor life performance and infertility in animals (Sherchand and Pradhan, 1997).

Bacterial diseases have been reported to form the major bottleneck and causes of morbidity and mortality in animals (Chauhan *et al.*, 1994). According to Porter (1997), stable settlements and proximity to animals cause pathogens to thrive and spread between animals

and humans, and diseases become prominent in influencing life and death. Food of animal origin can also be a major vehicle for animal pathogens and their spread can be amplified by market globalization (Pantosti, 2012).

There is increasing demand for intensive animal farming involving large numbers of animals, different species in the same area, and the use of growth promoters and antibiotics, in order to feed the world's growing human population (Pantosti, 2012). These practices can facilitate the emergence of new pathogens, including antimicrobial-resistant ones, and their transmission to humans.

Prevention of mainly respiratory and digestive disorders can be done by treating the animals in a known risk period during the production cycle and healthy animals sharing same space or barn with diseased ones may be treated (Cattray *et al.*, 2010). But there is a dearth of published information on livestock management practices in animals in the northeast Nigeria, which prompted this research. This paper was therefore aimed to assess the management practices and mortality profile in livestock production in northeast Nigeria.

Materials and methods

The study area covers Adamawa, Borno and Gombe states, with a land mass of over 136,000 km², located in North Eastern Nigeria. The area falls within the conventional arid zone and lies between latitudes 10 and 13°N and longitudes 11 and 15° E. The arid-zone is the largest part of Nigeria, covering the Sudan and Sahel savannah vegetations. The Sahel savannah lies to the north and occupies about 40% of the study area, while the Sudan savannah occupies the southern 60%. The climate here is Sahelian, with three distinct seasons, a rainy season which starts from June to September, with a mean annual rainfall of about 600 mm, the dry



Fig 3.1: Map of Nigeria showing the study area. (Area shaded sky-blue is the arid – zone region survey areas are 3 states marked red boxes (Adamawa, Borno and Gombe).

cold or harmattan season from October to February, characterized by low temperatures between 16 – 29oc and the dry hot season from March to May, with temperatures in the range 46-48oc (Adejuwon, 2005). The main occupation of the people within this area is farming, both arable and livestock (Figure 3.1 describes the study area).

The survey instrument was a Questionnaire which consisted of close-ended questions constructed to retrieve information from livestock farmers relative to farmer/animal demographics, livestock management practices and profile of livestock mortality. The research questionnaire was pre-tested on a sample of farmers randomly selected from two of the three areas by a pilot study, during January to February 2013. The questionnaire was sent to colleagues who are experts in statistics, who then provided suggestions on improving the questionnaire, and comments and suggestions from the pre-test were used to make the final copy of the questionnaire.

Data collection

The questionnaire was used in face – to – face interviews carried out between March and December 2013 in three states Adamawa, Borno and Gombe of the northeast region of Nigeria. The researcher with the assistance of trained veterinary and livestock workers from each of the clusters visited the respective farmers who have orally

consented to the study, and administered the questionnaire on them. The researcher maintained confidentiality throughout the study. Total number of respondents included 200 livestock farmers, keeping any of cattle, sheep, goat, pig and chicken (considered in this study as the livestock).

Data Analysis

Data collected were entered into a personal computer and analyzed using an SPSS data package software version 14 (2016) for descriptive statistic. Rates were computed by cluster and the results were tested for responses using chi-square test. P value was considered significant at $P < 0.05$.

Results

A total of 200 copies of the questionnaire were administered on farmers and 100% were completely or partially answered.

Demographics and animal ownership characteristics of respondents

The results on demographics and animal ownership characteristics of the respondents are presented in tables 4.1 and 4.2. The results revealed that male generally constituted majority (54%) of the respondents than females (46%). However, in cluster 2 females (55%) were more than males (45%)

respondents. Over 80% of the respondents were above 30 years of age, while 19.5% were between 15 and 30 years of age. The majority of respondents (66.5%) had ≤ 5 years of animal rearing or management experience, while 33.5% had more than 5 years management experience. In cluster 2 more respondents (51.7%) had more than 5 years of animal management experience, whilst 48.3% had ≤ 5 years of animal rearing experience compared to other clusters. On the possession of any of the animals cattle, sheep, goat, pig and chicken studied, the respondents indicated ownership of one (47.0%), two (28.0%), three (10.5%) and four (11.5%) of the animals, whilst, 3.0% of the respondents indicated owning all the five animals. Majority (71.4%) of respondents in cluster 1 possessed one of the five animals and 13.3% of respondents in cluster 2 possessed three, whilst 38.6% of the respondents in cluster 3 possessed two of the animals.

The result on animal-ownership characteristics of respondents (Table 4.2) showed that 74.5% indicated sole ownership, 17.5% indicated family ownership, 6.0% were joint ownership and 2.0% indicated others (either large scale farms, Government farms, Association farms, or Institutional farms). The result also showed that 51.5% of the respondents owned chickens, 49.5% owned goats, 37% owned sheep, 33% owned pigs, 31% owned cattle, and 35.5% in addition to owning these animals, also owned other animals (like fishes, ducks, geese, pets etc.). Further result showed that 16.5% of the respondents owned pigs only, 15% owned chickens only, 11% owned sheep only, 4% owned goats only and 2.5% owned cattle only. More farmers in cluster 1 were sole owners of the animals (84.3%), more farmers in cluster 2 were joint owners (7.1%) and more farmers in cluster 3 indicated family ownership (30.0%). High percentages of farmers in cluster 1 owned sheep (45.7%) and chickens (65.7%), high percentages of farmers in cluster 2 owned pigs (46.7%) and other animals (50.0%), whereas, high percentages of farmers in cluster 3 owned goats (68.6%) and cattle (40.0%). The majority (25.7%) of farmers in cluster 1 owned sheep only and majority

of farmers in clusters 2 (21.7%) and cluster 3 (11.4%) respectively owned only pigs.

Livestock Management Practices

Housing practices

The result of Responses on housing practices by respondent farmers is presented in Table 4.3. The results showed that 54.5% respondents practiced total confinement of animals in enclosure, 15.5% housed animals in open buildings with no outside access, 24% housed animals in open buildings with outside access, 14.5% housed animals in lots with no hut or building and 7% practiced pasture with hut or no building. Further results indicated that 32.5% respondents housed all animals under same area or shade, 11% housed adult animals separate from young ones, 48.5% housed lactating animals together with young animals, and 43% housed sick animals separately, whilst, 37.5% housed animals in different areas according to their kind. Majority of respondents in clusters one (62.9%) and two (43.3%) indicated housing animals in total confinement, whilst in cluster three majority (91.4%) indicated housing sick animals separated from healthy ones and lactating animals together with their young ones. Respondents in cluster one that indicated housing lactating animals with their young constituted 62%, and 33.3% of the farmers in cluster two indicated housing animals separately according to their kind.

Feed and water practices

The responses on feeding and watering practices employed by respondent farmers in livestock production indicated that 29.5% fed their animals solely on commercial feeds, 49.5% supplemented their animals' feeds, whilst 21% allowed animals free grazing (Table 4.4). Respondents that fed young animals separate were 43%, those that fed both young and adult animals together were also (43%) and 15.5% respondents indicated that young animals were cared by their mothers. Respondent farmers in cluster one indicated feeding adult and young animals together in same trough (55.7%) and in cluster two 48.3% of the farmers indicated feeding young animals separate from adults,

Table 4.1 Questionnaire Survey on Livestock Management in Northeast Nigeria: Demographic Characteristics of Respondents (N = 200)

Demography	No (%) of responses			
	Cluster 1 (n = 70)	Cluster 2 (n = 60)	Cluster 3 (n = 70)	Total (N = 200)
Sex of respondent				
Male	39 (55.7)	27 (45.0)	42 (60.0)	108 (54.0)
Female	31 (44.3)	33 (55.0)	28 (40.0)	92 (46.0)
Age of respondent (yrs)				
15 – 30	14 (20.0)	17 (28.3)	8 (11.4)	39 (19.5)
> 30	56 (80.0)	43 (71.7)	62 (88.6)	161 (80.5)
Livestock management experience				
≤ 5 years	46 (65.7)	29 (48.3)	58 (82.9)	133 (66.5)
> 5 years	24 (34.3)	31 (51.7)	12 (17.1)	67 (33.5)
Possession of the types of animals studied				
One of the animals	50 (71.4)	25 (41.7)	19 (27.1)	94 (47.0)
Two of the animals	11 (15.7)	18 (30.0)	27 (38.6)	56 (28.0)
Three of the animals	5 (7.1)	8 (13.3)	8 (11.4)	21 (10.5)
Four of the animals	4 (5.7)	4 (6.7)	15 (21.4)	23 (11.5)
All the five animals	0 (0.0)	5 (8.3)	1 (1.4)	6 (3.0)

Table 4.2 Questionnaire Survey on Livestock Management in Northeast Nigeria: Animal-Ownership characteristics of the respondents (N = 200)

Demography	No (%) of responses			
	Cluster 1 (n = 70)	Cluster 2 (n = 60)	Cluster 3 (n = 70)	Total (N = 200)
Ownership of animals				
Sole ownership	59 (84.3)	35 (58.3)	55 (78.6)	149 (74.5)
Joint ownership	3 (4.3)	4 (6.7)	5 (7.1)	12 (6.0)
Family ownership	8 (11.4)	18 (30.0)	9 (12.9)	35 (17.5)
Others <input type="checkbox"/>	0 (0.0)	3 (5.0)	1 (1.4)	4 (2.0)
Types of animals owned				
Cattle	18 (25.7)	16 (26.7)	28 (40.0)	62 (31.0)
Sheep	32 (45.7)	12 (20.0)	30 (42.9)	74 (37.0)
Goats	31 (44.3)	20 (33.3)	48 (68.6)	99 (49.5)
Pigs	13 (18.6)	28 (46.7)	25 (35.7)	66 (33.0)
Chickens	46 (65.7)	29 (48.3)	28 (40.0)	103 (51.5)
Others <input type="checkbox"/>	20 (28.6)	30 (50.0)	21 (30.0)	71 (35.5)
Ownership of specific animal				
Cattle only	3 (4.3)	2 (3.3)	0 (0.0)	5 (2.5)
Sheep only	18 (25.7)	1 (1.7)	3 (4.3)	22 (11.0)

Demography	No (%) of responses			
	Cluster 1 (n = 70)	Cluster 2 (n = 60)	Cluster 3 (n = 70)	Total (N = 200)
Goats only	4 (5.7)	2 (3.3)	2 (2.9)	8 (4.0)
Pigs only	12 (17.4)	13 (21.7)	8 (11.4)	33 (16.5)
Chickens only	13 (18.6)	11 (18.3)	6 (8.6)	30 (15.0)

□ Government Institutions and Cooperatives; □pets, other poultry and fish

Table 4.3 Questionnaire Survey on Livestock Management in Northeast Nigeria: Livestock Housing Practices amongst farmers (N = 200)

Housing practice	Number (%) Responses			
	Cluster 1 (n = 70)	Cluster 2 (n = 60)	Cluster 3 (n = 70)	Total (N = 200)
Total confinement	44 (62.9)	26 (43.3)	39(55.7)	109(54.5)
Open building with no outside access	5 (7.1)	14 (23.3)	12 (17.1)	31 (15.5)
Open building with outside access	13 (18.6)	9 (15.0)	26(37.1)	48(24.0)
Lot with hut or no building	8 (11.4)	10 (16.7)	11 (15.7)	29 (14.5)
Pasture with hut or no building	0 (0.0)	9 (15.0)	5 (7.1)	14 (7.0)
All animals housed under same area	7 (10.0)	9 (15.0)	49(70.0)	65(32.5)
Adults housed separate from young	8 (11.4)	9 (15.0)	5 (7.1)	22 (11.0)
Lactating animals housed with young	44 (62.0)	3 (5.0)	50(71.4)	97(48.5)
Sick animals housed separately	3 (4.3)	19 (31.7)	64(91.4)	86(43.0)
Animals housed in different spaces	8 (11.4)	20 (33.3)	47(67.10)	75(37.5)

whilst 68.8% of the farmers in cluster three indicated the use of feed supplements on their animals.

Some respondents indicated using individual automatic water bowls for animals (5.5%), automatic water bowls shared by groups of animals (12.5%), water tank for multiple animals (52%) and occasional access to surface water (26.5%), whilst 26% indicated using water mixed in feed to their animals. Respondents who indicated well water as the source of water for their animals were 30%, Municipal water or borehole 31.5% and 40.5% indicated ground water (pond, stream, river etc.) as source of water for their animals. The use of water tank for multiple animals was observed to be commonly practiced by 57.1% of the farmers in cluster one, 26.7% of the farmers in cluster two and by 68.6% of the farmers in cluster three. So also 43.3% of

farmers in cluster one and 38.3% in cluster two used municipal or borehole as the source of water for animals, whilst majority of farmers (51.4%) in cluster 3 used ground or surface water for their animals (table 4.4).

Bio-security Management practices

Bio-security management practices involve control of animal housing environments including manure handling, quarantine process and vaccination practices. The results of the animal environment management practices are presented in table 4.5. Respondent farmers indicated utilizing the following methods in managing or handling animal manure, mechanical scrapper (32%), hand-cleaning (30.5%), pit-holding (14.5%), flush under slates (11.5%) and flush to open gutter (9.5%), whilst, 7% of the respondent farmers paid little or no attention to manure management. Hand-cleaning of

Table 4.4 Questionnaire Survey on Livestock Management in Northeast Nigeria: Livestock feeding and watering practices by farmers (N = 200)

Practice	Number (%) of Respondents practicing these methods			
	Cluster 1 (n = 70)	Cluster 2 (n = 60)	Cluster 3 (n = 70)	Total (N = 200)
Feeding practice				
Fed animals solely commercial feeds	25 (35.7)	21 (35.0)	13 (18.6)	59(29.5)
Supplement animal feeds	27 (38.6)	24 (40.0)	48 (68.8)	99 (49.5)
Animals left to free grazing	18 (25.7)	15 (25.0)	9 (12.9)	42 (21.0)
Young animals fed separate	27 (38.6)	29 (48.3)	30 (42.9)	86 (43.0)
Young animals fed with adults	39 (55.7)	18 (30.0)	29 (41.4)	86 (43.0)
Young animals cared by their mothers	4 (5.7)	13 (21.7)	14(20.0)	31(15.5)
Watering system				
Individual automatic water bowl	9 (12.9)	2 (3.3)	0 (0.0)	11 (5.5)
Automatic water bowl shared by group	0 (0.0)	14 (23.3)	11 (15.7)	25 (12.5)
Water tank for multiple animals	40 (57.1)	16 (26.7)	48(68.6)	104(52.0)
Occasional access to surface water	2 (2.9)	12 (20.0)	39 (55.7)	53 (26.5)
Water mixed in feed	19 (27.1)	16 (26.7)	17 (24.3)	52 (26.0)
Sources of water for animals				
Well	23 (32.9)	15 (25.0)	22 (31.4)	60 (30.0)
Municipal (borehole)	24 (43.3)	23 (38.3)	16 (22.9)	63 (31.5)
Ground water (pond, river, stream)	23 (32.0)	22 (36.7)	36 (51.4)	81 (40.5)

Table 4.5 Questionnaire Survey on Livestock Management in Northeast Nigeria: Animal Environment Management Practices (N = 200)

Practice	Number (%) Responses			
	Cluster 1 (n = 70)	Cluster 2 (n = 60)	Cluster 3 (n = 70)	Total (N = 200)
Manure handling practice				
Hand-cleaning	24 (34.3)	24 (40.0)	13 (18.6)	61 (30.5)
Use of mechanical scrapper	19 (27.1)	9 (15.0)	36 (51.4)	64 (32.0)
Pit-holding	5 (7.1)	15 (25.0)	9 (12.9)	29 (14.5)
Flush under slates	7 (10.0)	4 (6.7)	12 (17.4)	23 (11.5)
Flush to open gutter	6 (8.6)	6 (10.0)	7 (10.0)	19 (9.5)
Little or no attention to manure Management	9 (12.9)	2 (3.3)	3 (4.3)	14 (7.0)
Containment of animal environment				
Restrict entry of visitors onto operations	22 (31.4)	8 (13.3)	22 (31.4)	52 (26.0)
Do not restrict entry of visitors to operations	25 (35.7)	30 (50.0)	21 (30.0)	76 (38.0)
Requires attendants to shower before entry	10 (14.3)	4 (6.7)	6 (8.6)	20 (10.0)
Provides footbaths and aprons for attendants	10 (14.3)	18 (30.0)	17(24.3)	45(22.5)
Washed Animals pens regularly	3 (4.3)	0 (0.0)	4 (5.7)	7 (3.5)

manure was observed to be most common amongst farmers in clusters one (34.3%) and two (40%), while use of mechanical scrapper was the most common practice amongst farmers in cluster three.

Majority of farmers (38%) do not restrict entry of visitors onto the animal operations, whilst 26% restricted entry of visitors onto the operations. Farmers that required attendants to shower before entry onto operations constituted 10% of the respondents, whilst 22.5% of the respondents provided footbaths and aprons to attendants before entry onto operations, 3.5% of the farmers however washed animal pens regularly. Comparing between clusters, 31.4%, 13.3% and 31.4% of farmers in clusters one, two and three respectively restricted entry of visitors onto animal operations, whereas, 35.7%, 50% and 30% respectively do not restrict entry of visitors onto their operations. Also 14.3%, 30% and 24.3% of the farmers respectively provided footbaths and aprons to attendants before entry onto their operations.

Animal quarantine and vaccination practices

Table 4.6 shows the quarantine and vaccination practices in livestock husbandry in northeast Nigeria. The result shows that 74% of the farmers received or purchased new animals during operations and 33.8% of these always quarantined and 48% sometimes quarantined, whilst 18.2% never quarantined their newly received animals. Also 81.5% of the farmers have vaccinated their animals, while 18.5% have never vaccinated their animals. The frequency of vaccination indicated that 51% vaccinated their animals regularly, whilst 30.5% sometimes or seldom vaccinated their animals. In cluster one, 38% of respondent farmers have always quarantined and 42.9% sometimes quarantined their new arrival animals. Farmers that regularly vaccinated their animals constituted 40% and 38.6% sometimes vaccinated their animals. Among respondents in cluster two, 56.6% sometimes practiced quarantine process, 22% always and 22% never practiced quarantine process. Also 51.7% of the farmers regularly vaccinated and 26.7% sometimes vaccinated

their animals. In cluster three, 41% of the farmers always and 44.6% sometimes practiced quarantine process, whereas, majority (61.4%) of farmers regularly vaccinated and 25.7% sometimes vaccinated their animals.

Flock Mortality Profile Record

The profile of mortality amongst animals in the last three years prior to the present study as reported by respondents is presented in tables 4.7. Mortality rate of $\leq 5\%$ was observed by 55.0% of the farmers in their animals during the previous 3 years period prior to this study. Farmer who reported mortality rate between 5 and 10% constituted 24.5% and 3.5% of the farmers indicated mortality rate greater than 10%, while 17% indicated no mortality during the acclaimed period. Aborted foetuses and/or still birth were reported by 45% of the farmers, and 19.5% reported mortality in pregnant animals. More farmers reported pre-weaned mortality (49%) and mortality of adult animals (49%), and 34% indicated mortality of young/pre-weaned animals. Mortality in apparently healthy animals was reported by 17.5% of the respondent farmers, whilst (48.5%) reported mortality of apparently sick animals, 12% of the farmers reported mortality of only malnourished animals. Observation across clusters revealed that majority of the farmers 64.3% in cluster one, 60% in cluster two and 41.4% in cluster three indicated mortality rates of less than 5% amongst their animals in the previous three years prior to this study. So also, 44.3% of the farmers in cluster one, 35% in cluster two and 54.3% in cluster three indicated more mortality in terms of aborted foetuses/still birth. Age-wise mortality report indicates that mortality of pre-weaned or young animals was observed by 51.4% of the farmers in cluster one, 35% in cluster two and 58.6% in cluster three, and mortality of adult animals was observed by 51.4% of the farmers in cluster one, by 48.3% in cluster two and by 47.1% of the farmers in cluster three. Mortality in apparently sick animals was reported by majority of the farmers in cluster one (61.4%), cluster two (55%) and cluster three (30%) within the period under review.

Table 4.6 Questionnaire Survey on Livestock Management in Northeast Nigeria: Quarantine and Vaccination practices (N = 200)

Practice	Number (%) of Respondents practicing			
	Cluster 1 (n = 70)	Cluster 2 (n = 60)	Cluster 3 (n = 70)	Total (N = 200)
New Arrivals (animals)				
Received or purchased new animals	42 (60.0)	50 (83.3)	56(80.0)	148(74.0)
Not received or purchased new animal	28 (40.0)	10 (16.7)	14 (20.0)	52 (26.0)
Frequency of Quarantine process				
Always	16 (38.1)	11 (22.0)	23(41.1)	50 (33.8)
Sometimes	18 (42.9)	28 (56.0)	25 (44.6)	71(48.0)
Never	8 (19.0)	11 (22.0)	8 (14.3)	27 (18.2)
Animal vaccine				
Vaccinated their animals	55 (78.6)	47 (78.3)	61 (87.1)	163(81.5)
Never vaccinated animals	15 (21.4)	13 (21.7)	9 (12.9)	37 (18.5)
Frequency of vaccinations				
Regularly	28 (40.0)	31 (51.7)	43 (61.4)	102(51.0)
Sometimes	27 (38.6)	16 (26.7)	18 (25.7)	61 (30.5)
Never	15 (21.4)	13 (21.7)	9 (12.9)	37 (18.5)

Table 4.7 Questionnaire on livestock management in northeast Nigeria: Rate, Gestational (reproductive status),Age/sex and Health-wise Mortality records for the last three years prior to the study (N = 200)

Practice	Number (%) Responses			
	Cluster 1 (n = 70)	Cluster 2 (n = 60)	Cluster 3 (n = 70)	Total (N = 200)
Mortality Rate				
Less than 5%	45 (64.3)	36 (60.0)	29 (41.4)	110 (55.0)
5 – 10%	14 (20.0)	11 (18.3)	24 (34.3)	49 (24.5)
Greater than 10%	2 (2.9)	2 (3.3)	3 (4.3)	7 (3.5)
No mortality recorded	9 (12.9)	11 (18.3)	14 (20.0)	34 (17.0)
Gestational mortality				
Abortion/still birth	31 (44.3)	21 (35.0)	38 (54.3)	90 (45.0)
Lactating/suckling animals	38 (54.3)	35 (58.3)	40 (57.1)	113 (56.5)
Pregnant animals	13 (18.6)	9 (15.0)	17 (24.3)	39 (19.5)
Age/sex-wise mortality				
Pre-weaned or young mortality	36 (51.4)	21 (35.0)	41 (58.6)	98 (49.0)
Mortality in adult animals	36 (51.4)	29 (48.3)	33 (47.1)	98 (49.0)
More often in neonates than dams	39 (55.7)	23 (38.3)	29 (41.4)	91 (45.5)
More often in dams than neonates	22 (31.4)	26 (43.3)	27 (38.6)	75 (37.5)
More often in males than females	13 (18.6)	6 (10.0)	30 (42.9)	49 (24.5)
More often in females than males	47 (67.1)	43 (71.7)	26 (37.1)	116 (58.0)

Practice	Number (%) Responses			
	Cluster 1 (n = 70)	Cluster 2 (n = 60)	Cluster 3 (n = 70)	Total (N = 200)
Health-wise mortality				
Apparently healthy animals	6 (8.6)	11 (18.3)	18 (25.7)	35 (17.5)
Apparently sick animals	43 (61.4)	33 (55.0)	21 (30.0)	97 (48.5)
Malnourished animals	12 (17.1)	5 (8.3)	7 (10.0)	24 (12.0)

Generally, mortality was also observed to be more often in lactating animals (56.5%) than in non-lactating animals (26.5%), more often in neonates (45.5%) than in dams (37.5%), and more often occurred in females (58.0%) than in males (24.5%). Across clusters 67.1% of the farmers in cluster one, 71.7% in cluster two, and 37.1% in cluster three indicated that mortality was more often in female than in male animals. Other details can be read in table 4.7.

Discussion

The present study was undertaken to investigate the management practices and mortality profile among livestock in the northeast region of Nigeria. Management practices have a direct or indirect linkage to the health of livestock.

The result of the present survey revealed that the majority of the livestock farmers were males constituting 54% of the respondent farmers. This implies that males more than females were more likely ready to respond to questionnaires, and perhaps because they are the heads of their families and are responsible for their households including their properties or possessions. This confirmed the experience during the interview where many females referred the researcher to their spouses for response to the questionnaire. This finding concurs with a recent study conducted on commercial poultry layer farmers in southwest Nigeria (Adebowale *et al.*, 2016), in which the majority of respondent farmers constituted of 71.8% men and 14.6% women. The present study also reports that higher percentage (80.5%) of the respondent

farmers were above 30 years of age (adults), as at the time of data collection, and they were observed to form the major livestock farmers or large holders of livestock operations. This might suggest that age is significant in livestock management. Similarly, Adebowale *et al.* (2016) reported that 83.5% of the respondent poultry farmers were have acquired tertiary education: One must be an adult to acquire this level of education. However, age is insignificant in livestock management when experience is the point for consideration. It was observed in this study that majority of the farmers had < 5 years of livestock management experience. More percentage of the respondents in cluster two appeared to have had more duration of livestock management experience (>5 years) compared to the respondents in clusters one and three. In a similar study in poultry production in Ghana (Boamah *et al.*, 2016), majority of poultry farmers were reported to have had more than 5 years management experience. Findings in the present study also showed that the highest percentage of the respondents kept only one of the five animals; cattle, sheep, goat, pig or chicken and the lowest percentage kept all the 5 animals. This may likely suggest that the choice of keeping a number of animal species may be determined by factors like economic status of the farmer, availability of space owned by the farmer, ease of management, religion, and the usefulness or economic importance of the animal as well as, the cost of the animal, amongst others. Possession of the types of animals was not associated with either age or livestock management experience, but was closely related to sex, as the percentage of females (46%) was close to percentage of farmers that possessed one type of animal

(47%). However, farmers keeping more than one type of animals in the same premises may have the tendency of using antibiotics for prophylaxis (Callens *et al.*, 2012), especially if such farmers are aware that cross-transmission of disease or infections occurs between animal species (Garforth, 2015). Also, farmers keeping more than one type of animal species were less likely to seek advice from veterinarians and the use of disinfectants (Boamah *et al.*, 2016), and were more likely also to use antibiotics without prescription than farmers that kept only one type of animal.

The present study also reports that majority of respondents solely owned the animals they kept, and there was no significant relationship between sole ownership, joint ownership and family ownership of animals ($P < 0.05$). Most of the respondents kept chickens and goats more frequently than any of the other animals studied. This may probably be due to the relative prices and management of these animals, which appeared to be much cheaper and easier compared to sheep, cattle and pigs. In the northern part of Nigeria goats are usually left to freely graze during the larger part of the season (dry season) and the first quarter of the rainy season, until all crop plantings are over, due to the relatively vast lands around this region. Goats, like chickens are also relatively cheaper and can be afforded by almost every low income household owner. The high percentage (51.5%) of the respondents owning chickens in this study may also be attributable to festivities such as Sallah (particularly during Islamic fasting periods) and Christmas celebrations, in which chickens are mostly used. The high percentage of respondents in clusters 1 and 3 that kept sheep indicates the importance of this animal (sheep are used for big Sallah festivities) in this part of the country. It is also probable that the patronage for sheep is likely to be more in these clusters, unlike pig which had the highest percentage of respondents that kept it in cluster 2. This may also be due to their patronage in this part of the northeast than in clusters 1 and 3. There appeared to be high percentage of respondents that owned only pigs

and no other animals, probably due to the high reproductive prolificacy of pigs (can produce up to 20 piglets per litter). Farm characteristics such as flock size, age, keeping more than one animals together and occurrence of infections, according to Boamah *et al.* (2016) may influence the use of antimicrobial agents.

Therefore, farmers in this study that kept more than one type of animals together might be influenced to use antimicrobials, and may likely to experience challenges of resistance amidst their animals. Although, in Nigeria information about similar studies encompassing both poultry and other livestock, at the disposal of the present survey is scarce, in the southern part of the country however, poultry, rather than other livestock, are mostly reared (Oluwasile *et al.*, 2014; Adebowale *et al.*, 2016), due probably to commercial benefits and lucrative advantage of chicken which produce all year round (Adebowale *et al.*, 2016; Boamah *et al.*, 2016). Another possible reason may be lack of vast lands for grazing in the southern part unlike obtainable in the north.

In this study, about 49% of respondents housed lactating animals along with their young ones in a separate area and majority of animals were housed under total confinement. Similarly, In a study involving dairy cattle, high percentages of farmers (73.7% conventional farms; 34.4% organic farms) were reported to have housed lactating animals separate (Zwald *et al.*, 2004), and in another study involving swine, Halev (2009) reported that majority of animals were housed under total confinement. This concurs with the finding in the present study. Housing under total confinement has the advantage of ease of controlling infections in livestock. However, it has been observed that microorganisms can amplify very efficiently in a holding pen containing live animals (Gilchrist *et al.*, 2007). Several practices such as housing pre-weaned animals in an individual area and use of individual water bowls for animals at different stages of reproduction could contribute to the reduction of disease spread among animals (Zwald *et al.*, 2004). Total confinement has been in practice in poultry management in Maiduguri, northeast Nigeria, in the form of

deep litter system (Akidarju *et al.*, 2010). It was observed in this study that more respondents housed animals in open buildings with outside access compared to open buildings with no outside access. Halev (2009) also reported lower percentage of animals housed in open buildings with outside access compared to the findings in this study, but the difference was not statistically significant. Animals housed in open buildings with outside access has the chances of contact with other animals, and will have increased risk of disease spread in an outbreak. While some farmers in this study housed animals in different housing units according to their kinds, many farmers housed sick animals separate from apparently healthy ones. Previous study (Zwald *et al.*, 2004) also reported housing sick cows separated from healthy lactating cows. However, the use of separate facilities to house sick animals from others might not be influenced by type or kind of animals. A previous study (Meena *et al.* 2007) reported that 92% of farmers housed young animals along with their mothers compared to 48.5% reported in this study. These practices were strategies to curb cross-contamination and transmission of diseases from animals to other animals. In this study also, 32.5% of the farmers housed different animal species under same housing unit, compared to the previous study (Meena *et al.*, 2007) which reported that 93% of farmers housed different animals in the same housing unit or area. Housing all animal species in the same housing unit may lead to fight among the various kinds of animals, and if pregnant animals are housed together with other animals, they may be injured by non-pregnant ones or by males (Meena, *et al.*, 2007). This practice is also unhealthy because of the possibility of being a reservoir of disease transmission and cross-contamination. The concentration of animals in close proximity is also said to enhance potential transmission of microorganisms among members of the group and also creates greater potential for infecting surrounding life farms and even those of different species (Gilchrist *et al.*, 2007). Furthermore, housing different species of animals in same area or unit may attribute

to exposure of animals to different kinds of infections, and may necessitate the use of high amount of antimicrobials and frequency of administration of the antibiotics on such farms (Boamah *et al.*, 2016). This study indicated that about 30% of farmers fed their animals commercial feed, compared to 49.5% that supplemented their animals' feeds. Similarly, previous study (Meena *et al.*, 2007) reported 86.7% farmers that allowed their animals free grazing compared to 24% that supplemented their animal feeds using salts and mineral licks. According to Meena *et al.* (2007), home-made concentrate mixtures were for lactating animals and bullocks only, not for unproductive animals. In this study farmers that fed young animals separate did not differ significantly from those that fed young animals together with their adult ones ($P > 0.05$). Feeding young and adult animals together might result in under-feeding of the young animals, as adult animals being stronger with stamina might bully the younger ones, and might displace them from feeding troughs. Their feeding capacity also differs, as adult animals are more likely to feed faster and could grasp much feed into their mouths than the younger animals. Depending on the quantity of feed placed to the animals, feeding of young and adults together in the same tray or space may likely lead to under-nutrition or undernourishment of the young animals, and may likely result in some consequences including late maturity, high mortality, poor life performance and infertility, especially in cattle (Sherchand and Pradhan, 1997). Respondents that fed their animals solely on commercial feeds differed significantly from those that left their animals on free grazing. Akidarju *et al.* (2010) reported lower percentage of farmers (4.6%) that left their poultry on free range compared to the report in the present study, where 21% of the farmers left their animals on free range. However, free grazing has been a traditional method of livestock management in the past. Animals left to free grazing are most likely more delicious and tasty than those confined totally on commercial feeding. They are also more advantageous and healthy because of their less cholesterol content and

are unlikely to lead to heart problems in human consumers. Animal feeds containing animal tissues and by-products, like in commercially available livestock feeds, have been observed as a source of major concern, as spore-forming bacteria if present will persist even after processing, and pathogens tend to amplify in animals raised with concentrated commercial feeds, which appear to be more difficult to eliminate in meat packing processes (Gilchrist *et al.*, 2007). In addition, such products may contain high fat content loaded with cholesterol, an unsaturated fatty acid that can cause obesity in humans, (Gilchrist *et al.*, 2007), thus leaving free grazing to be of advantage. According to Meena *et al.* (2007), high altitude or topography, small size land-holding, among other factors serve as hindrances to animal husbandry *vis-a-vis* free grazing. In Free grazing animals choose what to feed on, and thus this practice can help solve the problem of food shortage *vis-a-vis* underfeeding by providing the animals with natural nutrients, since animal health problems have been found to be closely linked to nutrition and breed (Meena *et al.*, 2007; Hersom *et al.*, 2014). Shortage of quality feed has been observed to affect animal health adversely as nutritional stress contributes significantly to diseases (Meena *et al.*, 2007; Hersom *et al.*, 2014).

The present study also revealed that higher percentage of respondents (52%) used water tank for multiple animals. The use of water tank for multiple animals may have its consequences, as it has been observed to put some animals at risk, as shared water sources may serve as potential source of disease spread among herd-mates Zwald *et al.* (2004). It was observed that the respondents that used water mixed with feed for their animals and those where animals had occasional access to surface water did not differ statistically ($P>0.05$). It was reported that animals having access to outdoors usually access surface water (Zwald *et al.*, 2004). About 13% of the respondents in this study used automatic water bowl shared by group, and less than 6% used individual automatic water bowls. Similarly,

the use of individual automatic water bowls for animals was previously reported in milking cows (Zwald *et al.*, 2004). High percentages of farmers in this study indicated the use of well and municipal (borehole) water as sources of water for their animals; well and municipal water produce nearly clean water. Similarly, high percentage (80%) of poultry farmers in Ghana were found to rely on boreholes and wells as the source of water for their animals (Baomah *et al.*, 2016). Majority of farmers in this study indicated surface water as the source of water for their animals. Surface water might be a potential source of disease spread among animals. The use of surface water by majority of the respondent farmers in this study might be attributed to relative free access by animals, unlike well and municipal water which require human or mechanical efforts to provide for the animals. It is worthy of note that surface or ground water contamination is a health risk for both human and animals alike, and the associated ecosystem (Gilchrist *et al.*, 2007). Feed and water contaminated with faeces, urine or saliva are noted to be frequent causes of oral transmission of disease agents among both animals and humans (Hersom *et al.*, 2014).

All segments of livestock production might be potential contributors to zoonotic diseases, including transportation of livestock, manure handling practices, etc. Environmental management forms an integral part of animal production, as manure management is an important aspect especially in swine (Hoag and Roka, 1995), poultry and even dairy cattle production. Water, soil and air quality have also been found to be key environmental and public health issues in chicken meat and pork production systems. In the present study only 7% of the respondent farmers paid little or no attention to environmental management, whereas, the majority have considered environmental management as key issues in their production. Poor environmental management was recently reported in poultry production (Adebowale *et al.*, 2016; Kamini *et al.*, 2016; Boamah *et al.*, 2016) and in dairy cattle production (Sharma *et al.*, 2015). Inadequacies in livestock management practices may lead to increased exposure to infectious agents with a

resultant immune-suppression on the animals (Oluwasile *et al.*, 2014; Boamah *et al.*, 2016) and dependence on antibiotic therapy.

The type of manure management practices used may depend on the type of facilities on the animal operation system. In this study, mechanical scrapping was observed as the most common method of manure management by the livestock farmers, closely followed by hand-cleaning. However, there was statistically no significant difference ($P > 0.01$) between farmers that used mechanical scrapper and those that used hand-cleaning. Manure treatment methods may affect the possibility of surface water contamination and environmental odour. Manures that are often flushed into open gutter may likely have the ease to cause environmental odour and surface water contamination and consequently result to spread of infections. It was gathered in this study that pit-holding, flushing manure into open gutters and flushing under slates, to prevent environmental contamination and odour was less frequently practiced by the farmers. Similar findings were reported previously in swine production elsewhere (Losinger *et al.*, 1998) in which hand-cleaning (27%), mechanical scrapping (25%), pit-holding (23%), flushing into open gutter (3.4%) and flushing under slates (2.4%) were observed as manure handling practices. The practice of hand-cleaning may most likely potent an attendant implication in that apart from injuries to the hand, many infective agents from the manure particularly in a contaminated environment may stick to the finger nails which might require appropriate cleaning with expensive antiseptic.

The routes of disease transmission to animals can be controlled if the animal's environments are controlled. Many disease agents can survive for extended periods of time in soil or other organic material like bedding or manure or old feed, and can be transmitted through inhalation, via oral consumption or from direct contact with an infected animal or with fomites from the contaminated environment (Hersom *et al.*, 2014), for instance, coccidiosis and chronic respiratory disease in chickens get transmitted via contact with

poultry manure (Msoffe *et al.*, 2009; Mubito *et al.*, 2014). Poor management or no attention to manure management could compound this process. In this study, most farmers paid attention to manure management. However, 7% of the farmers paid little or no attention to manure management, thus this percentage of farmers are likely to experience disease transmission on their farms. Similarly, majority of poultry farmers paid attention to manure management in Ghana, with 35% changing their litters on quarterly basis as reported by a recent study (Baomah *et al.*, 2016). Solid tanks or reservoirs treatment rather than the methods earlier described have been recommended to prevent manure contamination of surface and underground water with infectious agents harbouring antibiotic resistance genes (Gilchrist *et al.*, 2007).

Bio-security management refers to management practices that protect the health of livestock herds by preventing introduction of pathogens and poisons that are considered potentially harmful to them. Insects, rodents, birds, wildlife, pets, unnecessary visitors or even other animals can carry disease agents into animal operations. The purpose of bio-security is to establish a preventive barrier to disease-causing agents and other threats by minimizing the movement of biological organisms and external threats into and within livestock operations. In this study, the results of investigations into bio-security measures practiced by the farmers in northeast Nigeria indicate that majority of respondents do not restrict entry of visitors into their animal operations. These farmers which probably are small holders of animal operations are most likely to risk their operations to pathogens. Previous study in swine production (Losinger *et al.* 1998) reported high percentage of farmers that restricted entry of visitors into their animal operations compared to the finding in this study. Introduction of pathogens into animal operations can result in severe consequences, such as high mortality and reduced production, economic losses to producers as well as their health risk (Miller *et al.*, 1995), especially in swine and poultry operations. High percentage

(23%) of the farmers in this study compared to 4.6% in the previous study (Losinger *et al.*, 1998) used footbaths and aprons for visitors before entry into their operations; although, disposable boot covers have been advocated recently as better replacements to footbaths in containing contamination from soil and manure (Hersom *et al.*, 2014). Also more farmers in this study (10%) than in the previous study (0.4%) (Losinger *et al.*, 1998) required animal attendants to undergo shower before entering their operations. It was observed in this study also that some (3.5%) of the farmers washed their operations regularly instead of subscribing to entry restrictions and use of footbaths. Although, regular washing is labour-intensive, some farmers perhaps found it cheaper and easier to wash the pens regularly than provide footbaths which is expensive, this also was observed in a previous study (Zwald *et al.*, 2004). Regular cleaning of facilities and equipment between groups of livestock during processing was also considered as a good management practice aimed at reducing pathogen transmission (Hersom *et al.*, 2014). Disease agents and the infections they produce cohesive in one common thing, the animal must be exposed to disease agents and infections in order to develop a disease. One potential threat to animal operations is the introduction of new animals which may be harbouring infections or disease agents (Losinger *et al.*, 1998), which can spread from one animal to another or from animal to humans and vice versa, through a variety of transmission routes. Aerosol transmission can occur simply when a disease agent contained in droplets passes through the air from one animal to another or from an animal to human or vice versa. Close proximity of infected and susceptible animals has been observed to encourage such method of disease transmission (Hersom *et al.*, 2014).

It was observed in this study that majority (74%) of the farmers have received or purchased new animals into their operations, of this 33.8% have always and 48% have sometimes quarantined the newly received animals. Similarly, in a previous study (Losinger *et al.*, 1998) majority of farmers have received

new animals into their operations, however, only few of them have ever quarantined the new arrivals. The most important step in disease control is limiting contact, co-mingling, and movement of livestock. This process according to Hersom *et al.* (2014) is of special importance for new animals arriving on the farm, including replacement animals, breeding animals and animals returning from livestock shows.

In this study, the frequency with which newly received or purchased animals were placed through quarantine process revealed that the majority (63.5%) of the farmers sometimes quarantined the new arrivals, while 20% of the farmers always and 16.5% never quarantined newly received animals in their operations. This finding corroborates with the previous study (Losinger *et al.*, 1998) in swine operations, in which it was observed that 50.5%, 37.9%, and 18.6% of the farmers always quarantined breeding male, breeding female and feeder pigs respectively, and 12.9%, 11.9%, and 8.7% sometimes quarantined breeding male, breeding female and feeder pigs respectively. The percentage of farmers that never quarantined new arrivals or newly received animals were 72.7%, 50.2%, 36.6% for feeder pigs, breeding female and breeding male pigs respectively, higher than percentage of farmers that never quarantined their newly received animals reported in this study. The knowledge of occurrence and pattern of animal diseases is vital in livestock management practices. The present study also indicated that high percentage (81.5%) of the farmers vaccinated their animals, and the frequency of vaccination was observed; 44.5% of the farmers always vaccinated, 30.5% sometimes vaccinated and 25% of the farmers never vaccinated their animals. The percentage of those that always vaccinated their animals was higher in this study compared to a previous study elsewhere (Meena *et al.*, 2007) where 7.5% of the farmers always or regularly vaccinate their animals and about 73% of the farmers never knew about vaccination, and therefore never vaccinated their animals. Similarly, in a study on poultry management in Maiduguri, Nigeria, Akidarju *et al.* (2010) reported that 27.8%

of the farmers always or regularly vaccinate their chickens, 56.7% sometimes vaccinate, whereas, 15.6% never vaccinate their chickens. This report concurs with the finding in the present study. The finding in this study suggests that farmers in northeast Nigeria had more knowledge about vaccination of animals, and are more likely to experience low mortality of animals than are former elsewhere reported in the previous study (Meena *et al.*, 2007). Timely vaccination and other practices such as good nutrition, traffic control, reduction of overcrowding and increasing farm hygiene amongst others, constitute non drug means of preventing diseases among livestock (Hersom *et al.*, 2014; Glisson, 1998). Lack of bio-security practices have contributed immensely to the dependence of antimicrobial agents in many poultry productions systems in southwest Nigeria (Oluwasile *et al.*, 2014).

Diseases have been observed as the bottleneck in livestock profitability, and bacterial and parasitic diseases have been reported as the main causes of livestock losses or mortality. This has been particularly observed in the dairy industry (Chauhan *et al.*, 1994). The mortality profile of animals recorded by the respondent farmers from three years prior to this study was investigated. Higher percentage of farmers recorded mortality of less than 5% in the last three years prior to this study and a significant percentage also reported casualty between 5 – 10%, some recorded mortality of greater than 10%, yet a significant percentage did not record mortality within the period investigated (table 4.15). Similarly, significant percentage (37%) of poultry farmers in Cameroon did not record mortality in their flock (Kamini *et al.*, 2016). This suggests that the farmers that did not experience mortality in their animals adhered to good management practices. The finding in this study revealed high percentage (49%) of farmers that experienced more mortality of pre-weaned or young animals than aborted fetuses and/or still birth. However, percentages of farmers that reported mortality of Pre-weaned or young animals and those that reported mortality of adult animals did not differ significantly ($P > 0.05$). High percentage of

farmers observed mortality in lactating animals more often than in non-lactating animals, and high level of mortality in neonate animals than in dams. Similarly, Chaudhary *et al.* (2013) reported high level of mortality in neonate animals followed by in the young, and the least mortality in adults. These findings support the assertion in a previous study (Meena *et al.*, 2007) that healthy neonates relate to healthy mothers and lack of care of neonate animal may lead to higher age of first parturition. Contaminated neonates may contaminate their dams; this describes the relationship in mortality of non-lactating female animals and lactating animals observed in this study. According to Chaudhary *et al.* (2013), young animals had low immunity and were not provided good feeding and management. Chaudhary *et al.* (2013) also observed high mortality in male (20.9%) than in female animals (4.8%) and suggested that females may be well cared for, due to their economic importance, than are males. Contrary to the finding of Chaudhary *et al.*, (2013), in this study mortality was observed more often in females (58%) than in male animals (24.5%). The incidence of reproductive diseases appears to be more in female animals than in males, purely due to hormonal imbalance leading to repeat breeding, which is capable of triggering reproductive diseases due to genital infections and improper hygiene according to (Chaudhary *et al.*, 2013). This statement most likely explains the finding in the present study. It is also possible that the farmers in the present study maintained or managed male animals very well for their economic benefits than for reproductive benefits, since for instance, in the northeast Nigeria bulls are highly utilized for harrowing and crop cultivation. This probably also explains the difference in mortality of male and female animals observed in the present study.

Conclusion

This study is to the best knowledge of the researcher the first to describe livestock management practices in northeast Nigeria. In conclusion, the findings in this study

revealed that farmers in north-eastern Nigeria managed male animals very well than female animals, possibly for their economic benefits. The practice of housing more than one type of animal species in the same housing unit observed in this study may influence the use of antimicrobials, as well as promoting cross-transfer of diseases between different species.

Conflict of interest

None to declare

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A GROSS AND LIGHT MICROSCOPIC STUDY OF THREE NEOPLASMS IN FARMED *Clarias gariepinus* AND ITS HYBRID

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Abstract

There are only a few reports of neoplasms and associated pathologies in teleosts. Three different tumours observed in three different fishes were examined grossly and histologically. In the first *C. gariepinus*, a large irregular, bristly, pinkish mass was observed to be protruding from the oral cavity. Histopathology of the mass revealed numerous denticles and an abundant fibrovascular stroma. Histopathologically, the tumour was diagnosed as a compound odontoma. In the second *C. gariepinus*, numerous pale yellow to whitish nodules were observed in the liver. Histopathology of the tumours revealed numerous atypical hepatocytes growing in thick trabeculae within nodules compressing adjacent normal hepatocytes. Individual hepatocytes were pleomorphic, large and vacuolated. The cytoplasmic vacuoles frequently contained globular hyaline bodies. These findings prompted a diagnosis of hepatocellular carcinoma. The ovaries of the female hybrid-*Clarias gariepinus* x *Heterobranchus Longifilis*, were observed to be severely distorted by numerous whitish, irregular, nodules. Histopathologically, the tumour revealed sheets and nests of oval to round cells split into lobules by a fibrous stroma. Each cell possessed scanty to moderately abundant eosinophilic to amphophilic cytoplasm, hyperchromatic round to oval central nucleus with coarse chromatin and multiple nucleoli. Mitotic figures were frequently observed. The tumour was presumptively diagnosed as a dysgerminoma. The aetiologies of these tumours were not determined.

Key words: neoplasm, fish, histopathology, dysgerminoma, odontoma, carcinoma

UNE ETUDE PAR EXAMEN MACROSCOPIQUE ET OPTIQUE DE TROIS NEOPLASMES CHEZ LE CLARIAS GARIEPINUS D'ELEVAGE ET SON HYBRIDE

Résumé

Les rapports sur les néoplasmes et les pathologies associées chez les téléostéens sont rares. Trois tumeurs différentes observées chez trois poissons différents ont été soumises à un examen macroscopique et histologique. Chez le premier *C. gariepinus*, une grande masse irrégulière, rosâtre et rosâtre a été observée en saillie dans la cavité buccale. L'histopathologie de la masse a révélé de nombreux denticules et un stroma fibrovasculaire abondant. Sur le plan histopathologique, la tumeur a été diagnostiquée comme étant un odontome composé. Chez le second *C. gariepinus*, de nombreux nodules jaune pâle à blanchâtre ont été observés dans le foie. L'histopathologie des tumeurs a révélé de nombreux hépatocytes atypiques se développant dans des travées épaisses à l'intérieur des nodules comprimant des hépatocytes normaux adjacents. Les hépatocytes individuels étaient pléomorphes, grands et vacuolisés. Les vacuoles cytoplasmiques contenaient fréquemment des corps hyalins globulaires. Ces résultats ont conduit à un diagnostic de carcinome hépatocellulaire. Les ovaires de l'hybride femelle-*Clarias gariepinus* x *Heterobranchus Longifilis* étaient fortement déformés par de nombreux nodules blanchâtres irréguliers. Sur le plan histopathologique, la tumeur a révélé des feuilles et des nids de cellules ovales à rondes divisées en lobules par un stroma fibreux. Chaque cellule possédait un cytoplasme éosinophile à amphophile maigre à modérément abondant, un noyau central hyperchromatique rond à oval avec une chromatine grossière et de multiples nucléoles. Des figures mitotiques ont été fréquemment observées. La tumeur a été diagnostiquée par présomption comme étant un dysgerminome. Les étiologies de ces tumeurs n'ont pas été déterminées.

Mots-clés : néoplasme, poisson, histopathologie, dysgerminome, odontome, carcinome

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Introduction

The occurrence and pathology of neoplastic and non-neoplastic tumour-like growths in teleosts have been widely studied by various researchers worldwide. Some of the neoplasms previously reported in teleosts include Walleye dermal sarcoma, leiomyoma and fibroma/fibrosarcoma (Walker, 1969; Herman and Landolt, 1975; Anders et al. 1991)

Although the aetiology of most neoplasms in fish is multifactorial and not fully elucidated (Roberts, 2012), Anders and Yoshimizu (1994) were able to provide strong evidence linking viruses with the induction of skin neoplasms in teleosts. Other factors that have been associated with or suspected to have a role in the induction of neoplasms in teleosts include hormones, toxins, physical agents, as well as the age, sex, genetic predisposition and immunological status of the host (Roberts, 2012)

Clariid catfish species that comprise of *Clarias gariepinus*, *Heterobranchus longifilis*, *H. bidorsalis*, and the reciprocal hybrids of these two genera are the predominantly cultured fish in Nigeria (Oladosu et al. 1993). Due to the paucity of information on the occurrence and pathology of tumours in pond cultured African catfish and its hybrids, it is pertinent that studies be carried out in observed tumour cases. Information gleaned from such studies would be crucial in efforts aimed at formulating preventive and chemoprophylactic measures in combating this menace.

This study outlines the gross and light microscopic findings of three different tumours in two adult *C. gariepinus* males and an adult female hybrid (*C. gariepinus* female x *H. longifilis* male).

Materials and Methods

Two adult *C. gariepinus* males and a female hybrid catfish (*C. gariepinus* female x *H. longifilis* male) weighing 800g, 2.2kg and 3 kg respectively were observed either during feeding or sequel to being sacrificed for dry stripping or smoke drying to have abnormal

growths in various organs and tissues. The fishes were raised in a commercial catfish farm in Ibadan, Nigeria. One of the male *C. gariepinus* with an abnormal growth protruding from its mouth was submitted to the Department of Veterinary Pathology, University of Ibadan for necropsy. It was sacrificed by cervical transection followed by pithing prior to necropsy (Noga, 2010; Underwood et al. 2013). Only the affected organs from the two remaining catfishes were submitted for gross and histopathological examination. Following necropsy, all the abnormal tissues were excised and fixed in 10% neutral buffered formalin for routine processing for histology as described by Luna (1968).

Results

Gross and histopathology

An adult male *C. gariepinus* (Fish 1) had a pink, irregular, mass measuring 1x3x7cm protruding from its oral cavity. The mass was sessile, firmly attached to the lower band of teeth and lower lip, pink and studded with numerous tiny, sharp bristles. The cut surface of the tumour was firm, pink and bristly. The liver was diffusely pale. No other lesions found in other organs.

Histopathology revealed an irregular mass composed of numerous nests of abnormally shaped tooth-like structures (denticles) at various stages of development. Some of the denticles had a central pulp-like zone made up of mesenchymal elements and an odontogenic epithelium. Other structures of the well-formed denticles included a homogenous, pink acellular dentine either bounded by a thin rim of partially mineralized enamel or an empty space and an outer rim of palisading columnar cells (ameloblasts). In some areas the denticles consisted of abnormally shaped, acellular eosinophilic structures (dentine) partially enveloping pulp-like mesenchyme. Several structures resembling developing tooth buds were also scattered within the abundant loose fibrovascular stroma of the tumour. The oral tumour was thus diagnosed as a compound odontoma.

Grossly, the liver from another male *Clarias gariepinus* (Fish 2) had numerous pale yellow to whitish, firm, irregular nodules on the parietal and visceral surfaces of the liver. The sizes of the nodules ranged from 0.1cm to 1.8cm in diameter. Histologically the liver tumour was composed of clear cells organized in multiple nodules compressing adjacent hepatocytes. Within the nodules, the cells were arranged in thick trabecular pattern with sinusoids appearing slit-like or often completely obliterated by proliferating neoplastic cells. Individual neoplastic cells were large, round to polygonal, possessed abundant clear cytoplasm and a centrally located nucleus with a single large central nucleolus. In some

areas, the cytoplasm of the neoplastic cells often contained either light brown granules (Lipofucsin) or globular hyaline bodies. In a few areas, there were large, irregular channels in between trabeculae containing abundant homogenous eosinophilic material. There was also a marked increase in the number and sizes of melanomacrophage centres in the liver parenchyma. Taking the gross and histological findings into consideration, the liver tumour was diagnosed as a hepatocellular carcinoma (HCC).

Grossly, the ovaries of a hybrid catfish (cross of a female *C. gariepinus* and a male *H. longifilis*), (Fish 3) had severely distorted architecture. The ovaries were composed of

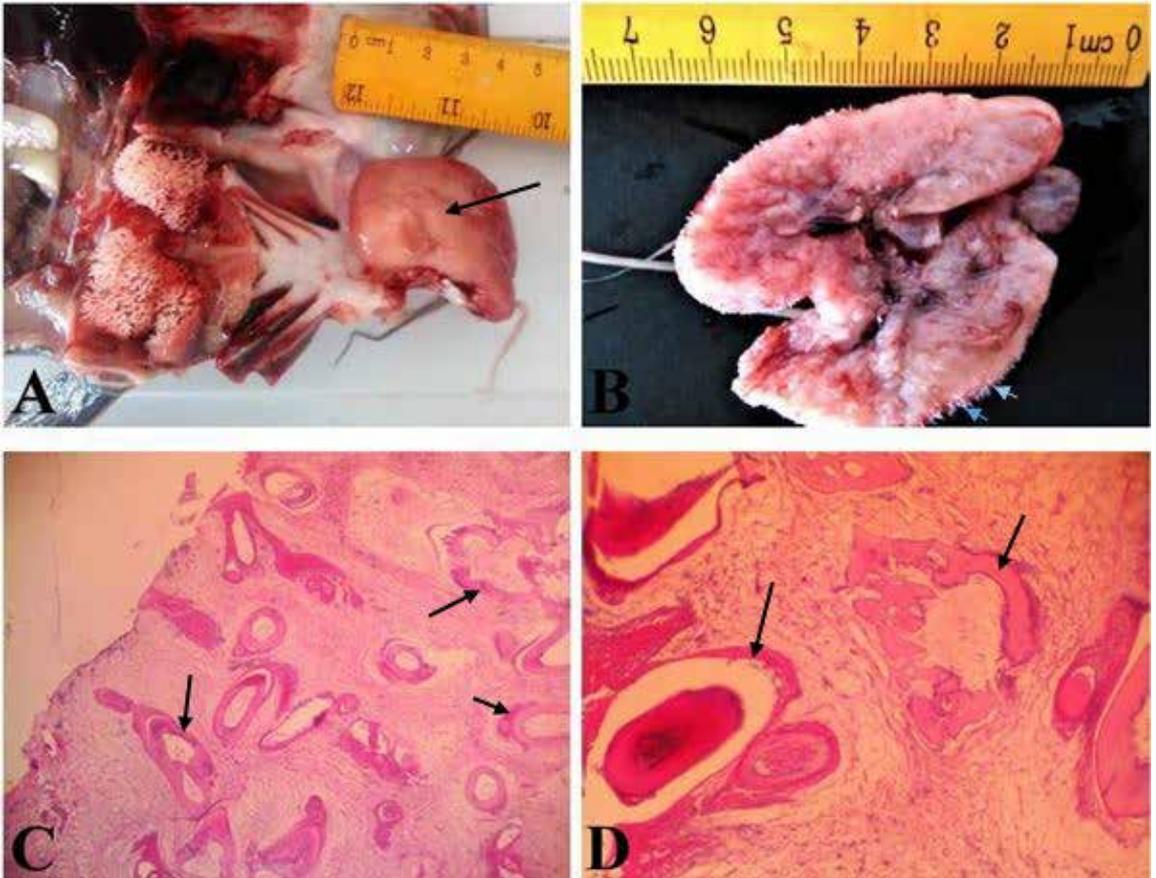


PLATE I: Oral odontoma A. Oral cavity, Adult *Clarias gariepinus*. Note the pink mass (arrow) arising from the lower band of teeth. B. Cut surface of mass excised from the oral cavity of the adult *C. gariepinus*. The cut surface is pale pink. Note the bristly surface of the mass (arrows). C. Photomicrograph of dental mass showing numerous irregular toothlike structures (arrows) in an abundant loose fibrovascular stroma. H&E, x40. D. Higher magnification of the dental mass showing irregular toothlike structures (arrows) H&E, x400

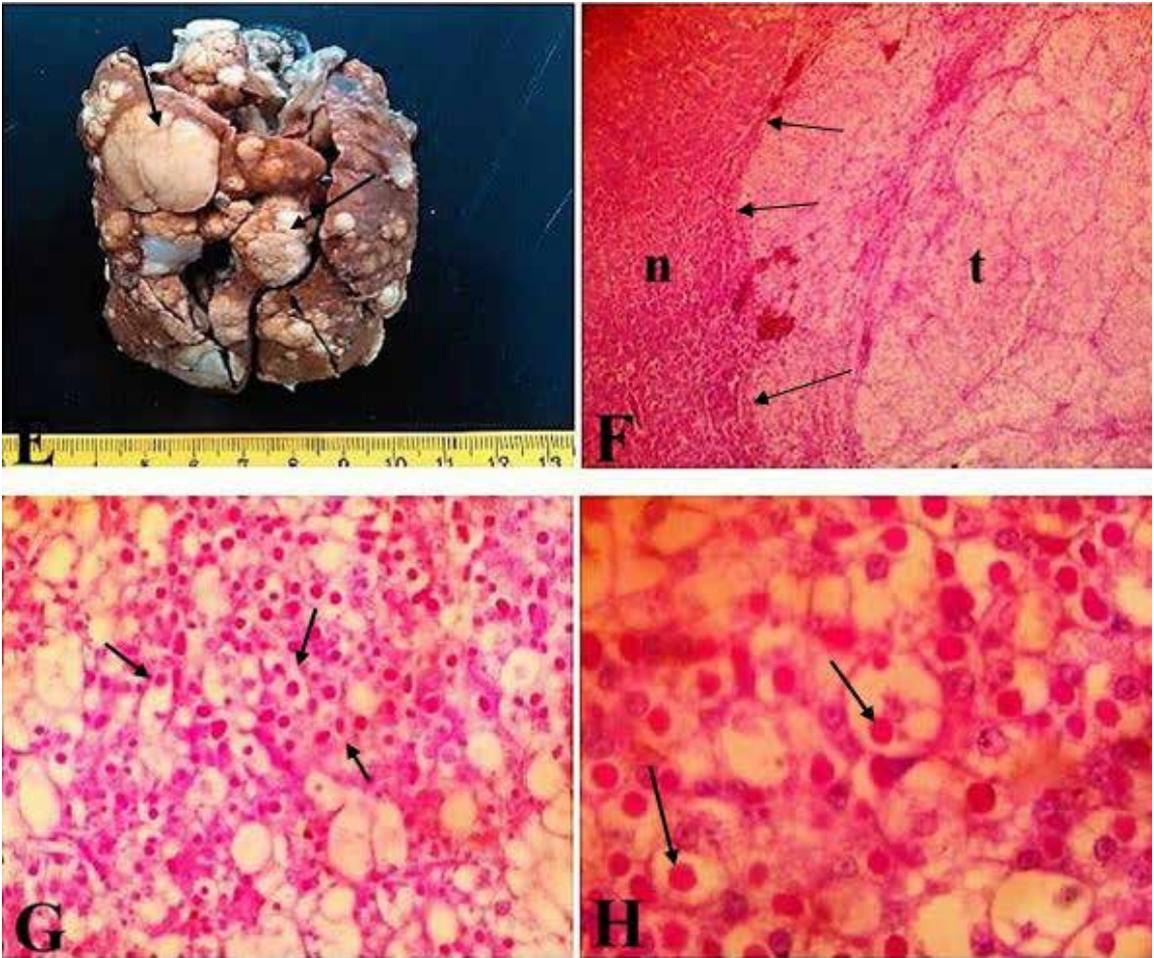


PLATE 2: Hepatocellular carcinoma. E. Formalin fixed liver of adult *C. gariepinus* showing numerous variably sized pale yellow to whitish nodules (arrows). F. Photomicrograph of liver tumour showing the pale staining, neoplastic hepatocytes (t) compressing (arrows) adjacent normal hepatocytes (n). H&E, x40. G. Photomicrograph of liver tumour showing numerous swollen, vacuolated hepatocytes with cytoplasmic globular hyaline bodies (arrows). H&E, x400. H. Photomicrograph of liver tumour showing numerous swollen, vacuolated hepatocytes with cytoplasmic globular hyaline bodies (arrows). H&E, x1000

numerous, variably sized, irregular, creamy white, firm nodules and very few apparently normal follicles. The ovaries had a botryoid appearance. The size of individual nodules ranged from 0.5x0.4x0.2cm to 7x6x5cm. Histologically, the tumour consisted of sheets of small to large pleomorphic cells often split into lobules by a fibrous stroma. The individual cell shape ranged from oval to round and polygonal. Individual cells possessed scanty to moderately abundant eosinophilic to amphophilic cytoplasm, vesicular and hyperchromatic round to oval central nucleus with coarse chromatin. Each nucleus

often possessed multiple nucleoli. There were a few small lymphocyte aggregates scattered within the tumour. Numerous mitotic figures were also observed.

Discussion

The oral tumour in fish I was diagnosed as a compound odontoma due to the findings of denticles that had well-formed but abnormally shaped components of odontogenesis. Compound odontomas are made up of well-formed components of teeth

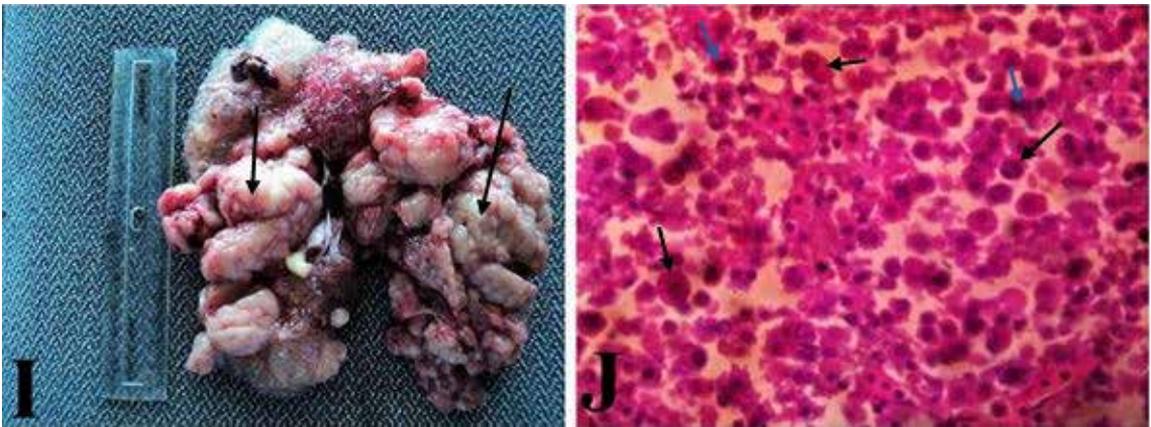


PLATE 3: Ovarian tumour. I. Ovaries from adult *C. gariepinus* x *H. longifilis* hybrid showing severe architectural distortion by numerous variably sized, irregular whitish nodules (arrows). J. Photomicrograph of ovarian tumour showing neoplastic cells occurring singly and in small nests. Individual cells are variably sized and round to oval with scanty to abundant eosinophilic cytoplasm and hyperchromatic nucleus (Black arrows). There are also a few mitotic figures (blue arrows) H&E, x1000

such as enamel, dentine and pulp configured to form abnormally shaped tooth-like structures. (de Oliveira et al. 2001; Nelson and Thompson, 2010; Meuten, 2016). Some authors have suggested that odontomas are hamartomas rather than true neoplasms (Nelson and Thompson, 2010; Gupta et al. 2014). The occurrence and pathology of odontoma in man has been reported by several authors including Nelson and Thompson (2010) and Gupta et al. (2014). However, there are only a few reports of this neoplasm in teleosts. Coffee et al. (2013) reported the presence of a pharyngeal odontoma in an adult Walleye (*Sander vitreus*). Similarly, Vijayakumar et al. (2015) reported the incidence of odontoma in the oral cavity of the marine fish *Sphyrna jello*. There is no report of the occurrence of this neoplasm in *Clarias gariepinus* and its hybrids. The aetiology of odontoma is unknown. However, some authors have associated the occurrence of this neoplasm with trauma and subsequent infection of damaged tissues (de Oliveira et al. 2001). In the same vein, Vijayakumar et al. (2015) was able to isolate four bacteria from an odontoma in the marine fish *Sphyrna jello*. The bacteria isolated included *Bacillus* sp., *Pontibacter* sp., *Burkholderia* sp. and *Macrocooccus* sp. However it was not proven that these pathogens were the causative agents. The fish presented in

this case had no evidence of trauma; neither did it show histological evidence of infection with pathogens that may be associated with oncogenesis.

The aetiology of the hepatocellular carcinoma in fish 2 was not identified. Several researchers have associated environmental exposure to polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) with the induction of hepatic tumours in teleosts (Hawkins et al. 1988; Dawe, 1990; Black and Baumann, 1991; Tuvikene, 1995). Furthermore the role of exposure to aflatoxin B1 contaminated feed in the induction of hepatocellular carcinoma in the rainbow trout has been reported (Sinnhuber et al. 1977; Wales et al. 1978). The fish in this study had no known exposure to aflatoxin and other environmental carcinogens. Further investigation in this regard was not carried out. The molecular basis of HCC in teleosts has not been fully elucidated. Mutations in the teleost *ras* gene has been found to play a role in tumourigenesis in some fish species environmentally exposed to high levels of hydrocarbons (WVirgin et al. 1989; McMahon et al. 1990; Rotchell et al. 2001). In the same vein, a study aimed at determining the role of *ras* oncogene in initiating tumour formation in hepatocellular carcinomas in transgenic zebrafish was carried out by Nguyen

et al. (2011). In that study, they reported that only a high level of krasV12 expression initiated liver tumorigenesis which progressed to the formation of hepatocellular carcinoma.

The occurrence and pathology of dysgerminoma in man and other mammals is well documented (Jackson et al. 1985; Chandra et al. 1998; Gimelli et al. 2009). However, there are only a few reports of this neoplasm in teleosts (Masahito et al. 1984; Harada et al. 1991; Jafarey et al. 2015). The ovarian tumour in fish 3 was presumptively diagnosed as dysgerminoma based on the gross appearance and histopathological features. An earlier study conducted on the reproductive capacity of *C. gariepinus*, *H. longifilis* and their reciprocal hybrids by Legendre et al. (1992) revealed numerous abnormalities in gonadal development of the hybrids, with intra-ovarian tumours observed in 20% of the hybrids examined. This is a pointer to possible genetic basis for the occurrence of neoplasm in the gonads of the hybrid catfish.

The fish and fish organ samples examined in this study were sourced from a farm in Ibadan, Nigeria over a period of four months. The frequency of occurrence of these tumours in fish cultured in this farm is worthy of note. The aetiology of these tumours were not determined. This study brings to light the occurrence and pathology of these rare neoplasms in cultured *Clarias gariepinus* and its hybrid.

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EFFECT OF PROBIOTIC MIXTURE ON SOME HAEMATOLOGICAL PARAMETRES IN *ESCHERICHIA COLI* O157:H7 EXPERIMENTALLY INFECTED YANKASSA LAMBS.

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Abstract

This study was designed to determine the effect of probiotics mixture on some haematological parameters in lambs experimentally infected with Shiga-toxin *Escherichia coli* (STEC) O157:H7. Fifteen Yankassa breed of lambs aged between 3-4 weeks old were used. The lambs were divided into three groups of five lambs each (n=5). Group A: Neither probiotics nor STEC O157:H7 were administered as they served as the control, Group B: lambs were administered viable STEC O157:H7 cells at 6X10⁸CFU/ml in 1ml normal saline together with daily administration of a mixture of probiotics (*Lactobacillus acidophilus*, *Bacillus pumilus*, *Bacillus subtilis* and *Bacillus licheniformis*) at 4.5 x10¹⁰ CFU/ml, Group C: lambs were administered only viable STEC O157:H7 cells at 6x10⁸ CFU/ml without probiotics. Both the mixtures of probiotics and STEC O157:H7 cells were mixed in 1ml of normal saline and administered orally to the lambs. Following oral inoculation of the lambs with STEC cells, blood samples were collected once weekly for six weeks: blood samples were collected for the determination of total white blood cell count (TWBCC) and differential counts. The mean TWBCC, neutrophil and lymphocyte counts of groups B and C lambs rose significantly (P<0.05) in the first week post infection and gradually decreased during the period of study. However, the values of the mean TWBCC, neutrophil and lymphocyte counts of Group C lambs were significantly higher (P<0.05) than that of groups A and B lambs over the six weeks period. There was no significant difference in the mean absolute eosinophil and monocyte counts among the three experimental groups. In conclusion, lower values of mean TWBCC, neutrophil and lymphocytes was observed in the lambs administered probiotics mixture as compared to the lambs that did not receive probiotics mixture. This was possibly due to the lower level of infection in those groups of lambs as a result of the probiotics administered. It was therefore recommended that probiotics should be administered to lambs to help control STEC O157:H7.

Keywords: Shiga-toxin producing *Escherichia coli*, Total white blood cells, *Lymphocytes*, Neutrophils, Probiotics, Yankassa lambs.

EFFET DU MÉLANGE PROBIOTIQUE SUR CERTAINS PARAMÈTRES HÉMATOLOGIQUES CHEZ DES AGNEAUX YANKASSA EXPÉRIMENTALEMENT INFECTÉS AVEC *ESCHERICHIA COLI* O157: H7

Resume

Cette étude a été conçue dans le but de déterminer l'effet d'un mélange de probiotiques sur certains paramètres hématologiques chez des agneaux infectés expérimentalement avec *Escherichia coli* (STEC) O157: H7. Quinze agneaux de race Yankassa âgées de 3-4 semaines ont été utilisés. Les agneaux ont été répartis en trois groupes de cinq agneaux chacun (n = 5). Pour le Groupe A, on n'a administré ni probiotiques ni STEC O157: H7 car ils ont servi de témoins. Concernant le Groupe B, on a administré aux agneaux des cellules viables STEC O157: H7 à raison de 6X10⁸CFU/ml dans 1 ml de solution saline normale avec administration quotidienne d'un mélange de probiotiques (*Lactobacillus acidophilus*, *Bacillus pumilus*, *Bacillus subtilis* et *Bacillus licheniformis*) à 4,5 x 10¹⁰ CFU / ml. Quant au Groupe C, les agneaux ont seulement

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reçu des cellules viables STEC O157:H7 à raison de 6×10^8 UFC / ml sans probiotiques. Les mélanges de probiotiques et de cellules STEC O157:H7 ont été ajoutés à 1 ml de solution saline normale et administrés par voie orale aux agneaux. Après l'inoculation orale des agneaux avec des cellules STEC, des échantillons de sang ont été prélevés une fois par semaine pendant six semaines, dans le but de déterminer le nombre total de globules blancs (TWBCC) et les numérations différentielles. Les nombres moyens de TWBCC, de neutrophiles et de *lymphocytes* des agneaux des groupes B et C ont augmenté significativement ($P < 0,05$) pendant la première semaine après l'infection et ont diminué progressivement tout au long de la période d'étude. Cependant, les valeurs moyennes des nombres de TWBCC, de neutrophiles et de *lymphocytes* des agneaux du groupe C étaient significativement plus élevées ($P < 0,05$) que celles des agneaux des groupes A et B au cours de la période de six semaines. On n'a pas noté de différence significative au niveau du nombre moyen absolu d'éosinophiles et de monocytes entre les trois groupes expérimentaux. En conclusion, des valeurs moyennes faibles de TWBCC, de neutrophiles et de *lymphocytes* ont été observées chez les agneaux recevant des probiotiques par rapport aux agneaux n'ayant pas reçu de mélange de probiotiques. Ceci était peut-être attribuable au faible niveau d'infection de ces groupes d'agneaux résultant de l'administration de probiotiques. Il a donc été recommandé d'administrer des probiotiques aux agneaux pour aider dans le contrôle de la STEC O157:H7.

Mots-clés : *Escherichia coli* produisant des toxines Shiga, numération totale des cellules blanches, *lymphocytes*, neutrophiles, probiotiques, agneaux Yankassa

Introduction

In most farms, antibiotics are added in feed in sub-therapeutic levels as a way of combating pathogens and improving production (FAO, 2009). This has led to development of antibiotic resistance organisms at levels which could pose a serious problem in the near future (Williamson, 2002). Hence, there is a growing need to control this pathogenic bacterium in ruminants so as to improve production, through the use of safer alternative technology such as probiotics. Probiotics are live organisms with the capacity to benefit the gastrointestinal tract microflora by promoting health or preventing diseases in the host (Borriello *et al.*, 2003; Rook and Brunet, 2005; Papadimitriou *et al.*, 2007). Probiotics benefit the host by improving microbial balance which includes the elimination or reduction of pathogenic microorganisms that are carried by the host and are harmful to humans (Zhao *et al.*, 1998). Several reports exist on the efficacy of probiotics in the control of Shiga-toxin producing *Escherichia coli* (STEC) O157:H7 (Iema *et al.* 2001; Avila *et al.* 2001; Everlon *et al.*, 2013). Shiga-like toxin-producing *Escherichia coli* (STEC), is a food borne pathogen primarily transmitted to humans through the consumption of contaminated water or food (Caprioli *et al.*, 2005). Ruminants such as cattle, sheep and goats are regarded as the main animal

reservoirs of STEC (La Ragione, 2009). Adult ruminants are usually asymptomatic carriers of these pathogens. However, this pathogen has been proven to cause diarrhoea in calves and lambs which can sometimes be complicated by other opportunistic organisms thereby affecting the survivability of these animals (Cray and Moon 1995; Dean-Nystrom *et al.* 1997). White blood cells such as *Lymphocytes* and Neutrophils, plays an important role in the control of bacterial infection in the body. The analysis of blood indices has proven to be a valuable approach for analyzing the health status of farmed animals (Bahmani *et al.* 2001). So, this study was designed to evaluate the effect of probiotics mixture on the total white blood cell and differential counts of lambs experimentally infected with STEC O157:H7.

Materials and Methods

This study was carried out at the Livestock Investigation Division of the National Veterinary

Research Institute, Vom, Plateau State, Nigeria. The State is located in the North Central Geopolitical zone of Nigeria and has a land area of 26,899 square kilometres (NPC, 2006).

Experimental Animals

Fifteen (15) Yankassa breed of lambs, aged between 1 to 2 weeks old were used for this research. The lambs were sourced from the Small Ruminant Section of the Livestock Investigation Division of the National Veterinary Research Institute, Vom, Plateau State.

The lambs were tagged and allocated into pens. They were confined with their dams to enable them suckle. The dams were fed on concentrate and hay. Water was provided *ad libitum*

Escherichia coli Strain Used in the Study

Shiga-toxin producing (STEC) *Escherichia coli* O157:H7 strain, which is under the Enterohaemorrhagic Serogroup of *E. coli* was used for this study and was sourced from the National Veterinary Research Institute, Vom, Plateau state, Nigeria.

Preparation

Cultures for use as inocula were produced in 10 ml of 0.9% saline solution after an overnight incubation on Sorbitol MacConkey agar supplemented with Cefixime Tellurite (Oxoid, UK) at 37°C for 24 hours. Cell numbers were determined spectrophotometrically using the McFarland standard and were adjusted to contain 6×10^8 CFU/ml.

Probiotics Used in the Study

Sky-flo® Probiotics containing *Lactobacillus acidophilus* and Sanolife® PRO-F Probiotics containing a balanced mixture of *Bacillus pumilus*, *Bacillus licheniformis* and *Bacillus subtilis* were used for this research. It was manufactured by Inve Aquaculture, Belgium.

Preparation of the daily doses of probiotics mixture

To prepare the daily doses of the Probiotic strain used, individual tube containing the lyophilized bacteria, were inoculated into 9 ml of *Lactobacillus* selective broth de Mann, Ragosa and Sharpe (MRS) for selective enrichment and incubated at 37°C for 24 hours. After the period of incubation a loop full of the positive broth was then streaked on MRS agar plates and incubated at 37°C for

24 hours (Plate II). Colonies that grew were re-suspended in 10 ml of saline to generate a suspension containing *Lactobacillus acidophilus* 4.5×10^8 CFU/ml, *Bacillus subtilis* 4.5×10^8 CFU/ml, *Bacillus licheniformis* and *Bacillus pumilus* 4.5×10^8 CFU/ml using the McFarland standard.

Experimental Design

Animal groupings

Lambs were divided into three (3) groups (A, B, and C) of five (5) lambs each and confined in separate pens. The animals were all ear tagged for the purpose of identification.

Pre- Experimental management of animals

The lambs were allowed to acclimatize to the environment for two weeks prior to the commencement of this study, during which the dams were de-wormed with albendazole (10 mg/kg) per os and administered ivermectin (0.2 mg/kg) sub-cutaneously to control ectoparasites. The lambs were weaned before the commencement of the study at four weeks of age. The lambs were fed concentrate and water during the study period.

Ethics

Ethical clearance was obtained from the Ahmadu Bello University Zaria committee on Animal Use and Care, with approval number ABUCAUC/2016/Vet.Medicine/003.

Pre-infection data.

Two millilitres (2ml) of blood sample was also collected from the jugular vein of each lambs into EDTA sample bottles, using sterile needle and syringe for haematological analysis pre-infection. The values obtained served as the base line data before the feeding trial and challenge.

Animal groupings and treatment regimen

At the end of the two weeks acclimatization period and after the screening of all the lambs for the presence of *E. coli* O157:H7 as described by Chapman et al (1994). They were subsequently, subjected to different treatment regimens as follows.

Group A: No Probiotic was administered neither *Escherichia coli* (STEC O157:H7) as they served as control.

Group B: Lambs were administered viable STEC O157:H7 (6×10^8 CFU/ml) together with a mixture of probiotics (*L. acidophilus*, *B. pumilus*, *B. subtilis* and *B. licheniformis*) at 4.5×10^8 CFU/ml given daily throughout the research period. Both were administered in a 1ml normal saline, orally through the use of sterile 5ml syringe directly into the mouth.

Group C: Lambs were administered 1ml inoculum containing viable STEC O157:H7 (6×10^8 CFU/ml) without probiotics, through the use of sterile 5ml syringe directly into the mouth.

Blood Sample Collection and Evaluation of Haemogramme Post-Infection

Blood samples were collected from each lamb once weekly for six weeks post-infection. Two millilitres (2 ml) of blood was aseptically collected from the jugular vein of each lamb using a sterile 21 G needle mounted on 5ml syringe and transferred into sample bottles containing EDTA as an anticoagulant. The samples were then appropriately labelled and taken to the laboratory for the evaluation of haematological parameters which included: Total White Blood Cell (WBC) counts and differential counts. These parameters were determined using the automatic blood analyzer (Improved Neubauer haemocytometer).

Data Analysis

The data obtained were expressed as mean \pm SEM (Standard Error of Mean) and presented in tables and graphs. One way ANOVA with Tukey's post hoc test using SPSS version 20 for windows was used to determine significant difference in the haematological parameters among the groups. Values of $p < 0.05$ were considered significant at 95% confidence interval.

Results

Pre-infection Baseline Data

The data collected were assessed to be clinically normal. The mean rectal temperature, pulse rate and respiratory rate were 37.6 $^{\circ}$ C, 80 beats / minute and 20 cycles / minute respectively all within normal range. All the lambs in the three experimental groups were confirmed to be negative for *E. coli* O157:H7 (STEC) following faecal laboratory examination.

Haematological parameters pre-infection

The mean Total White Blood Cell counts (TWBC) and Differential Leucocyte count of the three experimental groups pre-infection are presented in Table 1.

Total white blood cell counts post- infection

The mean Total White Blood Cell (TWBC) counts for each group for the six weeks post infection is shown in Figure 1

There was a significant increase ($P < 0.05$) in the mean TWBC of lambs in

Table 1: Pre-infection Mean \pm SEM of haematological values for the three experimental groups of lambs.

Parametres	Group C	Group B	Group A
Total Leucocyte count (10 ⁹ /L)	8.98 \pm 2.12	9.75 \pm 1.88	8.14 \pm 0.91
Neutrophils (10 ⁹ /L)	4.06 \pm 1.08	4.24 \pm 1.00	3.66 \pm 0.16
Lymphocytes (10 ⁹ /L)	4.80 \pm 1.11	5.29 \pm 0.83	4.40 \pm 0.43
Monocytes (10 ⁹ /L)	0.03 \pm 0.00	0.02 \pm 0.00	0.02 \pm 0.00
Eosinophils (10 ⁹ /L)	0.09 \pm 0.01	0.21 \pm 0.03	0.06 \pm 0.00

Key:

Group A: Control

Group B: Lambs inoculated with STEC cells together with probiotics administered daily.

Group C: lambs inoculated with STEC cells only without probiotics administration.

SEM: Standard Error of Mean

Group B from $9.75 \pm 1.88 \times 10^9/L$ pre-infection to $14.88 \pm 4.46 \times 10^9/L$ in the first week post infection and subsequently decreased significantly ($P > 0.05$) to $12.45 \pm 3.99 \times 10^9/L$ in the second week, $8.59 \pm 0.66 \times 10^9/L$ in the third week and increased slightly to $9.03 \pm 0.45 \times 10^9/L$ in the fourth week and decreased further to $8.49 \pm 0.36 \times 10^9/L$ in the fifth week and $8.40 \pm 0.30 \times 10^9/L$ in the sixth week (Figure 1). Group C lambs showed a significant increase ($P < 0.05$) in the mean TWBC from $8.98 \pm 2.12 \times 10^9/L$ pre infection to $40.14 \pm 3.91 \times 10^9/L$ in the first week post infection, and subsequently decreased to $14.15 \pm 2.80 \times 10^9/L$ during the second week, $13.41 \pm 2.43 \times 10^9/L$ in the third week, $10.54 \pm 0.37 \times 10^9/L$ in the fourth week, $9.74 \pm 0.24 \times 10^9/L$ in the fifth week and $9.68 \pm 0.20 \times 10^9/L$ in the sixth week (Figure 1). Lambs in Group A (Control) showed no significant difference ($P > 0.05$) in the mean Total White Blood cell count throughout the six weeks post infection (Figure 1).

Neutrophils counts

Figure 2 shows the Mean Neutrophil values of all the lambs in the three experimental groups for six weeks post infection.

There was no significant difference ($P > 0.05$) in the values of the mean Neutrophil counts of lambs in group A over the six week period of the study. However, there was a significant increase ($P < 0.05$) in the mean Neutrophils count of lambs in Group B from $4.24 \pm 1.00 \times 10^9/L$ pre-infection to $5.59 \pm 1.85 \times 10^9/L$ in the first week post infection and increased further to $7.21 \pm 2.31 \times 10^9/L$ in the second week and subsequently decreased to $4.96 \pm 0.40 \times 10^9/L$ in the third week, $4.76 \pm 0.20 \times 10^9/L$ in the fourth week, $4.42 \pm 0.18 \times 10^9/L$ in the fifth week and $4.24 \pm 0.16 \times 10^9/L$ in the sixth week (Figure 2). Group C lambs showed a significant increase ($P < 0.05$) in mean Neutrophil counts from $4.06 \pm 1.08 \times 10^9/L$ pre- infection to $15.09 \pm 1.49 \times 10^9/L$ in the first week post infection and then decreased to $9.13 \pm 1.65 \times 10^9/L$ in the second week, $7.43 \pm 1.03 \times 10^9/L$ in the third week, $5.89 \pm 0.33 \times 10^9/L$ in the fourth week, $5.46 \pm 0.27 \times 10^9/L$ in the fifth week and $5.40 \pm$

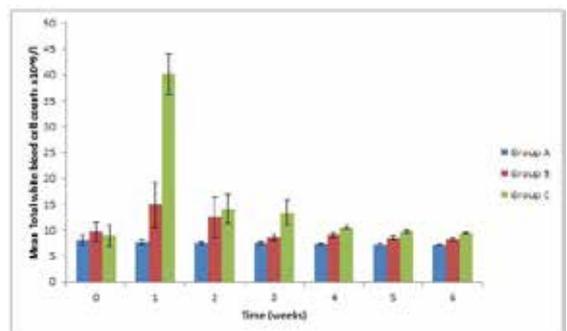
$0.23 \times 10^9/L$ in the sixth week (Figure 2).

There was statistically significant difference ($P < 0.05$) in the mean Neutrophil values between the three groups in the first five weeks post infection (Fig 2).

Lymphocytes

Figure 3 shows the Mean Lymphocyte values for all the lambs in the three experimental groups for the six weeks post infection.

Lambs in Group B showed a significant increase ($P < 0.05$) in the absolute Lymphocyte counts from $5.29 \pm 0.83 \times 10^9/L$ pre-infection to $9.07 \pm 2.57 \times 10^9/L$ in the first week post infection and then decreased ($P > 0.05$) to $5.06 \pm 1.64 \times 10^9/L$ in the second week, $3.55 \pm 0.32 \times 10^9/L$ in the third week and increased slightly to $4.18 \pm 0.38 \times 10^9/L$ the fourth week and decreased further to $3.84 \pm 0.21 \times 10^9/L$ in the fifth week and $3.82 \pm 0.20 \times 10^9/L$ in the sixth week (Figure 3). Similarly, there was a significant increase ($P < 0.05$) in the mean Lymphocyte values for Group C lambs from $4.80 \pm 1.11 \times 10^9/L$ pre-infection to $23.89 \pm 2.51 \times 10^9/L$ in the first week post infection and subsequently decreased further to $5.97 \pm 1.03 \times 10^9/L$ in the second week, $5.06 \pm 0.98 \times 10^9/L$ in the third week, $4.53 \pm 0.32 \times 10^9/L$ in the fourth week, $4.16 \pm 0.24 \times 10^9/L$ in the fifth week and $4.12 \pm 0.20 \times 10^9/L$ in the sixth week respectively



Key:

Group A: Control

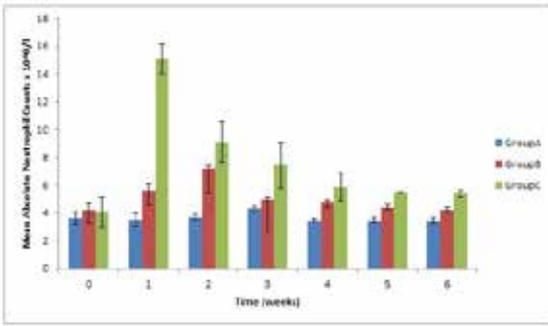
Group B: Lambs inoculated with STEC cells together with probiotics administered daily.

Group C: lambs inoculated with STEC cells only

-1 week represents pre-infection period

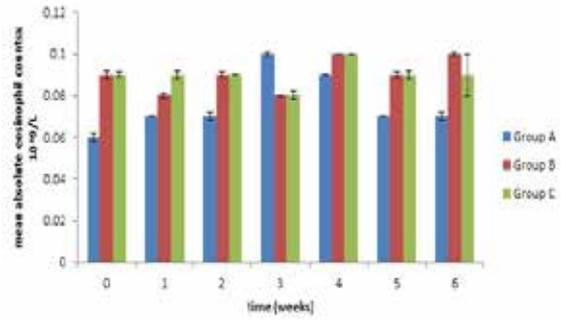
1-6th weeks represents post- infection period.

Figure 1 Mean Total White Blood Cell counts of the three experimental groups of lamb pre and post- infection.



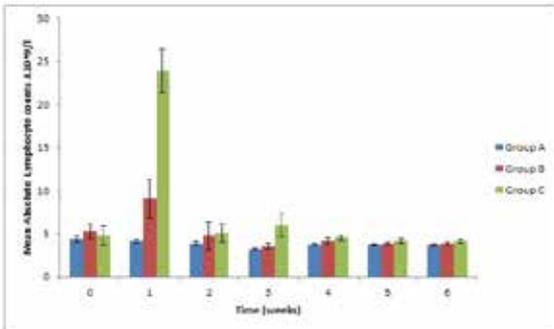
Key:
 Group A: Control
 Group B: Lambs inoculated with STEC cells together with probiotics administered daily.
 Group C: lambs inoculated with STEC cells only
 -1 week represents pre-infection period
 0-5th weeks represents post infection period.

Figure 2: Mean ± SEM Neutrophil counts of the three experimental groups of lambs pre and post -infection



Key:
 Group A: Control
 Group B: Lambs inoculated with STEC cells together with probiotics administered daily.
 Group C: lambs inoculated with STEC cells only
 0 week represents pre-infection period
 1-6th weeks represents post infection period

Figure 4: Mean ± SEM of Absolute Eosinophil Counts of the three experimental groups of lambs pre and post-infection



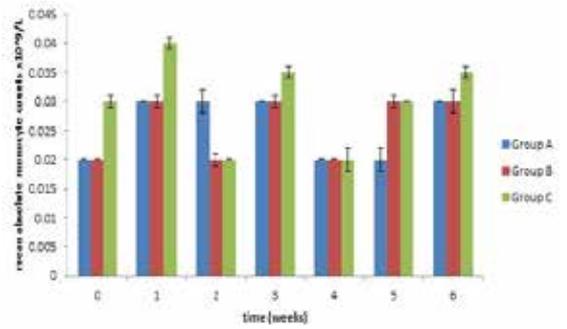
Key:
 Group A: Control
 Group B: Lambs inoculated with STEC cells together with probiotics administered daily.
 Group C: lambs inoculated with STEC cells only without administration of probiotics
 0 week represents pre-infection period
 1-6th weeks represents post infection period

Figure 3: Mean ± SEM absolute Lymphocyte counts of the three experimental groups of lambs pre and post-infection

(Fig. 3). However, lambs in Group A showed no significant difference ($P>0.05$) in the values of the mean Lymphocyte counts over the six weeks period of study (Figure 3).

Eosinophils

The mean absolute Eosinophil counts for the three experimental groups six weeks post infection is shown in Figure 4. The mean Eosinophil count for lambs in Group B was



Key:
 Group A: Control
 Group B: Lambs inoculated with STEC cells together with probiotics administered daily.
 Group C: lambs inoculated with STEC cells only
 0 week represents pre-infection period
 0-6th weeks represents post infection period

Figure 5: Mean ± SEM of Absolute Monocyte count of the three experimental groups of lambs pre and post-infection.

$0.08 \pm 0.02 \times 10^9/L$ pre-infection and rose slightly to $0.10 \pm 0.01 \times 10^9/L$ by the sixth week. While that for Group C lambs was $0.38 \pm 0.15 \times 10^9/L$ pre-infection and decreased to $0.09 \pm 0.01 \times 10^9/L$ in the sixth week. Lambs in Group A showed no significant difference throughout the six weeks of study. There was no significant difference ($P>0.05$) between the three groups over the six weeks post infection.

Monocytes

The mean absolute monocyte counts for the three experimental groups six weeks post infection is shown in Figure 5. The mean monocyte count for lambs in Group B was $0.15 \pm 0.02 \times 10^9/L$ pre-infection and decreased slightly to $0.10 \pm 0.01 \times 10^9/L$ by the sixth week. While that of Group C lambs was $0.58 \pm 0.02 \times 10^9/L$ pre-infection and decreased to $0.02 \pm 0.00 \times 10^9/L$ in the sixth week. Lambs in Group A showed no significant difference throughout the six weeks of the study. There was no significant difference ($P > 0.05$) between the three groups over the six weeks post infection (Fig.5).

Discussion

The increase in the mean Total White Blood Cell (TWBC) count observed first week post-infection in the lambs in Group B and C ($p < 0.05$), was principally due to neutrophilia and lymphocytosis. This could be an indicator of an immune response to bacterial infection (STEC O157:H7). The immune system mobilizes white blood cells such as neutrophils and *lymphocytes* in the early stage of bacteria invasion into the system to engulf and eliminate such pathogens (Blood *et al.*, 1983a). Similarly, the progressive decrease in the mean values of the TWBC, neutrophil and *lymphocytes* values, after the first week post-infection in group B and C lambs could, perhaps, be attributed to the gradual elimination of the pathogen from the body by the immune cells, thereby leading to decrease in the level of infection. This agrees with the reports of Dean-Nystrom *et al.* (1997) and Pablo *et al.* (2009), where an increase in neutrophils and lymphocyte values were observed post-infection in neonatal calves experimentally infected with STEC O157:H7.

The higher values of the mean *lymphocytes* and neutrophil counts observed in Group C lambs that received only a viable inoculum of STEC O157:H7 cells without the administration of a mixture of probiotics, could be attributed to the more established infection in this group, as no form of intervention was given, consequently the STEC O157:H7 cells

perhaps, adhered and proliferated successfully, which probably produced more toxins in the system. Consequently, the immune system mobilized more *lymphocytes* to bind to the toxins produced by these pathogens and eliminate them from the system. Meanwhile, the lower values of the mean TWBC, neutrophil and lymphocyte counts observed in Group B lambs that were inoculated with viable STEC O157:H7 cells together with daily administration of a mixture of probiotics, could be as a result of the intervention given in the form of a mixture of probiotics which was effective in controlling these pathogens. The probiotics mixture might have hindered the adhesion and multiplication of the bacterial pathogens (STEC cells) thereby reducing the level of infection (Everlon *et al.*, 2013). So therefore, the values of the total white blood cells, neutrophil and *lymphocytes* observed were lower than those in group C lambs that were inoculated with STEC cells without probiotics mixture.

There was no significant difference in the values of Mean absolute eosinophil and monocyte count among the three experimental groups. This could be because these groups of white blood cells are not activated during an early stage of an immune response to bacterial infection (Blood *et al.*, 1983a).

Conclusion

Probiotics was effective in the control of STEC O157:H7 as there was significant difference in the values of the Mean Total White Blood cells ($14.88 \pm 4.46 \times 10^9/L$), *Lymphocytes* ($9.07 \pm 2.57 \times 10^9/L$) and Neutrophils ($5.59 \pm 1.85 \times 10^9/L$) counts between the lambs experimentally infected with STEC O157:H7 and administered a mixture of probiotics strains at 4.5×10^8 CFU/ml and the lambs that were administered only STEC cells, Mean total white blood cell ($40.14 \pm 3.9 \times 10^9/L$), *Lymphocyte* ($23.89 \pm 2.51 \times 10^9/L$) and Neutrophil ($15.09 \pm 1.49 \times 10^9/L$) counts first week post-infection.

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PREVALENCE AND DIVERSITY OF GASTROINTESTINAL NEMATODES OF CATTLES IN AND AROUND JIMMA TOWN, SOUTH WESTERN ETHIOPIA

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Abstract

Cross sectional study was conducted from November 2015 to April 2016 to assess the prevalence of bovine gastrointestinal nematodes and its associated risk factors in and around Jimma town, southwestern Ethiopia. A total of 384 faecal samples for the coproscopic examination were collected and processed using direct faecal floatation method in parasitology laboratory of Jimma University, School of Veterinary medicine. Out of the total sampled cattle, 190 (49.5%) had a gastrointestinal nematode infection. Coprological investigation revealed that strongyles (37.5%) were the most prevalent genera than *Trichuris* (32.3%), *Toxocara* (15.4%) and *strongyloides* (11.7%). The eggs per gram count was determined by using McMaster egg counting technique showed that 42 (22.1%), 122 (64.2%) and 26 (13.7%) of the cattle were lightly, moderately and heavily infested, respectively. Parasite burden (EPG) of gastrointestinal nematodes in the current study shown that a statistically significant difference was found between ages and body condition scores ($P < 0.05$), but not between the sex groups. Significant difference was observed in the prevalence of parasites within monthly occurrence of the disease ($P < 0.05$) in the study area. Sex and breeds of cattle were found to have no statistically significant association ($P > 0.05$) with prevalence of gastrointestinal nematodes infections. Prevention of cattle from these nematode infection using strategic deworming and an improved feeding and management of cattle should be attempted.

Keywords: Faecal sample, McMaster, Risk factors, Strongyles, Strongyloides, *Toxocara*, *Trichuris*

PRÉVALENCE ET DIVERSITÉ DES NÉMATODES GASTRO-INTESTINAUX DE BOVINS DANS ET AUTOUR DE LA VILLE DE JIMMA DANS LE SUD-OUEST DE L'ÉTHIOPIE

Resume

Une étude transversale a été menée de novembre 2015 à avril 2016 dans le but d'évaluer la prévalence des nématodes gastro-intestinaux des bovins et de leurs facteurs de risque associés dans et autour de la ville de Jimma, dans le sud-ouest de l'Éthiopie. Un total de 384 échantillons fécaux pour examen coproscopique ont été prélevés et traités en utilisant la méthode de flottation fécale directe dans le laboratoire de parasitologie de la Faculté de médecine vétérinaire de l'Université de Jimma. De l'ensemble des bovins échantillonnés, 190 (49,5%) présentaient une infection à nématodes gastro-intestinaux. L'étude coprologique a révélé que les strongles (37,5%) étaient les genres les plus répandus, par rapport à *Trichuris* (32,3%), *Toxocara* (15,4%) et *Strongyloides* (11,7%). La numération d'œufs par gramme a été déterminée à l'aide de la technique de comptage des œufs de McMaster, qui a révélé que 42 (22,1%), 122 (64,2%) et 26 (13,7%) des bovins étaient respectivement infectés légèrement, modérément et fortement. La charge parasitaire (EPG) des nématodes gastro-intestinaux dans la présente étude montre qu'une différence statistiquement significative a été notée entre les âges et les scores de l'état corporel ($P < 0,05$), mais pas entre les sexes. Une différence significative a été observée au niveau de la prévalence des parasites dans les manifestations mensuelles de la maladie ($P < 0,05$) dans la zone d'étude. Le sexe et la race des bovins n'ont pas montré d'association statistiquement significative ($p > 0,05$) avec la prévalence d'infections gastro-intestinales. Il faudrait essayer de prévenir les infections de ces bovins par l'administration de vermifuges stratégiques et l'amélioration de l'alimentation et de la gestion des bovins.

Mots-clés : échantillon fécal, McMaster, facteurs de risque, Strongyles, Strongyloides, *Toxocara*, *Trichuris*

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Introduction

Ethiopia is one of the country from the tropical latitudes of Africa, and has an extremely diverse topography, a wide range of climatic features and a multitude of agro-ecological zones, which makes the country suitable for different agricultural production systems. This results in the existence of a large diversity of farm animal genetic resources in the country (Anon., 2004). The country has the largest livestock and draft animal population in the African continent which is approximately 56,706,389 cattle, 29,332,382 sheep, 29,112,963 goats, 2,033,115 horses, 400,329 mules, 7,428,037 donkeys, 1,164,106 camels and 56,866,719 chickens are found in the country (CSA, 2014). This figure indicates a huge potential of the country in the sector. Although Ethiopia has large livestock population, the economic benefits remain marginal due to prevailing diseases, poor nutrition, poor animal production systems, reproductive inefficiency, management constraints and general lack of veterinary care. Diseases of livestock have numerous negative impacts on productivity and fertility of herds (MOA, 2013).

Parasite infestation is one of the most common problem affecting cattle of all ages and breeds (Rafullah *et al.*, 2011). Internal parasites interfere with nutrition, growth and the production of the cattle (Pilarczyk *et al.*, 2009; Khan *et al.*, 2013). Helminths infection can result in losses in productivity through a reduction of feed intake and feed Conversion efficiency, loss of blood and even death (Tsotetsi and Mbat, 2003). Helminths cause great economic loss in livestock in Africa and Asia, and can be categorized as either direct or indirect losses (Vidya and Sukumar, 2002). Endoparasites are responsible for the death of one third of calves, lambs and kids, and considerable losses of parts of carcasses condemned during meat inspection (Anon, 2000).

Parasites from the phylum nematoda cause numerous diseases in humans, animals and plants placing major burdens on agricultural production and global health. Infections by these pathogens cause extensive suffering

in humans and animals and major losses in agricultural production due to disease and the cost of implementing control programs (Jasmer *et al.*, 2003). Gastrointestinal nematode (GIN) infections in cattle are of considerable economic importance, causing clinical disease and mortalities, but more importantly, by causing subclinical chronic production losses as a result of weight loss, reduced weight gain, and reduced milk production (Over *et al.*, 1992). There is compelling evidence from Europe and Canada that GI nematode infections have negative effects on milk production from dairy cows (Charlier *et al.*, 2009; Sanchez *et al.*, 2004). Forbes *et al.* (2004) reported a drop in milk yield in untreated, naturally infected dairy cows when compared with treated controls. The milk yield after anthelmintic treatment on pastured dairy cattle in the Netherlands was estimated to increase by 1 kg/cow/day (Charlier *et al.*, 2009). There is limited information on the economic impacts of GIN on milk production in Africa.

The environmental conditions such as low lying water filled grazing land, humidity, environmental temperature of this area are suitable for growth and survivable of parasites. However, no study has been conducted on the status and significance of bovine gastrointestinal nematode infection in the study area. Therefore, the objectives of this study were to investigate the prevalence of bovine gastrointestinal nematodes, to assess worm burden of nematode parasites and to determine the effect of different risk factors on the occurrence of the parasites.

Materials and Methods

Study Area

The study was conducted in Jimma town and its surrounding villages from November, 2015 to April, 2016. Jimma town, which is the capital city of Jimma zone, is located in Oromia Regional State at 352 km South West of Addis Ababa. The town has latitude of about 7°36' to 8°N and longitude of about 35°52' to 37°37' E, and an elevation ranging from 880 to 3360 m above sea level.

Very currently Jimma Zone is divided in to 17 districts hosting a total population of over 2.4 million (CSA, 2008), an agro-ecological setting of highlands (15%), midlands (67%) and lowlands (18%) (Dechassa, 2000). Farmers in the area practices mixed farming system. The zone is one of the major coffee growing areas of southwest Ethiopia; cultivated and wild coffee is a main cash crop of the area. The area receives a mean annual rainfall of about 1,530 mm, which comes from long and short rainy seasons. The average minimum and maximum annual temperature ranges between 14.4 and 26.7°C, respectively (Alemu *et al.*, 2011). The total livestock population of Jimma zone is estimated to constitute, 2.02 million cattle, 288,411 goats, 942,908 sheep, 152434 equines, 1,139,735 poultry and 418,831 beehives (CSA, 2008).

Study Design

A cross sectional study was conducted to determine the prevalence of gastrointestinal nematodes during the study period and to investigate the main risk factors influencing the prevalence and intensity of parasite infection in cattle.

Study Population

All cattle presented to the Jimma University, College of Agriculture and Veterinary Medicine open air clinic, Serbo clinic and dedo clinic having different health problems during the study periods were considered as study animals for the presence of gastrointestinal nematodes. Animals those admitted to three clinics were selected using a simple random sampling method. The ages of the animals were estimated using the definition described by De-Lahunta and Hable (1986). Animals were divided into two groups, namely young (≤ 2 year old) and adult animals (>2 year old). Body condition score was made by the scoring system described by Tennant *et al.* (2002) in cattle.

Sample Size Determination

As previous study has not been conducted on bovine gastrointestinal

nematodes in the study area, the expected prevalence was assumed to be 50%. Therefore, the sample size calculated at 50% expected prevalence rate with a desired precision of 5% and 95% confidence interval was determined by using the formula given in Thrusfield (2005).

$$n = \frac{1.96^2 \times P_{exp} (1-P_{exp})}{d^2}$$

Where n = required sample size, P_{exp} = expected prevalence, d = desired absolute precision. Therefore based on the above formula a total of 384 cattle's were examined.

Sample Collection and Examination

Samples were obtained directly from animal rectum as per Kanyari *et al.* (2010) method and a few from the ground when fresh and clean. Faecal samples were placed into vial containing 10 % formalin. The vials were labeled with animal Identity number, Sex, Age, Body condition, breed and District and placed into cool box. After this, each sample was transported to Jimma University, College of Agriculture and Veterinary Medicine Veterinary parasitology laboratory for Coprological examination.

In the laboratory the samples were subjected to fecal flotation method as described by Karki (2008) and eggs of the different nematode parasites were identified on the basis of morphological appearance and size of eggs as described by Van Wyk and Mayhew (2013). Positive fecal samples were subjected to McMaster egg counting technique as described by Karki (2008) and the degree of faecal egg output per gram was determined as described by Taylor *et al.* (2007) in mixed infection with different GI nematode species.

Data Analysis

The information and data collected on GI nematodes of cattle during the period were recorded in excel Sheet and analyzed using SPSS version 20. Prevalence was calculated using percentage. The significance of association between and among the considered variables was determined using P-value, chi square (χ^2)

test statistics. Association between variables was said to exist if the calculated level of significance is less than 5% ($P < 0.05$) at 95% confidence level.

Results

Prevalence of gastrointestinal nematodes of cattle encountered in the study area

The coprological examination conducted on 384 fecal samples revealed an overall prevalence of gastrointestinal nematodes infection of 190 (49.5%). Out Of the total positive cases, 144 (37.5%) were infected with genera of strongyles, 59 (15.4%) were infected with *Toxocara*, 124 (32.3%) were infected with *Trichuris* and 45 (11.7%) were infected with strongyloides (Table 1).

Association of gastrointestinal nematode parasites prevalence and risk factors

The prevalence of gastro-intestinal nematodes in the current study were 50% in male and 48.9% in female, 50.1% in local and 41.4% in cross breed cattle. But the difference was not statistically significant ($P > 0.05$) (Table 2). Adult and young animals were found to be infested with a prevalence of 45.8% and 57.5%, respectively with statistically significant difference ($P < 0.05$) (Table 2). Infection prevalence was significantly higher in animal with poor body condition when compared to that of medium and good body condition scores ($P < 0.05$). The overall infection prevalence according to body condition grades, 54.7%, 53.8% and 34.4% with poor, medium and good, respectively (Table 2).

Table 1: Prevalence of gastrointestinal nematodes of cattle encountered in the study area

Parasite egg type	No. of animal examined	Positive samples	Prevalence (%)
Strongyles	384	144	37.5%
Trichuris	384	124	32.3%
Toxocara	384	59	15.4%
strongyloides	384	45	11.7%

Table 2: Prevalence of GI nematode parasites in relation to sex, age, body condition and breed.

Risk factors	No. of animal examined	No. of Positive (%)	χ^2	P-value
Age				
Young	120	69 (57.5%)		
Adult	264	121 (45.8%)	4.492a	0.034
sex				
Male	204	102 (50%)		
Female	180	88 (48.9%)	0.047a	0.828
Body condition				
Poor	159	87 (54.7%)		
Medium	132	71 (53.8%)		
Good	93	32 (34.4%)	11.18a	0.004
breed				
Local	355	178 (50.1%)		
Cross	29	12 (41.4%)	0.823a	0.364
Total	384	190 (49.5%)		

Different prevalence of gastrointestinal nematodes was found for different months: 37 (57.8%) in November, 28 (43.8%) in December, 23 (35.9%) in January, 18(28.1%) in February, 40 (62.5%) in March and 44 (68.8%) in April. The seasonal difference was statistically significant ($P<0.05$) (Table 3).

Degree of gastrointestinal nematode parasites infestation

A total of 190 fecal samples that were positive by qualitative parasitological techniques were subjected to EPG count using McMaster egg counting technique. Accordingly, 42 (22.1%), 122 (64.2%) and 26 (13.7%) were found to be lightly (50-200), moderately (201-700) and massively (>700) infested, respectively

and levels of infestation were according to Taylor et al. (2007). Most of the infected cattle had a faecal egg count in a range of 201 to 700 EPG and less than this range (Table 4).

Body condition of the animals was statistically significant ($P<0.05$) with parasite burden. This can be shown by the fact that severe and moderate infestations were high in animals with poor body condition as compared to medium and good body conditioned animals. As well, the difference in the degree of EPG between young and adult cattle was statistically significant ($P<0.05$), younger animals were found to harbor heavier parasite load than adult ones. On the other hand, sex had no significant association with EPG ($P>0.05$) in the study (Table 5).

Table 3: Monthly prevalence of gastrointestinal nematodes of cattle in study area

Months	animals examined	No. of positive	Prevalence	χ^2	P-value
November	64	37	57.8%		
December	64	28	43.8%		
January	64	23	35.9%	32.84 ^a	0.000
February	64	18	28.1%		
March	64	40	62.5%		
April	64	44	68.8%		

Table 4: Level of infection based on egg per gram (EPG) count of examined positive animals for nematode eggs at the study area

Intensity of infection	No. of Examined samples (%)	EPG
Light	42 (22.1%)	50-200
Moderate	122 (64.2%)	201-700
Heavy	26 (13.7%)	>700
Total	190	

Table 5. Degree of gastrointestinal nematodes infection (EPG) Category (%) with different risk factors

Risk factors	Degree of Infestation			χ^2	P-value
	Light (%)	Moderate (%)	Heavy (%)		
Sex					
Male	23 (22.5%)	67(65.7%)	12 (11.8%)	0.732 ^a	0.693
Female	20 (21.7%)	54 (61.4%)	14 (15.9%)		
Age					
Young	13 (18.5%)	38 (55.1%)	18 (26.1%)	14.13 ^a	0.001
Adult	30 (24.8%)	83 (68.6%)	8 (6.6%)		
Body condition					

Risk factors	Degree of Infestation			χ^2	P-value
	Light (%)	Moderate (%)	Heavy (%)		
Poor	10 (11.5%)	60 (69%)	17 (19.5%)	17.164 ^a	0.002
Medium	19 (26.8%)	45 (63.4%)	7 (9.9%)		
Good	14 (43.8%)	16 (50%)	2 (6.2%)		

Discussion

The coprological examination done for this study using direct faecal floatation method revealed an overall prevalence of 49.5% of gastrointestinal nematode parasites in cattle originating from this area which were being parasitized with one or more species of nematode parasites. This report is in agreement with previous studies by coprological examination of GIN in some areas of Ethiopia by Yimer et al. (2015), who reported an overall prevalence of 41.15% in Dire Dawa, Bacha and Haftu (2014), who reported an overall prevalence of 49% in Arsi, Hiko and Wondimu (2010), who reported an overall prevalence of 54% in Haramaya District and Pfukenyi et al. (2007) reported an overall prevalence of 43% in Zimbabwe this might be due to similarity in study design and ecology of animal.

In contrast to the present result, Waruiru et al. (1998) reported an overall prevalence of 86.8% in Kenya which was recorded the highest prevalence. The variation may be due to sample size difference, the presence of sufficient rainfall and moisture during the study period was favored the survival of infective larvae in pasture and higher probability of uptake of the infective larvae leading to higher prevalence. The other possible explanation for high prevalence may be due to the fact that cattle are managed under extensive managements system with the high stocking density, where large numbers of animals graze together throughout the year in communal grazing land, topography, deworming practices, inadequate nutritional status and lack of community awareness. However, it is not in agreement with Tigist et al. (2012), who reported an overall prevalence of 27.57% in Gondar which is lower than the

prevalence determined in the present work. The variation might be due to difference in optimum temperature and moisture content which favors the growth and development of larvae on pasture.

According to the current study result which indicated the prevalent nematodes egg with respect to their genera were Strongyle (37.5%), Toxocara (15.4%), Trichuris (32.3%), and strongyloides (11.7%). In the case of strongyles, the prevalence agrees with reports of Telila et al. (2013), in East Showa Zone (41%) and that of Kemal and Terefe (2013) in Gedebano Gutazer Wolene district (37.9%). In the case of toxocara, the prevalence is lower than reports of Kemal and Terefe (2013) in Gedebano Gutazer Wolene district (22.4%) and relatively higher than the report of Telila et al. (2013), in East Showa Zone (7.7%). The prevalence of trichuris (16.82%) reported by Tigist et al. (2012) was lower than the current finding. The strongyloides prevalence (24.05%) reported by Yimer et al. (2015) was found higher than the current study finding. Therefore it seems obvious that differences in prevalent worm genera are dependent on geographical and climatic condition of each study area vary from one another in supporting infectivity of different parasite genera.

In the current study young were found more frequently infected than adult cattle. The statistically significant difference ($P < 0.05$) was recorded between the two age groups with prevalence being highest in young (57.5%) than in adult (45.8%) animals. This finding is in agreement with the earlier reports of Bacha and Haftu (2014), who reported 60.2% in young and 52.2% in adult animals and with that of Pfukenyi et al. (2007) report in Zimbabwe, which showed that the susceptibility and pathogenicity of nematode infections were

greater in young animals than in mature animals. This could be due to the fact that young animals do not have strong immunity to parasitic infection during the first year in pasture, due to a limited previous exposure and immaturity of the immune system that resulted in higher development of the parasite. Adult animals may acquire immunity to the parasites through frequent challenge and expel the ingested parasite before they establish infection (Dunn, 1987, Fishes, 1989).

The present study revealed that sex of the studied animal did not show significant association ($P > 0.05$) with the prevalence of GI nematodes in cattle. The absence of significant association between sex and prevalence agrees with that of Tigist et al. (2012) in Gondar. The prevalence of GIN in current study was 48.9% and 50% in females and males, respectively. This finding is in line with previous findings which was reported by Yimer et al. (2015) as 41.4% and 45.86% in females and males respectively in Dire Dawa, Bacha and Haftu (2014), who reported 46.7% and 52.2% in females and males, respectively in Arsi and Kemal and Terefe (2013), who reported 39.1% and 40.7% in females and males, respectively in Gedebano Gutazer Wolene district. This similarity in exposure to GIN among both sexes was more probably due to similar management under the same environmental condition of the two sexes in this area.

The present study showed that, body condition score is statistically significant ($P < 0.05$) with gastrointestinal nematode infection such that shedding of nematodes eggs increased with poor body conditioned animal (54.7%) than medium and good body (53.8%) and (34.4%), respectively. This finding is in agreement with the earlier reports of Bacha and Haftu (2014), who reported 62.7%, 55.7% and 37.2% in poor, medium and good body conditioned cattle, respectively. This could be due to the fact that increased weight gain is the most often observed and best-documented benefit of GI nematode control since well-fed animals develop good immunity that suppresses the fecundity of the parasites (Charlier et al., 2009).

The study further revealed that breed of the animal did not show significant association with the prevalence of the nematodes parasites. Local breeds' animals have higher prevalence than cross breed animals 50.1% and 41.4%, respectively. However, the limited number of cross breed was included in this study could not be taken a definite reflection of breed susceptibility. This finding is in line with previous findings of Tigist et al. (2012) in Gondar, who explained that infections with gastrointestinal nematodes were higher in the local breed compared to the cross breeds. This is due to the fact that local breed in the study area was managed extensively which increase the chance to graze in marshy, swamp areas which may be more concentrated with the nematode eggs or larvae than cross breed kept intensively with less access to marshy area. Another fact which needs to be considered in relation to difference is that in the present study, maximum sample collection was made from local breed on the basis of its availability as the population density of local breed was found maximum in the study area.

In the present study significant difference ($P < 0.05$) was recorded between the prevalence and months. The results also revealed that the high prevalence was observed during April (68.8%), March (62.5%) and November (57.8%) months. Alternatively, lower prevalence was observed in December (43.8%), January (35.9%), February (28.1%) than the other. This result is in agreement with Kemal and Terefe (2013), who reported high prevalence 65.5%, 50.0 % in March and April, respectively. Waruiru et al. (2001) also reported high nematodes worm burden during short rainy season from March to June and lower burden in dry season between January and February which in line with the current results. This could be due to the fact that during the rainy season, the rainfall and temperature are favourable for the development, survival and translation of infective larvae on herbage. These conditions result in increased availability of infective larvae on pastures, so the chances of cattle picking up infective stages of the parasites whilst grazing are high, leading to a buildup of high worm

burdens in the host (Pfukenyi & Mukaratirwa, 2013).

Majority of infected animals (64.2%) had fecal egg count in the range of > 200 to 700 EPG and few proportions of animals (13.7%) had fecal egg count over 700 which in line with report of Kemal and Terefe (2013). In the current finding, even if EPG was slightly greater in males than females for light and moderate infestation there was no statistically significant difference between the two sexes which is in agreement with that of Kemal and Terefe (2013) in Ethiopia and Keyyu et al. (2003) in Tanzania. In the present study, the EPG were high in young with significant difference from adult age group. This follows with the result reported by Pfukenyi et al. (2007), Keyyu et al. (2003) and Maichomo et al. (2004), who suggested that younger animals were prone to more worm burden as compared to old ones. This could be due to the fact that healthy adult cattle generally have a well developed immunity, resulting in lower worm burdens than in young animals with typically less developed immunity (Reinemeyer, 1990; Gibbs, 1979). Adult animals may acquire immunity to the parasites through frequent challenge and expel the ingested parasite before they establish infection (Shah-Fischer and Say, 1989). In the current finding, heavier and moderate degrees of EPG were recorded high in poor than good and medium body condition and significantly different. This result corresponds with reports of Keyyu et al. (2003).

Conclusion

Gastrointestinal nematodes were prevalent in the study area and cattle were infected with diversified gastrointestinal nematodes that can seriously affect the health and productivity of the animals. The predominant gastrointestinal nematode parasites identified were strongyles, strongyloides, toxocara and trichuris species. These parasites affect all age and sex groups and fluctuation of gastrointestinal nematode infections were associated with seasonal changes, exhibiting highest prevalence in wet season. Therefore,

this study identified the potential risk factors associated with high prevalence rate and high degree of egg per gram faecal sample (EPG) enabling to design strategic control of gastrointestinal nematodes of cattle in areas with similar ecological features to current study. Strategic anthelmintic treatment need to be applied in the area to control the infestation risk in cattle.

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RELAPSE FOLLOWING CHEMOTHERAPY OF HUMAN AND ANIMAL AFRICAN TRYPANOSOMOSSES: A REVIEW

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Abstract

An effective treatment regimen depends on detecting cases of African trypanosomosis, is a highly desirable and dependable control options. The last new veterinary drug, diminazene aceturate (Berenil) and that for treating human African trypanosomosis, alpha-Difluoromethylornithine or eflornithine (□-DFMO) that were patented 55 and 40 years ago, respectively showed the dearth of anti-trypanosome drugs. A major setback is ineffectiveness of treatment often attributed to the following factors: (i) wide spread resistance, which cut across the disease ecological areas involving all the pathogenic trypanosome species and available trypanocides. The increasing reports of multiple and cross resistance could worsen the already deplorable situation with very few drugs of choice, and (ii) parasites are shielded from trypanocidal drug actions in "privileged" sites; eyes, testes adipose tissue and brain. Advances in molecular biology have afforded the application of polymorphism to identify drug resistant genes. Lately, RNA interference used in gene silencing have allowed more insight into mode of drug actions and mechanism of resistance. Most trypanocides (excluding tryparsamide, melamingly arsenicals, nitrofurans and DFMO cannot cross the blood-tight (the blood-brain/blood-cerebrospinal fluid) barriers. Similarly, the lack of detectable levels of drugs in adipose tissue, spleen, skeletal muscle and lungs could result in relapse. Occult infection with amastigote and sphaeromastigote stages of *Trypanosoma brucei* lodged in the choroids plexus has remained controversial. Strict adherence to established protocol for rational drug use has been advocated as possible panacea to limit emergence and spread of induced drug resistance. Effective management of HAT cases will depend on accurate diagnosis and clinical staging of the disease, will inform using pentamidine and suramin or merlasoprol or a combination of these drugs for early and late stage infections. Other operational factors that promote occurrence of relapse includes increase in defective or adulterated drugs, inappropriate handling resulting in under dosage and pharmacokinetic limitations. The areas requiring research attentions and ways to minimize relapse are highlighted.

Key words: Cryptic infection, Drug-resistance, Privileged sites, Recrudescence, Relapse, Trypanocides, Trypanosomosis.

RECHUTE APRES LA CHIMIOThERAPIE DES TRYPANOSOMOSSES HUMAINE ET ANIMALE AFRICAINES : UN EXAMEN

Resume

Un régime de traitement efficace dépend de la détection des cas de trypanosomose africaine, et constitue une des options de contrôle hautement souhaitable et fiable. Le dernier nouveau médicament vétérinaire, l'acéturate de diminazène (Berenil) et celui destiné au traitement de la trypanosomose humaine africaine, l'alpha-difluorométhylornithine ou l'orthoornithine (□-DFMO) brevetés il y a 55 et 40 ans, ont respectivement montré la rareté des médicaments de lutte contre les trypanosomes. L'inconvénient majeur est l'inefficacité du traitement, souvent attribuée aux facteurs suivants : (i) la résistance à grande échelle, qui concerne les zones écologiques de la maladie impliquant toutes les espèces de trypanosomes pathogènes et les trypanocides disponibles. Les rapports croissants faisant état de résistance multiple et croisée pourraient aggraver la situation déjà déplorable où très peu de médicaments de choix sont disponibles ; et (ii) les parasites sont protégés contre les actions des trypanocides dans les sites « privilégiés » : les yeux, les testicules, le tissu adipeux et le cerveau. Les progrès de la biologie moléculaire ont permis l'application du polymorphisme pour identifier les gènes résistants aux médicaments. Dernièrement, l'interférence ARN

utilisée dans l'inhibition génique a permis de mieux comprendre le mode d'action des médicaments et le mécanisme de résistance. La plupart des trypanocides (à l'exception du tryparsamide, des mélamingly arsenicaux, du nitrofurane et du DFMO) ne peuvent pas franchir les barrières d'étanchéité au sang (sang-cerveau / liquide céphalo-rachidien). De même, l'absence de taux détectables de médicaments dans le tissu adipeux, la rate, le muscle squelettique et les poumons peut conduire à une rechute. L'infection occulte avec des stades amastigotes et sphaéromastigotes de *Trypanosoma brucei* logés dans le plexus des choroïdes reste controversée. L'adhésion stricte au protocole établi pour une utilisation rationnelle des médicaments a été préconisée comme une panacée éventuelle capable de limiter l'émergence et la propagation de la résistance induite par les médicaments. La prise en charge effective des cas de THA dépendra d'un diagnostic précis et d'une stadification clinique de la maladie, elle guidera l'utilisation de la pentamidine et de la suramine ou du merlasoprol ou d'une combinaison de ces médicaments pour les infections précoces et tardives. Les facteurs opérationnels qui favorisent l'apparition des rechutes comprennent les médicaments défectueux ou frelatés, la manipulation inappropriée entraînant le sous-dosage et les limitations pharmacocinétiques. Les domaines nécessitant des attentions en matière de recherche et les moyens de minimiser les rechutes sont mis en évidence.

Introduction

African Trypanosomiasis is a disease complex affecting man (known as sleeping sickness) and his domestic animals (sammore or nagana in cattle). Present prospect for vaccine production is bleak since the parasite, *Trypanosoma* species exhibit a phenomenon known as antigenic variation, which is a change in the surface-coat glycoprotein termed variant surface-coat glycoprotein, VSG (1). This enables the parasite to escape subsequent host immune assault (2). Hence, control measures are directed at eliminating the vector, tsetse flies (*Glossina* species) or prophylactic and/or curative treatment to prevent the establishment and destruction of host reservoir. Huge doses (estimated at 25 – 30 million) of trypanocides, 10 times less than the projected threshold are used annually (3). The drug repertoire is very few and their use is limited by their toxicity and development of relapse due to natural or acquired resistance and refractoriness arising from inappropriate usage, poor sensitivity of some species or as a result of cryptic infections in tissues inaccessible to drugs (4, 5, 6, 7, 8). Most often when there is relapse, it is misconstrued to mean resistance of the infecting strains to the drug used (9). Relapse is infrequently mentioned in publication relating to chemotherapy or treatment of the disease thereby making critical evaluation of its impact on control underestimated. Typical example is the high rates of relapse cases occurring

during epidemics as observed in Ugandan by (10). Risk was higher in men than women, and patients diagnosed with trypanosome(s) in CSF. Possible reasons were attributed to biases of selection or over-diagnosis, poor application of treatment protocol, degraded melarsoprol because of poor storage conditions and could possibly be due to development of resistance.

To address this gap in information, this review is aimed at collating research reports relevant to this topic in view to identify the problems and highlight prospects, and proffer solutions or necessary remedies. Difference in treatment failures as a result of drug resistance and relapse is largely due to other factors that are clearly verified based on empirical evidence available in literature from research studies. Information obtained about the drug resistance cases documented were based on different assays. Therefore, the need for standardization of test for drug resistance (11) still remains valid even now than before to enhance multifaceted control strategy. The trypanocidal drugs are the most applied method that farmers use to treat or prevent animal trypanosomiasis and they represent 20-40% of veterinary drug market in West Africa (12) Dia et al 2004). Conservative estimate of average purchase of UU\$1.0 per dose and UU\$35 million per year were spent by African farmers (13). The low cost of trypanocidal drugs coupled with their availability in the open market has empowered farmer affordable (0.5 USD) and access. According to Swallow et al.

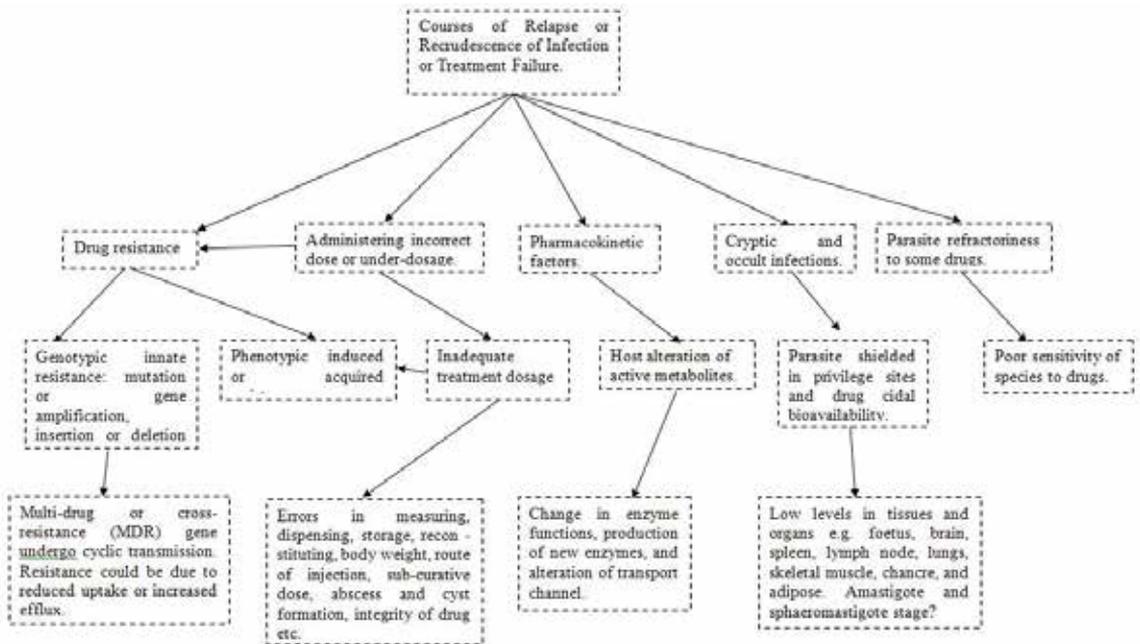


Figure 1: Factors causing relapse in the chemotherapy of African trypanosomoses

(14), assuming each cattle is treated twice per year, 17.5 million out of the 46 million cattle representing two-third of cattle at risk are not treated. A general practice well entrenched in livestock farming management whereby the herdsmen/herd owners do treat their animals without relying on competent professionals. This will no double give rise to misuse leading to under dosage problem.

Interaction of Factors Causing Relapse

For practical purpose and contextual clarity, the word relapse connote treatment failure consequent to using the recommended dose. It is defined as ineffectiveness of treatment or recrudescence of parasitaemia after treatment (15) or the reappearance of a disease during convalescence. There are major interacting factors of the 4R words of relapse, resistance, refractoriness and recrudescence. The interaction of these components in contributing to the occurrence of treatment failure or relapse is represented in a simple flow-chart on Figure 1. Inappropriate use of anti-trypanosomal drugs or trypanocides leading to sub-dosage can result into drug-induced resistant and/or cause of treatment

failure.

There is claim of widespread misapplication of trypanocides (16) making their judicious use through controlled drug policy a matter of importance (6). Another factor that will be reviewed in this treatise include drug resistance, differences in host and trypanosome species' refractoriness to drugs, and the level of bioavailability of drugs in certain tissue or organs and inaccessibility of drug to certain organs. The ability of some trypanosome species to invade tissues and to assume transformation into intracellular parasitism is also discussed. So far the treatment for the early or haemo-lymphatic stage of HAT involves the drugs pentamidine and suramin which have been very successful (1) Nok 2003). Human African trypanosomiasis have four main drugs of choice with pentamidine, suramin and □-Difloromethyl-ornithine (DFMO) for early stage and Merlasoprol for late stage treatment.

Drug Resistance

Of all the factors attributed to relapse, the most widely research into, is trypanocidal drug resistance. This phenomenon, from



Figure 2: Spread of drug resistance trypanosomes in sub-Saharan Africa countries.

empirical evidence, could be natural (innate or genetically controlled) or arise from antigenically susceptible strains (17). Its spread is facilitated by mechanical and cyclical transmission (through many cycles) while still retaining its resistant trait (18, 19). Over the years, the problem of drug resistance, including multiple or cross resistance has been reported to be on the increase (15, 20, 21). Delespaux et al. (22, 23) had projected that it occurs in not less than 18 endemic countries as shown on Figure 2. The two main parasite species, *T. vivax* and *T. congolense* that mainly infect cattle are involved and have assumed serious economic concern and consequence. The cell membrane efflux pump, P-glycoprotein (Pgp), appears to contribute to anti-helminthic drug resistance.

Two contrasting mechanism of drug resistance are the reduced uptake in some species and in other ones with increased efflux. Based on experimentally over expressed P-glycoprotein in *T. brucei*, indicate possible involvement in the mechanism of drug resistance in this parasite (24). The drug resistance trend is linked to genotypic or phenotypic modification generally responsible for microbial drug-resistance. They include but not limited to the following: (i) production of drug destructive enzyme (ii) altered permeability to drug (iii) change of structural target (iv) altered metabolic pathway, and

(v) altered enzyme function less affected in resistant than in the susceptible organisms (25). In the past, research to elucidate the role of these mechanisms in trypanosomiasis had been limited by lack of simple in vitro cultivation method. Dependence on in vivo or use of infected and treated experimental animals was hindered by inability to control breakdown of active drug compound through metabolism.

Importance of understanding the mechanism underlying selective drug action and resistance have remained largely unknown (26). Latest advances in RNA interference target sequencing (RIT-seq) screens has been applied for *T. brucei* to reveal the transporters, organelles, enzymes and metabolic pathways that function to facilitate anti-trypanosomal drug action. Secondly, there is now a feeder cell layer free culture system for *T. congolense* and *T. vivax* (27, 28). This development paved way for testing drug resistance of *T. vivax* that is non-infective to small laboratory animals and different stages of the parasite in the vector and host can now be tested (29). With the kit for in vitro isolation (KIVI), isolation and culture of stocks from mammals has been made easy and successful (30). The mode of drug entry into trypanosomes and how it causes the structural malfunctions had previously remained enigmatic. Studies of endocytosis have practical application in the understanding of drug entry and identification of target organelles (31, 32, 33). With endonuclease digestion, different chromosome-size fragments can now be obtained (34) and as small as 15 base pair oligonucleotide can be produced in vitro using polymerase chain reaction, PCR. Separation and analysis of DNA fragment is accomplished by the pulsed field zonal electrophoresis (35). RIT-sequencing profiling have identified known drug importers and the only known pro-drug activators, and linked more than fifty additional genes to drug actions (26). The authors reported that suramin uptake was mediated by stage-specific invariant surface glycoprotein (ISG75), and API adaptin complex, lysosomal proteases and major transmembrane protein, as well as speridine and N-aetylglucosamine biosynthesis. Others are obiquinone availability to nitro-drug

action, plasma membrane P-type H⁺-ATPases to pentamidine action, and trypanothione and several putative kinases to melarsoprol cross-resistance. A non-targeted metabolomics-based approach had shown that Eflornithine, a polyamine pathway inhibitor, and nifurtimox, whose mode of action involves its metabolic activation, are currently used in combination as first line of treatment against stage 2 (36). Eflornithine changed polyamine pathway, arginase activity and N-acetylated ornithine and putrescine. Nifurtimox was shown to be converted to a trinitrile metabolite indicative of metabolic activation and those involved in carbohydrate and nucleotide metabolism.

An identified 6-kilo base (kb) extra-chromosomal DNA element from multidrug resistant (MDR) *T. brucei* strain and the gene likely responsible for encoding the MDR-like protein in *T. brucei* and *T. vivax* were investigated at ILRAD (37). DNA probe diagnostic is being adapted for field use (38). Resistance of *T. b. brucei* to Berenil is attributed to reduced uptake due to lack of P2 type adenosine transporter-1 encoded by TbAT1 gene has been cloned in yeast (39, 40). Another mutation in transport surface of cells that confers resistance to pentamidine by *T. b. gambiense* is the aquaglyceroporin 2 (AQP2) channel (41, 42). A similar gene with 99.7% identity with TbAT1 gene has been cloned and sequenced from *T. evansi* (TevAT1) by (43). These authors have shown that induction of RNAi resulted in 10-fold depletion of TbevAT1 mRNA, with concomitant resistance to diminazine aceturate. A two point mutation within the central fragment of the gene could be used to distinguish between TbAT1s and TbAT1r. Diminazene was rapidly accumulated through single transporter which is dose dependently inhibited by pentamidine and adenosine. For more elaborate information about the enzymes that confers resistance to trypanocidal drugs in humans and animal trypanosomosis was recently reviewed by (44). The authors cited Eflornithine resistance has been associated with loss of amino acid transporter family member AAT6 (26, 38, 45). Suramin was linked to invariant surface

glycoprotein ISG75, melarsoprol to adenosine transporter TbAT1/P2 (46, 47) pentamidine to P-type H⁺-ATPases that maintain the proton-motive force across the plasma membrane, and pentamidine/melarsoprol cross-resistance to aquaglyceroporins, specifically to aquaglyceroporin 2, which encodes the High Affinity Pentamidine Transporter (HAPT1) is said to controls the susceptibility to both drugs (48, 49, 50).

Stability, Reversibility and Bypassing the Mechanism of Drug Resistance in Trypanosomes

Stock of trypanosomes within an area subjected to enzyme analysis proved that they belong to the same zymodene that was spreading and non-importation of new strain from outside the focus. Another experiment with cyclic transmission of drug resistance strain of the parasite still maintained its resistance trait despite several cycle of transmission. This confirms the stability of genetically acquired resistance, which remained unaltered irrespective of moving between its digenetic hosts (51). The value of current trypanocides will be enhanced if a compound capable of reversing drug resistance in trypanosome is available as obtains in cancer cells and malaria (37). Reversing agents such as the calcium-channel blocker Verapanil (VPL) reverses resistance of *T. cruzi* to Nifurtimox and *Leishmania donovani* to stibogluconate (52, 53). It is not effective in trypanosome probably due to difference in mechanism of drug resistance. Recently, (42)-Broceta et al. reported the development of chitosan nanoparticles loaded with the trypanocidal drug pentamidine and coated by a single domain nanobody that specifically targets the surface of African trypanosomes. Once loaded into this nanocarrier, pentamidine enters trypanosomes through endocytosis instead of via classical cell surface transporters like AT-2 and AQP2. The curative dose of pentamidine-loaded nanobody-chitosan nanoparticles was 100-fold lower than pentamidine alone in a murine model of acute African trypanosomiasis.

Table 1: Some drug-resistance cases in Human African Trypanosomiasis

S/No	Isolates: Cured/Total Treated	Drug Resistant (Sensitive)	Country	Authors
<i>T. b. gambiense:</i>				
1.	All 17 late stages.	Mel B (Nifurtimox)	Sudan	(58)
2.	16/18 late stage.	Mel B (DFMO)	Sudan	(59)
3.	All 100 late stages	Mel B (DFMO)	Sudan	(60)
4.	All the 12 late stages	Mel B (DFMO)	Cote d' Ivoire	(61)
5.	21/26 late stage (5 death)	Mel B (DFMO)	Zaire	(62)
6.	51 out of the 86 late stages	Mel B (DFMO)	Zaire	(63)
7.	132/145 late stage (13 died)	Mel B (DFMO)		(60)
Frequency of relapse		Treatment		
8.	5.6% (31/553) 1982-1985	Melarsoprol	Democratic Republic of Congo	(64)
9.	6.8% (35/512) 1986-1989			
10.	4.5% (18/398) 1990-1993			
11.	11.4% (34/299) 1994-1997			
12.	5.0% (17/343) 1998-2000			
13.	42 relapsed patients in first and 2 relapse to second combination treatment.	Merlasoprol (comination with DFMO)		(65)
<i>T. b. Rhodesiense</i>				
13.	7/7 (isolates)	Mel B (Suramin)	Uganda	(66)
14.	3/3 L/S	Suramin, Mel B (DFMO)	Kenya	(67)
15.	4 and 5/16 (isolates)	Mel B and Nifurtimox respectively	Kenya	(68)
16.	2/32 <i>T. rhodesiense</i> isolates	Merlassoprol, DIM and Suramin and ISMM.	Tanzania	(69)

Geographic and Demographic Spread of Drug Resistance

It is generally acclaimed that drug resistance is wide spread cutting across the disease ecological areas involving all pathogenic species and current trypanocides, (6, 54). Some documented cases of drug resistance for human and animal infective species are listed in Tables 1 and 2, respectively. In the past, the use of trypanamide and antrycide (re-introduced) were discontinued while Francophone countries suspended the use of homidium due to reports of widespread drug-resistant development (8). Numerous cases of arseno-resistance abound and only

current alternative treatment of the Gambian disease form is alpha-difluoromethyl ornithine, (α -DFMO) approved in 1990 for human use after many years of clinical trials. This remained the last drug to be approved and patented for human use since no new drug has been developed. The situation is even more hopeless as the economic returns for drugs against neglected tropical disease is not attractive to pharmaceutical companies (11). The Rhodesian disease form is left to less effective Nifurtimox. An in vitro drug sensitivity test in feeder layer free medium (55, 56) and empirical assay based on thymidine 3H-hypoxanthine incorporation (57) had shown some promise.

Table 2: Some documented cases of drug-resistance among animal-infective species

S/No	Resistant Isolates	Country	Drugs: Resistant (Sensitive)	Authors
1.	T. vivax outbreak in a ranch	Kenya	Cross-resistant to Samorin, Homidium and Quinapyramine (Berenil)	(70)
2.	7 T. vivax stocks	Kenya and Somalia	Cross resistant to samorin, Homidium and Quinapyramine (Berenil)	(20)
3.	6 T. congolense naturally infected cattle	Tanzania	Berenil (Samorin)	(71)
4.	6 T. congolense and 2 T. brucei; 10 T. congolense, 3 T. brucei subspecies (cattle and pigs)		Berenil (Samorin) Homidium (Samorin), Berenil and Samorin Berenil	(15) (72) (73)
5.	2 T. congolense and 1 T. vivax stocks 4 T. congolense and 1 T. vivax isolated from bovine 3 and 5 out of 11 T. brucei isolated from dogs.	Nigeria	Diminazene aceturate and Isometamidium (double dose isometamidium)	(74)
6.	12 T. vivax stocks 11 T. vivax stocks	Ethiopia	Berenil, Samorin and Homidium	(37)
7.	T. evansi infection in 61 camels	Kenya	Naganol and quinapyramin sulphate (merlasomine) (1992)	(76)
8.	10 isolates of T. congolense in cattle.		Diminazene aceturate (Berenil), Isometamidium chloride (Samorin) and homidium chloride (Novidium)	(77)
9.	24 out of 31 (77.4%) and 19 of the 62 (30.6%) T. congolense isolates.	Mali	Isometamidium and Diminazene (2007)	(78)
10.	24 out of 71 T. congolense 2 T. vivax and 2 T. brucei isolates. 8 and 1 of the 71 isolates cattle bovine, respectively.		ISMM, Diminazene aceturate and to drugs.	(79)
11.	Two group of T. congolense isolated in 1996 (5/39, 12.8%) and 2003 (24/38, 63.2%) were resistant	Zambia	Diminazene aceturate (Berenil)	(80)

Though they can circumvent the limitation due to non-infectivity of *T. vivax* to rodents and bioavailability of mouse drug sensitivity test, it will not be useful when drug is effective only in a metabolized state. Concerted effort can be made to minimize drug resistance through prevention of drug-induced resistance

by strictly practicing a regime of rational drug use (RDU) that is being widely advocated. In a study by (81) showed that resistance to both isometamidium and diminazene was widespread infecting cattle in Kéné Dougou Province of Burkina Faso. However, there was considerable variation between villages in drug-

resistance parameters, with the proportion of treated cattle with trypanosome infections 3 months after isometamidium prophylaxis varying from 6.9 to 63.8% and the proportion of cattle having infections 2 weeks after treatment with diminazene varying from 0 to 36.8%. The situation is the same in east Africa as reported from Ethiopia by (82) and in many other sub-Saharan African countries as shown on Tab. 2.

Subdose from Inappropriate Drug Use

The main reasons given for spread of drug resistance including the occurrence of multiple drug resistance has been widely reported in ten African countries are under dosing and unsystematic program of treatments are the predisposing factors for the development of drug resistance (83). In practice, the sensitization of trypanosomes exposed to sub dose can occur. This is facilitated by inaccurate measuring equipment (syringes, measuring cylinder and faulty weighing balance), underestimation of host body weight, formation of abscess at drug inoculation site, long interval between treatment, poor storage leading to break down of cidal compound and the use of prophylactic for curative treatment (8, 84). There are inherent diverse chemical and physical characteristics of anti-trypanosome drug that interplays important roles leading to resistance development. Typical among these factors, Berenil is sensitive to light, Novidium is sparingly soluble in cold water, suramin is

hygroscopic and pentamidine is hygroscopic and easily forms toxic amide.

In addition, the route of administration (73, 85), level of challenge, obvious size between animal breeds and differences in pharmacokinetic and pharmacodynamics (84) have profound effect on recommended dose. Drug regimen should take into cognizance the maximum tolerated (MTD) and maximum cidal dose (MCD) that will be suitable for a given field situation (86). Rather than sticking to manufacturers recommended dose, which in most cases, is not backed by extensive field trial. There are variable results using different treatment schedules (87). These are differences in species response to drugs. Presence of diminazene generic substandard products in the open market that poses a serious concern to stakeholders are competing with quality drugs. The significant qualitative and quantitative differences were found in products from two manufacturers and there was significant differences occurred in the content of ISM and of the ammonia chloride impurity in different batches from one of the manufacturers (88). The probability of having new drug from new molecules in the near future seem to be very slim.

This brings to question when there are reported cases of drug resistance, does it really represent a true or false case scenario. Therefore, a national control policy to monitor and regulate the standard of imported veterinary drugs including anti-trypanosome

Table 3: Preparation of trypanocide solutions:

• Diminazenes:	Berenil type
	• 1 bag of 2.36 g; diluted in 12.5 ml = 15 ml, 7%
	• 1 bag of 23.6 g; diluted in 125 ml = 150 ml, 7%
• Diminazenes:	Veriben type:
	• 1 bag of 2,36 g; diluted in 15 ml = > 15 ml; 6,25%
	• 1 bag of 23,6 g; diluted in 150 ml = > 150 ml; 6,25%
• Isometamidium:	Samorin
	• 1 bag of 125 mg; diluted in 12,5 ml = sol 1%
	• 1 bag of 125 mg; diluted in 6,25 ml = sol 2%
	• 1 bag of 1g; diluted in 100 ml = sol 1%
	• 1 bag of 1g; diluted in 50 ml = sol 2%

drugs as obtained for human medicines is of utmost importance. Other equally important confounding factors are those of measuring body weight, calculating drug dosage and adequate reconstitution of drug according to manufacturers' recommendation as shown on Table 3. Other are improper storage of drug and the integrity of drug depending on the source from reputable manufacturer. An attempt to minimize all these shortcomings has led to development of a chart for estimating animal body weight, sachet and bottles with marked line for diluting to the required quantity of sterile water among others (Diall unpublished document).

Trypanosome Species Refractory to Drugs

Most trypanocides in current use lack broad spectrum activity. Only Berenil is effective for most species with variation of dose from 3.5mg/kg to 7.0mg/kg body weight for treatment of *T. brucei* subspecies. In practice, when treatment is carried out in the field without identifying the species involved could result into using the wrong dosage. For convenience, the trypanocides are grouped as those effective for treatment of Trypanozoon species or Duttonella and Nannomonas species (86). The use of trypanocides have been ably reviewed (7, 8, 54). The identification of infecting species is vital to determining the course of treatment (Tab. 4). There is melarsoprol/pentamidine cross-resistance (MPXR). Baker et al. (89) has shown that a locus encoding two closely related aquaglyceroporins, AQP2 and AQP3, was linked to MPXR in a high-throughput loss-of-function screen. The AQP2 is said to serve as an unconventional "selectivity filter."

Suramin is most effective for *T. simiae*, which cannot be differentiated from *T. congolense* (6). Most trypanocidal drugs have low activity against *T. evansi* (90), which is indistinguishable from other *T. brucei* subspecies using motility in wet blood film and morphology in stained thin blood film. This could account for treatment failures in the field. There are now species-specific identification

systems using DNA probes (19) and antigen capture enzyme linked immunosorbent assay (ELISA) (91) and their adaptation for field use will help determine course of treatment. The innate lack of susceptibility of *T. b. rhodesiense* to DL \square -DFMO (92). Out of the twelve Trypanozoon stocks isolated by (74) 3 were resistance to both standard and higher doses of Berenil and 2 were consistently resistant to both higher doses of Samorin and the trypanolytic action of human plasma.

Routine testing for drug resistance for *T. brucei* and *T. congolense* that are infective to rats and mice could not be carried out simply because of cost. Classically this involves using multiple drug doses to determine dose that can cure 50 percent of animals (CD50), it required at least 30 mice per drug. In order to overcome this limitation, (11) developed a simplified protocol for trypanocidal drug sensitivity testing using just 3 doses for isometamidium (0.1, 1.0 and 10 mg/kg body weight or diminazene (1, 20 and 40 mg/kg b.w.). This innovation cannot be applied to *T. vivax*, which is not infective to small laboratory animals. A solution to this problem has already be found with the use of the KIVI as elaborated above under the drug resistance.

Cryptic Infections

Trypanosoma brucei subspecies have propensity for tissue invasiveness (93) with disease progressing from haemolymphatic to central nervous system CNS involvement (94, 95, 96, 97). Unexplained cause of CNS involvement by the less tissue invasive species have been observed in *T. vivax* (98) and *T. congolense* (99) infected cattle, *T. vivax* infected goats (100) and *T. congolense* infected calves, sheep and goats (101) and *T. simiae* infected pigs (102). It could be as a result of the drug action with or without host immune responses. This calls for follow-up after treatment, the practice restricted to human African trypanosomosis. Phenanthridium, isometamidium, diamidine and ethidium drugs clears trypanosomes from brain, including choroids plexus and interstitium (103) not when parasite have transversed the capillary wall surrounded by layer of glial cells

(blood-brain) and the choroids plexus epithelial (blood-CSF) barriers. Only trypanamide, melaminyl arsenicals, nitrofurans and DFMO can penetrate these barriers in required cidal levels. Early treatment is the panacea to prevent CNS involvement (102). The poor economic position of majority of small-scale pastoralist and endemic countries can not guarantee desired regular systematic surveillance. Most research into control of the disease in endemic sub-Sahara Africa countries still remains a service for public good.

Parasites are shielded from drug cidal action not only in the brain as previously believed (100) with *T. vivax* disseminated in the aqueous humour of the eye. The ability of drugs to clear parasites from this site remained to be investigated. With transplacental transmission (104, 105) ability of existing drugs to clear parasite from an infected foetus have to be examined. Chancre at maximum size on treatment results in relapse (106). Presence of abnormal trypomastigote stages, amastigote and sphaeromastigote (“Latent bodies”) remains controversial as it is seen only in *T. brucei* infected mice (107, 108, 109) and not in others (95, 110, 111). The reported intracellular (cerebral intraepithelial) localization of the stages in some ependymal cells of the choroid plexus (112, 113) and ventricular ependymal cells of rats (114) have been criticized. (115) had cautioned that cerebral extravascular and extracellular persisters can be confused for intracellular trypanosomes which can not be differentiated using homogenate transfer experiment. The size of these stages is large compared to protein molecules which permeate the plasma membrane (95). The sensitivity of these stages is unknown (109) and absent in *T. vivax* and *T. congolense*, so is likely a transient stage in *T. brucei*.

Bioavailability Of Drug

Drugs are distributed and disposed differently in various tissues or organs. A situation where cidal levels is not attained as reported for adipose tissues, spleen, skeletal muscle and lungs 4 and 12 weeks after

isometamidium treatment of experimental goats (116) and tissue from cattle slaughtered at abattoir (117) makes studies into their sequential drug levels imperative. Imaging data from mouse models of infection suggest that trypanosomes sequester to major organs such as the spleen, liver and brain (118, 119) and recent evidence has demonstrated trypanosomes in extravascular adipose tissue (120). These adipose-associated trypanosomes appear to be a new life-cycle stage with a distinct transcriptional profile. Bioavailability of intramuscular dose of 58% and major route of excretion was in faeces. Approximately 80% of intravenous dose given was excreted within 21 days, of which only 18% was through urine. Total residue is said to account for 15% of dose given. Drug residues remained high in organs with excretory functions, including the liver and kidney. The reason for relapses in *T. b. gambiense* epidemics were not known (58). Recently, the role of the skin as reservoir for trypanosomes in both animal and human infection has been reported (121). There are descriptions of cutaneous symptoms associated with African trypanosomiasis and distinct ‘trypanid’ skin lesions (122). The presence of trypanosomes in the skin matrix reported by (123) has shown the broader role of skin-dwelling trypanosomes in transmission remains unclear. The report of the investigation carried out by (121) on a possible anatomical reservoir in the skin of the mammalian host provide conclusive evidence of *T. b. brucei*, (a causative agent of animal trypanosomiasis) and the human-infective trypanosome, *T. b. gambiense*, invading the extravascular tissue of the skin (including but not restricted to the adipose tissue) and undergoing onward transmission despite undetected vascular parasitaemia. They suggested that other parasite-related factors might be involved, e.g. affinity to extravascular sites other than the CNS which are less accessible to the drug. Moreover, a combination of factors rather than a single one may be responsible for the phenomenon of melarsoprol treatment failures in *T. b. gambiense* patients.

The pharmacokinetic and pharmacodynamic study of trypanocides which hitherto received little attention due to lack of good analytical tools will now depend on available modern biochemical techniques such as high performance liquid chromatography (HPLC), mass spectrometry, nuclear magnetic resonance (NMR) and paired ion extraction (124). These techniques have allowed not only pharmacokinetic and pharmacodynamic study of existing drugs; they are also useful to examine inter-subject variation in drug response and the influence of diet and other extraneous factors on drug bioavailability (125, 126).

Moreover, there are variations in levels of drugs between species of host as demonstrated by higher isometamidium levels in goats than sheep after seven days of drug administration. This is an indication that isometamidium metabolism differs between the sheep and goats (127). There is prospect in the use of ELISA to measure drug levels in tissues as reported for plasma isometamidium and Mel B concentration (128-130) for monitoring drug resistance in the field. Also, determination of Berenil (131) and homidium (bromide) in serum of treated animals (132, 133) will be invaluable in investigating drug distribution which is an important factor to determine complete parasite clearance. Basically, the drug in the test samples and the developed drug-horseradish peroxidase compete for antibodies to the drug raised in rabbit and immobilized on microtiter plates. The method has been shown to be highly repeatable and reproducible with several advantages over the previous assay techniques for the drug. According to (134) it may not be possible to maintain constant drug concentrations in serum of patients as was the case with *in vitro* experiments. The authors found that minimum inhibitory concentration (MIC) of 0.072 microg/ml exhibited by one of the isolates from Northwest Uganda was above levels attainable in CSF indicating that this isolate would probably not be eliminated from CSF of treated patients. On the hand, cure was defined as absence of trypanosomes in all body fluids and ≥ 20 leucocytes in CSF at 22 months post-treatment; and relapse as

trypanosomes detected in any body fluid or ≥ 50 CSF leucocytes/mL anytime (135).

A central issue in human African Trypanosomosis (HAT) treatment is diagnosing the stage of the disease. Examination of CSF plays an essential role in the diagnosis, selection of treatment and post-treatment follow-up (136). The cut-off point of white blood cell count in HAT has been set at five cells/ μ L in CSF. At any time point, the criterion @ trypanosomes present and/or a cerebrospinal white blood cell count $>$ or $=50/\mu$ L allowed accurate timely detection of HAT relapse, irrespective of the disease stage (137). Patients with cell counts higher than 5/ μ L are considered in the meningo-encephalitic stage are recommended to be treated by WHO (138). Prioto et al. (139) defined second stage as finding of trypanosomes in blood, lymph nodes or CSF, with ≥ 20 leucocytes/ μ L in CSF. Pentamidine and Merlasoprol are the two main front line drugs used for treatment of first or haemolymphatic and central nervous system (CSF) or second stage infection, respectively (140). Merlasoprol is an arsenical derivative that required hospitalization for a 10 day short course and 25-36 days long treatment regime (140, 141). Difloromethylornithine (DFMO) or eflornithine is less toxic and proof more effective in the treatment of second stage HAT. It had an adjusted risk of death of 0.2% and only fewer cutaneous and neurologic adverse effect than patients treated with merlasoprol (142). There is no clear cut agreement on the level of CSF white blood cell. Although Doua et al. (143) study in Côte d'Ivoire showed that Pentamidine was found to be effective in "intermediate" cases between 6 and 20 WBC cells/mm³ in CSF. On the contrary, (144) in Uganda noted there was a higher risk of relapse for these patients treated with pentamidine, they thereby recommended a value of 10 cells/mm³ as the cut off. According to (145) pentamidine treatment of patients having CSF with WBC of 6-10 cells/mm³ did not result in relapse. A non-invasive approach such as the assay of serum anti-myelin antibody assay using ELISA (146) need to be evaluated to replace and/or complement routine lumbar puncture

for CSF analysis.

Historically, a relapse rate of 5 per cent is observed in patients treated with melarsoprol, an arsenical derivative used for both late *T. b. gambiense* and *T. b. rhodesiense*. Relapse rate of upto 30 percent were recorded in Angola, Sudan and Ugandan (147). Ticket rats *Grammomys surdaster* were more susceptible to *T. b. gambiense* and grow faster after inoculation than in typical laboratory rodents. A simple and sensitive test for identification of parasites resistant to melarsoprol, which are defective in plasma membrane transporter responsible for drug uptake will be help in case management (148). The same transporter carries the fluorescent dimidine DB99 (2, 5-bis-(4-amidinophenyl)-3, 4-dimethylfuran) into trypanosomes. The nucleus and kinetoplast begin to fluoresce within 1 minute of introduction of DB99, unless the trypanosome is drug resistant. According to (149), the cumulated incidence of relapse among patients who attended at least one follow-up visit 1 year after discharge was 8.1% (11/136) for those treated with eflornithine, 14% (36/258) for those treated with standard melarsoprol and 15.5% (9/58) for those treated with short course melarsoprol.

Pharmacokinetics studies in patients by (150) showed that oral DFMO at the dose of 125 mg/Kg body weight given every 6 h for 14 days may not produce adequate therapeutic plasma and CSF levels in late stage *T. b. gambiense* sleeping sickness. A case of resistant to DFMO in a 5 years old girl with high CSF cell count (313.4/mm³) was successfully treated with melarsoprol (151). In such situation, combining two or three drugs in the management of HAT cases proved highly effective. This brings to the fore the need for judicious application of sanative treatment. Pepin (65) has defined endpoint for monitoring after treatment of early or late-stage Gambian trypanosomiasis, patients should be followed with a lumbar puncture every 6 months for 2 years. Patients whose symptoms have not improved 3 months after treatment should have a lumbar puncture then, without waiting any longer. Similarly, patients who develop symptoms compatible

with a relapse (headaches for more than 2 weeks, somnolence) between the routine interval should have a lumbar puncture sooner. Two years after treatment, the incidence of relapses is not higher than that of new cases among other inhabitants of the same foci and it is not useful to perform systematic lumbar punctures any more. The authors are of the view that in *T. b. rhodesiense* trypanosomiasis, relapsing cases progress more rapidly so that lumbar puncture should be performed every 3 months during the first year, and every 6 months during the second year. This definition is similar to that defined relapse (or failure to have been cured at 1 year) as: death due to any cause after discharge, recurrence of parasites in any body fluid, white cell count in cerebrospinal fluid >50 cells/mm³ and at least doubled from the previous measurement, or white cell count in cerebrospinal fluid 20–49 cells/mm³ with a significant increase from the previous measurement and/or symptoms suggestive of disease. Jennings et al. (152) has proven that there is no doubt that the brain is a major source of relapse was experimentally demonstrated in mice.

Conclusion

The control/eradication of the disease will rely on integrated approaches including those targeted at eliminating relapse. The following areas deserve due attention: (a) Adaptation of in vitro assays and culture system for field use to facilitate identification of isolates to guide the course of treatment, provides easy drug sensitivity test and studies into drug-parasite interaction. (b) The prevailing poor economic situation in endemic countries necessitate call for external support from developed countries and donor agencies like World Health Organization, WHO and Food and Agricultural Organization, FAO in form of funds and inputs to sustain regular systematic surveillance, which is the panacea to early case detection and treatment before CNS involvement sets in. (c) A standardized protocol to ascertain the threshold for early CNS involvement in determining late stage drug

administration is very pertinent in reducing frequent occurrence of relapse and associated increase in mortality rate. The rate of relapse is higher when treatment is administered without benefit of diagnosing CNS involvement. (d) In the absence of new drugs, management of existing ones through established drug policy is a matter of great concern. Enforce policy that will discourage the indiscriminate use of two drugs interchangeably as “Sanative” pairs, periodic change of drug and drug synergy have been advocated as ways by which effective value of these drugs can be enhanced.

All the above constitute short-and medium-term approach to curtail frequency of occurrence of relapse. Intensified research effort in screening for novel therapeutic compounds will lead to emergence of ideal trypanocides with therapeutic and broad spectrum activity, with little or no toxicity and effective orally against both stages, will overcome limitation to control imposed by treatment failure. The recent development of NECT in 2009 by the Drug for Neglected Diseases initiative (DNDi), Médecins Sans Frontières/Doctors without Borders (MSF), and partners. NECT replaced an old, arsenic-based medicine, and today the vast majority of all late-stage sleeping sickness patients receive this combination as first-line treatment. Secondly, the announcement of success in the Phase I study, conducted in France, that assessed the safety, tolerability, pharmacokinetics and pharmacodynamics of SCYX-7158 after single oral ascending doses in 128 healthy human volunteers of sub-Saharan origin is highly welcome. It allowed for the therapeutic dose to be determined at 960 mg administered once as three tablets, with a favourable safety profile. As the drug has a long half-life (400 hours), the study was extended to ensure extensive safety monitoring of the healthy volunteers up to 210 days. This pharmacological finding has the advantage of translating into prolonged exposure with just one dose. With this development, there seems to be bright prospect for future drive in effective control of HAT by overcoming the serious limitation posed by relapse.

Competing Interest:

The author declared there is no personal competing interest neither from the affiliate institution.

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PHENOTYPIC DIVERSITY AND PHYLOGENETIC RELATIONSHIP BETWEEN THE BAKOSI/BAWERI AND OTHER PIG BREEDS (*SUS SCROFA DOMESTICUS*) IN THE HUMID FOREST WITH MONOMODAL RAINFALL AGRO-ECOLOGICAL ZONE OF CAMEROON.

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Abstract

The present study was conducted from April to May 2017 in the humid forest with monomodal rainfall (agro-ecological) zone of Cameroon with the following geographical coordinates: 44°10'00"-5°50'00"LN and 9°10'00"-9°30'00"LE. The objective was to describe and determine the morphology, biometric characteristics, prediction equation of live weight and the genetic variability of this pig population of the humid forest with monomodal rainfall agro-ecological zone of Cameroon. For this purpose, a data collection scope of 19 traits (quantitative and qualitative) was conducted on a total of 208 pigs including males and females from two divisions and six sub-divisions of the South West Region of Cameroon. The main results show that the black coat colour (44.7%) is dominant with majority of pigs having black (51.06%) skin pigmentation. Moreover, majority of pigs are rectilinear (48.6%) with large (53.4%) erect (48.1%) ears which are mostly oriented forwards (39.9). The pigs are mostly docile (53.8%) and have curly tails (55.3%). The main body measurements (in cm) gave the following values: body length (87.28±2.18), heart girth (79.59±1.89), height at withers (58.36±1.22), eye distance (16.85±0.30), ear length (21.48±0.55), head length (30.00±6.59), snout length (13.60±0.30), hock circumference (20.36±0.47), tail length (27.37±0.63) and the average live weight (in kg 61.56±5.03). The hearth girth best predict the live weight of pigs in the humid forest with monomodal rainfall agro-ecological zone of Cameroon. The polynomial equations with highest coefficients ($LW = 0.039HG^2 - 6.259HG + 305.7$ $R^2=0.930$, $LW = 0.028HG^2 - 3.498HG + 132$ $R^2=0.899$) best predict the live weight of the exotic and crossbreeds respectively while the power equation with the highest coefficient ($LW = 3E-06HG^3.782$ $R^2=0.229$) best predict that of Bakosi/Bakweri breeds. The principal component analysis (PCA) revealed that the first three components explain 77.34% of the genetic variability in the studied population. The discriminant analysis (DA) suggested that the population is made of three genetic types (I, II and III) with genetic type III having the highest characteristics. The dendrogram showed that type I and II are closest and type II and III are genetically more distant. In conclusion, the genetic variability obtained within the population offers possibilities for their genetic improvement by convention methods of selection and crossing.

Key words: biodiversity, biometric characteristics, Cameroon, pig.

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Introduction

Pig is a livestock of great importance with huge potential (Ndebi et al. 2004; Ndebi and Ongla, 2006; Bime et al., 2014) because of its high prolificacy and feed efficiency (Devendra and Thomas, 2002). Pig production is one of the most important agro-businesses of rural dwellers in Cameroon (Manjeli et al., 1992; Devendra et al., 2000). It directly influences the socio-economic status and acts as living capital and insurance to the most deprived populations.

Smallholder pig production in Cameroon is mostly under the semi-intensive and/or scavenging management system with occasional castration (Keambou et al., 2010; Ritchill et al., 2013, Defang et al., 2014). This system is common in the industrial plantation zones of the South West Region of Cameroon, where pigs show diverse phenotypic attributes (Njoya et al., 1996). There has been no in-depth investigation carried out to characterize nor to evaluate the phenotypic and performances of indigenous pig breeds in Cameroon, in spite of the fact that they continue to thrive under poor management and harsh climatic conditions (Subalini et al., 2010 Borkotoky et al., 2014). Despite the decreasing trend of pigs in Cameroon, the indigenous or native breed still represent a valuable component of local genetic resources that has to be conserved for future improvement (Subalini et al., 2010).

The indigenous pig in Cameroon is almost in extinction. This decline has been reported by Devendra (1980) and Eusebio (1980). The need to maximize profits has resulted in farmers diverting their interest to rear hybrids of exotic pig breeds which are said to be highly productive. Hence, evaluating and assessing the phenotypic variation among indigenous pig populations in Cameroon will serve as a base line study for the conservation and improvement of the pig industry in Cameroon. This is important to identify the uniqueness of populations and the possible gene flow between wild, local domesticated and exotic pig populations. Further, as there is diversity in the pig population across the

country, the production system also varies from one farmer to another making it very difficult to characterize the pig population as reported by (Meffeja, 2006) and (FAO, 2008). The global objective of this study is to contribute to a better understanding of indigenous pig biodiversity in Cameroon with the view of developing conservation and genetic improvement strategies.

Material and methods

Site characteristics

This study was conducted from April to May 2017 in the South West Region of Cameroon (figure 1). The motivation is to retrace the indigenous Bakosi/Bakweri pig biodiversity in this agro-ecological zone.

Household and animal level survey

A total of 45 households' were selected and surveyed using a snow ball test. The selection of these households was also done with the help of the local extension services of the Ministry of livestock, fisheries and animal industries of the South-West Region and local authorities. The animal level survey was adapted from that of Lesnoff et al., (2010) designed to estimate various demographic parameters in tropical livestock populations, and the phenotypic characterization using FAO (2013) livestock descriptors. A total of 208 mature pigs were sampled (110 females and 98 males) across six sub-divisions of the Region. These pigs were chosen randomly in the selected herds.

Phenotypic characterization of pig populations

The phenotypic traits were observed and recorded by visual observation and body measurements. Descriptive traits included breed, age, Coat colour, skin pigmentation, ear orientation, ear size, tail shape, head shape, ear type and hair. Biometrical characterization included information on body length (BL), ear length (EL), head length (HL), heart girth (HG), height at withers (HW), snout length (SL), tail length (TL), pelvic width (PW), hock circumference (HC) and the eye distance (ED).

All animals were measured standing symmetrically on a flat solid surface using a measuring tape. The description of these measurements is presented in table 1 and illustrated by figure 2.

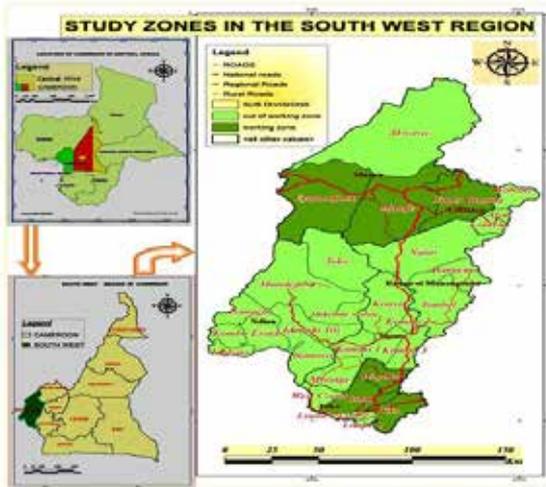


Figure 1: Map of the South West Region. Source: National Institute for Cartography - Cameroon

Table 1: Body measurements of pigs

Trait	Description
Body length (BL)	Distance from the external occipital protuberance to the base of the tail on the dorsal line; distance between tip of scapula and ischium, measured as the distance between the point of shoulder and the pin bone
Eye distance (ED)	Inter orbital distance
Ear length (EL)	From central point of the base to the vertex; from the base of the notch to the most distant point of the margin of the pinna (external ear)
Head length (HL)	From the external occipital protuberance occipital to tip of nasal bone
Heart girth (HG)	Total distance around the animal (circumference) measured directly behind the front leg; total distance around the animal (circumference) measured directly behind the front leg;
Height at withers (HW)	Distance from the surface of a platform to the top of the shoulder
Snout length (SL)	Tip of the nasal bone to coronal suture; From the frontal-nasal suture to the point of the snout
Tail length (TL)	From insertion of the tail to the tail tip
LW	$(HG*HG*BL)/400$

The biometric indices were calculated from body measurements as described by Lauvergne (1993) and Bourzat et al. (1993):

- Atria index (AI): $AI = EL/HW$, is the length of the ear (EL) over the height at the withers (HW)
- Size index (SI): $SI = BL/HW$; is the body length (BL) over the height at withers (HW)
- Compactness index (CI): $CI = HG/BL$, is the hearth girth(HG) over the body length (BL)
- Mass index (MI): $MI = HG/HW$, is the hearth girth (HG) over the height at withers (HW)
- Skeleton index (Skl): $Skl = HC/HW$, is the hock circumference (HC) over the height at withers (HW).
- Dactylo-thoracic index (DTI): $DTI = HC/HG$, is the hock circumference (HC) over the heart girth (HG)
- Caudal index (Col): $Col = TL/HW$, is the tail length (TL) over the height at withers (HW)

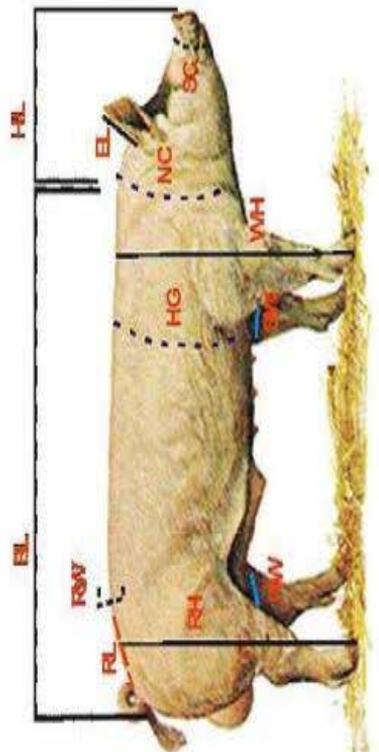


Figure 2: body measurements of pigs, Source: Adeola et al., (2013)

Data analysis

The morphological traits were analyzed using descriptive statistics. The biometric variables were subjected to a one way ANOVA analysis. Distances between breeds were carried out using morphology and measurements by sex, calculating dissimilarity and distance using UPGMA (Unweighted Pair Group Method Arithmetic Mean) to generate the dendrogram. The type and degree of association of quantitative parameters were tested by the Pearson correlation coefficient at the 1% and 5% significant level. The principal component analysis (PCA) helped to determine the level of similarity and differences among individuals of the population as well as their genetic variability. Discriminant analysis (DA)

was applied to determine the structure of the population. The relation between the different genetic types in this population was established to construct the phylogenetic tree following the protocol of the hierarchical ascending classification. All these analysis were done using SPSS 20.0 and XLSTAT.

Results and Discussion

Morphological characteristics of pigs in the humid forest with monomodal rainfall agro-ecological zone of Cameroon

Table 2 summarises the morphological characteristics of pigs in the humid forest with monomodal rainfall agro-ecological zone of Cameroon.

Table 2: Frequency distribution of qualitative traits of pigs

Parameters	Characteristics	Sample size	Frequency
Coat colour	White	35	16.8
	Black	93	44.7
	Dark red	14	6.7
	Pink	16	7.7
	Spotted	50	24.0
	Total	208	100.0
Skin pigmentation	White	63	30.3
	Black	106	51.0
	pink	22	10.6
	Dark red	17	8.2
	Total	208	100.0
Head shape	Concave	76	36.5
	Straight	101	48.6
	Convex	31	14.9
	Total	208	100.0
Ear type	Droopy	79	38.0
	Erect	100	48.1
	Semi-lop	29	13.9
	Total	208	100.0
Ear orientation	Forwards	83	39.9
	Backwards	47	22.6
	Upwards	78	37.5
	Total	208	100.0

Parameters	Characteristics	Sample size	Frequency
Ear size	Small	48	23.1
	Medium	49	23.6
	Large	111	53.4
	Total	208	100.0
Tail shape	Straight	93	44.7
	Curly	115	55.3
	Total	208	100.0

There is a phenotypic variability among pigs in the humid forest with monomodal rainfall agro-ecological zone of Cameroon. Five different coat colours are observed with black (44.7%) and spotted (24.0%) being the most abundant. The skin pigmentation of pigs is mostly black (51%) and white (30.3%) while

most of them are rectilinear (48.6%). Majority of the pigs has large ears (53.4%). The ears are mostly erected (48.1%), droopy (38.0) and are predominantly oriented forwards (39.9%). The short and dense (40.4 %) hair structure is abundant in the pig population. The pigs mostly have a straight tail shape (55.3%).



Typical mature Bakosi/bakweri sow



Pre-mature Bakosi/bakweri sow



Crossbreed



Red mankon sow



Red mankon boar



Crossbreed



Exotic breeds piglets



Exotic mature sow

Figure 3: Few pig phenotypes from the humid forest with monomodal rainfall agro-ecological zone of Cameroon

Biometric characteristics of pig's population of the humid forest with monomodal rainfall agro-ecological zone of Cameroon.

Body measurements.

Body measurements with respect to localities

Table 3 gives records of the mean values of the body measurements of pigs' populations according to the localities in the humid forest with monomodal rainfall agro-ecological zone of Cameroon. The analysis of variance showed that the locality significantly affect ($P < 0.05$) the body measurements.

Pigs of Mamfe have higher body measurements than those of other localities. Irrespective of the head length, snoot length and hock circumference which have higher coefficient of variation in Eyumojock, all the other body measurements had higher coefficients in Upper-Bayang making the pigs of this locality to be more diversified. The ear distance had no significant ($P > 0.05$) effect with respect to localities.

Body measurements with respect to breeds

The analysis of variance showed that the breeds significantly affect ($P < 0.05$) the body measurements. The table 4 gives record of the mean values of the body measurements of pigs' populations according to the breeds in the humid forest with monomodal rainfall agro-ecological zone of Cameroon.

The exotic pigs have the highest body length (96.81cm) with the highest coefficient of variance. Also, they record the highest values in all the other body measurements when compared with the other breeds. However, there is no significant difference ($p \leq 0.01$) between the pigs with respect to the eye distance, ear length and hock circumference. Also, all the body measurements (except the eye distance) of cross breeds are higher than those of Bakosi/Bakweri pigs.

Pearson's correlation between body measurements of pigs

Table 5, 6 and 7 present respectively correlation coefficients between body

measurements for the general pig population, for Bakosi/Bakweri and exotic pigs, and for crossbreed pigs in the humid forest with monomodal rainfall agro-ecological zone of Cameroon

In general, the body measurements are positively and significantly ($p > 0.01$) correlated among each other. The live weight correlates positively and significantly ($p > 0.01$) with all the other body measurements. However, the highest correlation which is positively significant ($p > 0.01$) is that between the live weight and the heart girth ($r = 0.87$), followed by the correlation with the body length ($r = 0.83$).

Considering the breeds, all the correlation coefficient between body measurements are positive whatever the breed. There exist many significant correlations and most of them vary from weak to moderate. However, whatever the breed, the highest significant correlation coefficient was found between the live weight and the height at withers ($r = 0.90$ to 0.92) and between the live weight and the body length ($r = 0.82$ to 0.88).

Body indexes of pigs.

Body indexes with respect to localities

Table 8 gives record of the Means and coefficient of variation of the body indexes of pigs' populations according to locality in the humid forest with monomodal rainfall agro-ecological zone of Cameroon.

The mean size index of the studied pigs is 1.51 with a variation coefficient of 20.51%. The Mamfe locality has the significantly ($p > 0.05$) highest value while Eyumojock has the lowest, the later pigs being also more variable for this index. The compactness index has a mean value of 0.94 with the highest value found in Tiko and the lowest in Upper-Bayang. The mean value of the mass index is 1.39 with a variation coefficient of 23.02%. The lowest index is found in Buea and the pigs of this locality are more variable. The skeletal index has a mean value of 0.36. The pigs of Tiko and Upper-Bayang both have the same value (0.37) which the highest. But when compared with respect to their variation coefficient, the pigs of Upper-

Table 3: Means and coefficient of variation of the body measurements of pigs according to the Localities in the humid forest with monomodal rainfall agro-ecological zone of Cameroon

Traits	Localities					Total n=208	
	Tiko n=91	Buea n=10	Muyuka n=40	Eyumojoek n=20	Mamfe n=10		Upper-Bayang n=37
BL(cm)	81.21±3.12 ^a (31.63)	87.00±9.41 ^a (36.63)	92.20±4.70 ^a (34.74)	75.85±6.65 ^a (25.31)	128.40±9.41 ^b (36.64)	92.05±4.89 ^a (37.73)	87.28±2.18 (35.94)
HG(cm)	82.30±2.74 ^a (27.07)	75.70±8.27 ^a (19.89)	76.10±4.14 ^a (38.04)	69.75±5.85 ^a (29.75)	113.80±8.27 ^b (35.18)	73.84±4.30 ^a (42.98)	79.59±1.89 (34.31)
HW(cm)	56.46±1.84 ^b (27.68)	62.40±5.55 ^{ab} (25.71)	59.25±2.78 ^a (33.91)	56.90±3.92 ^a (30.05)	71.60±5.55 ^b (28.18)	58.16±2.89 ^a (32.67)	58.36±1.22 (30.26)
ED (cm)	16.03±0.44 ^a (25.45)	16.40±1.34 ^a (31.52)	16.93±0.66 ^a (24.47)	17.75±0.95 ^a (23.77)	19.00±1.34 ^a (36.35)	17.81±0.70 ^a (42.91)	16.85±0.30 (25.34)
EL (cm)	19.44±0.79 ^a (31.33)	23.40±2.37 ^{ab} (40.17)	24.85±1.18 ^b (32.11)	19.95±1.67 ^{ab} (31.18)	29.90±2.37 ^c (36.35)	20.86±1.22 ^{ab} (42.91)	21.48±0.55 (36.82)
HL (cm)	29.12±0.89 ^a (22.05)	28.50±2.67 ^a (17.30)	29.65±1.34 ^a (26.27)	31.80±1.89 ^a (48.40)	37.20±2.67 ^b (24.19)	30.00±1.39 ^a (29.80)	30.00±0.59 (28.47)
SL(cm)	12.54 ± 0.42 ^a (27.27)	13.80 ± 1.28 ^a (22.90)	14.22 ± 0.64 ^a (28.01)	14.30 ± 0.90 ^a (32.10)	18.30 ± 1.28 ^b (39.07)	13.81 ± 0.66 ^a (31.21)	13.60±0.30 (30.88)
TL(cm)	28.07±0.93 ^a (31.14)	30.80±2.80 ^a (22.18)	25.13±1.40 ^a (36.35)	25.15±1.98 ^a (36.65)	36.60±2.80 ^b (25.14)	25.87±1.46 ^a (34.13)	27.37±0.63 (33.32)
PW(cm)	37.96±0.23 ^{ab} (37.14)	43.10±0.24 ^b (17.75)	40.58±0.19 ^{ab} (30.48)	32.55±0.27 ^a (28.39)	51.70±0.35 ^c (25.74)	34.43±0.32 ^{ab} (37.53)	38.22±0.93 (35.00)
HC (cm)	20.07 ± 0.71 ^{ab} (32.44)	21.10 ± 2.16 ^{ab} (17.25)	19.42±1.08 ^a (25.64)	20.25 ± 1.53 ^{ab} (49.93)	24.60 ± 2.16 ^b (25.37)	20.81 ± 1.12 ^{ab} (30.06)	20.36±0.47 (33.60)
LW(kg)	46.02 ±5.65 ^a (78.44)	38.03±17.05 ^a (65.08)	50.54±8.53 ^a (95.96)	29.83 ± 12.06 ^a (76.94)	153.49 ±17.05 ^b (84.87)	51.43 ± 8.87 ^a (114.27)	61.56± 5.03 ^a (114.29)

n = sample size, ^{a,b,c} in the same line, the means with the same superscripts show no significant difference (P ≤ 0.05) between the localities, () coefficient of variations, BL= body length, HG= heart girth, HW= height at withers, CV (%) = variation coefficient, ED= Eye distance, EL= ear length, HL= head length, SL= snout length, TL= tail length, PW= pelvic width, HC= hock circumference, LW= live weight

Table 4: Means and coefficient of variation of the body measurements of pigs according to the breeds in the humid forest with monomodal rainfall agro-ecological zone of Cameroon

Traits	Breeds			Total n=208
	Bakosi/Bakweri n=91	Exotic n=59	Crossbreed n=58	
BL(cm)	83.99±3.24 ^a (31.74)	96.81±4.03 ^b (38.46)	82.76±4.06 ^a (36.44)	87.28±2.18 (35.94)
HG(cm)	74.14±2.83 ^a (33.41)	84.76±3.52 ^{ab} (37.75)	82.88±3.55 ^a (29.86)	79.59±1.89 (34.31)
HW(cm)	55.66±1.82 ^a (33.56)	64.20±2.26 ^b (26.53)	56.64±2.28 ^a (27.19)	58.36±1.22 (30.26)
EL (cm)	18.75±0.76 ^a (32.27)	26.36±0.95 ^b (34.75)	20.79±0.95 ^a (32.90)	21.48±0.55 (36.82)
ED(cm)	16.88±0.45 ^a (24.05)	17.36±0.56 ^a (25.00)	16.28±0.56 ^a (26.23)	16.85±0.30 (25.34)
HL (cm)	28.60±0.89 ^a (36.01)	31.96±1.10 ^b (20.59)	30.22±1.11 ^{ab} (22.44)	30.00±0.59 (28.47)
SL(cm)	13.69±0.44 ^a (31.33)	13.97±0.55 ^a (30.49)	13.06±0.55 ^a (30.63)	13.60±0.30 (30.88)
TL(cm)	23.47±0.89 ^a (29.36)	30.19±1.11 ^b (32.20)	30.62±1.11 ^b (30.63)	27.37±0.63 (33.32)
PW(cm)	33.68±1.35 ^a (37.35)	41.71±1.67 ^b (27.12)	41.79±1.68 ^b (34.84)	38.22±0.93 (35.00)
HC (cm)	19.70±0.72 ^a (37.11)	21.31±0.89 ^a (34.63)	20.43±0.80 ^a (33.48)	20.36±0.47 (33.60)
LW(kg)	41.85±6.05 ^a (123.23)	41.85±6.05 ^a (111.17)	67.03±7.5 ^b (93.29)	61.56± 5.03 ^a (114.29)

n= sample size, a, b, c in the same line, the means with the same superscripts show no significant difference ($P \leq 0.05$) between the localities, () coefficient of variance, BL= body length, HG= heart girth, HW= height at withers, ED= Eye distance, EL= ear length, HL= head length, SL= snoot length, TL= tail length, HC= hock circumference, PW= pelvic width, LW= live weight

Bayang have the highest value. Nevertheless, no significant difference ($p \leq 0.05$) is observed among all the localities for this index.

The dactylo-thoracic index (DTI) has a mean value of 0.28 with a variation coefficient of 64.29%. The highest variation coefficient is recorded in Upper-Bayang locality. This implies that the pigs here are more diversified for this index compared to those of Muyuka. However, no significant difference ($p \leq 0.05$) is observed in the different subdivisions. The mean value of the caudal index is 0.48 with a variation coefficient of 27.08%. Just like the dactylo-thoracic index, no significant difference is observed among

all the localities. However, the pigs of Tiko are more heterogeneous, followed by those of Eyumojock. The atria index has a mean value of 0.30 with a variation coefficient of 23.33. The highest variation coefficient is found in Muyuka locality and the lowest in Eyumojock.

Body indexes with respect to breeds

Table 9 gives record of the mean values of the body indexes of pig population according to the breeds in the humid forest with monomodal rainfall agro-ecological zone of Cameroon.

Table 5: Overall correlation coefficients between body measurements of pigs in the humid forest with monomodal rainfall agro-ecological zone of Cameroon.

	BL	HG	HW	ED	EL	HL	SL	TL	PW	HC	LW
BL	1										
HG	0.76**	1									
HW	0.81**	0.70**	1								
ED	0.73**	0.60**	0.74**	1							
EL	0.67**	0.48**	0.69**	0.62**	1						
HL	0.60**	0.62**	0.70**	0.59**	0.50**	1					
SL	0.71**	0.61**	0.76**	0.69**	0.70**	0.66**	1				
TL	0.53**	0.65**	0.59**	0.52**	0.43**	0.56**	0.47**	1			
PW	0.61**	0.59**	0.71**	0.43**	0.60**	0.53**	0.59**	0.52**	1		
HC	0.40**	0.47**	0.53**	0.46**	0.40**	0.41**	0.48**	0.47**	0.40**	1	
LW	0.83**	0.87**	0.64**	0.52**	0.50**	0.58**	0.60**	0.51**	0.53**	0.38**	1

** = $P \leq 0.01$, BL= body length, HG= heart girth, HW= height at withers, ED= eye distance, EL= ear length, HL= head length, SL= snout length, TL= tail length, PW= pelvic with, HC= hock circumference, LW= live weight.

Table 6. Correlation coefficients between body measurements of Bakosi/Bakweri (below diagonal) and exotic (above diagonal) pigs in the humid forest with monomodal rainfall agro-ecological zone of Cameroon

	BL	HG	HW	ED	EL	HL	SL	TL	PW	HC	LW
BL	1	0.63**	0.84**	0.47**	0.68**	0.67**	0.78**	0.28	0.77**	0.42*	0.82**
HG	0.91**	1	0.30	0.07	0.16	0.65**	0.46**	0.47**	0.69**	0.50**	0.92**
HW	0.75**	0.72**	1	0.62**	0.78**	0.70**	0.74**	0.15	0.57**	0.32	0.520**
ED	0.56**	0.52**	0.62**	1	0.53**	0.29	0.51**	0.23	0.13	0.21	0.20
EL	0.41**	0.28*	0.52**	0.50**	1	0.46**	0.71**	0.12	0.42*	0.32	0.39*
HL	0.66**	0.67**	0.54**	0.57**	0.31*	1	0.69**	0.32	0.69**	0.50**	0.71**
SL	0.53**	0.45**	0.63**	0.61**	0.73**	0.44**	1	0.28	0.57**	0.40*	0.69**
TL	0.31*	0.36**	0.44**	0.54**	0.23	0.62**	0.22	1	0.23	0.37*	0.41*
PW	0.53**	0.46**	0.55**	0.42**	0.72**	0.39**	0.55**	0.30*	1	0.53**	0.77**
HC	0.11	0.04	0.23	0.36**	0.17	0.10	0.29*	0.28*	0.02	1	0.51**
LW	0.88**	0.90**	0.57**	0.39**	0.19	0.68**	0.34**	0.26	0.39**	0.01	1

** = $P \leq 0.01$, * = $P \leq 0.05$, BL= Body length, HG= heart girth, HW= height at withers, ED= ear distance, EL= ear length, TL= tail length, HL= head length, SL= snout length, PW= pelvic width, HC= hock circumference, LW= live weight.

The analysis of variance showed that the size index, skeletal index, dactyl-thoracix index are not significantly affected ($P \leq 0.05$) by the breed effect.

The Bakosi/Bakweri pigs have the highest atria index, while crossbreeding significantly ($P > 0.05$) improves the compactness, massivity and caudal indices.

Pearson's correlation between body indexes of pigs in the humid forest with monomodal rainfall agro-ecological zone of Cameroon.

Table 10, 11 and 12 show the correlation coefficients between the biometric indices of pigs' respectively in a general populations, in Bakosi/Bakweri, and in crossbreeds from the humid forest with monomodal rainfall agro-ecological zone of Cameroon.

Table 7. Overall correlation coefficients between body measurements of crossbreed pigs in the humid forest with monomodal rainfall agro-ecological zone of Cameroon.

	BL	HG	HW	ED	EL	TL	HL	SL	PW	HC	LW
BL	1										
HG	0.64**	1									
HW	0.67**	0.69**	1								
ED	0.73**	0.53**	0.58**	1							
EL	0.63**	0.35*	0.59**	0.51**	1						
TL	0.26	0.33*	0.30*	0.25	0.23	1					
HL	0.46**	0.55**	0.66**	0.34*	0.49**	0.45**	1				
SL	0.66**	0.56**	0.61**	0.43**	0.60**	0.33*	0.67**	1			
PW	0.36*	0.34*	0.73**	0.13	0.36*	0.10	0.46**	0.37**	1		
HC	0.03	0.34*	0.49**	0.03	0.09	0.14	0.43**	0.18	0.42**	1	
LW	0.84**	0.90**	0.70**	0.64**	0.51**	0.35*	0.61**	0.72**	0.38**	0.22	1

** = $P \leq 0.01$, BL= body length, HG= heart girth, HW= height at withers, ED= eye distance, EL= ear length, HL= head length, SL= snout length, TL= tail length, PW= pelvic with, HC= hock circumference, LW= live weight.

Table 8: Means and coefficient of variation of the body indexes of pig population according to locality in the humid forest with monomodal rainfall agro-ecological zone of Cameroon

Biometric indexes	Localities						Total n=208
	Tiko n = 91	Buea n = 10	Muyuka n = 40	Eyumojoek n = 20	Mamfe n = 10	Upper-Bayang n = 37	
SI	1.46±0.03 ^a (20.55)	1.40±0.09 ^a (25.00)	1.59±0.05 ^{ab} (18.35)	1.38±0.07 ^a (27.74)	1.76±0.09 ^b (14.20)	1.59±0.05 ^{ab} (17.61)	1.51± 0.02 (20.51)
CI	1.04±0.02 ^b (21.15)	0.95±0.06 ^{ab} (32.63)	0.83±0.03 ^a (18.07)	0.94±0.05 ^{ab} (25.53)	0.90±0.06 ^a (13.33)	0.81±0.03 ^a (20.98)	0.94±0.02 (23.40)
MI	1.48±0.32 ^{bc} (16.89)	1.25±0.10 ^a (22.40)	1.31±0.05 ^{ab} (25.19)	1.28±0.07 ^{ab} (28.91)	1.57±0.10 ^c (15.29)	1.30±0.05 ^{ab} (30.00)	1.39± 0.02 (23.02)
Skl	0.37±0.01 ^a (29.73)	0.36±0.03 ^a (30.56)	0.35±0.17 ^a (28.57)	0.36±0.02 ^a (33.33)	0.35±0.03 ^a (33.33)	0.37±0.01 ^a (35.14)	0.36± 0.01 (30.56)
DTI	0.25±0.02 ^a (28.00)	0.28±0.06 ^a (24.14)	0.27±0.03 ^a (22.22)	0.30±0.04 ^a (43.33)	0.23±0.06 ^a (30.43)	0.35±0.03 ^a (108.57)	0.28±0.01 (64.29)
Col	0.51±0.01 ^a (29.41)	0.50±0.04 ^a (18.00)	0.44±0.02 ^a (25.00)	0.45±0.03 ^a (28.89)	0.53±0.04 ^a (20.75)	0.45±0.02 ^a (26.67)	0.48± 0.01 (27.08)
AI	0.29±0.01 ^{ab} (20.69)	0.27±0.02 ^a (29.63)	0.30±0.01 ^{ab} (30.00)	0.32±0.01 ^b (12.50)	0.27±0.21 ^a (18.52)	0.32±0.01 ^b (15.63)	0.30±0.00 (23.33)

^{a, b, c} on the same row, means with the same superscripts are not significantly different ($P \leq 0.05$), n = sample size, () = coefficient of variation, SI= size index, CI= compactness index, MI= massivity index, Skl= skeleton index, DTI= dathylo-thoracic index, Col=Caudal index, AI= atria index.

Generally, the body indexes of pigs' populations from the humid forest with monomodal rainfall agro-ecological zone of Cameroon have variable types of correlations, but all of them vary from very weak negative and non-significant, to moderate strongly significant.

The highest significantly positive ($p > 0.01$) correlation coefficient is observed between the mass index and the compactness index. The highest negative significant ($p > 0.01$) correlation coefficient is observed between the dathylo-thoracic index and the massivity index.

Table 9: Means and coefficient of variation of the body indexes of pigs according to breeds in the humid forest with monomodal rainfall agro-ecological zone of Cameroon

Traits	Breeds			Total n=208
	Bakosi/Bakweri n=91	Exotic n=59	Crossbreed n=58	
SI	1.56±0.32 ^a (18.58)	1.48±0.40 ^a (19.60)	1.46±0.40 ^a (22.60)	1.51± 0.02 (20.51)
CI	0.89±0.22 ^a (15.73)	0.91±0.28 ^a (27.47)	1.05±0.28 ^b (23.40)	0.94±0.02 (23.40)
MI	1.38±0.33 ^{ab} (22.46)	1.32±0.33 ^a (27.27)	1.47±0.42 ^b (19.05)	1.39± 0.02 (23.02)
SkI	0.38±0.01 ^a (31.58)	0.34±0.14 ^a (26.47)	0.37±0.14 ^a (27.03)	0.36± 0.01 (30.56)
DTI	0.30±0.02 ^a (83.33)	0.27±0.23 ^a (29.63)	0.26±0.23 ^a (26.92)	0.28±0.01 (64.29)
Col	0.44±0.13 ^a (22.73)	0.48±0.16 ^a (27.08)	0.55±0.16 ^b (27.27)	0.48± 0.01 (27.08)
AI	0.32±0.01 ^b (21.88)	0.28±0.01 ^a (17.86)	0.30±0.01 ^a (23.33)	0.30±0.00 (23.33)

^{a,b,c} on the same row, means with the same superscripts are not significantly different ($P \leq 0.05$), n = sample size, () = coefficient of variation, SI= size index, CI= compacty index MI= massivity index, SkI= skeleton index, DTI= dacthlyo-thoracic index, Col= caudal index, AI= atria index.

Table 10: Overall correlation coefficient between body indexes of pigs' populations from the humid forest with monomodal rainfall agro-ecological zone of Cameroon

	SI	CI	MI	SkI	DTI	Col	AI
SI	1						
CI	-0.43**	1					
MI	0.48**	0.56**	1				
SI	0.123	0.22**	0.32**	1			
DTI	-0.08	-0.34**	-0.45**	0.27**	1		
Col	0.15*	0.35**	0.49**	0.33**	-0.10	1	
AI	0.45**	-0.07	0.35**	0.41**	0.01	0.27**	1

** = $P \leq 0.01$; * = $P \leq 0.05$. SI= size index, CI= compacty index, MI= massivity index, SkI= skeleton index, DTI= dactyl-thoracic index, Col= caudal index, AI= atria index

Table 11: Correlation coefficients between body indexes of Bakosi/Bakweri (below diagonal) and exotic (above diagonal) pigs in the humid forest with monomodal rainfall agro-ecological zone of Cameroon

	SI	CI	MI	SkI	DTI	Col	AI
SI	1	-0.14	0.47**	0.22	-0.21	0.10	-0.10
CI	-0.27*	1	0.80**	0.35*	-0.36*	0.55**	0.11
MI	0.78**	0.39**	1	0.47**	-0.41*	0.57**	0.06
SkI	0.23	-0.11	0.15	1	0.60**	0.36*	0.04
DTI	-0.13	-0.28*	-0.31*	0.88**	1	-0.11	0.01
Col	0.20	0.29*	0.36**	0.41**	0.25	1	0.35
AI	0.45**	0.04	0.47**	0.56**	0.30*	0.62**	1

** = $P \leq 0.01$; * = $P \leq 0.05$. SI= size index, CI= compacty index, MI= massivity index, SkI= skeleton index, DTI= dactyl-thoracic index, Col= caudal index, AI= atria index

Table 12: Overall correlation coefficient between body indexes of crossbreed pigs in the humid forest with monomodal rainfall agro-ecological zone of Cameroon

	SI	CI	MI	DTI	Col	AI
SI	1					
CI	-0.77**	1				
MI	0.33*	0.29*	1			
DTI	-0.49**	0.21	-0.45**	1		
COI	0.14	0.05	0.41**	-0.04	1	
AI	0.63**	-0.40**	0.31*	-0.32*	0.24	1

** = $P \leq 0.01$; * = $P \leq 0.05$, SI selection index, CI=compacity index, MI=massivity index, SkI= skeletal index, DTI= datylo-thoracic index, Col=Caudal index, AI= atria index.

Considering the breeds, the relations between the different body indexes are very variable, extending from weakly significantly negative to significantly highly positive.

In all the breeds, the CI-SI correlations are negative, ranging from weak on Bakosi/Bakweri ($r = -0.27^*$) and exotic pigs ($r = 0.14$) to highly significantly negative the crossbreeds ($r = -0.77^{**}$). Similar observations are made for various correlations.

The highest significantly positive correlations were that of DTI-Skl ($r = 0.88^{**}$) in Bakosi//Bakweri pigs, CI-MI ($r = 0.80^{**}$) in exotic pigs and AI-SI ($r = 0.63^{**}$) in the crossbreeds.

Prediction equations of live weight in pigs populations of the humid forest with monomodal rainfall agro-ecological zone of Cameroon.

Table 13 gives records of the different types of equations that can help predict the live weight of the different breeds with respect to body measurements.

The heart girth appears to be the best predictor of the live weight of crossbreed pigs in the humid forest with monomodal rainfall agro-ecological zone of Cameroon. The best type of equation for that prediction of the live weight is the polynomial one: $LW = 0.028HG^2 - 3.498HG + 132$ $R^2 = 0.899$.

The heart girth appears to be the best predictor of the live weight of exotic pigs. The best type of equation for that prediction of the exotic pigs' live weight is the polynomial one: $LW = 0.039HG^2 - 6.259HG + 305.7$ $R^2 = 0.930$.

It comes from the interpretation of the very low coefficient of determination that

the live weight of indigenous Bakosi/Bakweri pigs is not easily predictable from its body measurement. This denotes existence of a high variability and the ever absence of any selection programme among this population. However, despite these low values of R^2 , the heart girth will still be the best predictor of the live weight of Bakosi/Bakweri pigs. In such case, the best equation type of equation of prediction will be the power one: $LW = 3E-06HG^{3.782}$ $R^2 = 0.229$.

Genetic variability and structure of pig population in the humid forest with monomodal rainfall agro-ecological zone of Cameroon.

Genetic variability

In order to determine the genetic variability within the pig population of the humid forest with monomodal rainfall agro-ecological zone of Cameroon, a Principal Component Analysis was run based on the 11 body measurements under study. Table 14 illustrates the contribution of the 11 variables (body measurements) to the analysis of observed variability in pig's population of the humid forest with monomodal rainfall agro-ecological zone of Cameroon.

The body length is the major component determining the variability among pig population of the humid forest with monomodal rainfall agro-ecological zone of Cameroon, contribution alone to 62.99% of the variability. Additionally, the first three components (body length, heart girth and height at withers) revealed 77.34% of the total phenotypic variation observed in the studied population.

Table 13: Prediction equations of the live weight of exotic pigs in the humid forest with monomodal rainfall agro-ecological zone of Cameroon

Types of equations	Body measurements	Pigs' breeds		
		Exotic Equations	Crossbreed Equations	Bakosi/Bakweri Equations
Linear	Body length	LW= 2.188BL - 156.9	LW= 1.363BL - 63.01	LW= 5.955BL - 40856
	Height at withers	LW= 3.465HW - 150.1	LW= 2.323HW - 81.46	LW= 13.242HW - 66726
	Heart girth	LW= 3.084HG - 222.8	LW= 2.197HG - 142.5	LW= 6.220HG - 38214
	Body length	LW= 259.3ln(BL) - 112.9	LW= 123.3ln(BL) - 490.0	LW= 701.35ln(BL) - 306.6
	Heart girth	LW= 342.6ln(HG) - 148.6	LW= 201.2ln(HG) - 846.6	LW= 650.24ln(HG) - 306.1
Exponential	Height at withers	LW= 247.0ln(HW) - 953.9	LW = 141.6ln(HW) - 518.5	LW= 740.64ln(HW) - 306.9
	Body length	LW= 12.49e0.016BL	LW = 9.052e0.018BL	LW= 2.295e0.033BL
	Heart girth	LW = 7.439e0.023HG	LW = 3.026e0.030HG	LW= 1.881e0.039HG
Power	Height at withers	LW = 12.59e0.026HW	LW = 6.545e0.033HW	LW= 2.272e0.051HW
	Body length	LW= 0.005BL2.033	LW= 0.017BL1.779	LW= 4E-06BL3.656
	Heart girth	LW = 0.000HG2.670	LW= 0.000HG2.902	LW= 3E-06HG3.782
	Height at withers	LW= 0.023HW1.925	LW = 0.009HW2.096	LW = 0.000HW2.989
	Body length	LW= 0.020BL2 - 3.222BL + 184.9	LW = 0.011BL2 - 0.897BL + 43.56	LW= -115.2BL2 + 32314BL - 206.9
Polynomial	Heart girth	LW= 0.039HG2 - 6.259HG + 305.7	LW= 0.028HG2 - 3.498HG + 132	LW= -117.7HG2 + 30387HG - 206.3
	Height at withers	LW= 0.149HW2 - 19.10HW + 682.6	LW = 0.027HW2 - 1.260HW + 31.02	LW= 235.9HW2 - 16770HW + 232.02
		R ²	R ²	R ²
		0.670	0.714	0.015
		0.270	0.494	0.031
		0.851	0.812	0.012
		0.592	0.640	0.019
		0.797	0.736	0.016
		0.249	0.464	0.027
		0.691	0.753	0.210
		0.896	0.855	0.213
		0.291	0.559	0.203
		0.672	0.738	0.227
		0.893	0.848	0.229
		0.279	0.564	0.190
		0.755	0.762	0.028
		0.930	0.899	0.022
		0.338	0.513	0.034

LW= heart girth, BL= body length, HW= height at withers

Table 14: Genetic variability observed based on the principal components within the pigs' populations in the humid forest with monomodal rainfall agro-ecological zone of Cameroon.

Components	Variables	Eigen values	Variance (%)	Cumulative variance (%)
PC1	Body length	6.93	62.99	62.992
PC2	Hearth girth	0.81	7.32	70.31
PC3	Height at withers	0.77	7.03	77.34
PC4	Eye distance	0.60	5.48	82.82
PC5	Ear length	0.53	4.79	87.61
PC6	Head length	0.44	3.97	91.59
PC7	Snoot length	0.30	2.74	94.32
PC8	Tail length	0.24	2.21	96.53
PC9	Pelvic width	0.18	1.62	98.14
PC10	Hock circumference	0.14	1.28	99.42
PC11	Live weight	0.06	0.58	100.00

PC= principal component.

The contribution of the different principal components to the variability within pig population of the humid monomodal rainfall region of Cameroon is illustrated by figure 4.

Structure of the population

Statistical analysis has revealed three (03) sub-populations of pigs in the humid forest with monomodal rainfall agro-ecological zone of Cameroon. The organisational structure of these population is presented in table 15 and illustrated by the scatter plot of figure 5 and the dendrogram of figure 6.

The hierarchical ascending classification helped to establish the relation between the three genetic types of our studied population. The results are represented in the dendrogram based on the dissimilarities among the individuals, Euclidian distances, the Ward method and an automatically truncation.

Genetic type 2, though having lowest values are the most represented. This group is constituted by the Bakosi/Baweri indigenous pigs. They are followed by type 1 representing the crossbreeds in which the body values and indices have been improved, but remain lower than those of type 3. The individuals of type 1 turn to be more grouped around the barycentre while those of the other types are sparse. There exist a great distance between

genetic type 3 (exotic) and the others (local and crossbreeds).

The genetic distances between the different groups of pigs are presented in table 16, confirming that the highest distance is found between the local and the exotic pigs.

Table 16 confirm the intermediate position of the crossbreeds between the local and exotic pigs and denote the potential of crossbreeding in the improvement of body parameters of Bakosi/Bakweri pigs.

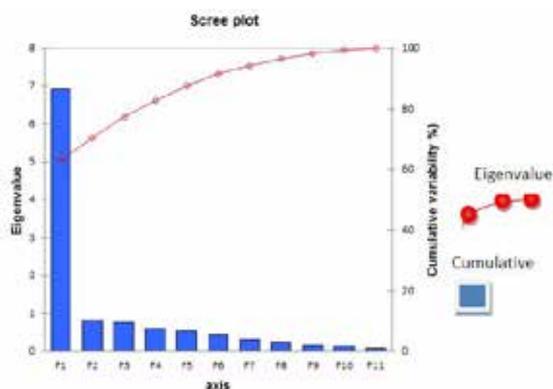


Figure 4: Contribution of principal components to the genetic variability of pig's population of the humid forest with monomodal rainfall agro-ecological zone of Cameroon

Table 15: Characteristics of the genetic types of pig’s population in the humid forest with monomodal rainfall agro-ecological zone of Cameroon

Variables	Type 1	Type 2	Type 3
BL	114.50	72.41	155.07
HG	101.72	66.95	140.57
HW	71.91	51.58	85.429
ED	19.98	15.35	22.43
EL	26.76	18.91	31.29
HL	33.50	27.62	43.57
SL	16.28	12.18	19.71
TL	31.48	24.88	40.21
PW	45.22	34.21	57.64
HC	21.78	19.22	27.93
LW	2953.57	917.63	7970.59

BL= body length, HG= heart girth, HW= height at withers, ED= eye distance, EL= ear length, HL= head length, SL= snout length, TL= tail length, PW= pelvic width, HC= hock circumference, LW= live weight.

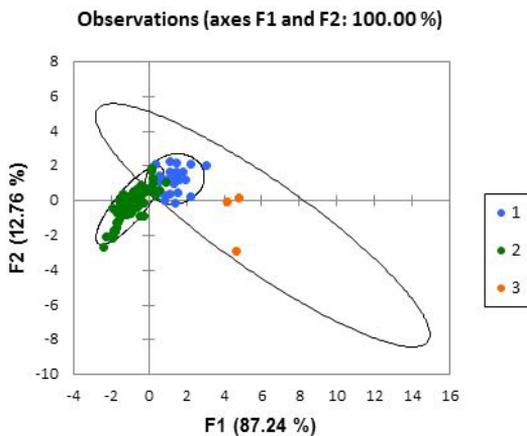


Figure 5: Population structure of pigs’ population of the humid forest with monomodal rainfall agro-ecological zone of Cameroon.

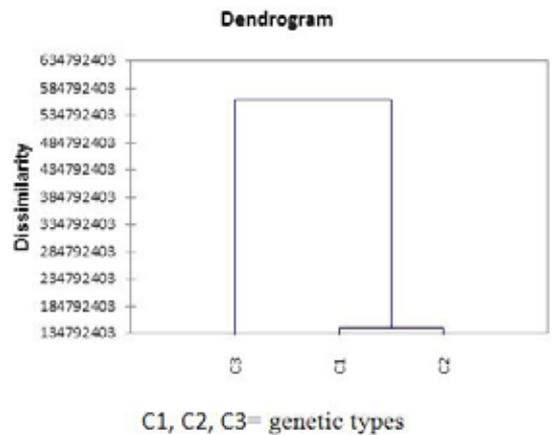


Figure 6: Dendrogram illustrating the phylogenetic relationship between the different genetic types of pig’s population of the humid forest with monomodal rainfall agro-ecological

Table 16: Distances between the genetic types of pig population in the humid forest with monomodal rainfall agro-ecological zone of Cameroon

Genetic types	Crossbreeds	Bakosi/Baweri	Exotic
Crossbreeds	0		
Bakosi/Baweri	2036.85	0	
Exotic	5017.40	7054.01	0

Discussion

The variability of coat colours and skin pigmentation in pigs of the humid forest with monomodal rain fall agro-ecological zone of Cameroon is similar to the result obtained by Subalini et al. (2010), Sahoo et al. (2012), Zaman et al. (2013), Prasanta et al. (2016) and Adjei et al. (2015). According to these authors, indigenous pigs could had a black coat colour changing from young stage (greyish brown) to adult stage, or being completely black colour with complete black skin pigmentation. Nevertheless, the percentage variability of coat colours and skin pigmentation in our studied population could be due to the introgression of exotic genotype. Many farmers do mix production, keeping both the exotic and Bakosi/Bakweri breed. Lauvergne (1993) explained that invasion of exotic genotypes in a locality or within a population is a source of colour variability in pigs.

The straight head shape prevalence is in accord with the works of Subalini et al. (2010), Ritchil et al. (2013), and Prasanta et al. (2016). It should be noted that the straight shape is peculiar to the local breed of pigs. The relatively high prevalence of straight head shape in the studied population can be due to the presence of Bakosi/Bakweri pigs who continue to maintain this trait when crossed with the exotic breed. The majority of the pigs have a curly tail. However, this does not reflect the observations recorded by Subalini et al. (2010) who reported that a majority of native pigs in Sri Lanka had a straight tail. The this characteristics may be due to the original genetic nature of the Bakossi/Bakweri Pigs, or link to the introduction of exotic breeds which may have transmitted this traits.

Biometric characterization is an important tool for livestock selection. Age had no significance difference ($p < 0.05$) on the body measurements of pigs. However, there exists a great variability of body measurements of pigs in our studied population with respect to localities and breeds.

Pigs of the Mamfe Sub-division have the highest body measurements. Their live weight

has a mean value of 153kg. This might be due to the fact that most exotic breeds are kept in this locality under intensive breeding. However, the live weight variation coefficient of pigs in Upper-Bayan is the highest, followed by Muyuka. This may tell that there is a great variability or diversity in these locality with respect to the live weight. This variability can be due to the fact that not only exotic breeds are kept there but also indigenous pigs. According to farmers of Upper-Bayang, Eyumojock, Muyuka and Tiko, keeping both Bakosi/Bakweri and exotic breed is important for profitability and resilience of the production system. The cross between these two gives animal with great performance which according to farmers may be more resistant to certain diseases such as erysipelas and African swine fever.

Furthermore, the study revealed that the exotic pigs of the studied population have higher body measurements than the Bakosi/Bakweri and crossbreeds. These findings are similar to those obtained by Adeola et al. (2013), Mbaga et al. (2005), Holnes (1991) and Lekule et al. (2003). Also, Holnes (1991) reported that most indigenous pigs having smaller sizes than the exotic breeds, gives them the ability to survive under harsh conditions (Lekule et al., 2003). Moreover, the longer snout, long head and erect ears observed in Bakosi/Bakweri pigs indicate that they represent a largely unselected group of pigs (Adeola et al., 2013).

The correlations coefficient among pig's body measurement in the studied population are in agreement with findings of McManus et al., (20120), Miserani et al., (2002). According to these authors, the differences observed might be influenced by the breeds, breeding management and the environment. The head traits are strongly related to breed while the body traits are strongly influenced by the environment, depending on feeding regime and rearing system.

The present study shows that pigs are greatly diversified in the different localities with respect to the body indexes. This is due to wide variation of coefficient of variance from one locality to another. Also, the Bakosi/Bakweri pigs have low body indices (except the skeletal

index) compared to the crossbreed. This shows that the cross between the Bakosi/Bakweri and exotic breed gives animals with greater carcass aptitudes. This might be influenced by the genetic make-up of the breeds.

The highest correlation ($p < 0.01$) was observed between the live weight and the heart girth which proves that the live can be predicted based on the heart girth. Results obtained from this study show that the heart girth is the best predictor of live weight of pigs in the humid forest with monomodal rainfall agro-ecological zone of Cameroon with the polynomial equations with the highest coefficient being the best for the exotic and crossbreed while the power equation is the best for Bakosi/Bakweri breeds. This is partially similar to those obtained by Edrian and Rodeza (2014) and Walugembe *et al.*, (2014). Nevertheless, Edrian and Rodeza (2014) reported that the heart girth and the body length best predict the live weight of Philippine native pigs with the linear equation being the best.

The principal component analysis based on 11 body measurements helped to determine the level of similarities and dissimilarities among individuals of the population as well as their genetic variability. The height at withers, tail length and live weight are all discriminant traits in the pigs' population of the humid forest with monomodal rainfall agro-ecological zone of Cameroon. This is similar to what was reported by Hayashi *et al.* (1984).

The hierarchical ascending classification gave us the phylogenetic tree allowing to establish the genetic distances that exist between the three genetic types of pigs' population of the humid forest with monomodal rainfall agro-ecological zone of Cameroon. This closeness might be due to the distance that exists between them and their origin. Jafe (2016) estimated that the close distances when existing between the two genetic types might be due to the fact that they have the same origin or belong to the same subgroup which later differentiated and gave two distinct genetic types.

Conclusion

There is a diversity of coat colours of pigs' populations with an abundance of the black colour. The pigs mostly have black skin pigmentation. Their heads are mostly straight with large erect ears which are mostly oriented forwards. They are mostly docile and have curly tails. The body measurements showed that there is a diversity of pigs in the humid monomodal rainfall agro-ecological zone of Cameroon. The exotic breeds have higher body measurements compared to the Bakosi/Bakweri breeds and the crossbreed. The live weight was the most diversified with respect to the localities. The heart girth is the best live weight predictor of pigs in the studied population. Discriminant analysis of the population structure revealed the existence of three genetic types with different characteristic values. Contrary to genetic type crossbreds and Bakosi/Bakweri, the distance between genetic type Bakosi/Bakweri and exotic is very high. The genetic diversity in the pig population of the humid forest with monomodal rainfall agro-ecological zone of Cameroon gives for possibilities of genetic improvement through selection and/or crossbreeding.

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CRYOSURVIVAL OF GOAT SPERMATOZOA IN TRIS-EGG YOLK EXTENDER SUPPLEMENTED WITH VITAMIN E

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Abstract

The effect of vitamin E supplementation in tris-egg yolk extender on sperm parameters of West African Dwarf (WAD) goat bucks was determined. Tris-egg yolk extenders supplemented with different levels of vitamin E (2, 4, 6 and 8 mM) were diluted with semen samples. The diluted semen samples were cryopreserved for 30 days and thereafter evaluated for sperm quality parameters. The results showed higher ($P<0.05$) sperm motility, acrosome integrity, membrane integrity, live sperm, acrosome reaction and capacitation, and reduced sperm abnormality in extenders supplemented with vitamin E compared to the control. These parameters were better sustained in extenders supplemented with 6 mM and 8 mM ($P<0.05$). Lower concentrations of malondialdehyde (MDA) were observed at 6 mM and 8 mM compared to the control ($P<0.05$). Higher percentages of acrosome reaction and capacitation were observed in extenders supplemented with vitamin E compared to the control ($P<0.05$). Optimal percent ($P<0.05$) of acrosome reaction and capacitation were observed in extenders supplemented with 6 mM and 8 mM of vitamin. The findings revealed that supplementation of vitamin E at 6 mM and 8 mM in tris-egg yolk extenders were effective for improving sperm parameters of WAD goat bucks during cryopreservation.

Keywords: Antioxidants, bucks, freezing, oxidative stress, sperm

Introduction

The possibility of using spermatozoa from proven donors and the use of stored semen over extended period of time are some important reasons of cryopreservation in for artificial insemination programme (Bagchi *et al.*, 2008; Rahman *et al.*, 2008; Abrishami, 2009). Freezing at low temperature however reduces sperm quality, loss of membrane and DNA integrities (Rama Raju *et al.*, 2006; Pegg, 2007; Bagchi *et al.*, 2008; Abrishami, 2009). In addition, during freezing the concentrations of antioxidant decrease and consequently increase reactive oxygen species (ROS) molecules (Andrabi, 2009; Kumar *et al.*, 2011). Therefore, supplementation of semen extender with antioxidant to remove the damaging effect of freezing is necessary (Correa *et al.*, 2006).

In vitro studies indicated that supplementation of some antioxidants to semen extenders improved sperm quality (Bilodeau *et al.*, 2001; Atessahin *et al.*, 2008; Bucak *et al.*, 2009). Vitamin E (α-tocopherol) is an antioxidant that protects cell integrity (Luo *et al.*, 2011). Information on the cryosurvival of semen obtained from WAD goat (*Capra hircus*) bucks following vitamin E supplementation in semen extender is limited. The objective of this study was therefore to determine the cryosurvival of spermatozoa obtained from WAD goat bucks in extender supplemented with vitamin E.

Materials and Methods

Experimental location and animal management

Goat Unit of Teaching and Research Farm, Federal University of Agriculture, in South-Western Nigeria was used for this study. Eight (8) intact WAD bucks aged 2.5-3 years kept under an intensive management system and fed with concentrate and guinea grass (*Panicum maximum*) were used for this study.

Semen collection and cryopreservation

A study repeated two times was carried out to determine the effect of adding different levels of vitamin E on sperm parameters of

semen obtained from WAD goat bucks. Eight semen samples collected with the aid of artificial vagina. The pooled semen samples were diluted at room temperature with a tris-based extender composed of two fractions. The Fraction one solution contained tris-hydroxymethyl-aminomethane (2.42 g), citric acid (1.36 g), glucose (1 g), penicillin (0.028 g), egg yolk (20 mL) and distilled water made up 100 mL as control. Glycerol (14.0%) was added to Fraction 1 solution to make Fraction 2 solution. The pooled ejaculate was split into five fragments, first diluted with the Fraction 1 solution and supplemented each with 0, 2, 4, 6 and 8 mM of vitamin E respectively at a final concentration of 589×10^6 sperm/mL and pH of 6.91. Fraction 2 solution was thereafter added. Diluted semen samples were then put into 2 mL plastic straws, sealed with polyvinyl, cooled to 4°C at a rate of 0.25°C/min and equilibrated at 4°C for 10 min in TYFSF Refrigerated Incubator (Model:SPX-7OB III, Hebei China). The straws were plunged into liquid nitrogen and stored for 30 days.

Microscopic evaluation of sperm motility

Microscopic evaluation of sperm motility was carried out as described by Bearden and Fuquay (1997). Semen was thawed in Clifton water bath (Model: 74178 by Nickel Electro Ltd, Weston-S-Mare Somerset, England) at 38 °C. A drop of semen sample was placed on a warmed microscope slide with cover slip and examining for progressively motile spermatozoa that moved forward in a straight line by four observers using Celestron PentaView LCD digital microscope (LCD-44348 by RoHS, China) at 400x magnification. The average of the ten successive observations was recorded as the final motility score.

Acrosome integrity

The method described by Ahmad *et al.* (2003) was used to evaluate acrosome integrity. Thawed semen sample (50 µL) was added to 500 µL of 96 mL 2.9% sodium citrate and 4 mL 37% formaldehyde. A drop of the mixture was placed on a microscope slide. Two hundred spermatozoa were counted using

Celestron PentaView LCD digital microscope (400x magnification) for sperm acrosome characterized by normal apical ridge.

Sperm membrane integrity

Sperm membrane integrity was carried out using hypo-osmotic solution (9 g fructose plus 4.9 g sodium citrate mixed with 1000 mL of distilled water) as described by Zubair et al. (2013). Semen sample (10 μ L) was incubated with 100 μ L hypo-osmotic solution at 37°C for 30 min and 0.1 mL of the mixture was spread over a warmed slide with a cover slip. Two hundred spermatozoa (200) spermatozoa that exhibited swelling and coiled tail were recorded as intact plasma membrane in Celestron PentaView LCD-44348 digital microscope (400x magnification).

Sperm abnormality and live sperm

The method earlier described by Bearden and Fuquay (1997) was used to evaluate sperm abnormality and live sperm. A drop of semen sample and eosin-nigrosin solution was drawn across the slide and dried. Two hundred and forty spermatozoa were observed for abnormal spermatozoa with defects in the tail, midpiece and head using Celestron PentaView LCD digital microscope (400x magnification) while spermatozoa that showed white colour were recorded as live sperm and those that pick up the stain were recorded as dead spermatozoa.

Malondialdehyde concentrations

Thiobarbituric acid reactive substances (TBARS) as described by Pipan et al. (2014) was used to evaluate malondialdehyde (MDA) concentration of the stored semen. Sperm suspension (0.1 mL) and 0.1 mL of 150 mM Tris-HCl (pH 7.1) were incubated for 20min at 37 °C. Thereafter, 1mL of 10% trichloroacetic acid and 2 mL of 0.375% thiobarbituric acid were added and further incubated in boiling water for 30 min and then centrifuged (3000 g) for 15 min. The absorbance was read in UV spectrophotometer (Surgifriend Medicals, England) at 532 nm. The concentration of MDA (nmol/mL) was estimated (MDA = AT –

AB/1.56 \times 10⁵; where AT = the absorbance of the sample, AB = the absorbance of the blank, 1.56 \times 10⁵ molar absorptivity of MDA).

In vitro acrosome reaction

Proportion of acrosome reaction was determined as described by Somanath and Gandhi (2002) with modification as follows: Stored semen samples were thawed at 38°C for 1min and the samples washed with phosphate-buffered saline, and the pellets were re-suspended in culture medium consisting of Calcium chloride dihydrate 265 mg/L, Magnesium chloride anhydrous 46 mg/L, Potassium chloride 200 mg/L, Sodium chloride 8000 mg/L, Sodium dihydrogen phosphate anhydrous 50 mg/L and D-Glucose 1000 mg/L. Following inclusion of 0.9% wt/vol phosphate-buffered saline, acrosome reaction was induced in by incubating spermatozoa with progesterone (2.5 mg/mL) at 38.5°C for 20 min (5% CO₂ in air; 100% humidity). Subsequently, an equal volume of phosphate-buffered saline was added in order to determine the proportion of spontaneous acrosome reaction. One hundred sperm cells were counted per slide in an upright Carl Zeiss Fluorescent Microscope (Primo Star, Germany) equipped with phase contrast and epifluorescence optics. Spermatozoa with no fluorescence or a dull fluorescence along the equatorial segment were classified as acrosome reacted while those with intense fluorescence over the acrosome were classified as acrosome intact.

In vitro capacitation

Chlortetracycline (CTC) fluorescence assay as described by Collin et al. (2000) was used to evaluate in vitro capacitation of the spermatozoa. CTC (750 μ M) was prepared in 20 mM Tris buffer containing 130 mM NaCl and 5 mM DL-cysteine (final pH 7.8). Cryopreserved semen suspension (5 μ L) was added to 5 μ L of CTC solution on a warmed slide for 30 s and 5 μ L of 0.2% glutaraldehyde in 0.5 M Tris (pH 7.4) was subsequently added. Thereafter, 10% phosphate-buffered saline (pH adjusted to 8.6) and 5 μ L of 90% glycerol were added to retard fluorescence fading. A drop of the mixture

was placed on a microscope slide with cover slip. One hundred sperm cells were counted per slide in an upright Carl Zeiss Fluorescent Microscope (Primo Star, Germany) equipped with phase contrast and epifluorescence optics. The cryopreserved spermatozoa that showed bright anterior head and faint fluorescence in the post-acrosomal region were classified as capacitated spermatozoa.

Statistical analysis

The study was repeated two times and estimations were performed for the pooled semen samples for each treatment consisted of two straws. The results analyzed using a two-way analysis of variance in SAS 1999 package were expressed as the means +SEM. Duncan multiple range test (Duncan, 1955) was used to separate significantly different means ($P < 0.05$). The model used is shown below:

$$Y_{ijkl} = \mu + J_i + R_j + T_k + \square_{ijkl}$$

Where Y_{ijkl} = Dependent variable; μ = population mean; J_i = i th effect due to level of vitamin E inclusion, $i = 0, 2, 4, 6, 8$; R_j = j th effect due to number of experiment, $j = 1, 2$; T_k = k th effect due to number of straw, $k = 1, 2$; \square_{ijkl} = Experimental error.

Results

The results (Figures 1-4) showed higher ($P < 0.05$) sperm motility, acrosome integrity, membrane integrity and live sperm in extenders supplemented with vitamin E compared to the control. Optimal improvement in these parameters was observed at 6 mM and 8 mM of vitamin E supplementation ($P < 0.05$). Similarly, reduced ($P < 0.05$) sperm abnormalities (Figure 5) were observed in extenders supplemented with vitamin E compared to the control and 6 mM and 8 mM of vitamin E supplementation had the lowest ($P < 0.05$) sperm abnormalities.

The results showed lower ($P < 0.05$) concentrations of MDA (Figure 6) in extenders supplemented with vitamin E compared to the control and 6 mM and 8 mM of vitamin E supplementation had the lowest ($P < 0.05$)

concentrations of MDA.

The results ((Figures 7 and 8)) showed that cryopreserved spermatozoa in extenders supplemented with vitamin E had higher acrosome reaction and capacitation compared to the control ($P < 0.05$). Optimal percent ($P < 0.05$) of acrosome reaction and capacitation were observed in extenders supplemented with 6 mM and 8 mM of vitamin E.

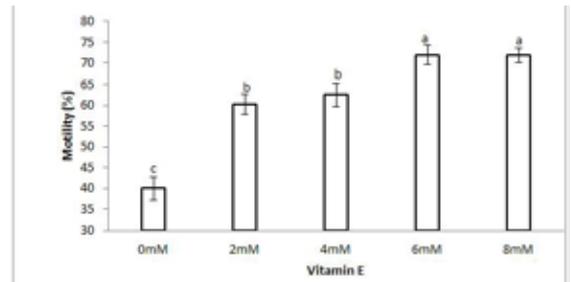


Figure 1: Motility (%) of buck semen cryopreserved with different levels of vitamin E ($n = 20$). a, b, c Means with different superscripts differ significantly ($P < 0.05$).

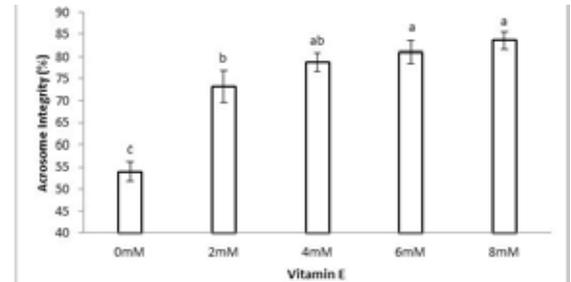


Figure 2: Acrosome integrity (%) of buck semen cryopreserved with different levels of vitamin E ($n = 16$). a, b, c, Means with different superscripts differ significantly ($P < 0.05$).

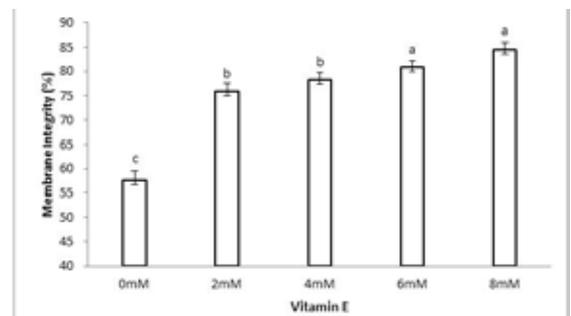


Figure 3: Membrane integrity (%) of buck semen cryopreserved with different levels of vitamin E ($n = 16$). a, b, c, Means with different superscripts differ significantly ($P < 0.05$).

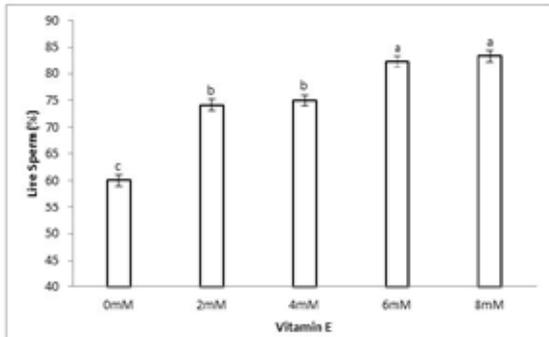


Figure 4: Live sperm (%) of buck semen cryopreserved with different levels of vitamin E (n=16). a,b,c, Means with different superscripts differ significantly (P<0.05).

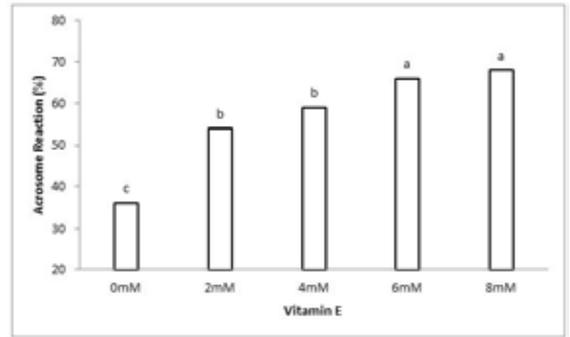


Figure 7: Acrosome reaction (%) of buck semen cryopreserved with different levels of vitamin E (n=16). a,b,c,d, Means with different superscripts differ significantly (P<0.05).

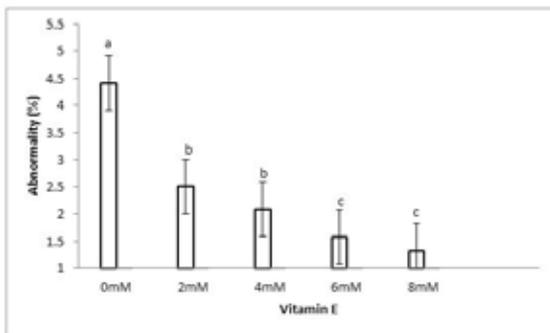


Figure 5: Abnormality (%) of buck semen cryopreserved with different levels of vitamin E (n=16). a,b,c, Means with different superscripts differ significantly (P<0.05).

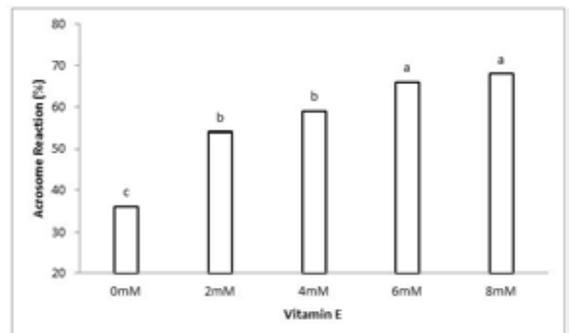


Figure 8: Sperm capacitation (%) of buck semen cryopreserved with different levels of vitamin E (n=16). a,b,c,d, Means with different superscripts differ significantly (P<0.05).

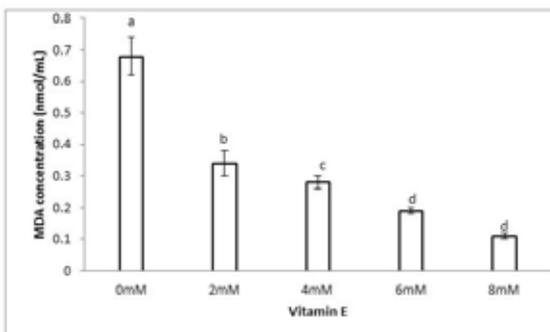


Figure 6: MDA concentration (nmol/mL) of buck semen cryopreserved with different levels of vitamin E (n=16). a,b,c,d, Means with different superscripts differ significantly (P<0.05).

Discussion

The improved motility of cryopreserved spermatozoa in extenders supplemented with vitamin E in the present study indicated the beneficial effect of vitamin E, and agreed with previous studies (Yousef *et al.*, 2003; Maia *et al.*, 2010). Vitamin E acts as intracellular antioxidant and help to scavenge free reactive oxygen and lipid hydroperoxides (Kheradmand and Babaei, 2006). This action was reported to help maintain the function of membrane phospholipids against oxidative damage (Smith and Akinbamiyo, 2000). Furthermore, the improved sperm motility could be attributed to possible reduction or prevention of cryodamage to spermatozoa metabolism by antioxidative ability of vitamin E (Anghel *et al.*, 2009).

In addition, the improvement in acrosome integrity and membrane integrity in extenders supplemented with vitamin E observed in the present study further substantiated the protective role of vitamin E. The finding was in consonant with earlier reports (Sarlos *et al.*, 2002; Michael *et al.*, 2009) that vitamin E had protective effect on acrosome integrity and membrane integrity. Vitamin E as primary component of antioxidant system of the spermatozoa protects the membrane against ROS and peroxidation of polyunsaturated fatty acids (PUFA) contained in the cellular and sub-cellular membrane phospholipids because of its lipid solubility (Yousef *et al.* 2003; Horton *et al.* 2002). The results agreed with (Bansal and Bilaspuri, 2009) that vitamin E promotes sperm membrane integrity and increases the live sperm percent. The mechanism by which vitamin E protects the cells against oxidative stress is via the inhibition of ROS reactions and lipid peroxides (Yue *et al.*, 2010). Vitamin E has been shown to inhibit the free-radical-induced damage to sensitive cell membranes as it is a major chain-breaking antioxidant (Sinclair, 2000).

The improved sperm parameters in the present study could be linked to antioxidant ability of vitamin E, suggesting a possible role of vitamin E in reducing seminal oxidative stress. Addition of vitamin E to cryopreservation media during freeze-thaw process has been reported to reduce lipid peroxidation and apoptosis of boar spermatozoa (Roca *et al.*, 2007), injuries to ram spermatozoa and oxidative stress to rat spermatozoa (Bucak *et al.*, 2007). Vitamin E has also been observed to increase sperm viability and reduced lipid peroxidation when subjected to oxidative stress inducer (Bansal and Bilaspuri, 2009).

MDA concentration is an index of seminal oxidative stress and has been used to determine adverse effects of seminal oxidative stress on sperm quality (Agarwal and Prabakaran, 2005). Pasqualotto *et al.* (2000) furthermore reported an association between high seminal reactive oxygen species levels and reduced percentage motile sperm. The degree of oxidative damage to spermatozoa is

reflected in higher MDA concentrations (Piyali *et al.*, 2009). The low MDA concentrations in vitamin E extenders compared to the control showed the advantage of vitamin E as an antioxidant on quality of spermatozoa. The action of vitamin E against peroxidation of PUFAs is linked to its lipid solubility (Horton *et al.*, 2002). Vitamin E is a major chain-breaking antioxidant in membranes known to directly neutralize superoxide anion, hydrogen peroxide and hydroxyl radical (Sharma and Agarwal, 1996). Vitamin E has been shown to inhibit the free-radical-induced damage to sensitive cell membranes as it is a major chain-breaking antioxidant (Sinclair, 2000). The present findings on reduced MDA supported the role of vitamin E as a chain-breaking antioxidant (Luo *et al.*, 2011). Inhibition of fructolysis and respiration, intracellular enzyme binding and damage to plasma membrane structure, especially on the acrosome are consequences of peroxidation on sperm motility (Kumar *et al.*, 2003). The mechanism by which vitamin E protect membranes has been linked to its ability to capture free radicals, break the peroxidation reaction by releasing hydrogen ions with electrons (Wahjuningsih and Rachmawati, 2012).

Conclusion

In conclusion, the findings revealed that supplementation of vitamin E at 6 mM and 8 mM in tris-egg yolk extenders improved sperm parameters of WAD goat bucks during cryopreservation. Supplementing semen extenders of goat buck with vitamin E could be of practical importance for sperm cryopreservation in artificial insemination programme.

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PHYTOCHEMICAL AND ANTI-MICROBIAL ACTIVITY OF METHANOLIC EXTRACT OF *Moringa oleifera*, *Aspilia africana* and *Azadirachta indica* LEAVES ON RABBIT INTESTINAL PATHOGENS

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Abstract

The antibacterial activity of leaf extracts of *Moringa oleifera* Lam, *Azadirachta indica*, *Aspilia africana* (Pers) C. D. Adams against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus saprophyticus* were determined using agar diffusion method. The minimum inhibitory concentration (MIC) was determined. The minimum inhibitory concentration (MIC) was determined. The result showed that the MIC ranges from 21.3-42.5 mg, 42.5-85.0 mg and 85.0 mg for *M. oleifera*, *A. indica* and *A. africana* leaf extracts respectively against *E. coli* and *S. aureus*. The phytochemical screening of the leaves revealed the presence of secondary metabolites such as tannin, saponin, alkaloid, flavonoid and phenol. The secondary metabolites detected in the leaves are responsible for the observed antibacterial activity of the plant and hence, its potential use as medicinal herb in the treatment of infections caused by the test organisms.

Keywords: Antibacterial activity, Minimum Inhibitory Concentration, Phytochemical, Moringa, Neem, Aspilia.

ACTIVITÉ PHYTOCHIMIQUE ET ANTI-MICROBIENNE DE L'EXTRAIT MÉTHANOLIQUE DES FEUILLES DE *Moringa oleifera*, *Aspilia africana* et *Azadirachta indica* SUR LES PATHOGENES INTESTINAUX DU LAPIN

Résumé

La présente étude a déterminé l'activité antibactérienne des extraits de feuille de *Moringa oleifera* Lam, *Azadirachta indica*, *Aspilia africana* (Pers) C. D. Adams contre *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus saprophyticus*, en utilisant la méthode de diffusion sur gélose. La concentration minimale inhibitrice (CMI) a été déterminée. Le résultat a montré que la CMI variait de 21,3 à 42,5 mg, de 42,5 à 85,0 mg et 85,0 mg respectivement pour les extraits de feuilles de *M. oleifera*, d'*A. Indica* et d'*A. Africana* contre *E. coli* et *S. aureus*. L'examen phytochimique des feuilles a révélé la présence de métabolites secondaires tels que le tanin, la saponine, l'alcaloïde, le flavonoïde et le phénol. Les métabolites secondaires détectés dans les feuilles sont responsables de l'activité antibactérienne observée de la plante, d'où son utilisation potentielle comme herbe médicinale dans le traitement des infections causées par les organismes testés.

Mots-clés : Activité antibactérienne, Concentration inhibitrice minimale, Phytochimique, Moringa, Neem, Aspilia

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Introduction

Plants contain many biologically active compounds which have potential for development as medicinal agents. Herbal medicines already form the basis of therapeutic use in the developing countries, but of recent, there has been an increase in the use of herbal medicines in the developed world too (De and Ifeoma, 2002; El-Mahmood *et al.*, 2010). Medicinal plants have been found useful in the cure of a number of diseases including bacterial diseases as the plants are a rich source of antimicrobial agents (Mahesh and Satish *et al.*, 2008) that are naturally toxic to bacteria organisms (Basile *et al.*, 1999). These active compounds are generally called phytochemical. Presence of certain phytochemical components such as alkaloids, saponins, tannins, polyphenols, are the bioactive bases for the medicinal properties. *Azadirachta indica* is one of such plants belonging to the Meliaceae family. It is popularly known as Neem (English) or “dongoyaro” (Yoruba-Western Nigeria) in Nigeria. The plant is perhaps one of the most studied and widely used medicinal plants of all ages. *Aspilia africana* (Pers) C. D. Adams in the family of Asteraceae. It is used in African ethno-medicine for the treatment of haemorrhage (Katsayal, 2002). *Moringa oleifera* popularly called the “miracle tree” is a monogeneric plant in the family Moringaceae. In ethno medicine, *Moringa oleifera* leaves have been used by local traditional healers in treatment of various ailments. The leaves have also been found to possess anti-tumour, anti-pyretic, anti-epileptic, anti-inflammatory, anti-ulcer, anti-spasmodic, diuretic, antihypertensive and antioxidant properties (Bukar *et al.*, 2010). Due to wide uses of these leaves, the present study was planned to investigate the antibacterial potential of the leaf extracts on *E. coli*, *P. aeruginosa*, *S. aureus* and *S. saprophyticus* in an attempt to provide a profile that gives a scientific backing to various tradomedical claims and the uses.

Materials and Methods

Plant Material and Identification

Fresh leaves of *M. oleifera* Lam, *A. africana* (Pers.) C. D. Adams and *A. indica* were harvested during dry season within the arboretum of Ogun State, Nigeria state. The leaves were identified taxonomically in the herbarium of the Department of Pasture and Range Management, Federal University of Agriculture, Abeokuta, Ogun State.

Extraction of leaf material

The leaves were air-dried and milled to obtain a leaf meal product. Methanolic extraction of *M. oleifera*, *A. africana* and *A. indica* was prepared in cold condition. In cold extraction, the coarse dried powdered leaves were macerated. The extract was done at 25°C for 72 hrs. These were carried out by suspending 250 g of the powdered leaves were soaked into 1.5 L of methanol in a conical flask cover with rubber cork with periodic shaking. The extracts were then decanted and filtered through a Whatman filter paper. The filtrate was evaporated to dryness in a water bath at 25°C which gives a semi-solid residue.

Source of test organisms

Caecal content of rabbits were aseptically collected after slaughtering and cultured overnight on nutrient agar. It was incubated at 37°C for 18-24 hrs at ambient temperature. Each bacterium isolate was biochemically characterized and identified appropriately according to Cowan and Steel (1996). These include at least four isolates: *E. coli*, *P. aeruginosa*, *S. aureus* and *S. saprophyticus* as the test organisms.

Screening of the Extracts for Antibacterial Activity

Agar disc-diffusion assay was performed to evaluate the antibacterial activity of *M. oleifera*, *A. africana* and *A. indica* extract. Twenty ml of molten sterile nutrient agar was poured into petri dishes. After solidification, an overnight broth culture of *E. coli*, *P. aeruginosa*, *S. aureus* and *S. saprophyticus* was introduced unto the surface of the sterile plate each and

sterile glass spreader were used for even distribution. Wells were made aseptically with 7 mm sterile cork borer and 1 g of each extract was reconstituted in normal saline to obtain extract concentration of 100 mg/ml i.e. 1000 mg in 10 mls of normal saline. The plates were incubated aerobically for 24 hours at 37°C and were examined for zone of inhibition, which indicate the degree of susceptibility of the test organism. The same procedure was carried out using a standard disc antibiotic (procaine penicillin) as the positive control and the prepared extract was used as negative control.

Determination of Minimum Inhibitory concentration (MIC) of the Extracts

The MIC of the extract on isolates was determined by micro broth dilution assay following the recommendation of (Kumar et al., 2007). Equal volume of 1000 mg/ml concentrations of each extracts and broth medium were mixed in Mueller-Hinton broth by serial dilutions to make up 100 mg of solution were assayed against the test organisms and incubated for 24 hrs at 37°C.

The experiment was conducted in duplicate for all the test isolates. Tubes of Mueller-Hinton broth containing only the 100 mg/ml suspension of the test organisms without the extract, and the tubes of Mueller-Hinton broth containing different concentrations of the extract without test organisms, were used as controls. The lowest concentration of the extract to inhibit the growth of microorganisms after incubation period was taken as the MIC.

Phytochemical analysis

The extract were subjected to quantitative phytochemical analyses for secondary metabolites to assess the presence of tannins, saponins, alkaloids, flavonoids, phenol, following the standard method (Harborne, 1998; Houghton and Raman, 1998 and Parekh, 2006).

Statistical Analysis

Data collected were subjected to one way analysis of variance (ANOVA) in a Completely Randomized Design using SPSS

(Release 20.0) statistical package (SPSS, 2011). Treatment means were separated using Duncan's Multiple Range Test.

Results

The results of the antibacterial effectiveness of the methanol leaf extracts as compared with the activity of standard antibiotics (*proclaim penicillin*) was shown in Table 1. The extracts inhibited the growth of two Gram negative (*E. coli* and *P. aeruginosa*) and positive (*S. saprophyticus* and *S. aureus*). The antibacterial activity of the antibiotic inhibited higher zones of inhibition than the plant extracts. The antibiotics and extracts of *M. oleifera*, *A. indica* and *A. africana* produced inhibitory zone of 20, 13 and 11 mm against *E. coli* respectively. The extract of *M. oleifera* showed high activity with the diameter of Zone of inhibition of 17 cm against *S. aureus* and least activity of 11 cm against *P. aeruginosa*. Mild effect of the extract of *A. africana* on *E. coli* was in line with the findings of (13) who reported a mild effect of *A. africana* leaf extract against *E. coli*. The leaf extract of *A. indica* had a mild effect against *E. coli*, *P. aeruginosa* 13, 14 mm and a susceptible effect on *S. aureus* and *S. Saprophyticu* 18 mm for each respectively. Table 2 shows the minimum inhibitory concentration (MIC) of the methanol extraction of *M. oleifera*, *A. africana* and *A. indica* leaves against *E. coli* and *S. aureus*. The MIC antibacterial activity of *A. Indica* recorded the highest value when compared to that of *M. oleifera* and *A. africana*. The result revealed that the MIC of the methanol leaf extract of *M. oleifera* on *E. coli* and *S. aureus* were 42.5 mg/ml and 21.3 mg/ml respectively. The MIC of *A. africana* leaf extract on *E. coli* and *S. aureus* were 42.5 mg/ml and 85 mg/ml respectively. However, the extract of *A. indica* at 85 mg/ml concentration exhibited antibacterial activities against the test organisms. The data obtained in Table 3 shows the presence of secondary metabolites such as alkaloid, tannin, saponin, flavonoid and phenol. *A. indica* contained significantly ($P < 0.05$) higher concentrations of tannin (1.25%) and alkaloid (2.74%) than *M. oleifera* and *A. africana* leaves.

Table 1: Antimicrobial activity of the leaf extracts of *M. oleifera*, *A. africana* and

Gram	Bacteria isolates	Procaine penicillin	Methanol based Extract		
			<i>M. oleifera</i>	<i>A. indica</i>	<i>A. africana</i>
			Diameter zone of inhibition (mm)		
Negative	<i>Escherichia coli</i>	20	13	13	11
Negative	<i>Pseudomonas aeruginosa</i>	19	11	14	18
Positive	<i>Staphylococcus saphrophyticus</i>	22	16	18	16
Positive	<i>Staphylococcus aureus</i>	20	17	18	16

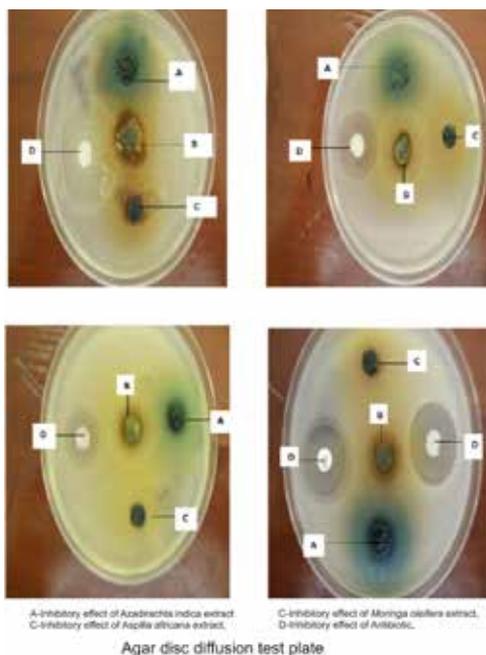
Table 2: Minimum Inhibition Concentration of the leaf extracts of *M. oleifera*, *A. africana* and *A. indica* (mg/mL)

Test organism	Methanol based Extract			Gram
	<i>M. oleifera</i>	<i>A. africana</i>	<i>A. indica</i>	
<i>Escherichia coli</i>	42.5	42.5	85.0	Negative
<i>Staphylococcus aureus</i>	21.3	85.0	85.0	Positive

Table 3: Phytochemical content of dried leaves of the selected browse plants

Components	Dried leaves of the selected browse plants			SEM
	<i>M. oleifera</i>	<i>A. Africana</i>	<i>A. indica</i>	
Tannin	0.45 ^c	0.93 ^b	1.25 ^a	0.02
Flavonoid	2.74 ^a	1.92 ^b	1.63 ^c	0.01
Alkaloid	0.85 ^c	1.47 ^b	2.74 ^a	0.01
Phenol	0.59 ^a	0.30 ^c	0.37 ^b	0.00
Saponin	0.74 ^b	0.86 ^a	0.38 ^c	0.01

^{abc} Means along the same row with different superscripts are significantly different ($P < 0.05$). SEM- Standard Error of Mean.



Higher concentration of 0.86% was recorded for saponins in *A. africana* than *M. oleifera* and *A. indica* leading to a significant ($P < 0.05$) difference with least value (0.38%). The phenol content in this study ranged between 0.30-0.59%.

Discussion

The data obtained from this study indicates the sensitivity of *S. aureus*, *S. saphrophyticus* and *P. aeruginosa* to the extracts of *A. africana* in this study corresponds with the work of Adeniyi and Odufowora (2000) that showed that extracts of *A. africana* possessed a broad spectrum antibacterial activity against both Gram positive and negative bacteria. Failure of some of the extract to exert antibacterial effect on the test organism is not enough to conclude that the leaf does not

contain substances that can exert antibacterial activity against the test organisms because the potency of extract depends on the method used to obtain the extract (Unaeze and Abarikwa, 1986). The sensitivity of the leaf extract shown in this study, was only able to inhibit the growth of organisms but did not exert a killing effect on the bacteria isolates. This shows that the methanolic extract was bacteriostatic and not bactericidal. The minimum inhibition concentration (MIC) shows the effectiveness of the extract concentration against *E. coli* and *S. aureus*. Generally, the test organisms had the same MIC value of 85 mg/ml for *A. indica*. This indicated that the leaf of *A. indica* has similar potency on *S. aureus* and *E. coli*. This is similar to the findings of National Library of Medicine at the National Institutes of Health who reported that in test tubes *A. indica* has been shown to have significant effects on both Gram positive and negative organisms and other bacteria that cause a wide array of human and animal diseases. The secondary metabolites of the leaves of these study plants revealed the presence of alkaloid, saponin, tannin, phenols and flavonoid as the major phytochemical components (Veeramuthu et al., 2006). Table 3. This is in agreement with the work of Geyid et al., (2005) ; Tedong et al., (2006) who reported that plant have variety of secondary metabolites as mentioned above. The phytochemical composition level of the selected browse plants studied are below 2.5% recommended by (NRC, 1985) with the exception of *M. oleifera* and *A. indica* which recorded little above the recommended value in flavonoid and alkaloid. *A. indica* recorded ($P < 0.05$) higher concentrations of tannin (1.25%) and alkaloid (2.74%) than *M. oleifera* and *A. africana* leaves. Tannin has inhibitory effect on many enzyme due to protein precipitation and the presence could be the reason why the observed plants are used locally as treatment of wound and skin disease (Bruneton, 1999; Trease and Evans, 2002). Higher concentration of 0.86% was recorded for saponins in *A. africana* leading to a significant ($P < 0.05$) difference. The presence of saponin in plant leaves confirmed the plant as anti-inflammatory, antifungal, anti parasitic

(Sprag et al., 2004) and antimicrobial activity (Barile et al., 2007; Ayoola et al., 2008).

Cheeke (1971) and Eleazu et al. (2010) reported that saponin have effect on erythrocyte, haemolysis, reduction of blood and liver cholesterol, depression of growth rate, bloat inhibition of smooth muscle activity, enzyme inhibition and reduction in nutrient absorption. High levels of saponin in feed affect feed intake and growth rate in animal (Sim et al., 1984; Dei et al., 2007) and bitterness (Sodipo et al. (2000). However, the concentrations of flavonoid and phenol also vary among the leaves with highest values (2.74%) and (0.59%) recorded for *M. oleifera* respectively. The level of alkaloid and flavonoid recorded for *M. oleifera* fell below the report listed above but higher than the report (0.42%) alkaloid reported by Ojiako (2014). Phenol and their oxidative products are corrosive to living bacteria cells. Baker and Breach (1980) reported that phenols are considered to be potentially toxic to the growth and development of pathogens (Singh and Sawhney, 1998). The value reported is similar to the findings of Njidda (2010) who reported 0.24-0.65% but lower than that of (Osuga et al., 2006; Ojiako, 2014).

Conclusion

The methanol leaf extracts of *M. oleifera*, *A. indica* and *A. africana* possessed good antibacterial activity confirming an appreciable inhibitory activity against the test organisms. The activity of the leaf extract against Gram positive (*S. saprophyticus* and *S. aureus*) were more sensitive than negative (*E. coli* and *P. aeruginosa*) bacteria. The inhibitory effect of the extract is an indication of the presence of broad-spectrum bioactive compounds in the leaf. It is believed that the leaves used in this research could be potential sources of drugs if the active ingredients are identified and adequately characterized. Therefore, the leaves could be a promising natural antimicrobial agent with potential applications in livestock industry for controlling bacteria used in this work. Further studies using other extracts may be carried out to find out the active constituents.

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PREVALENCE OF CATTLE TRYPANOSOMIASIS AND BABESIOSIS IN THE WESTERN HIGHLANDS OF CAMEROON

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Abstract

The prevalence of trypanosomiasis was studied in the Sudano-Guinean highlands of western Cameroon in 107 cattle through parasitological examination and serological analysis. Parasitological examination revealed the presence of trypanosomes in cattle with a prevalence of 16.82%, regardless of cattle breed. The highest prevalences were obtained from cross-breeds (23.33%) and Red Fulani (21.74%), followed by Gudali cattle (6.67%). Conversely, serological analysis highlighted antibodies against *T. brucei* in 42.17 % of cattle. The highest seroprevalences were identified in White Fulani (60.00%), followed in order by the Gudali (37.14%), the Red Fulani (48.15%) and the crossbreeds (21.05 %). Seroprevalence obtained were significantly higher than the parasitological prevalence. The dilutions performed on the positive sera have proven that regardless cattle breed, the level of antibodies produced was generally low. Infestations in trypanosomes have not significantly affected the neither haematocrit nor blood cell counts. Concerning babesiosis, parasitological search revealed 11.20% of cattle infected, with 6.67, 10.87, 6.67 and 20.00% respectively for the white Fulani, red Fulani, gudali and crossbreeds. Infections with *Babesia* sp did not affect significantly blood parameters studied. Concerning babesiosis, parasitological search revealed 11.20% of cattle infected, with 6.67, 10.87, 6.67 and 20.00% respectively for the white Fulani, red Fulani, gudali and crossbreeds. Infections with *Babesia* sp did not affect significantly blood parameters studied.

Keywords: prevalence, trypanosomiasis, babesiosis, cattle

PREVALENCE DE LA TRYPANOSOMOSE BOVINE ET DE LA BABESIOSE DANS LES HAUTS PLATEAUX OCCIDENTAUX DU CAMEROUN

Résumé

La prévalence des trypanosomoses a été étudiée dans la zone soudano-guinéenne d'altitude de l'Ouest-Cameroun chez 107 bovins par l'examen parasitologique et analyse sérologique. L'examen parasitologique a révélé la présence des trypanosomes chez les bovins avec une prévalence de 16,82%, indépendamment des races. Les prévalences les plus élevées ont été obtenues chez les races croisées (23,33%) et Red Fulani (21,74%), suivis par les Gudali (6,67%). L'analyse sérologique a par contre mis en évidence les anticorps dirigés contre *T. brucei* chez 42,17 de bovins. Les séroprévalences les plus élevées ont été recensées chez les White Fulani (60,00%), suivis dans l'ordre par les Gudali (37,14%), les Red Fulani (48,15%) et les croisés (21,05%). Les séroprévalences obtenues étaient significativement supérieures aux prévalences parasitologiques. Les dilutions effectuées sur les sera positifs ont révélés que quel que soit la race bovine, le taux d'anticorps produits a été généralement faible. Les infestations à trypanosomes n'ont affecté significativement ni l'hématocrite, ni la numération globulaire. La recherche parasitologique des

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babésioses a révélé 11.20 de bovins infectés avec 6.67, 10.87, 6.67 et 20.00% respectivement pour les races White Fulani, Red Fulani, Gudali et les croisés. Les infections à Babésia sp n'ont affecté significativement ni l'hématocrite, ni la numération globulaire, ni la formule leucocytaire. La recherche parasitologique des babésioses a révélé 11.20 de bovins infectés avec 6.67, 10.87, 6.67 et 20.00% respectivement pour les races White Fulani, Red Fulani, Gudali et les croisés. Les infections à Babésia sp n'ont affecté significativement ni l'hématocrite, ni la numération globulaire, ni la formule leucocytaire.

Mots-clés : prévalence, trypanosomose, babesiose, bovins

Introduction

There are 31 species of tsetse flies that invading one-third of Africa (Tasew and Duguma, 2012). They transmit trypanosomes to humans and their livestock, hence overshadow and impair the public health and agriculture sector in 38 African countries, exposing 160 million cattle to the risk of anaemia, emaciation and death and 60 million people to the risk of sleeping sickness (Rogers and Robnson, 2004).

Cattle play an important role in the supply of animal protein in developing countries. In these countries, this species is the main source of supply of meat and milk, while it contributes to the improvement of cropping systems by traction and organic fertilization. Despite their importance, the production and productivity of domestic cattle breeds in the tropics in general and African countries in particular remain weak for various reasons (Ngole *et al.*, 2003). Among the major constraints to productivity of cattle in Cameroon include blood parasitic diseases and gastrointestinal whose extent in the humid tropics is considerable (Ukoli, 1991).

Cattle trypanosomiasis is one of the main pathological constraints to the development of cattle production in sub-Saharan Africa (Swallow 2000; Michael *et al.*, 2002, Abenga *et al.*, 2004) and causes yearly losses estimated at US\$ 1 billion (Dehaan and Bekure, 1991). Cattle trypanosomiasis known to cause not less than 3 million livestock deaths every year, 20% loss in calving, 25% reduction in milk yields, 50% reduction in livestock numbers (PAAT, 2000) and reduces work efficiency of cattle hence hampering crop production (Swallow, 2000). African trypanosomiasis also known as a major cause in the depopulation.

Cattle babesiosis is also known by various names such as piroplasmosis, cattle tick

fever, or red water fever is a haemolytic disease caused by intraerythrocytic protozoans of the genus *Babesia* sp. It is a common tick-borne disease in tropical and subtropical regions (Jongejan and Uilenberg, 2004). Ticks of the family Ixodidae transmit it and they are prevalent in the Western Highlands of Cameroon and all over the country where ecological conditions are particularly favorable for the survival of parasites and their vectors. Though they often only cause sub-clinical sickness, they have a significant economic effect on the cattle industry (Niazi *et al.*, 2008; Onoja *et al.*, 2013), which is characterized by high fever and intravascular haemolysis, leading to anaemia, icterus, haemoglobinuria and death. Diagnosis of babesial infections plays an important role in monitoring, management and control of infection (Bashir, 2008).

The present study represents an attempt to investigate trypanosomiasis and babesiosis in cattle in the western highlands of Cameroon, for appropriate control measures that will permit the exploitation of the abundant vegetal diversity and water resource for large scale cattle production.

Material and Methods

The study was conducted in the Sudano Guinean Western Highlands of Cameroon (LN 05° 20' - 7° 00' and LE 10° 03' - 12° 00'). 5ml blood samples were collected from the jugular vein in 107 cattle in the municipal slaughterhouse of Dschang and in its neighboring farms in North Bafou (high cold mountain area) and Santchou (lowland flood plains area). All animals were sampled based on their availability.

Blood was collected in a sterile tube labeled with anticoagulant and sent directly to the laboratory for immediate analysis or

delayed analysis within a maximum of 72 hours. Information was also recorded on the age, sex and breed. 3ml of blood from 83 cattle were centrifuged at 1500 rev/min for 15 minutes and the obtained plasma was collected in a sterile vial, labeled and stored frozen until analysis.

The direct detection of trypanosomes in the blood was performed by the method of "buffy coat" described by Murray et al (1977). Thick films were performed according to the method described by Penchenier and Laveissiere (2000). The search for parasites of the genus *Babesia* was done by microscopic observation at 1000X magnification with immersion oil blood smears previously fixed and stained with May-Grünwald-Giemsa.

Evidence of antibodies against *Trypanosoma b. g.* in blood was carried out by the method of the Card Agglutination Test for Trypanosomiasis (CATT) described by Magnus et al (1978). The positive samples underwent up to 1:32 dilution series to determine the title of the CATT. The hematocrit was evaluated by the technique of micro hematocrit of Levy-Lambert (1973) and differential count (basophils, eosinophils, neutrophils, lymphocytes and monocytes) established by the same author. The number of white and red blood cells was determined by dilution in Lazarus and Marcano solutions respectively according to the WHO (1982).

The Chi-square test was used to evaluate the influence of breed, sex and age on the prevalence of infestation, while the influence of parasites on blood parameters was evaluated by the Student's test in the SPSS software, version 11.0.

Results

Parasitological prevalence of trypanosome and Babesia infections in cattle in the Western Highlands of Cameroon

Cattle of the Western Highlands of Cameroon are infested with the two types of parasites. The prevalence of *Babesia* infestation in cattle is 11.2%, against 16.8% of trypanosomes infection rate. The number of animals infected with both types of haemoparasites is relatively

low (less than 5%).

Parasitological prevalence of trypanosome and Babesia infections according cattle breeds in the Western Highlands of Cameroon

The prevalence of trypanosome and *Babesia* infections according cattle breeds in the Western Highlands of Cameroon are illustrated by Figure 1. All the cattle breeds are infested with the two types of parasites. Regardless of the type of parasite, the prevalence of infestation in crossbreeds (46.7%) and Red Fulani (37.0%) were significantly higher ($P < 0.05$) as compared to those of White Fulani (13.3%) and Gudali (13.3%).

For the same parasite, trypanosome prevalence was significantly higher ($P < 0.05$) in the Crossbreeds (23.3%) and Red Fulani (21.7%), followed by Gudali (6.67%). Single infestations by trypanosomes were not identified in White Fulani. Infestations in *Babesia* have comparable levels of prevalence ($P \geq 0.05$) in all breeds, being 6.67, 10.8, 6.67 and 20.0% respectively for the White Fulani, Red Fulani and crossed Gudali. Whatever the breed, the prevalence of trypanosomes and *Babesia* are comparable ($P \geq 0.05$), or 21.7% and 10.9 for the Red Fulani, 6.67 and 6.67% for Gudali, 23.3 and 20.0% for the Crossbreeds.

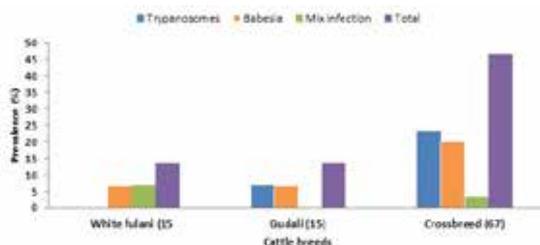


Figure 1: Prevalences of trypanosome and *Babesia* infestations according to the cattle breed in the Western Highlands of Cameroon

The prevalence of mixed infections (trypanosomes and *Babesia*) are comparable ($P \geq 0.05$) in White Fulani (6.67%), the Red Fulani (4.35%) and the crossbreeds (3.33%).

Parasitological prevalence of trypanosome Babesia infections according to the age and sex in cattle in the Western Highlands of Cameroon

The Prevalence of infestation trypanosome and Babesia according to age and sex in cattle of the High Lands Western Cameroon as illustrated in Figure 2 show that all age groups and sexes are infested by the two types of blood parasites, apart from females under 3 years which showed no infestation and males aged 5 years and above who are infested only by Babesia. Regardless of the type of parasite and sex, infestation rates were significantly higher ($P < 0.05$) in cattle of more than 5 years of age (34.9%) and those of 3-4 years (38.1%) compared to cattle of 0-2 years (13.6%). Whatever the age and sex, the prevalence of trypanosomes is comparable to that of Babesia.

In the same age group, males younger than 5 years were significantly more infested ($P < 0.05$) than females. By cons, in animals of more than 5 years, the infestation prevalence of males (33.33%) and females (35.0%) were comparable ($P \geq 0.05$). In cattle of 0-2 years, the rate of infestation by trypanosomes was significantly higher in males compared to females, while the prevalence of infestation Babesia is comparable ($P \geq 0.05$) in both sexes. The prevalence of infections caused by trypanosomes and Babesia for males and females of 3-4 years are statistically identical. In cattle aged 5 years and above, the prevalence of trypanosome infections was significantly higher ($P < 0.05$) in females (17.5%) compared to males, whereas on the contrary, that of Babesia in both sexes (respectively 17.2 and 7.69% for males and females) is comparable.

For the same sex, infestation rates of males of 5 years and above and those of 3-4 years (44.8%) are comparable from one hand, on the other hand significantly higher ($P < 0.05$) than male of 0-2 years (14.2%). By cons, prevalence levels in females of more than 5 years of age (35.0%) and those of 3-4 years (23.1%) were comparable ($P \geq 0.05$); females of 0-2 years did not show any infestation. Mixed infestations are absent in cattle under 3 years while they are present at comparable prevalence ($P \geq 0.05$) in

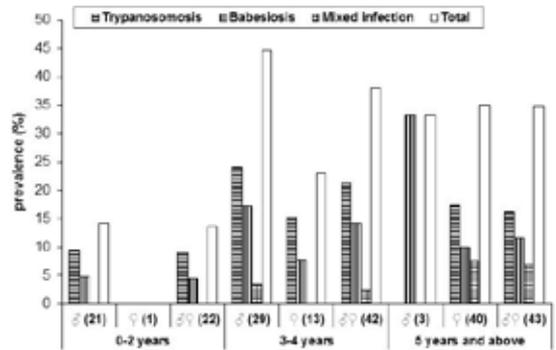


Figure 2: Prevalence of trypanosome and Babesia infestations according to age and sex of cattle in the Western Highlands of Cameroon

cattle of 3-4 years (2.38%) and in those over 5 years (6.98%).

An analysis of the test results of trypanosomes infestation in cattle according to type of diagnosis used showed that serological tests show antibodies against *T. brucei gambiense* where the parasitic test is less accurate. Serological prevalence *T. brucei gambiense* in cattle of the Western Highlands of Cameroon was significantly higher ($P < 0.05$) compared to the parasitological prevalence (16.8%).

Influence of trypanosome and Babesia infestations on the hematocrit and blood cell count in cattle from the Western Highlands of Cameroon.

It follows from table 1 that the hematocrit, the number of red and white cells in animals infected with trypanosomes or babesia are comparable ($P \geq 0.05$) to those of the non-infected animals. In cattle infected by both trypanosomes and babesia, hematocrit was significantly lower ($P < 0.05$) than non-infected animals. In the contrary, the number of white blood cells in these animals was significantly higher ($P < 0.05$) than that of uninfected animals.

Influence of Trypanosoma infestation on hematocrit and blood cell count according to age and sex in cattle from the Western Highlands of Cameroon

The influence infections with *Trypanosoma* on hematocrit and blood cell count according to age and sex in cattle is presented in Table 2. It appears that apart from the white blood cells of the female aged

5 years and above infected by trypanosomes which is significantly higher ($P < 0.05$), no significant differences ($P \geq 0.05$) was found between hematocrit and the number of blood cells (white and red) of cattle infested and uninfested, regardless of age and sex of cattle.

Influence of Babesia infestation on hematocrit and blood cell count according to age and sex in cattle from the Western Highlands of Cameroon

The influence infections with Babesia on hematocrit and blood cell count according to age and sex in cattle as presented in Table 3 shows that, whatever the age and cattle sex, hematocrit and the number of blood cells (white and red) remain comparable ($P \geq 0.05$) between animals infested and uninfested, apart from male aged 0-2 years whose number of

leukocytes in animals infected is significantly higher compared to that of uninfested ones.

Influence of trypanosome and Babesia infections on leucogrammes depending on the age and sex of cattle in the Western Highlands of Cameroon

The influence of trypanosome and Babesia infections on leucogrammes according to the age and sex (Figures 3 and 4) shows that neutrophils and lymphocytes are the types of leukocyte prevailing among uninfested, cattle.

In general, the shape of leucogrammes of infested animals is comparable to that of uninfested ones, apart from remarkable but not significant variations ($P \geq 0.05$): In male cattle of 0-2 years infested by trypanosomes (15a) and females of 3-4 years infested with babesia (16c), the lymphocyte rate is dominant while in uninfested, animals, neutrophils predominate.

Table 1: Influence of trypanosome and babesia infestations on the hematocrit and blood cell count in cattle from the Western Highlands of Cameroon

Type of infestation	Hematocrit (%)		Leucocytes ($10^3/\text{mm}^3$)		Erythrocytes ($10^6/\text{mm}^3$)	
	Non-infested	Infested	Non-infested	Infested	Non-infested	Infested
Trypanosome	38.6±04.0	37.7±5.19	6.58 ± 2.08	7.82±2.59	6.51±2.24	6.43±2.11
Babesia	38.4±04.6	37.9±5.08	7.06±2.08	7.34±3.25	6.87±4.00	6.80±1.94
Trypanosomes and Babesias	38.3±04.5*	33.1±7.4	6.76±2.32	9.20±3.68*	6.79±2.28	6.48±2.54

*: significantly high ($P \leq 0.05$)

Table 2: Influence of Trypanosoma infestation on hematocrit and blood cell count according to age and sex in cattle from the Western Highlands of Cameroon

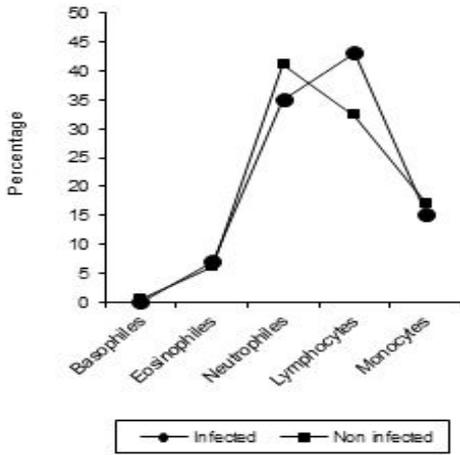
Age and sex of cattle	Hematocrit (%)		Leucocytes ($10^3/\text{mm}^3$)		Erythrocytes ($10^6/\text{mm}^3$)	
	Non-infested	Infested	Non-infested	Infested	Non-infested	Infested
0 - 2 years (Mâles)	39.2±4.77	34.2±0.30	6.37±2.51	8.38±2.37	6.04±2.42	6.40±3.82
3 - 4 years						
Males	38.6±4.01	40.7±3.37	6.79±2.06	7.81±2.75	7.14±2.33	6.26±2.41
Females	38.9±2.96	43.6±1.82	6.88±1.64	7.27±2.03	6.37±1.63	5.73±1.32
Males/Females	38.7±0.21	34.4±4.86	6.83±0.06	7.54±0.38	6.75±0.54	5.99±0.37
≥ 5 ans (Females)	37.9±3.90	42.2±2.08	6.22±1.78	7.61±2.82*	6.07±1.96	6.69±2.00

*: significantly high ($P \leq 0.05$)

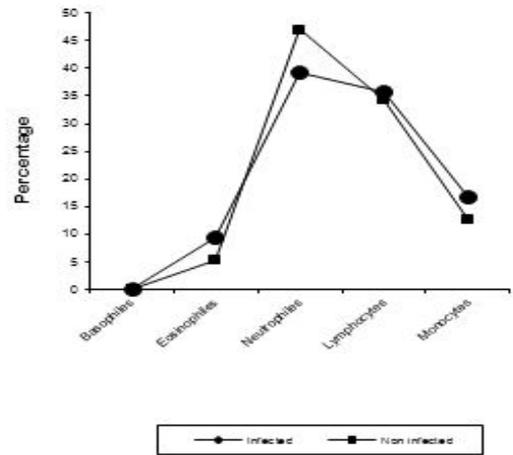
Table 3. Influence of Babesia infestation on hematocrit and blood cell count according to age and sex in cattle from the Western Highlands of Cameroon

Age and sex of cattle	Hematocrit (%)		Leucocytes (10 ³ /mm ³)		Erythrocytes (10 ⁶ /mm ³)	
	Non-infested	Infested	Non-infested	Infested	Non-infested	Infested
0 - 2 years (Mâles)	39.6±5.65	35.09±0.00	6.37±1.59	15.80±0.00*	7.34±7.42	9.07±0.00
3 - 4 years						
Males	38.7±4.02	40.9±3.09	7.69±2.29	5.47±0.78	7.15±2.43	6.08±1.42
Females	39.8±3.35	38.3±0.00	7.49±1.69	7.90±0.00	6.13±1.53	8.12±0.00
Males/Females	39.2±0.75	39.6±1.85	7.58±0.15	6.68±1.72	6.64±0.72	7.10±1.42
≥ 5 ans (Females)	36.7±4.97	35.0±5.72	6.87±2.23	7.73±3.19	6.38±1.93	6.61±1.98

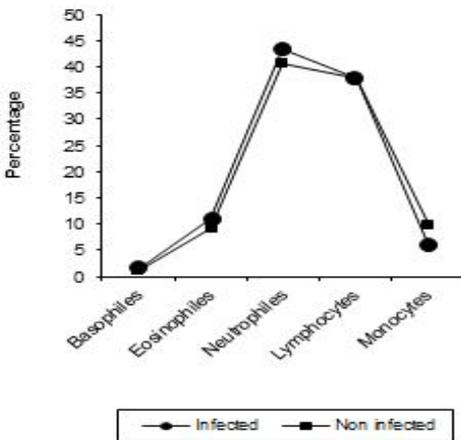
*: significantly high ($P \leq 0.05$)



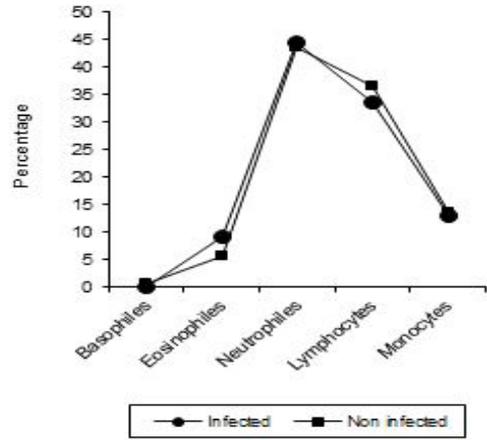
3_a : Males of 0-2 years



3_b : Males of 3-4 years



3_c : females of 3-4 years



3_d : females of 5 years and above

Figure 3: Influence of trypanosome infestations on leucogrammes according to age and sex in cattle in the Western Highlands of Cameroon

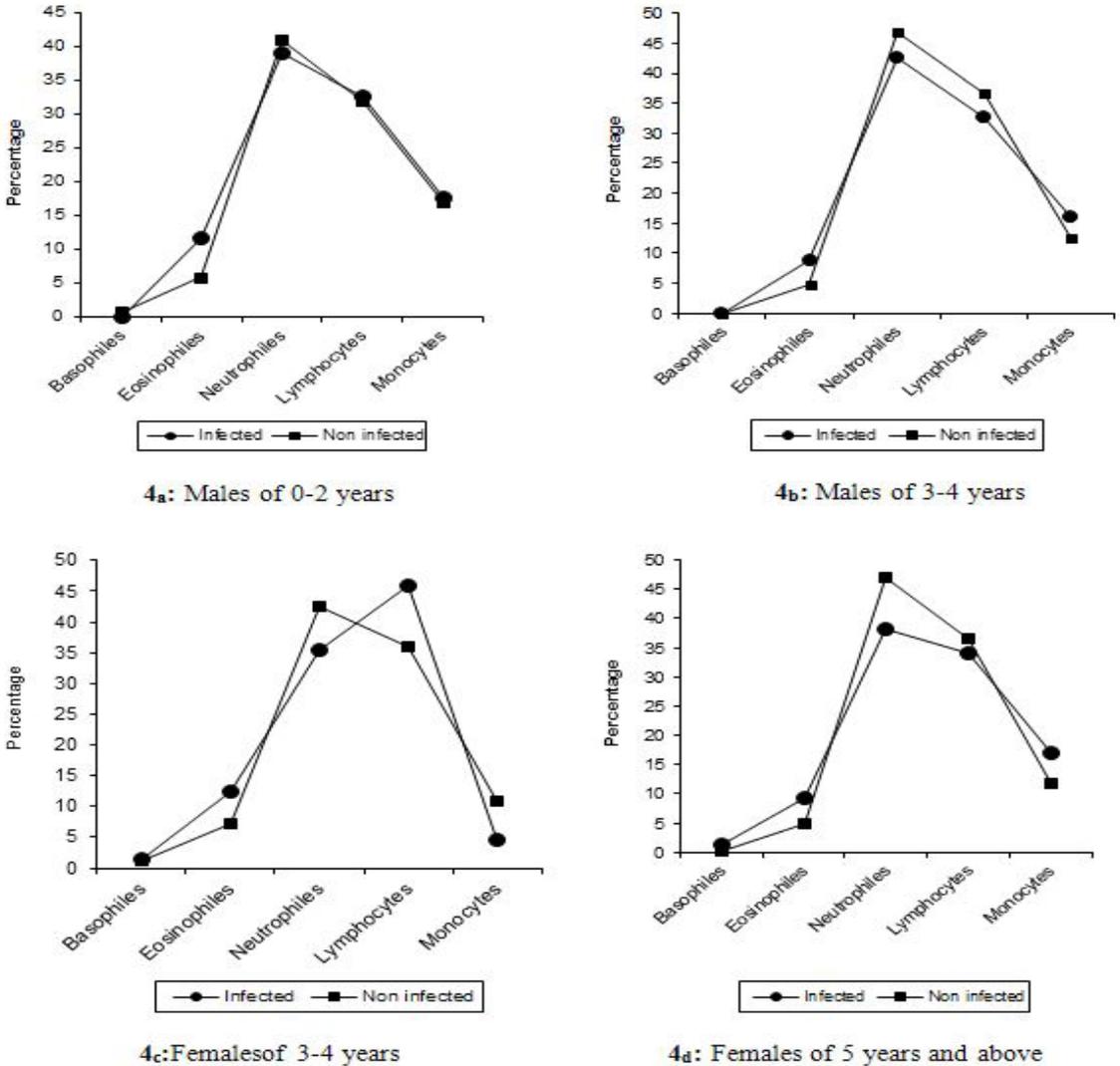


Figure 4: Influence of Babesia infestations on leucogrammes according to age and sex in cattle in the Western Highlands of Cameroon.

Seroprevalence and intensity of anti Trypanosoma antibodies in cattle of the Western Highlands of Cameroon.

The distribution of antibody levels revealed that animals with low level of antibodies (1:1 and 1:2) are the most common, as compared to those with high antibody production intensity (1:16 and 1 : 32). Only 7.14% of cattle have high antibody production intensities.

Seroprevalence and intensity of anti Trypanosoma antibodies according to breed in cattle of the Western Highlands of Cameroon

The seroprevalence and levels of production of antibodies against Trypanosoma sp according to breed of cattle as reflected in Figure 5 show that antibodies against Trypanosoma have been identified in all breed of cattle. The seroprevalence in Red Fulani (48.15%) and White Fulani (60.00%) and Gudali (57.14%) are comparable on one hand and significantly higher (P <0.05) to that of the crossbreds (21.05%) on the other hand.



Figure 5: Seroprevalences of trypanosomoses according to cattle breeds in the Western Highlands of Cameroon

The distribution of levels of antibodies against *Trypanosoma* sp according to cattle breed is illustrated in Figure 6. It appears that regardless of breed, animals with low rate of antibody production (tracks 1: 1: 2) are the most represented (88.9%). Whatever the breed, animals having the highest rate of production (1:32) have been identified in only a small proportion of Red Fulani (7.69%) and Gudali (25.0%).

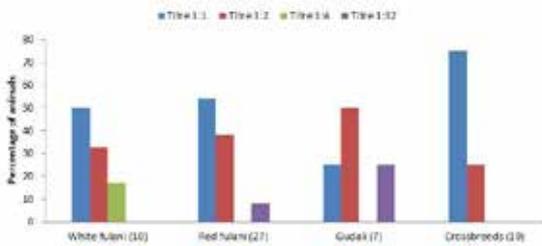


Figure 6: The distribution of levels of antibodies against *Trypanosoma* sp according to cattle breed in the western Highlands of Cameroon.

Seroprevalence and levels of antibodies against Trypanosoma sp according to the age and sex in cattle of the Western Highlands of Cameroon

The seroprevalence of antibodies against *Trypanosoma* sp. according to the age and sex of cattle (Figure 7) is similar ($P \geq 0.05$) as well for different age groups than sex.

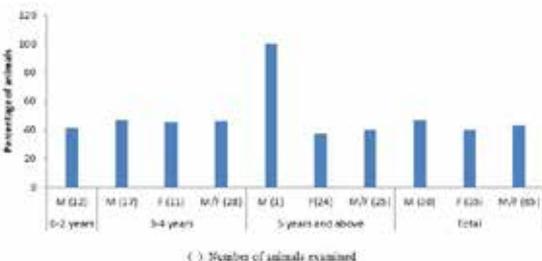


Figure 7: Seroprevalence of antibodies against *Trypanosoma* sp according to the age and sex in cattle of the Western Highlands of Cameroon

The distribution of antibody levels according to the age and sex of cattle (Figure 8) shows that regardless of age and sex groups, the animals with the highest antibody (1: 32) are the least represented (7.14%) as compared to those with very low levels of antibodies.

Regardless of age, cattle with low antibody production rates are also represented both in males than in females. Whatever the age and sex, animals producing antibodies at very low rates are the most represented, compared to those with a level of antibodies considered to be very high (1/32), which are represented only in males of 3-4 years (25.0%).

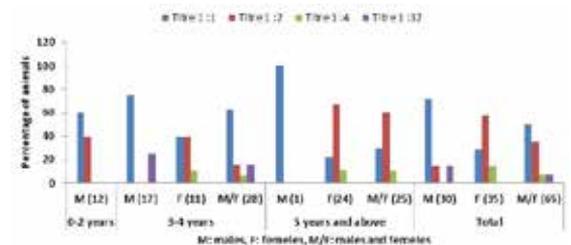


Figure 8: Distribution of antibody levels according to the age and sex of cattle of the Western Highlands of Cameroon

Concordances of immunological and parasitological results

Table 4 shows that the degree of agreement among the immunological and parasitological results in cattle amounts to 48.44%.

Table 4: Degree of agreement among the immunological and parasitological results in cattle

Concordances	CATT	Trypanosomes	Total
Concordances	+	+	4
	-	-	27
Total			31 (48.44%)
Discordances	+	-	24
	-	+	9
Total			33 (51.56%)

Discussion

This study revealed the presence of trypanosomes and babesia among cattle breeds of the Western Highlands of Cameroon. Here, the prevalence of bovine trypanosomes was by nearly 13% higher than that obtained in Buea in the South West of Cameroon by Ngole et al. (2003). Cattle from Buea slaughterhouse having almost the same origin as those in our study (Adamawa and North-west regions), the difference between the results could be related to the method of analysis. Indeed, they have used the technique of thick film while we added to it the “buffy coat” technique which is more sensitive.

The prevalence of *Babesia* infections found in cattle is lower than that obtained by El Haj et al. (2002) in cattle in Morocco. This could result from regular treatment of the animals or be related to the analytical methods used. Indeed, the authors quoted above had used the ELISA test which is an immunological test highlighting antibodies against babesia in infected individuals, but also treated ones or those which have been in contact with the parasite.

It comes from this study that breed, age and sex influence the prevalence of cattle infestation. This result is consistent with that of Itard (2000) and El Haj et al. (2002). In fact Itard (2000) discovered that some breeds showed a natural or artificial resistance to certain parasitic diseases. Thus, it has been shown that some African cattle breeds such as N'Dama, forest Mutturaurines such as Bakweri are trypanotolerant. Other authors (Omotainse et al., 2000; Ngole et al., 2003) also highlighted the

influence of age and gender on the prevalence of infestation. These variations could be linked to a wide range of intrinsic or environmental factors.

Immunological analyzes by card agglutination showed antibodies against *T. brucei gambiense* in all cattle breeds. This observation confirms the findings of parasitological examinations. Indeed, the parasitological examination reveals only the parasite in the blood while immunological tests show antibodies, even in the absence of parasitism. However, after repeated treatments against trypanosomes, antigens disappear quickly from the bloodstream while the antibody level is still high shortly after disinfection (Luckins, 1992). Thus, the presence of antibodies does not imply that of the parasites. It was also demonstrated that the CATT, although directed against the antibody to *T. b. gambiense* react also has a positive with other molecules around them (Itard, 2000).

The intensity of antibody production in animals of our study was influenced significantly ($P \leq 0.05$) by breed, age and sex. Rates of relatively very low antibody translate perhaps low infection intensity, an infection or a simple early degeneration of antibody shortly after treatment. However, with the lowest dilutions (1:32 and 1:16), the antibodies were evidenced in the Red Fulani, the Gudali and older animals of both sexes. These high levels of antibodies are associated with heavy infestations, to prolonged infestations in the absence of treatment or to a high-capacity of antibody production in these animals. However, the existence of animals that could produce high levels of antibodies is of particular interest

from the perspective of the development of a vaccine against trypanosomiasis. However, multiple spontaneous variations of variant surface glycoproteins (VSG) during an infestation by trypanosome make improbable the development of a vaccine against the blood forms of African trypanosomes (Itard, 2000).

Infestations by trypanosomes and babesia did not induce significant effect on hematocrit, blood cell count and differential leukocyte count. This result is consistent with that of D'leteren et al. (1992) obtained on cattle, and could be explained by the existence of multiple endogenous factors (species, breed, age, sex ...) and exogenous (environment, season, food ...) that may influence blood parameters in animals. In fact it has been shown that a better nutritional status of the animal could allow him to escape the symptoms of certain infestations (Ogunsanmi et al. 2000). On the contrary, our observations are opposed to those of Ngole et al. (2003) on slaughterhouse cattle. These authors noticed a significant difference between the blood parameters in animals infested and uninfested ones. The lack of influence could also be associated with the fact that animals have developed adaptations or some tolerance to parasites, thereby allowing their escape from the symptoms of these infections (Roitt et al., 1989). Unlike simple infestations, mixed infestations in cattle caused a significant decrease in hematocrit and a significant increase in the number of white blood cells.

Conclusion

Trypanosomiasis present in the Western Highlands of Cameroon. The prevalence of infection in cattle is influenced by breed. These infestations have shown no effect on hematocrit and blood cell count. The antibodies directed against *T b gambiense* were found in all cattle breeds of the study area and seroprevalence obtained were superior to parasitological prevalence.

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PREVALENCE, RISK FACTORS AND PARASITIC DISEASES CO-INFECTION OF PIGS IN OSUN STATE, SOUTH-WEST NIGERIA, 2006-2015.

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Abstract

Parasitic diseases are the most devastating diseases of pigs in the tropics after African swine fever. It causes a significant economic setback to the swine industry in Africa and Nigeria. The objective of the study was to assess the prevalence, associated risk factors and parasitic diseases co-infections of pigs over a ten year (2006 - 2015) period. A retrospective study on parasitic diseases of pigs was conducted in Osun state, Nigeria. Records from the four major veterinary clinics in the state were used for the study. Statistical package for social sciences (SPSS) version 22 was used to analyse the Chi square (χ^2) test and its odd ratios, while graphs were drawn using Microsoft Excel, 2010. Mange, helminthoses, lice infestation, tick infestation, eperythrozoonosis and babesiosis were the parasitic diseases diagnosed in pigs during the period under review. Mange (296/875; 33.83 %) was the most prevalent and babesiosis (4/875; 0.46 %) the least prevalent parasitic disease. There was no defined pattern in the yearly and monthly prevalence of parasitic diseases. Season of the year was the risk factor significantly associated ($p < 0.05$) with the prevalence of mange and helminthoses. There was a strong correlation in the co-infection of tick infestation with babesiosis; and lice infestation with eperythrozoonosis. This study showed that parasitism is a major disease condition affecting pigs in Osun state and so there is a great need for increased input in terms of sanitary practices, prevention and control measures against these diseases so as to improve productivity in the swine industry of the state and country.

Key words: helminthoses, mange, Nigeria, Osun state, parasitic diseases, pigs,

PREVALENCE, FACTEURS DE RISQUE ET CO-INFECTION PAR DES MALADIES PARASITAIRES DES PORCS DANS L'ÉTAT D'OSUN DANS LE SUD-OUEST DU NIGERIA, 2006-2015

Résumé

Les maladies parasitaires sont les maladies les plus dévastatrices des porcs en milieu tropical, après la peste porcine africaine. Elles sont responsables d'un frein économique important à l'industrie porcine en Afrique et au Nigéria. L'objectif de l'étude était d'évaluer la prévalence, les facteurs de risque associés et les co-infections de maladies parasitaires des porcs sur une période de dix ans (2006 - 2015). Une étude rétrospective sur les maladies parasitaires des porcs a été réalisée dans l'Etat d'Osun, au Nigeria. Les dossiers des quatre principales cliniques vétérinaires de l'État ont été utilisés pour l'étude. La version 22 du logiciel SPSS (progiciel statistique pour les sciences sociales) a été utilisée pour analyser le test Chi 9 carré (χ^2) et ses rapports de cotes (Odd Ratios – OR), tandis que des graphiques ont été tracés à l'aide de Microsoft Excel 2010. Les maladies parasitaires diagnostiquées chez les porcs au cours de la période considérée comprennent la gale, les helminthoses, l'infestation par les poux, l'infestation par les tiques, l'éperythrozoonose et la babésiose. La gale (296/875; 33,83%) était la maladie la plus répandue, tandis que la babésiose (4/875; 0,46%) était la maladie parasitaire la moins prédominante. On n'a pas identifié de modèle défini dans la prévalence annuelle et mensuelle des maladies parasitaires. La saison de l'année était le facteur de risque significativement ($p < 0,05$) associé à la prévalence de la gale et des helminthes. Une forte corrélation a été notée entre la coinfestation de l'infestation par les tiques et la babésiose ; et l'infestation par les poux et l'éperythrozoonose. Cette étude a montré que le parasitisme est une pathologie majeure qui affecte les porcs dans l'état d'Osun, et il y a donc un grand besoin d'un apport accru en termes de pratiques

sanitaires, de mesures de prévention et de lutte contre ces maladies afin d'améliorer la productivité de l'industrie porcine de l'État et du pays.

Mots-clés : helminthes, gale, Nigeria, Etat d'Osun, maladies parasitaires, porcs

Introduction

Swine production is lucrative with quick turnovers and it forms an integral part of the rural economy in many parts of the world (Seid and Abebaw, 2008). It occupies an essential part in the livestock sector in Nigeria and most parts of the world. Pigs are among the abundant livestock potential of Nigeria, as they are a source of meat, leather and its similar products and employment (Aiyedun, 2014). In the last decades, the swine industry has experienced an unparalleled increase in terms of production and consumption. This positive development has resulted in an increase in provision of animal protein for human consumption, employment generation and poverty reduction, and has contributed to the Nation's Gross Domestic Product and general economic growth (Pam *et al.*, 2013; Aiyedun, 2014). Despite the religious taboo of swine in some quarters, its high fecundity, short generation interval, early maturity and relatively small space requirement for their production, makes them viable sources of economic benefits to farmers (Wosu, 2015).

Parasitism exerts a negative pressure on livestock production throughout the world. It has a devastating impact on human and animal health worldwide particularly in developing countries (Ellis *et al.*, 2003). Swine parasitic diseases are considered to be next in economic importance after African swine fever and it causes a major obstacle to the growth of the pig industry (Sangeeta and Prasad, 2002). Parasitic disease infections are associated with productive drawbacks, substantial reproductive loss and poor reproductive performance in the swine industry thereby posing a major hindrance to profitable pig production in Nigeria (Karaye *et al.*, 2016). The direct losses caused by parasites (ecto and endo) are attributed to restlessness, pruritus, anemia, diarrhea, acute illness and death, premature

slaughter and rejection of some body parts at meat inspection. Indirect losses include the reduction of productive potential such as ineffective feed conversion abilities, decreased growth rate, weight loss in young growing animal (pigs) and late maturity of slaughter stock (Ademola and Onyiche, 2013).

Most survey studies on pig parasites carried out in Nigeria are centered on gastrointestinal parasites and haemoparasites with very few on ectoparasites. For example, Nwoha and Ekwurike (2011) and Agumah *et al.* (2015) conducted studies on gastrointestinal parasites of pigs in Umuahia (south-east Nigeria) and Pankshin (north-central Nigeria) respectively, while Usip (2014) and Ademola and Onyiche (2013) did studies on haemoparasites in Uyo (south-south Nigeria) and Ibadan (south-west Nigeria) respectively. Among the few studies on ectoparasites of pigs is the one done by Odo *et al.* (2016) in Emene (south-east Nigeria).

Retrospective study of animal diseases is a quick and inexpensive means of identifying the strategy for effective disease control when analyzed statistically (Abiola *et al.*, 2016). Therefore the objectives of this study are to determine the prevalence, associated risk factors and diseases co-infection of different parasitic conditions of pigs presented to the major Veterinary clinics in Osun state, southwest Nigeria. The knowledge of these findings will assist in the formulation of government policies for a better management, prevention and control measures against parasitic diseases of pigs in the state and the south-west region of Nigeria. This study also aimed at appending and updating current literature on parasitic diseases of pigs prevalent in Osun state.

Materials and Methods

Study location

The study was carried out in Osun

state, south-west Nigeria. The state covers an area of 9,026 square kilometers and is located between latitude 7° 30'N and longitude 4° 30'E with an altitude of 246 metres above sea level. Osun state is characterised by a tropical wet (March – November) and dry (December – February and August “August break from rains”) climate with a lowland tropical rain forest vegetation. The state has a mean annual rainfall of between 127.77 cm and 159.76 cm and an average annual temperature ranging from 21.1 °C to 31.9 °C. The minimum and maximum annual relative humidity are 58.7 % and 79.6 % (National Bureau of Statistics, 2012). Administratively, the state has 30 local government areas that are grouped into 6 zones. The zones are Osogbo, Ede, Iwo, Ikirun, Ilesha and Ife. Osun state is bordered in the north by Kwara state, in the east partly by Ekiti state and partly by Ondo state, in the south by Ogun state and in the west by Oyo state.

Study design and data collection

Clinical records were retrieved from the record archives of the major state Veterinary clinics located in Osogbo, Ilesa, Ede and Ikirun. Case records between January 2006 and December 2015 were examined and relevant data associated with parasitic diseases of pigs were extracted. These included information such as the presence or absence of parasitic disease conditions, age and sex of the animal and the date of presentation to the clinic. Diagnosis of each parasitic disease was carried out in the clinic based on case history, physical examination and clinical signs. Haemoparasitic cases were confirmed using a thin blood smear technique as described by Jain (1986), while the detection of the presence or absence of helminth eggs was carried out using the simple floatation method as described by Soulsby (1982). Ectoparasites were identified using the morphological characteristics as described by Soulsby (1982) with the aid of a stereomicroscope.

Data management and analysis

Data collected were analyzed to establish the prevalence of the different parasitic

disease conditions as well as the prevalence of their co-infections. The prevalence of each and co-infections of parasitic disease(s) was calculated by dividing the number of positive cases by the total number of cases examined and the result expressed in whole numbers and as percentages. The univariate analysis (χ^2) test and odds ratios with its 95% confidence interval were used to determine the association between each epidemiological factor and the parasitic diseases with more than 50 cases (helminthoses and mange). The odds ratios were calculated with respect to a reference category as indicated in the respective tables. All statistical tests were conducted using statistical package for social sciences (SPSS) version 22 (SPSS Inc., Chicago). Significance was determined at $p \leq 0.05$. Graphs were drawn using Microsoft Excel, 2010.

Results

A total of 875 pigs were presented in the four veterinary clinics during the period under review. Approximately 62.50 % (544) of the pigs presented were diagnosed with one parasitic disease condition or the other. Mange, with 296 cases was the most prevalent parasitic disease diagnosed representing 33.83 % (95 % CI: 30.75 – 37.01) of the population. Babesiosis (4; 0.46 %; 95 % CI: 0.15 – 1.10) was the least prevalent parasitic disease. The prevalence of other parasitic diseases was helminthoses (210; 24.00 %; 95 % CI: 21.26 – 26.91); tick infestation (22; 2.51 %; 95 % CI: 1.62 – 3.72); lice infestation (6; 0.69 %; 95 % CI: 0.28 – 1.42) and Eperythrozoonosis (6; 0.69 %; 95 % CI: 0.28 – 1.42) (Table 1).

The yearly and monthly prevalence of the various parasitic diseases is presented in Figures 1 and 2 respectively. Mange was the only parasitic disease that was diagnosed all through the reviewed period with peak prevalence seen in 2011 (58.82 %) and 2012 (57.14 %). Helminthoses was diagnosed in 2006 (37.95 %); 2009 (8.88 %); 2010 (4.08 %); 2013 (16.46 %); 2014 (16.81 %) and 2015 (32.65 %). Tick infestation was diagnosed in 2010 (16.33 %) and 2013 (8.86 %) and eperythrozoonosis in 2010

(8.16 %) and 2013(1.27 %). Lice infestation and Babesiosis were only diagnosed in 2014 (5.04 %) and 2013 (2.53 %) respectively. Mange was most prevalent in April (57.69 %) and October (57.14 %) and least prevalent in August (10.85 %) and December (6.59 %). Helminthoses; tick infestation and eperythrozoonosis were most prevalent in December (48.35 %); September (18.18 %) and August (3.10 %) respectively. The diagnosis of lice infestation (7.79 %) and babesiosis (5.20%) was only observed in the month of September.

Mange was more prevalent in young (216; 34.29 %), female (184; 36.51 %) and during the wet season (208; 46.02 %) compared to adult (80; 32.65 %), male (112; 30.19 %) and dry season (88; 20.80 %) respectively. Statistically, season was significantly associated with the prevalence of mange ($p < 0.05$), while age and sex were not significantly associated ($p > 0.05$). Young, male and wet season categories were 1.08 (95 % CI: 0.79 – 1.48); 0.75 (95% CI:

0.56 – 1.00) and 3.24 (95 % CI: 2.41 – 4.38) times likely to be infested with mange (mite infestation) than adult; female and dry season categories respectively (Table 2).

Higher prevalence of helminthoses was observed in adult pigs (60; 24.49 %) than young (150; 23.81 %), male (100; 26.95 %) than female (110; 21.83 %) and during the dry season (154; 36.41 %) than the wet season (56; 12.39 %). Statistically, age and sex were not significantly associated with the prevalence of helminthoses ($p > 0.05$), while season was significantly associated ($p < 0.05$). Young, male and wet season categories were 0.96 (95 % CI: 0.69 – 1.37); 1.32 (95 % CI: 0.97 – 1.81) and 0.25 (95 % CI: 0.18 – 0.35) times likely to be infected with helminthoses than adult; female and dry season categories respectively (Table 3).

Analysis of parasitic disease(s) single and co-infections among pigs from this study (Table 4) revealed that mange was the most

Table 1: Prevalence (%) of parasitic diseases of pigs in Osun State, Nigeria, 2006-2015.

Disease Condition	Number positive (%)	95% CI
Helminthoses	210 (24.00)	21.26 – 26.91
Tick infestation	22 (2.51)	1.62 – 3.72
Mange	296 (33.83)	30.75 – 37.01
Lice infestation	6 (0.69)	0.28 – 1.42
Eperythrozoonosis	6 (0.69)	0.28 – 1.42
Babesiosis	4 (0.46)	0.15 – 1.10
Total	544 (62.17)	58.92 – 65.34

Table 2: Univariate association between age, sex and season with the prevalence of mange among pigs in Osun State, Nigeria, 2006-2015.

Variables	Positive (%)	Negative (%)	Total	OR	95% CI	p
Age						
Young	216 (34.29)	414 (65.71)	630	1.08	0.79 – 1.48	0.65
Adult ^a	80 (32.65)	165 (67.35)	245	1.00		
Sex						
Male	112 (30.19)	259 (69.81)	371	0.75	0.56 – 1.00	0.05
Female ^a	184 (36.51)	320 (63.49)	504	1.00		
Season						
Wet	208 (46.02)	244 (53.98)	452	3.24	2.41 – 4.38	<0.01*
Dry ^a	88 (20.80)	335 (79.20)	423	1.00		

^a Reference category, * Significant, OR = Odds Ratio, CI = Confidence Interval

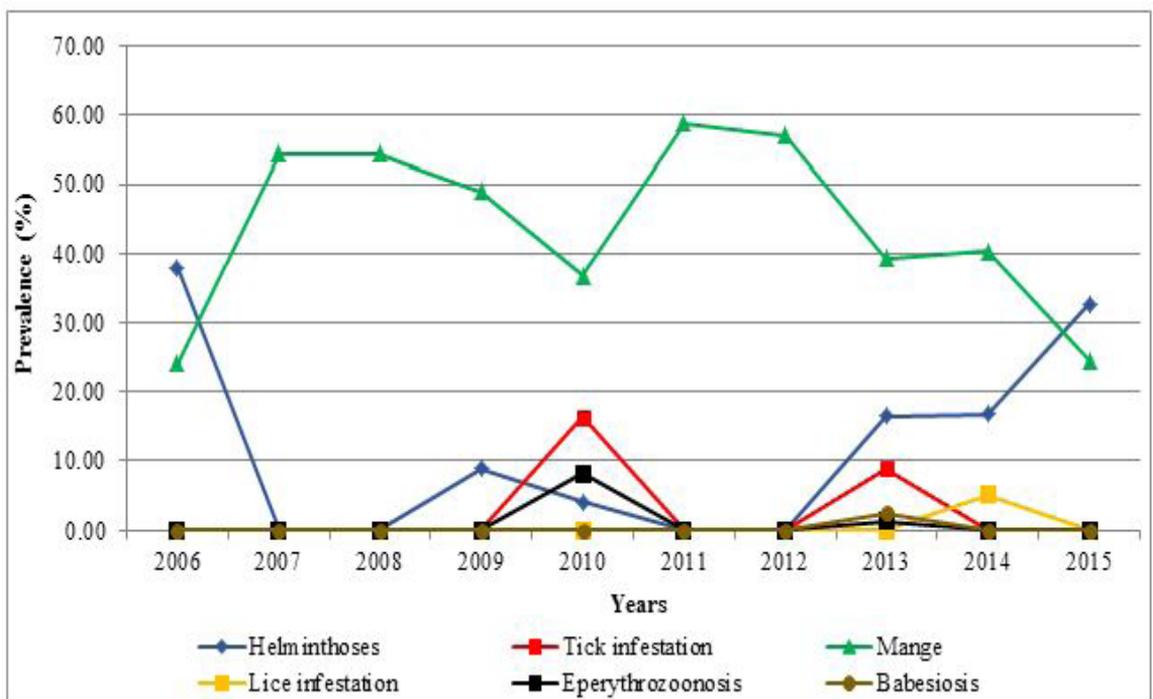
Table 3: Univariate association between age, sex and season with the prevalence of helminthoses among pigs in Osun State, Nigeria, 2006-2015.

Variables	Positive (%)	Negative (%)	Total	OR	95% CI	p
Age						
Young	150 (23.81)	480 (76.19)	630	0.96	0.69 – 1.37	0.83
Adult ^a	60 (24.49)	185 (75.51)	245	1.00		
Sex						
Male	100 (26.95)	271 (73.05)	371	1.32	0.97 – 1.81	0.08
Female ^a	110 (21.83)	394 (78.17)	504	1.00		
Season						
Wet	56 (12.39)	396 (87.61)	452	0.25	0.18 – 0.35	<0.01*
Dry ^a	154 (36.41)	269 (63.59)	423	1.00		

^a Reference category, * Significant, OR = Odds Ratio, CI = Confidence Interval

Table 4: Prevalence (%) of parasitic diseases co-infection among pigs in Osun State, Nigeria, 2006-2015.

Disease co-infection(s)	Number positive (%)	95% CI
Helminthoses alone	182 (20.80)	18.21 – 23.59
Tick infestation alone	18 (2.06)	1.26 – 3.17
Mange alone	268 (30.63)	27.64 – 33.75
Helminthoses and mange	28 (3.20)	2.18 – 4.53
Tick infestation and babesiosis	4 (0.46)	0.15 – 1.10
Lick infestation and eperythrozoonosis	6 (0.69)	0.28 – 1.42

**Figure 1:** Yearly Prevalence (%) of parasitic diseases of pigs in in Osun State, Nigeria, 2006-2015.

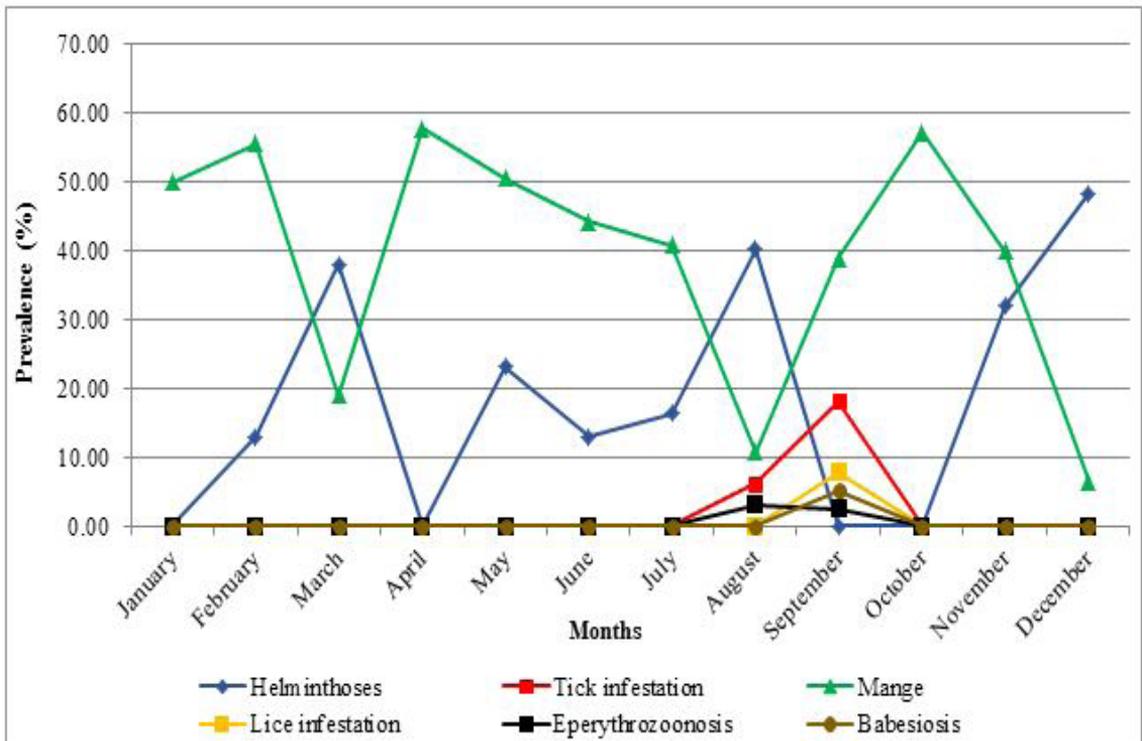


Figure 2: Monthly Prevalence (%) of parasitic diseases of pigs in in Osun State, Nigeria, 2006-2015.

prevalent single disease condition (268; 30.63 %), while sole infections with tick infestation the most uncommon (18; 2.06 %). Helminthoses and mange co-infection was the most prevalent form of double parasitic disease condition (28; 3.20 %), whereas tick infestation and babesiosis co-infection was the least encountered (4; 0.46 %). All cases of eperythrozoonosis and babesiosis were co-infected with lice and tick infestation respectively.

Discussion

Parasitism is among the major cause of setback to swine production in Sub-saharan Africa and the world, hence its study is very important. The overall prevalence of 62.17 % for the total parasitic diseases diagnosed in this study confirms that parasitism is a major disease condition affecting pigs in Osun state. Among the parasitic diseases diagnosed, mange and helminthoses were the most prevalent. Mange has been reported to be the most prevalent ectoparasitic disease affecting pigs in

Nigeria with 36.80 % prevalence (Odo *et al.*, 2016); Ethiopia, 16.20 % prevalence (Abdu and Gashaw, 2010); Tanzania, between 21.00 % and 91.00 % prevalence (Kambarage *et al.*, 1990); Germany, 45.40 % prevalence (Damriyasa *et al.*, 2004); Spain, 37.00 % prevalence (Alonso de Vega *et al.*, 1998); India, 37.50 % prevalence (Maiti *et al.*, 2004) and Romania, 70.00 % prevalence (Cozma *et al.*, 1997). This shows that mange is a worldwide disease of pigs, affecting both intensively and extensively raised pigs. Mange in swine is caused by sarcoptic (*Sarcoptes scabiei* var. *suis*) and demodectic (*Demodex phylloides*) mites (Soulsby, 1982; Urquhart *et al.*, 1996). However, sarcoptic mites is the most common cause of swine mange and it is associated with a more serious form of the disease (Das *et al.*, 2010). The high prevalence of mange from this study may be attributed to the unique size (small tiny about, 0.40 mm long and 0.30 mm wide) of mites and the fact that they burrow into the skin of their host as against other ectoparasites that are found on the surface of their host, making them to survive

severe environmental conditions. Studies on swine helminthoses, reported a prevalence of 47.50% in Nigeria (Karaye *et al.*, 2016), 13.50% in Ghana (Atawalna *et al.*, 2016) and 92.70% in Burkina Faso (Tamboura *et al.*, 2006). This shows that helminthoses is a common problem of the pig industry all over the world. Management system, nutritional status, sanitary practices, environmental and climatic conditions and regular medical attention (eg deworming) play a significant role in the prevalence of helminthoses in animals. Prevalence of 1.70 % and 5.12 % has been reported for *Babesia* species and *Eperythrozoon* species respectively (Ogbaje *et al.*, 2015), 3.20 % for *Haematopinus suis* (louse) infestation (Odo *et al.*, 2016). This reported prevalence may be the reason for the low prevalence recorded in this study. The thick skin layer and the underlining fat seen in pig skin may discourage lice and ticks from attaching; thereby resulting to low prevalence of the ectoparasites and the haemoparasitic diseases they transmit. Based on clinical records, pigs infested with ticks were raised with other animals/pet in the same farm.

There was no consistent yearly and monthly pattern in the prevalence of parasitic diseases. Although, mange was the only parasitic disease that was diagnosed throughout the study period and within the year, this confirms its high prevalence among pigs in Osun state. Other parasitic diseases with exception to helminthoses recorded one or two occurrences throughout the study years and months, confirming their low prevalence among pigs in the state.

The prevalence of mange decreased with increase in age, this could be as a result of immunity acquired from previous infection by adult pigs and also due to the soft skin possessed by young pigs making it easier for mites to burrow into the skin of the young. Prevalence of mange was higher in females than male pigs. This could be attributed to the stress of breeding, milking and cyclical hormonal changes in the female. The absence of significant difference in the prevalence of mange in relation to age and sex has been reported in Nigeria by Odo *et al.* (2016) and in Ethiopia by

Abdu and Gashaw (2010). Significantly, mange is about 3.3 times more likely to occur during the wet season than the dry season; this could be attributed to the high moisture content and lower temperature which is seen during the wet seasons, and this may favour the growth and development of the parasite. Temperature between 7 °C to 18 °C and a relative humidity of 65 to 75 % favours the survival of mite for 12 days outside the host (Jacobson *et al.*, 1999).

Season was the only risk factor significantly associated with the prevalence of helminthoses in this study. A 4 times tendency for helminthoses to occur during the dry season as against the wet season was observed. The abundance of flukes (metacercariae) in the pasture at the end of the rainy season (Kemal and Terefe, 2013) could be the reason for higher prevalence of helminthoses during the dry season in our study. A non-significant difference in the prevalence of helminthoses in relation to age and sex has been reported in Nigeria (Wosu, 2015).

Helminthoses and mange co-infection was the most prevalent form of double parasitic disease condition. The depression of the immune system caused by both parasitic diseases could have resulted in their high co-infection rate. The high correlation in the co-infection of tick infestation with babesiosis; and lice infestation with eperythrozoonosis could be attributed to the fact that ticks and lice are the main vectors for porcine babesiosis and eperythrozoonosis respectively.

Conclusion

The study has established the presence of both ecto and endo (haemo and gastrointestinal) parasitic diseases in pigs of Osun state. Mange is the most prevalent parasitic disease of pigs and it occurs all year round with a striking prevalence. Helminthoses is also a common parasitic disease of pigs with tick infestation, lice infestation, eperythrozoonosis and babesiosis been less commonly diagnosed among pigs in the state. The effect of parasitism is usually manifested in production and economic losses. There is therefore a need for

increased input in terms of sanitary practices, prevention and control measures so as to have a zero level of parasitic diseases prevalence which will translate to enhanced productivity in the swine sector of the state and Nigeria in general.

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SEROLOGICAL PREVALENCE AND ASSOCIATED RISK FACTORS OF *SALMONELLA GALLINARUM* IN COMMERCIAL CHICKENS IN BENUE STATE, NIGERIA

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Abstract

Fowl typhoid caused by *S. Gallinarum* is an acute septicemic bacteria disease of economic importance to poultry worldwide. A cross sectional study was carried out to establish the sero-prevalence and associated risk factors of *S. Gallinarum* in commercial chickens in Makurdi, Gboko and Otukpo local Government areas of Benue State from March to July 2015. Five hundred and eighty eight (588) blood samples were randomly collected from chickens and tested for *S. Gallinarum* antibodies using Serum plate agglutination test. Direct flock observation, and a short structured questionnaire administered in forty nine (49) flocks was used to collate data on poultry farms, to identify risk factors associated with *Salmonella* infections in chickens. The overall sero-prevalence established using serum plate agglutination test was 16.7% (98/588). Using a univariate logistic analysis, factors significantly associated with *Salmonella* infections at $p < 0.05$ were presence of other birds in poultry farms (OR = 7.2; 95 % CI = 1.9334 - 26.8181), movement of farm attendant from one pen to another (OR = 4.5; 95 C.I = 1.3034 – 15.6602), presence of other farm animals and rodents (OR=2.8000 0.6649-11.7915). Effective equipment washing and disinfection and the use of foot bath with disinfectant when entering poultry house significantly reduced the risk of testing positive for *Salmonella* in chickens ($p < 0.05$). In conclusion, this study established the seroprevalence and identified risk factors associated with *S. Gallinarum* infections in chickens in three major towns of Benue State. Therefore, to prevent *Salmonella* infections in commercial chickens, proper implementation of biosecurity measures, hygiene practices and rodents control in poultry farms are recommended.

Key words: Serological prevalence, *S. Gallinarum*, chickens, *Salmonella* infection and risk factors.

PREVALENCE SEROLOGIQUE ET FACTEURS DE RISQUE ASSOCIES DE *SALMONELLA GALLINARUM* CHEZ LES POULETS COMMERCIAUX DE L'ÉTAT DE BENUE AU NIGERIA

Résumé

La fièvre typhoïde causée par *S. Gallinarum* est une maladie bactérienne septicémique aiguë d'importance économique pour la volaille dans le monde entier. Une étude transversale a été réalisée pour déterminer la séroprévalence et les facteurs de risque associés à *S. Gallinarum* chez les poulets commerciaux des zones de la collectivité locale de Makurdi, Gboko et Otukpo de l'État de Benue, de mars à juillet 2015. Cinq cent quatre-vingt-huit (588) échantillons de sang ont été prélevés de manière aléatoire chez des poulets et testés pour rechercher la présence d'anticorps dirigés contre *S. Gallinarum* en utilisant un test de séro-agglutination sur lame. L'observation directe des troupeaux et un court questionnaire structuré administré dans quarante-neuf fermes ont été utilisés pour recueillir les données sur les élevages de volailles afin d'identifier les facteurs de risque associés aux infections à *Salmonella* chez les poulets. La séroprévalence globale établie à l'aide du test de séro-agglutination sur lame était de 16,7% (98/588). L'analyse logistique univariée a révélé que les facteurs significativement associés aux infections à *salmonella*

à $p < 0,05$ comprennent la présence d'autres oiseaux dans les exploitations avicoles (OR = 7,2; 95% IC = 1,9334-26,8181), le déplacement de l'agent avicole d'un logement de volaille à un autre (OR = 4,5 ; 95 C.I = 1.3034 – 15.6602), la présence d'autres animaux de ferme et de rongeurs (OR = 2,8000 0,6649-11,7915). Le nettoyage et une désinfection efficaces du matériel ainsi que l'utilisation d'un bain de pieds avec un désinfectant lors de l'entrée dans les poulaillers réduisent considérablement le risque de dépistage positif de *Salmonella* chez les poulets ($p < 0,05$). En conclusion, cette étude a établi la séroprévalence et identifié les facteurs de risque associés aux infections à *S. Gallinarum* chez les poulets dans trois grandes villes de l'Etat de Benue. Ainsi, pour prévenir les infections à *Salmonella* chez les poulets commerciaux, il est recommandé de mettre en œuvre correctement les mesures de biosécurité, les pratiques d'hygiène et le contrôle des rongeurs dans les fermes avicoles.

Mots-clés : prévalence sérologique, *S. Gallinarum*, poulets, infection à *Salmonella* et facteurs de risque.

Introduction

The poultry industry in Nigeria is fast growing, transforming from the tradition husbandry system where chickens are reared in small numbers in backyards into a commercial husbandry system that is large scale, highly specialized and profit oriented. *Salmonella* infections in chickens are caused by the non-motile *S. Gallinarum* and *S. Pullorum* serotypes and the various kinds of motile, non-host adapted serotypes such as *S. Enteritidis*, *S. Kentucky*, *S. Typhimurium*, *S. Ziga*, *S. Nima* and *S. Livingstone* which are commonly referred to as paratyphoid *Salmonellae* (Gast, 1997; Ameh et al., 2016).

Salmonella enterica sub spp *enterica* serotype *Gallinarum* is the causative agent of fowl typhoid, an acute septiceamic bacteria disease of chickens characterized by sudden death, diarrhoea, anemia, fever, increase thirst, abnormal respiratory signs and high mortality in adult chickens (Okwori et al., 2007; Abdu, 2014). Fowl typhoid has worldwide distribution but has been eradicated from commercial poultry in most developed countries of the world; Australia, Canada, USA and Japan (OIE, 2005). In some developing countries, the disease was controlled through the implementation of co-ordinated policies of standard hygienic practice, good biosecurity measures and periodic serological testing and slaughter of positive reactors (Barrow et al., 1999). Recently, fowl typhoid has reoccurred in many European countries (Ivanics et al., 2008). Since the discovery of the disease, a lot of efforts have been made to control and prevent

its occurrence in commercial poultry; yet, outbreaks of fowl typhoid still pose a serious economic challenge in places where control measures are not enough or areas where the climatic conditions support the environmental spread of *Salmonella* organism (Barrow and Freitas, 2011).

In Africa, fowl typhoid has been reported in countries like Nigeria (Sa'idu et al., 1994; Okwori et al. 2007; Ezema et al., 2009; Mbuko et al., 2009), Uganda (Ojok, 1993), Tanzania (Msami and Mtie, 1996; Mdegela et al., 2000; Bura et al., 2014), Senegal (Arbelot et al., 1997) and Zambia (Sharma et al., 1991).

Fowl typhoid is an important cause of economic loss in commercial poultry in Nigeria. Outbreak of the disease is often associated with high mortality, in Udi South East Nigeria, fowl typhoid outbreak was reported in commercial laying hens affecting 11,000 birds with mortality rate of up to 25% (Ezema et al., 2009). Despite its economic importance, there appear to be a dearth of information on the disease in the country. Periodic serological testing which helps in dictating and estimating the prevalence of *Salmonella* infection in flocks as well as the removal of positive reactor is seldom not practice. Hence, this study was designed to determine the serological prevalence of *S. Gallinarum* and investigate risk factors that are associated with *Salmonella* infections in commercial chickens in the study area. Thus provide baseline data that may assist in the design and deployment of preventive and control measures.

Materials and Method

Study area

This study was carried out in Makurdi, Otukpo and Gboko towns of Benue State. The State lies within the lower river Benue trough in the Middle Belt Region of Nigeria. Its geographic coordinates are longitude 7° 47' and 10° 0' East; Latitude 6° 25' and 8° 8' North, and shares boundaries with five other states namely Nasarawa to the North, Taraba State to the East, Cross River to the South, Kogi State to the West and Enugu to the South West. The State also shares a common boundary with the Republic of Cameroon on the South-East. According to the 2006 census, it has a population of 4,253,641 and occupies a landmass of 32,518 square kilometres. Farming and livestock husbandry are the main source of livelihood of Benue people (Gilbert, 1980).

The study design and sampling

A cross sectional study was carried out to determine the sero-prevalence and associated risk factors of *S. Gallinarum* infection in commercial chickens in Makurdi, Otukpo and Gboko local Government areas of Benue State from March to July 2015. Forty nine (49) flocks comprising of broilers and layers were randomly selected based on data provided by the Benue State Ministry of Agriculture. The flock ages were grouped into starter (day old – 8wks old), growers (9 – 20 weeks) and layers (>20wks). Sample size was determined based on the formula recommended by Thrusfield (1997).

$$N = \frac{Z^2 PQ}{d^2}$$

Where n= sample size

Z= confidence level (1.96)

P= prevalence of previous study (25% prevalence reported by Ezema et al. (2009) was used),

Q = 1-P,

d = allowable or standard error (Allowable error was decrease from 0.05 to 0.035 to obtained large sample size thereby increasing

the precision).

Therefore;

$$N = \frac{(1.96)^2 \times (0.25) \times (1-0.25)}{(0.035)^2}$$

$$n = 588$$

A short structured questionnaire was designed to collect information on general farm practice and house level factors that may be associated with responsible risk of *Salmonella* infection in chickens. This include information on production type, flock size, movement from one pen to another, source of feed and water, feed storage, use of foot bath/ equipment washing and disinfection, multiple flocks, vaccination status against fowl typhoid, sighting of rodents on farms, presence of other farm animals and farm attendants. All the variables were selected based on literature review on Fowl typhoid and associated risks factors of *Salmonella* in commercial chickens (Mbuko et al., 2009; Snow et al., 2010; Agada et al., 2014).

Each farm was visited twice and a total of 588 blood samples were randomly collected from 264 layers and 324 broilers from poultry farms within Makurdi (n=288), Otukpo (n=150) and Gboko (n=150). Approximately 2ml of blood were drawn from the wing vein aseptically into sterile sample bottles, all samples collected were transported in cool thermos to the laboratory on the day of collection. Blood samples were kept in slant position and allowed to clot at room temperature, stored overnight at 4°C and spun at 3,000 rpm for 10 minutes. Sera were harvested and stored frozen at –20°C in vials until analyzed for serum plate agglutination test.

Serum Plate Agglutination Test (SPAT)

The standard serum plate agglutination test was carried out according to the method described by OIE (2012). Antigen used was prepared stained *S. Gallinarum* antigen (Intervet International, Holland). Samples were analysed according to the instruction of the manufacturer. Briefly, the reagent and sera samples were allowed to warm up to room temperature prior to use. Using a sterile Pasteur

pipette, 0.02 ml of the sera was dispensed on a tile and 0.02 ml of the antigen was added using separate sterile pipette. The antigen and sera was properly mixed with wooden applicator sticks, the tile plate gently rocked for a few seconds and reaction read within 2 minutes. Identification of positive samples was based on formation of clumps (agglutination) within two minutes.

Investigation of risk factors for Salmonella in poultry farms

Interview using a short structured questionnaire was conducted to obtain information on the risk factors for *Salmonella* infection in all the sampled flocks. All terms used in the questionnaire were clarified by the interviewer to avoid misinterpretation by the respondent.

Data analysis

Data on sero-prevalence study were analysed using Microsoft® Office Excel 2010, professional edition and SAS software (version 9.3). Simple descriptive statistics such as percentages and frequency was used to express seroprevalence of *Salmonella*. Data generated from the questionnaire study on associated risk factors for *Salmonella* were entered, cleaned and analysed using Epi Info (version 7.3). A univariate logistic analysis was done,

odds ratio (OR) and probability (p-values) at 95% confidence interval were established for biological and statistical association between dependent and predictive investigated variables.

Results

Out of the 588 serum samples tested with serum plate agglutination test, 98 (16.7%) were positive for *Salmonella* antibodies. From the two hundred and eighty eight (288) chickens screened from Makurdi metropolis, 57 (19.79%) had positive reactions, while the 150 chickens screened each from Otukpo and Gboko had 32 (21.33%) and 9 (6%) positive reactions, respectively (Table 1). There was statistical significant difference ($p < 0.05$) in the seroprevalence of *Salmonella* antibodies in Gboko (6%) as compared to Otukpo and Makurdi, with Otukpo having the highest seroprevalence of 21.33%.

Response to questionnaire

All the 49 (100 per cent) farms sampled and investigated respond to the questionnaire on risk factors. Data collated shows variables that significantly influence prevalence of *Salmonella* infection at $P < 0.05$ during univariate analysis were; presence of other birds such as turkeys, ducks and local chickens in poultry farms (**OR = 7.2; 95 % CI = 1.9334 26.8181**)

Table 1: Distribution of serum reactivity to *S. Gallinarum* antigen among commercial chickens in three locations in Benue State.

location	No of samples	No of seropositive	Sero-prevalence %
Makurdi	288	57	19.97
Otukpo	150	32	21.33
Gboko	150	9	6.0
Total	588	98	16.66

Table 2: Distribution of serum reactivity to *S. Gallinarum* antigen among different age groups of commercial chickens in three locations in Benue State.

Age in weeks	No of sera samples tested	No of seropositive samples	Sero-prevalence (%)
1-8	123	006	4.88
9-12	201	023	11.44
>20wk	264	069	26.14
Total	588	98	16.66

Table 3: Distribution of serum reactivity to *S. Gallinarum* antigen based on type of chicken (production type) in three locations in Benue State.

Production type	No tested with SPAT	No positive	Sero-prevalence (%)
Broilers	324	029	8.95
Layers	264	069	26.14
Total	588	98	16.66

Table 4: Results of univariate analysis for variables considered as risks associated with *Salmonella* infection in chickens showing sero-prevalence, p – values and odd ratios

Variable	N	Percentage positive	p-Value	OR (95% CI)
All in all out				
Yes	36	33.3	0.7394	0.8000(0.2148-2.9792)
No	13	38.0		
Bird type				
Layers	22	40.9	0.1629	2.4231 (0.6990-8.400)
Broilers	27	22.2		
Flock size				
< 1000	27	22.2	0.0166	0.2078 (0.0575-0.7515)
1000-2999	19	57.9		
3000-5000	3	33.3	0.2000	0.00
Movement from one pen to another				
Yes	18	61.1	0.0174	4.5179 (1.3034-1566)
No	31	25.8		
Source of feed				
Commercial	31	33.3	0.7530	1.2222 (0.3503-42649)
Home made	18	29.0		
Storage of feed				
Within pen	11	45.5	0.3979	1.8056 (0.4589-7.044)
Stores	38	31.6		
Equipment washing and disinfection				
Yes	33	24.2	0.0317	0.2489 (0.7000-0.8851)
No	16	56.2		
Use of foot bath				
Yes	33	24.2	0.0317	0.2489 (0.700-0.8851)
No	16	56.2		
Multiple flocks				
Yes	27	44.4	0.1176	2.7200 (0.7768 – 9.5243)
No	22	22.7		

Variable	N	Percentage positive	p-Value	OR (95% CI)
Presence of rodent				
Yes	36	41.7	0.0241	2.3810 (0.5583-10.1546)
No	13	23.1		
Presence of other farm animals				
Yes	34	41.2	0.0160	2.8000 (0.6649-11.7915)
No	15	20		
Other birds				
Yes	20	60.0	0.0033	7.2000(1.9334-268131)
No	29	17.2		

increases the chance of contracting *Salmonella* infection by 7.2 fold, movement from one flock to another (**OR = 4.5; 95 C.I = 1.3034 – 15.6602**) increases the likelihood of *Salmonella* infection in chickens by 4.5 folds and similarly, poultry farms with presence of other farm animals like pigs, goats and sheep (OR= 2.8, 95% C.I =0.6649-11.7915) are 2 times more likely to test positive for *Salmonella* than flocks reared in the absence of these animals table 4.

Discussion

This study has established a sero-prevalence of *S. Gallinarum* infection and risk factors for *Salmonella* in commercial chickens in Benue State. Sero-prevalence of *Salmonella* infection in chickens has been reported in various parts of the world (Islam *et al.*, 2006; Hossain *et al.*, 2010). While Islam *et al.* (2006) reported a 43.4% sero-prevalence in Bangladesh, Hossain *et al.* (2010) reported 25.3% and Waiswa *et al.* (2006) reported 27.9% sero-prevalence of *S. Gallinarum* antibodies in Tanzania. These authors have reported a higher sero-prevalence than that of this study. The difference in results could be attributed to lack of awareness on the prevention and control of fowl typhoid, poor management practices and difference in geographical locations of the study areas. The 16.7% sero-prevalence reported in this work, is higher than the 9.4% overall sero-prevalence of *S. Gallinarum* reported by Okwori *et al.* (2007)

in Jos, and much higher than the 3.2% reported by Onunkwo and Onovarian (1978) in Plateau state Nigeria. In this study, there was statistical significant difference $p (<0.05)$ in the sero-prevalence of *Salmonella* antibodies in Gboko (6%) as compared to Otukpo and Makurdi, with Otukpo having the highest sero-prevalence of 21.33%. The high sero-prevalence recorded in Otukpo and Makurdi could be due to poor management practices and poor biosecurity implementation as observed in most of the farms sampled and investigated. The low sero-prevalence recorded in Gboko could be attributed to better management practice, as information gathered from the questionnaire study reveal that farms investigated in the area have provision for the use of foot bath before entering poultry houses and also wash their equipment with disinfectant. However, the result of this study is relatively similar to the reports of Mdegela *et al.* (2000) and Bura *et al.* (2014) who reported 18.4% and 15% sero-prevalence of *S. Gallinarum* respectively in Tanzania. In addition, it was observed in this study that sero-prevalence of *S. Gallinarum* increased with the age of the chickens. This finding is in agreement with the reports of Islam *et al.* (2006) who reported 72.9% sero-prevalence in layers of > 60wks and low sero-prevalence of 4.2% in starter (0-8wks) layers. This could be attributed to the longer stay of layers.

The survey on associated risk

factors for *Salmonella* in commercial chickens investigated several variables that were considered to have significant association with *Salmonella* infections in chickens. We found that, poultry farms whose attendant move from one flock or farm to another was found to be significantly associated with an increase chance of testing positive for *Salmonella* (O.R 4.5179, 95% C.I= 1.3034 – 156). This finding is in agreement with reports of Fris and Van. (1995) who reported that the risk for a flock to be infected by *Salmonella* increase through hands, clothing and farm equipment when poultry attendant move from one pen to the other and from one poultry farm to the other. Also, study by Agada et al. (2014) showed significant association between movement from one pen to the other and the likelihood of *Salmonella* contamination.

In this survey, there was no significance association between farms that used commercial feed and those that used locally made feed ($p>0.05$). However, sero-prevalence of *S. Gallinarum* infection was found to be a little higher in flocks that use commercial feed (33, 3%) against flocks that are feed with locally made feed (29%). Corrry et al. (2002) and Okonkwo et al. (2010) reported commercial feed as potential sources of *Salmonella* infection for poultry flocks. Also, studies by Snow et al. (2010) found an association between the use of commercial feed and an increase likelihood of *Salmonella* infection in commercial chicken.

This study found a significant protective effect of equipment washing and disinfection as well as the use of foot bath with disinfectant before entering poultry houses (O.R 0.25) to be associated with lower odds of testing positive for *Salmonella*. This finding corroborate studies by Davies and Breslin (2003a) which shows that good cleaning and disinfecting practices are effective in reducing *Salmonella* infection. Disinfection alone without effective washing and cleaning was shown not to have significant protective effect on *Salmonella* (Snow et al. 2010). This was possibly due to the failure of disinfectants to penetrate organic matters left in the poultry houses previously contaminated (Davies and Breslin 2003b). Other studies have

established that disinfectant in foot bath may not effectively kill microorganisms, but using a boot brush to remove organic matters from the boots may be more effective (Amass et al., 2000; Snow et al., 2010).

The presence of multiple flocks of layers in the same farm house has been shown to be significantly associated with an increased risk of *S. Gallinarum* infection (Bura et al., 2014). This is in agreement with the findings of this survey (O.R 2.72, 95% C.I 0.7768-9.52). This finding is a pointer that horizontal transmission can occur between one flock and another due to poor biosecurity implementation measures.

Presence of rodents and other farm animals in poultry farms were found to be significantly associated with increased risk of *Salmonella* in chickens (OR 2.38, 95% C.I= 0.5583-10.1546). Infected rodents may contaminate poultry feed and water, which can then be a potential source of *Salmonella* infection when the contaminated water and feed are ingested by susceptible chickens. This finding has been corroborated by reports of other studies (Agada et al., 2014). Likewise, other animal carriers of *Salmonella* in the premises of poultry farms can serve as carriers and thus, contaminate poultry feed and water, thereby promoting horizontal transmission of *Salmonella* in chickens.

The findings of this survey showed a very significant association between *Salmonella* infection and the presence of other birds ($p<0.05$). Poultry farms with other birds such as local chickens, ducks, guinea fowl and turkeys are 7 times more likely to test positive for *Salmonella* than farms without the presence of other birds. This finding is in line with report of Bura et al. (2014) where they reported that the presence of other birds increases the risk of *Salmonella* infection. Generally, in poultry farms where biosecurity measures are poorly implemented, horizontal transmission from an infected chicken to a susceptible chicken or flock is more likely to occur. In conclusion, this study has established the presence of *S. Gallinarum* antibody in chickens in the study area. Also, the result of this study shows that effective equipment washing and disinfection

is associated with lower risk of *Salmonella* infection in chickens. Thus, support previous research work on the importance of high standard of hygienic practice and proper implementation of biosecurity measures in poultry farms (Davies and Breslin 2003b; Snow *et al.*, 2010).

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EXPERIMENTAL STUDY ON ALTERNATIONS IN GROWTH PERFORMANCE AND SERUM BIOCHEMICAL ANALYTES IN BROILER CHICKS EXPOSED TO VARYING LEVEL OF CALCIUM IN STANDARD POULTRY RATION IN ADDIS ABABA, ETHIOPIA

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Abstract

The current experimental study was conducted to evaluate the growth performance responses and serum biochemical alteration in broiler chicks exposed to different dietary calcium level in standard poultry ration in Addis Ababa, Ethiopia. Randomized Complete Block Design (RCBD) was employed from January to June 2015 for a period of six weeks on a total of 80 day-old Cobb 500 broiler chicks breed (40 females and 40 males) which were randomly allocated into four equal treatment groups each comprising 20 broiler chicks. Accordingly, the four treatment groups designated as A (kept as control), B, C and D received dietary calcium levels 8.5 g Ca/Kg, 17 g Ca/Kg, 25.5 g Ca/Kg and 36.5 g Ca/Kg, respectively. Similarly, each treatment group was blocked on the basis of sex (10 males and 10 females). Serum samples from all treatment groups were obtained and subjected to biochemical analysis using spectrophotometer auto analyzer. Growth performance responses namely feed intake, feed conversion ratio, live body weight gain and carcass yield were evaluated. Results of the present experiment revealed that treatment groups (C and D) to have statistically significant increase ($P < 0.05$) in the mean serum level of uric acid and calcium as compared to control group and group B; however serum level of phosphorus showed statistically significant decrease ($P < 0.05$). Feed intake, live body weight gain, feed conversion ratio and carcass yield were statistically lower in the treatment groups (C and D) in comparison with control group (A) and treatment group (B). Treatment group (B) favored breast and drumstick yields which constituted the major retail value of dressed chickens but showed relatively lower serum biochemical alteration. From the above results, it was concurred that neither higher and nor lower calcium is appropriate for use as feed ingredient in broiler chickens' diets.

Keywords: Broiler Chicks, Dietary Calcium, Growth Performance, Serum Biochemical

ÉTUDE EXPÉRIMENTALE SUR LES ALTERATIONS DE LA PERFORMANCE DE CROISSANCE ET LES ANALYTES BIOCHIMIQUES SÉRIQUES CHEZ LES POUSSINS DE CHAIR EXPOSÉS A DIFFERENTS NIVEAUX DE CALCIUM DANS LA RATION STANDARD POUR VOLAILLE - ADDIS-ABEBA (ÉTHIOPIE)

Resume

La présente étude expérimentale a été menée dans le but d'évaluer les réponses de performance de croissance et l'altération biochimique sérique chez les poussins de chair exposés à différents niveaux de calcium alimentaire dans la ration standard de volaille, à Addis-Abeba en Éthiopie. Un dispositif en blocs complètement randomisé (RCBD) a été utilisé de janvier à juin 2015 pour une période de six semaines, sur un total de 500 poulets à griller Cobb âgés de 80 jours (40 femelles et 40 mâles) réparties aléatoirement en quatre groupes de traitement égal, comprenant chacun 20 poussins de chair. En conséquence, les quatre groupes de traitement désignés comme A (témoin), B, C et D ont reçu respectivement des taux de calcium alimentaire de 8,5 g de Ca / Kg, 17 g de Ca / Kg, 25,5 g de Ca / Kg et 36,5 g de Ca / Kg. De même, chaque groupe de traitement a été réparti en blocs sur base du sexe (10 mâles et 10 femelles). Des échantillons de sérum de tous les groupes de traitement ont été recueillis et soumis à une analyse biochimique en utilisant

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un analyseur automatique de spectrophotomètre. Les réponses de performance de croissance, à savoir la consommation alimentaire, le taux de conversion alimentaire, le gain de poids corporel et le rendement en carcasse, ont été évaluées. Les résultats de la présente expérience ont révélé que les groupes de traitement C et D avaient une augmentation statistiquement significative ($P < 0,05$) du taux sérique moyen d'acide urique et de calcium par rapport au groupe témoin et au groupe B ; cependant, le taux sérique de phosphore a montré une diminution statistiquement significative ($P < 0,05$). La consommation alimentaire, le gain de poids corporel vif, le taux de conversion alimentaire et le rendement carcasse étaient statistiquement plus faibles dans les groupes de traitement C et D par rapport au groupe témoin A et au groupe de traitement (B). Le groupe de traitement B a favorisé les rendements en poitrine et pilon, parties qui constituent la principale valeur au détail des poulets habillés, mais présentaient une altération biochimique sérique relativement plus faible. La prise alimentaire, la prise de poids corporel, le taux de conversion alimentaire et le rendement carcasse étaient statistiquement plus faibles dans les groupes de traitement (C et D) par rapport au groupe témoin (A) et au groupe de traitement (B). Le groupe de traitement (B) a favorisé les rendements de poitrine et de pilon qui constituaient la principale valeur au détail des poulets préparés, mais a montré une altération biochimique sérique relativement plus faible. Sur la base des résultats ci-dessus, il a été admis que le calcium (que son taux soit élevé ou faible) n'est pas approprié pour utilisation comme ingrédient alimentaire dans les régimes de poulets de chair.

Mots-clés : poulets de chair, Calcium diététique, performance de croissance, biochimie sérique

Introduction

Poultry is the class of domesticated fowl (birds) farmed for their meat and eggs. These most typically are members of the orders Galliformes (such as chickens and turkeys), and Anseriformes (waterfowl such as ducks and geese). Chicken is by far the most popular poultry species utilized for both meat and egg production. Poultry industry produces animal proteins for human consumption most effectively and economically within the shortest possible time. The basic role of poultry production is turning feed stuffs into meat (Hafez, 2011).

The chicken meat industry produces meat and uses a different type or breed of chicken than that used for egg production. Modern varieties of chicken are bred specifically for meat production, with an emphasis placed on the ratio of feed to meat produced by the animal. Chickens raised specifically for meat are called broilers. Broiler production plays a major role in food security for the rapidly increasing human population, their short production cycle and high feed efficiency (Ansar *et al.*, 2004)

Nowadays in our country the situation of poultry production especially the meat type (broiler) is widely expanding particularly in the urban regions. Moreover, chickens are

dominating the livestock production because of their small investment requirement, ease to raise and to sell. However, the development of poultry industry has faced serious setbacks of various types. Among others, mineral imbalance, particularly calcium is one of such problems responsible for economic losses to farm holders, who often formulate the poultry rations themselves (Richard and Julian, 2005).

Calcium is a macro-nutrient element with many important biological functions, constituting by far the greatest part of mineral matter in the bird body and eggshell (Williams *et al.*, 2000). Therefore, calcium is one of the major constituents to be supplemented in poultry ration. However, the beneficial effects of calcium can only be achieved when the recommended doses are administered. Many metabolic problems, such as kidney lesion, Urolithiasis, visceral gout, higher percentage excreta moisture have been reported to be associated with excessive dietary calcium (Guo *et al.*, 2005; Leeson *et al.*, 2008). In a study of (Ansar *et al.*, 2004) the broiler chicks with different ratios of calcium to phosphorous: 1: 0.5, 2:0.5 and 3:0.5% from 1st-42nd day reported that the chicks fed by high dietary calcium revealed the increase of serum calcium concentration, decrease of serum phosphorus concentration, increase of the ratio of kidney's weight to total body weight and ureters

dilation that is caused by urate accumulation. In the study conducted by (Patel *et al.*, 2007), the broiler chicks fed high dietary calcium from 1st day, they observed plasma uric acid triple the normal rate on the 15th day also some paste color urate deposition in viscera.

Excess dietary calcium will also contribute to decreased feed intake by reducing palatability (Shafey *et al.*, 1990), so as feed costs represent 60-70% of the total cost of broiler production, the efficient conversion of feed into live weight is essential for profitability, and small changes in feed conversion ratio at any given feed price can have a substantial impact on financial margins (FAO, 2006).

However, Most of the studies about the metabolic problems were focused on calcium deficiency instead of excess dietary calcium (Walk *et al.*, 2012b). In Ethiopia, no research has been conducted on the effects of excess dietary calcium level in the feed of growing broiler chicks.

Thus, before conducting the experimental trial, preliminary baseline data collection was performed using structured questionnaire survey and clinical observation in 40 poultry farms in the study area in order to assess the existence of similar problems. Most respondents (53%) reported that calcium and phosphorous imbalance is one of the major problems among others. Clinical observation of birds in the respondent's poultry flocks revealed clinical signs of excess calcium such as nervousness, emaciation, retarded growth, decreased feed intake, watery diarrhea, and stiff-legged gait. However, the findings of the preliminary survey in the study area suggested the frequent occurrence of this problem in small poultry farm holders and therefore it was found important to quantify the extent and magnitude of the problem in a controlled experimental study. Hence, the present experimental study was initiated and designed taking into account common mistakes of poultry diet formulation particularly calcium level to be critical factors in Ethiopian context.

Therefore, the present study was designed with the following specific objectives:

- To evaluate the performance responses

(feed intake, feed conversion ratio, live body weight gain and carcass yield) following exposure to different level of calcium in standard ration.

- To determine the effects of high dietary calcium on serum concentration of calcium, phosphorous and uric acid of broiler chicks following exposure to different level of calcium in standard ration.

Materials and Methods

Experimental site

The present experimental study was conducted in Addis Ababa. Addis Ababa is the capital city of Ethiopia, located at latitude and longitude of 13°29'N 39°28'E with an elevation of 2000 meters above sea levels. Climate is characterized by relatively high temperatures and evenly distributed precipitation throughout the year. The average high temp is 24°C, average low temp is 15°C (CSA, 2007)

Experimental Unit

Study animal were 80 apparently healthy day old Cobb 500 broiler chicks' breed (40 female and 40 male) meat type which were obtained from Debre Zeit private poultry farm.

Experimental Design

To evaluate effects of high dietary level of calcium Randomized Complete Block Design (RCBD) were conducted from January to June 2015 on a total of 80 day-old Cobb 500 broiler chicks breed (40 female and 40 male) which were randomly divided into four equal treatment groups each comprising 20 broiler chicks (A, kept as control, B, C and D) on the basis of dietary calcium levels and then each group was blocked based on sex (10 males and 10 females) using lottery method of randomization. In the present experimental work standard poultry ration was prepared according to NRC (1994) recommendations using Feedwin software and the nutritional contents were analyzed in national veterinary institute (NVI). The same standard poultry ration was used for all the four treatment

Table 1: Treatment groups and different amount of calcium (g/kg) in the standard poultry ration

Treatment groups	Amount of calcium
Group A	8.5 gram of Ca/Kg *
Group B	17 gram of Ca/Kg
Group C	25.5 gram of Ca/Kg
Group D	36.5 gram of Ca/Kg **

* = normal dietary calcium percentage which is given for the control group NRC (1994) ** the amount of dietary calcium from previous work which induced pathological lesions (Guo et al., 2008) and Adel Feiziet al., 2012).

groups with varying calcium in the ration and commercially available calcium powder was supplemented to fulfill the required amount of calcium in the ration of each treatment group (Table 1). Birds were fed crumbled starter diets from d 0 to 21 and pelleted grower diets from d 21 to 42. All management factors including light, temperature, vaccination program, ventilation rate, nutrition for all groups were the same but dietary calcium was different. Over the entire experimental period (day 1 to 42), the broilers were allowed *ad libitum* consumption of feed and water.

Data Collection

Growth Performance Response

The major growth performance response criteria were daily feed intake, daily weight gain, feed conversion ratio (FRC) and Final bodyweight. Carcass parameters were also determined. Feed intake was determined by offering a known quantity of feed (A) to each replicate, morning and evening and the left over (B) weight the following morning. The difference between A and B (A-B) gave the quantity of feed consumed. Daily weight gain was obtained by weighing birds individually from each group weekly. Mean of each group was taken (X) and that of the previous week (Y) was subtracted from it (X-Y). The difference between the two divided by seven days gave the daily weight gain for a particular day in a week i.e., $(X-Y)/7 =$ daily wt. gain (DWG). Feed conversion ratio was given by the ratio of feed intake (A) to bodyweight gain at a particular period i.e., A/BWG (g). Final body weight is the weight of each bird at the end of the study period.

Clinical observations and Mortality Percentage

The birds were inspected daily. Clinical signs, mortalities and abnormalities of color, shape, size, texture and hemorrhages were critically recorded from each group of birds.

Serum Biochemical Analysis

Blood samples were collected in vacutainer tubes by wing vein puncture before feeding at 8: 30 h and then subjected to centrifugation. Similarly serum samples were obtained from each treatment group at regular interval of two week until the end of the experiment. Then serum was stored at $\square 20^{\circ}\text{C}$ for analysis. A Spectrophotometric Auto-Analyzer were used to measure the concentrations of calcium, inorganic phosphorus and uric acid in the serum at Addis Ababa University Applied Chemistry Department (Bartholomew and Delaney, 1966; Beljan et al., 1971)

Data Analysis

To test the hypotheses the raw data stored in excel spread sheet was analyzed by SPSS statistical software, version 20. For quantitative variables measured two ways ANOVA was used to compares the mean differences between groups and the level of calcium in the feed and sex and interaction between independent variables on the dependent variable. For all statistical analysis $\square = 0.05$ was considered a significant level (Steel and Torrie, 1982).

Results

Growth Performance Response

Feed intake

In the experimental study to assess the effects of high dietary level of calcium in growing broiler chicks exposed to four different levels of calcium in standard poultry ration resulted statistically significant changes in feed consumption compared with the control group. The trend of feed consumption appeared to be decreasing with increasing dietary Ca, with group C and D being the least consumer but group A and B being the highest. There was no statistically significance ($P>0.05$) difference in feed consumption between group A and B and group C and D. There was statistically insignificant ($P>0.05$) difference between males and females birds in feed consumption throughout the whole experimental period and statistically insignificant ($P>0.05$) difference in the interaction effect of dietary calcium and sex in mean feed intake were observed (Table 3).

Live Body Weight

The comparison of body weight on the four groups during six weeks revealed that in the first week the mean body weight on the four groups were insignificantly ($P>0.05$) different

from each other but during second, third, fourth, fifth and sixth week of the experimental period the means live body weights on group A and B and between group C and D were statistically insignificantly ($P>0.05$) different from each other but group C and D showed significantly ($P<0.05$) lower value than group A and B in term of live body weight with their respective dietary level of calcium. This trend appeared to have followed feed consumption trend with group C and D being the least as compared with group A and B (Table 4).

Feed Conversion Ratio

Birds in the group A and B tended to convert the feed consumed more efficiently, into meat, than group C and D. However, no statistically significant ($P>0.05$) difference was observed between group A and B. (Table 2) in terms of feed conversion ratio. The low feed conversion ratio was due to high dietary calcium to experimental group birds while the comparatively better feed conversion ratio was obtained in group A. No significant interactions effects were obtained between sex and dietary Ca level for the variables of live body weight and feed conversion ratio.

Table 2: Average feed conversion ratio of broiler chicks subjected to four different calcium in the ration (Mean± SD)

Experimental groups	Sex	Feed conversion ratio	P- value
A(8.5 g Ca/kg)	Male	1.97 ± 0.53	0.12
	Female	1.96 ± 0.38	
	Total	1.95 ± 0.46a	
B(17 g Ca/kg)	Male	1.95 ± 0.63	0.15
	Female	1.94 ± 0.48	
	Total	1.95 ± 0.56a	
C(25.5 g Ca/kg)	Male	2.55 ± 1.03	0.25
	Female	2.55 ± 1.12	
	Total	2.55 ± 1.06b	
D(36.5 g Ca/kg)	Male	2.53 ± 0.98	0.07
	Female	2.52 ± 0.78	
	Total	2.53 ± 0.85b	

Values in each column within a parameter followed by different small letters are statistically different $p < 0.05$

Table 3: Feed intake (g) of male and female broilers fed diets containing 4 levels of calcium (Mean± SD)

Groups	sex	Experimental weeks				P-value		
		1st week	2nd week	3rd week	4th week		5th week	6th week
A(8.5 g Ca/kg)	Male	780.86±213.39	1086.21±242.17	1840.79±277.37	2885.93±176.45	3661.07±195.75	4462.29±163.53	0.94
	Female	778.79±215.18	1090.86±241.18	1845.07±235.42	2886.21±168.64	3668.50±192.64	4471.07±156.48	
	Total	779.83±214.29 ^a	1088.54±241.68 ^a	1842.93±256.39 ^a	2886.07±172.26 ^a	3664.79±194.19 ^a	4466.68±160.01	
B(17 g Ca/kg)	Male	785.35±218.03	1092.04±266.65	1856.00±232.23	2892.71±220.13	3680.86±260.86	4476.57±149.54	0.65
	Female	785.00±216.36	1089.25±250.42	1849.45±253.32	2863.00±231.90	3672.00±249.52	4460.00±147.66	
	Total	785.12±217.12 ^a	1090.65±258.54 ^a	1852.73±242.78 ^a	2877.86±226.02 ^a	3676.43±255.19 ^a	4468.29±148.60 ^a	
C(25.5 g Ca/kg)	Male	725.14±214.69	893.00±231.87	1674.71±311.48	2649.56±213.45	3428.87±168.54	3656.76±134.42	0.12
	Female	723.57±215.29	887.00±231.92	1669.73±310.26	2645.78±208.63	3418.04±192.56	3649.25±142.03	
	Total	724.36±214.99 ^b	890.00±231.89 ^b	1672.44±310.87 ^b	2647.67±211.04 ^b	3423.72±180.55 ^b	3653.01±138.23 ^b	
D(36.5 g Ca/kg)	Male	718.56±213.96	881.65±245.76	1668.45±297.34	2659.14±209.78	3415.29±189.80	3648.00±144.95	0.68
	Female	713.36±217.16	878.96±233.55	1670.05±268.86	2652.43±189.96	3411.00±190.42	3625.14±149.31	
	Total	715.92±215.56 ^b	880.31±239.67 ^b	1669.25±283.10 ^b	2655.79±199.87 ^b	3413.15±190.11 ^b	3636.57±147.13 ^b	

Values in each column within a parameter followed by different small letters are statistically different from each other; $p < 0.05$

Table 4: Live body weight (BW), of male and female broilers fed diets containing 4 levels of calcium (Mean± SD)

Groups	sex	Experimental weeks				P-value		
		1st week	2nd week	3rd week	4th week		5th week	6th week
A(8.5 g Ca/kg)	Male	156.72±6.57	411.64±13.69	803.18±12.57	1305.93±35.18	1804.24±71.86	2267.69±89.81	1.02
	Female	153.36 ±7.77	412.43±8.61	804.97±13.09	1314.25±14.00	1803.93±75.90	2281.23±84.19	
	Total	155.04 ±7.21 ^a	412.03±11.14 ^a	804.07±12.53 ^a	1310.09±26.40 ^a	1804.09±71.94 ^a	2259.46 ±87.62 ^a	
B(17 g Ca/kg)	Male	156.97 ±8.02	415.29±13.05	813.07±11.39	1323.50±14.21	1821.10±79.01 ³	2293.75±65.12	0.96
	Female	154.07 ±7.76	419.13±14.61	812.39±11.82	1316.80±19.59	1798.94±652.17	2288.03±76.78	
	Total	155.52 ±7.73 ^a	434.71 ±13.49 ^a	812.73±11.31 ^a	1320.15±17.01 ^a	1809.52±452.6 ^a	2290.39±69.70 ^a	
C(25.5 g Ca/kg)	Male	151.74 ±7.48	372.63±18.64	714.64±18.87	1072.44±295.53	1212.59±50.13	1436.69±42.83	1.05
	Female	153.27 ±4.84	371.43±22.22	715.40±25.48	992.58±14.63	1222.19±52.31	1468.46±32.93	
	Total	152.51 ± 6.18 ^a	372.02±19.97 ^b	715.02±21.82 ^b	1032.51±207.73 ^b	1217.39±50.11 ^b	1452.58±40.59 ^b	
D(36.5 g Ca/kg)	Male	155.22 ± 6.69	370.09±21.52	703.46±23.12	975.25±19.40	1228.79±36.23	1446.52±47.19	0.75
	Female	154.69 ±4.62	370.56±21.62	702.92±21.24	979.68±17.41	1193.32±42.72	1442.14±57.28	
	Total	154.96±5.60 ^a	370.32±20.99 ^b	703.19±21.61 ^b	977.47±18.b09	1211.06±42.63 ^b	1424.33±54.25 ^b	

Values in each column within a parameter followed by different small letters are statistically different from each other. p < 0.05

Table 5: Carcass yield of broiler chickens finished on varying calcium in the ration.

Groups	Sex	Carcasses yield (g)	P-value
A(8.5 g Ca/kg)	Male	1612.01±58.61	0.23
	Female	1608.64±61.47	
	Total	1610.32±58.48a	
B(17 g Ca/kg)	Male	1656.09±76.17	0.65
	Female	1639.83±48.12	
	Total	1695.46±62.17a	
C(25.5 g Ca/kg)	Male	1019.42±58.81	1.02
	Female	1020.12±30.33	
	Total	1019.77±45.55b	
D(36.5 g Ca/kg)	Male	998.36±51.11	0.84
	Female	1016.27±26.70	
	Total	1007.32±40.74b	

Values in each column within a parameter followed by different small letters are statistically different from each other $p < 0.05$

Carcass Yield

Carcass analysis (Table 5) revealed that, the dressed carcasses in groups A and B had statistically insignificant difference ($P > 0.05$). However, statistically significant difference ($P < 0.05$) was observed in the carcass yield of dressed birds in groups A and B than groups C and D. There was no statistically significant difference ($P > 0.05$) between group C and D in dressed carcass yields. No statistically significant difference ($P < 0.05$) between males and females and no statistically significant interactions effect were obtained between sex and calcium level for dressed carcasses yield.

Clinical Signs

No clinical signs and behavioral alterations were observed among group A and B their feed and water intake was found normal and had good body condition (figure 1A). However, no signs of impaired skeletal development were observed in treatments group A and B. Almost 100% birds in the groups C and D showed severe signs of depression, loss of appetite, watery diarrhea, and stiff-legged gait, ruffled feathering and birds progressively became emaciated and had prominent keel bone starting from the second week of the experiment (figure 1B) and they had a tendency to hide and appeared to be chilled. Additionally the affected chicks showed

leg weakness and distortion, difficulty standing, severe lameness and crooked keel, birds unable to rise or prefers to remain recumbent.

Mortality Rate

Mortality rate recorded in group D was the highest (35 %, 7/20), four male and three female followed by group C (25 %, 5/20), three female and two male. In group D death was recorded at the third (day 16 and 18), fourth (day 23 and 27) and fifth (day 31, 33 and 35) weeks, whereas, in group C at 3rd (day 18), fourth (day 27 and 28) and fifth (day 35) weeks of the experimental period. But no mortality was recorded in group A and B throughout the experimental period

Serum Biochemical Analysis

The results showed the rates of mean value of serum calcium, phosphorous and uric acid in four understudying groups that examined 3 times. The growers raised on group C and D diet had statistically significantly ($P < 0.05$) higher serum calcium (Table 6) and significantly ($P < 0.05$) lower serum phosphorous (table 8) in terms of dietary calcium levels, those have statistically meaningful difference compared with the control group. In our study serum phosphorus level was statistically significantly higher ($P < 0.05$) (Table 7) in chicks of control group and group B. But also there were

Table 6: Serum concentration of calcium in Cobb 500 broiler chicks breed subjected to four different levels of calcium in the ration (Mean±SD).

Groups	Sex	Experimental weeks			P-value
		2nd week	4rd weeks	6th weeks	
A(8.5 g Ca/kg)	Male	8.09±.73	8.52±.64	8.69±.67	>0.05
	Female	8.29±.65	8.78±.68	9.04±.82	
	Total	8.19±.68 ^a	8.65±.65 ^a	8.87±.75 ^a	
B(17 g Ca/kg)	Male	8.34±.94	8.96±.75	9.28±.52	> 0.05
	Female	8.56±.99	9.18±.98	9.57±.68	
	Total	8.45±.95 ^a	9.07±.86 ^a	9.43±.60 ^a	
C(25.5 g Ca/kg)	Male	10.22±.78	10.76±.64	11.80±.71	> 0.05
	Female	10.09±.67	10.26±.50	11.69±.68	
	Total	10.16±.71 ^c	10.51±.62 ^c	11.75±.68 ^c	
D(36.5 g Ca/kg)	Male	11.13±.65	11.89±.37	13.02±1.2	> 0.05
	Female	10.64±.68	11.92±1.01	13.15±1.25	
	Total	10.89±.69 ^d	11.91±.74 ^d	13.08±1.24 ^d	

Values in each column followed by different small letters are statistically different $p \leq 0.05$

Table 7: Serum concentration of phosphorous in Cobb 500 broiler chicks breed subjected to different levels of calcium in the ration (Mean±SD)

Groups	Sex	Experimental weeks			P-value
		2nd week	4rd weeks	6th weeks	
A(8.5 g Ca/kg)	Male	4.15 ±.43	3.96 ±.3	4.05 ±.37	>0.05
	Female	3.80 ±.47	3.88 ±.68	4.00 ±.43	
	Total	3.99 ±.47 ^a	3.92 ±.54 ^a	4.02 ±.39 ^a	
B(17 g Ca/kg)	Male	3.88±.45	3.75±.28	3.79±.14	>0.05
	Female	3.82±.41	3.58±.35	3.85±.44	
	Total	3.85±.42 ^a	3.67±.32 ^a	3.82±.32 ^a	
C(25.5 g Ca/kg)	Male	2.97 ±.41	2.92 ±.24	2.95 ± .20	>0.05
	Female	2.75 ±.55	2.93 ±.28	2.82 ±.34	
	Total	2.86 ±.49 ^c	2.93 ±.26 ^c	2.88 ±.28 ^c	
D(36.5 g Ca/kg)	Male	2.38 ±.17	2.50 ±.13	2.61 ±.27	>0.05
	Female	2.33 ±.19	2.56 ±.14	2.51 ±.18	
	Total	2.35 ±.18 ^d	2.53 ±.14 ^d	2.56 ±.23 ^d	

Values in each column followed by different small letters are statistically different $p \leq 0.05$

statistically significance ($P < 0.05$) difference in mean serum calcium and phosphorous concentration among treatment groups (C and D) based on their dietary calcium level within each examined period. However, there was no statistically significance difference between the control group and group B in terms of serum level of calcium and phosphorous. In the present experiment there was no statistically significant

difference ($P > 0.05$) in mean serum calcium and phosphorous concentration between males and females throughout the experimental period. There were no statistically significant ($P > 0.05$) interaction effects of dietary levels of calcium and sex (dietary level of calcium \times sex) in mean serum calcium and phosphorous concentration.

Table 8: Serum concentration of uric acid in Cobb 500 broiler chicks breed subjected to four different levels of calcium in the ration (Mean±SD).

Groups	Sex	Experimental weeks			P-value
		2nd week	4rd weeks	6th weeks	
A(8.5 g Ca/kg)	Male	5.19 ±.58	5.28 ±.54	5.72 ±.29	>0.05
	Female	5.24 ±.43	5.42 ±.49 ^a	5.81 ±.49	
	Total	5.22 ±.49 ^a	5.30 ±.52	5.76 ±.40 ^a	
B(17 g Ca/kg)	Male	5.30±.53	5.60±.42	6.03±.39	>0.05
	Female	5.54±.51	5.44±.38	6.29±.18	
	Total	5.42±.53 ^a	5.52±.40 ^a	6.16±.33 ^a	
C(25.5 g Ca/kg)	Male	7.85 ±.64	007.94 ±.54	10.24 ±.82	>0.05
	Female	7.82 ±.62	8.02 ±.84	10.39 ±.94	
	Total	7.84 ±.61 ^c	7.98 ±.69 ^c	10.31 ±.86 ^c	
D(36.5 g Ca/kg)	Male	9.25 ±.64	9.71 ±.96	12.14 ±1.37	>0.05
	Female	9.20 ±.55	9.80 ±.87	12.12 ±1.24	
	Total	9.23 ±.58 ^d	9.76 ±.89 ^d	12.13 ±1.27 ^d	

Values in each column followed by different small letters are statistically different $p \leq 0.05$

Statistical investigation of the rate of serum uric acid (table 8) revealed meaningful statistical difference b/n treatment group C and D in comparison with control groups; increased level of uric acid is seen throughout the experimental period with the respective dietary level of calcium. There were significance ($P < 0.05$) difference in mean serum uric acid among treatment groups (C and D) during the whole experimental period but there was no statistically significant difference ($P > 0.05$) in mean serum uric acid concentration between control group and group B and males and females ($P > 0.05$) broiler chicks during the experimental period and there were statistically insignificant difference ($P > 0.05$) interaction effect of dietary level of calcium and sex (dietary level of calcium \square sex) in mean serum concentration of uric acid.

Discussion

Calcium is a mineral that plays a central role in maintaining the homeostasis of birds, including muscle contraction, blood coagulation, enzyme activity, neural excitability and cell adhesion. Therefore, calcium is one of the major constituents to be supplemented

in poultry ration. However, the beneficial effects of calcium can only be achieved when the recommended doses are administered. In the current study, complications resulted of different levels of calcium in broiler chicks' dietary was investigated. Feed consumption varied significantly between the control group on one hand and the treatment groups on the other. The trend of feed consumption appeared to be decreasing with increasing dietary Ca with group A and B being the highest and group C and D being the least consumer. It can therefore be said that higher dietary Ca decrease appetite than lower dietary calcium which might be due to loss of palatability of the feed. The result, however, partly agreed with that of (Roland *et al.*, 1985; Rouesh, *et al.*, 1986; Robertson *et al.*, 2005) who reported significant inverse linear relationship between levels of dietary calcium and feed intake.

In the current experiment birds fed a diet contained 25.5 g Ca /kg and 36.5 g/kg calcium exhibited significant decrease in body weight gain, this trend appeared to have followed feed consumption trend with group C and D being the least as compared with group A and B. This implied that, higher dietary Ca support lower rate of gain in finishing broiler

chickens and this may be attributed to lower feed consumption. The present result agreed with the earlier report of (Cesar Coto, 2008) which showed that lower dietary Ca provided better growth rate than higher dietary calcium. There was no statistically significance ($P>0.05$) mean body weight difference between group A and B, this might be due to continued increase in growth rate of birds and the demand for increasing the daily recommended intake of mineral requirements, the result in agreement with the previous finding of (Bar *et al.*, 2003) which indicated a Ca requirement for optimum weight that was similar to or slightly higher than recommendation of 1.0% and (Driver, *et al.*, 2005) which recommended higher ratios of calcium and phosphorous than 2:1 for better gain, so this suggested that double dietary calcium to be optimal for grower finishing. In the present study birds in the group A and B tended to convert the feed consumed more efficiently, into meat, than group C and D. however, no statistically significant ($P>0.05$) difference was observed between group A and B in terms of feed conversion ratio. These findings disagree with the report of (Edwards, *et al.*, 2002) which observed improved feed efficiency with increasing dietary level of calcium; this observation suggests that a reliable evaluation could not be performed. However, these findings are in agreement to those of (Ansar *et al.*, 2004), who observed poor weight gain, decreased feed consumption and low feed conversion ratio values in birds given a high dietary calcium and phosphorus ratio. It also supported the work of (Cesar Coto, *et al.*, 2008) which reported that birds fed diet slightly more Ca than the 1 % had significantly better feed conversion ratio than birds fed the 1 %. The dressed carcasses in groups A and B had not statistically significance difference, however, significantly ($P<0.05$) heavier dressed birds produced than group C and D, may mean that the 1 % is may or may not adequate in finishing broiler chickens; this is a beneficial trait in broiler industry. In the present study double calcium in the ration favored breast and drumstick yields which constituted the major retail value of dressed chickens.

In the present experiments no clinical signs and behavioral alterations were observed in control group and in group B broiler chicks except depression in some chicks, suggesting this level as tolerable for the birds. In higher dose groups (25.5 g/kg and 36.5 g/kg) began to show signs of depression, loss of appetite, gradual emaciation, dehydration and loss body weight gain and watery diarrhea was observed this might be excess dietary calcium might have resulted in fluid moving into the gastrointestinal tract from other fluid compartments. These findings were in line with different studies in day old broiler chicks and commercial white leghorn layers (Guoet *et al.*, 2005)

Birds exposed for excess dietary calcium in poultry ration resulted in mortality varying from 25 % in group C and 35% in group D. Calcium effect on mortality might be due to necrotic enteritis-related intestinal impairment; this is consistent with previous result reported by (Paiva *et al.*, 2013) and Previous research showed that increasing dietary levels of Ca in the diet significantly increased small intestine pH (Shafey *et al.*, 1991; Walk *et al.*, 2012b). This increased in pH due to Ca supplementation likely creates a more favorable environment for clostridial growth (Williams, 2005). Ca had increased mortality as a result of an increased activity of α -toxin; the α -toxin is a phospholipase that acts on the enterocyte membrane, causing extensive damage to the intestinal lining and inducing a severe inflammatory response (Sakurai *et al.*, 2004; Van Immerseel *et al.*, 2004) and NetB activity may also be influenced by Ca levels. NetB causes cell lysis by creating pores in the cell membrane, resulting in an influx of Ca ions to the cytoplasm (Keyburn *et al.*, 2010). In addition to promoting cell lysis through an osmotic imbalance, the Ca influx caused by netB may also lead to a special type of programmed cell death. As (Kennedy *et al.*, 2009) reported that the pore-forming α -toxin of *Clostridium septicum* creates Ca permeable pores, which increase intracellular Ca.

In present study significantly increased levels of serum calcium were observed in birds fed, a diet contained 17 g Ca/kg, 25.5 g/kg and 36.5

g/kg dietary calcium. This finding is in agreement to those of (Ansar, *et al.*, 2004; Guo *et al.*, 2005; Patel *et al.*, 2007), who observed higher serum calcium concentrations in birds fed high dietary calcium and also in consistent with (Ansar, *et al.*, 2004) who obtained high serum calcium and lower serum phosphorous by feeding higher dietary calcium to phosphorus ratios for broiler chicks. In our study, serum phosphorus level was significantly higher ($P < 0.05$) in chicks of group A fed normal dietary calcium. These results indirectly indicate that a diet with increased calcium induces hypophosphataemia. Birds fed on the HC diet had hypercalcemia and hypophosphataemia suggesting that growers rose on this diet had higher rates of intestinal calcium absorption and lower rates of intestinal phosphorus absorption. These results are in line with the reports of (Patel *et al.*, 2007) in a study fed the broiler chicks with high calcium (2 and 3%) dietary. Similar results have been reported by (Ismail, 1989; Ogura, 1981), who studied pathology of the high dietary level of calcium in broiler chicks. Excessive calcium levels in poultry feed can also increase the pH in the gut resulting in decreased absorption of phosphorus from the intestines and may also lead to phosphorus deficiency by the formation of insoluble calcium phosphates in the digestive tract. (Tamim *et al.*, 2004; Plumstead *et al.*, 2008; Paiva *et al.*, 2013). The increase of serum calcium and decrease of serum phosphorous in the present study also conformed to findings of (Al-ankari, 2006). No sex influence was observed on mean value of serum calcium and phosphorous and there was no statically significant interaction effect of dietary calcium and sex observed but any data not obtained from other researchers previously.

Kidneys' uric acid clearance is used as an important index in functional evaluations of kidneys. Therefore, the rate of uric acid in blood sample is one of the indices for health of kidney. There were statistically significance ($P < 0.05$) difference in mean serum uric acid concentration among treatment groups (B, C and D) during the whole experimental period this might be due to impaired blood clearance of uric acid as a result of renal insufficiency. As

(Keshavarz *et al.*, 2001) reported the normal rate of uric acid is 5.8 mg/dl which conforms to the findings in control group of the present experimental study (5.22--5.76). However, in treatment groups C and D uric acid rate of blood was 2.5 times more than that of the control group on 42nd day which revealed renal insufficiency, this suggested that plasma uric acid concentrations are directly related to calcium-induced kidney damage. So the present finding also inconsistent with the report of (Patel *et al.*, 2007) fed the broilers with excess dietary calcium, they observed triple of the normal rate in uric acid on a day 15. For all parameters evaluated in this experiment there was no statistical significance difference between males and females broiler chicks, this indicate that they have equal susceptibility to excess dietary calcium.

Conclusion and Recommendations

Calcium (Ca) is a mineral that plays a central role in maintaining the homeostasis of birds. Therefore, calcium is one of the major constituents to be supplemented in poultry ration. However, the beneficial effects of calcium can only be achieved when the recommended doses are administered.

Based on the results of the present study, it can be concluded that different levels of calcium in the poultry ration has strong influence on the growth performance of broiler finisher chickens. The findings of this study disclosed that neither higher 25.5 g Ca/kg (3%) and 36.5 g Ca/kg (4%) nor lower 8.5 g Ca/kg (1%) calcium in the ration were appropriate for finishing broiler chickens. At double dietary calcium level 17 g Ca/Kg (2%) in the ration of chickens were heavier than the standard 1% (8.5 g Ca/kg of feed), this suggesting that 1% calcium level in the ration was may or may not be adequate for finishing broiler chickens. The double calcium level in the ration favored breast and drumstick yields which constituted the major retail value of dressed chickens so the double calcium level in the poultry ration seems to be optimum for finishing broiler chickens, therefore optimal Ca supplemented

ration earned more profit in terms of better growth performance.

However, Consistent data demonstrate that altered serum calcium, phosphorous and uric acid level markedly above the reference range for individual traits and contexts is associated with adverse health effects. In practice, there is a tendency of incorporating excess calcium in poultry ration which could predispose growing broiler chicks to various types of impaired growth and also alteration of serum biochemical analytes. Therefore, based on the above conclusion the following points are forwarded as recommendation.

- Re-evaluation of dietary calcium specifications should be workout by taking into consideration the difference in requirements between poultry breeds.
- Much attention must give in setting the broilers' ration formulation especially the rate of dietary calcium for preventing its consequences on growth performance
- Feed manufacturers and the farmer will all be careful in their action and be vigilant for any signs that could result in this type of problem
- Further intensive and monitoring studies should be proceed on metabolic diseases of chickens

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Bulletin of Animal Health and Production in Africa
Guide for Preparation of Papers
Notes to Authors

The Editor in Chief
January 2017

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The Bulletin of Animal Health and Production in Africa (BAHPA) of the African Union Interafrican Bureau for Animal Resources (AU-IBAR) is a scientific journal which publishes articles on research relevant to animal health and production including wildlife and fisheries contributing to the human wellbeing, food security, poverty alleviation and sustainable development in Africa. The bulletin disseminates technical recommendations on animal health and production to stakeholders, including policy makers, researchers and scientists in member states. The Bulletin is the African voice on animal resources issues specific to Africa.

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6. Materials and Methods should describe materials, methods, apparatus, experimental procedure and statistical methods (experimental design, data collection and data analysis) in sufficient detail to allow other authors to reproduce the results. This part may have subheadings. The experimental methods and treatments applied shall conform to the most recent guidelines on the animal's treatment and care. For manuscripts that report complex statistics, the Editor recommends statistical consultation (or at least expertise); a biostatistician may review such manuscripts during the review process. Cite only textbooks and published article references to support your choices of tests. Indicate any statistics software used.
7. Results should be presented clearly and concisely, in a non-

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8. Discussion of significance should be focused on in the interpretation of results. Subheadings are not accepted in this section.
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Examples of References

- **Journal Articles:** Ouyang D, Bartholic J, Selegean J, 2005. Assessing sediment loading from agricultural croplands in the Great Lakes basin. *Journal of American Science*, 1(2): 14-21.
- **Books:** Durbin R, Eddy SR, Krogh A, Mitchison G, 1999. *Biological Sequence Analysis: Probabilistic Models of Proteins and Nucleic Acids*. London, Cambridge University Press.

- *Chapter in a Book*: Leach J, 1993. Impacts of the Zebra Mussel (*Dreissena polymorpha*) on water quality and fish spawning reefs of Western Lake Erie. In *Zebra Mussels: Biology, Impacts and Control*, Eds., Nalepa T, Schloesser D, Ann Arbor, MI: Lewis Publishers, pp: 381-397.
- *Reports*: Makarewicz JC, Lewis T, Bertram P, 1995. Epilimnetic phytoplankton and zooplankton biomass and species composition in Lake Michigan, 1983-1992. US EPA Great Lakes National Program, Chicago, IL. EPA 905-R-95-009.
- *Conference Proceedings*: Stock A, 2004. Signal Transduction in Bacteria. In the Proceedings of the 2004 Markey Scholars Conference, pp: 80-89.
- *Thesis*: Strunk JL, 1991. The extraction of mercury from sediment and the geochemical partitioning of mercury in sediments from Lake Superior, Unpublished PhD thesis, Michigan State University, East Lansing, MI.
- *Web links*: Cerón-Muñoz M F, Tonhati H, Costa C N, Rojas-Sarmiento D and Solarte Portilla C 2004 Variance heterogeneity for milk yield in Brazilian and Colombian Holstein herds. Livestock Research for Rural Development. Volume 16, Article #20 Visited June 1, 2005, from <http://www.lrrd.org/lrrd16/4/cero16020.htm>

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