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HAEMATOLOGICAL, BIOCHEMICAL AND CLINICAL CHANGES IN DOMESTIC PIGS EXPERIMENTALLY INFECTED WITH AFRICAN SWINE FEVER VIRUS ISOLATES FROM UGANDA

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Abstract

African swine fever (ASF) is a highly contagious often fatal viral disease of pigs caused by asfivirus. The disease causes marked leucopaenia, depletion of lymphocytes in the lymphoid tissues, changes in biochemical parameters, haemorrhages and necrosis in multiple organs of the infected pigs. We studied the pathogenic effect of three different Ugandan ASF virus (ASFV) isolates on twelve infected and six uninfected pigs. Each pig in the infected group was inoculated per os with 2 mls of ASFV culture solution containing 1×10^8 H.A.D.U/ml of the viral culture solution while the control group were given 2 mls of uninfected porcine alveolar macrophages culture per-os. Clinical parameters were monitored daily and blood samples collected for leucocytes count and biochemical tests. In the present study, the incubation period of the disease ranged from 7 - 15 days and in average the clinical disease lasted for 5 days. On the eighth day post infection, all test pigs had significant leucopaenia ($p = 0.000$) and number of lymphocytes reduced significantly ($p = 0.001$). Band neutrophils progressively increased in number as the disease progressed, however when the changes in mean band neutrophils in the three groups were compared it was not statistically significant ($p = 0.52$). There were no significant variations in the mean basophils and eosinophil counts in all experimental groups during study period ($p = 0.30$ and $p = 0.32$ respectively). Nevertheless, mean monocytes counts significantly increased in infected pigs ($p = 0.01$), while in uninfected group there was no significant variation in the mean monocytes counts. The majority of the pigs, 83.3% ($n = 10$) in the test groups had elevated levels of gamma-glutamyl transferase (GGT). The Level of Alanine Amino Transferase (ALT) at 8 days post infection was elevated in all infected pigs in the three groups. In 66.7% ($n = 8$) infected pigs, Albumin (ALB) levels were elevated in the serum samples above the normal range of 18 – 33 g/l. The levels of other biochemicals in the serum samples such as Creatine kinase (CK), Creatinine (CREA), and Alkaline Phosphatase (ALKP) remained within the normal range (50- 3531 μ /L, 44 - 186 μ mol/L, 92 - 294 μ /L, respectively). We concluded that ASF causes significant deviation in leucocytes counts, increased levels of GGT, ALT and ALB and clinical parameters in pigs infected with Ugandan isolates of ASF virus.

Key words: African swine fever (ASF), Domestic pigs Haematological, biochemical and clinical parameters

MODIFICATIONS HÉMATOLOGIQUES, BIOCHIMIQUES ET CLINIQUES CHEZ LES PORCS DOMESTIQUES INFECTÉS EXPÉRIMENTALEMENT AVEC DES ISOLATS OUGANDAIS DU VIRUS DE LA PESTE PORCINE AFRICAINE

Résumé

La peste porcine africaine (PPA) est une maladie virale très contagieuse et souvent mortelle des porcs, causée par l'asfivirus. Chez les porcs infectés, la maladie provoque une leucopénie marquée, un épuisement des lymphocytes dans les tissus lymphoïdes, des modifications des paramètres biochimiques, des hémorragies et des nécroses dans plusieurs organes. Nous avons étudié l'effet pathogène de trois différents isolats ougandais du virus de la peste porcine africaine (VPPA) sur douze porcs infectés et six non infectés. Dans le groupe infecté, chaque porc a été inoculé par voie orale avec 2 ml de solution de culture du VPPA contenant 1×10^8 H.A.D.U/ml de solution de culture virale, tandis que les porcs du groupe témoin ont reçu par voie orale 2 ml de culture de macrophages alvéolaires de porc non infecté.

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Les paramètres cliniques ont été surveillés chaque jour, et des échantillons de sang prélevés en vue de déterminer la numération leucocytaire et d'effectuer des tests biochimiques. Dans la présente étude, la période d'incubation de la maladie variait entre 7 et 15 jours, et en moyenne la maladie clinique a duré 5 jours. Au huitième jour post-infection, tous les porcs étudiés présentaient une leucopénie significative ($p = 0,000$), et le nombre de lymphocytes avait considérablement baissé ($p = 0,001$). Le nombre de neutrophiles non segmentés augmentait progressivement au fur et à mesure que la maladie progressait, mais lorsqu'on a comparé les modifications au niveau des numérations moyennes des neutrophiles non segmentés dans les trois groupes, on a constaté que les modifications n'étaient pas statistiquement significatives ($p = 0,52$). On n'a pas relevé de variations significatives au niveau des numérations moyennes de basophiles et d'éosinophiles dans tous les groupes d'expérience pendant la période de l'étude (respectivement $p = 0,30$ et $p = 0,32$). Néanmoins, les numérations moyennes de monocytes ont sensiblement augmenté chez les porcs infectés ($p = 0,01$), alors qu'il n'y avait pas de variation significative des numérations moyennes de monocytes chez le groupe non infecté. La majorité des porcs, à savoir 83,3% ($n = 10$) dans les groupes étudiés, avait des taux élevés de gamma-glutamyl transférase (GGT). Le taux d'alanine amino-transférase (ALT) à 8 jours post-infection était élevé chez tous les porcs infectés dans les trois groupes. Chez 66,7% ($n = 8$) des porcs infectés, les taux d'albumine (ALB) étaient élevés dans les échantillons de sérum et se situaient au dessus de la plage normale de 18 - 33 g / l. Les taux d'autres substances biochimiques dans les échantillons de sérum tels que la créatine kinase (CK), la créatinine (CREA), et la phosphatase alcaline (ALKP) sont restés dans la fourchette normale (respectivement 50- 3531 μ /L, 44 -186 μ mol/L, 92 - 294 μ /L). Nous avons conclu que la PPA provoque un écart significatif au niveau des numérations de leucocytes, une augmentation des taux de GGT, d'ALT et d'ALB et des modifications des paramètres cliniques chez les porcs infectés avec des isolats ougandais du virus de la PPA.

Mots-clés : Peste porcine africaine (PPA), porcs domestiques, paramètres hématologiques, biochimiques et cliniques

Introduction

African swine fever (ASF) is a highly contagious disease of pigs caused by a large icosahedral DNA virus of the genus *Asfivirus* and family *asfarviridae* (Dixon *et al.*, 2005, Takamatsu *et al.*, 2011). The virus exhibits varying virulence between strains and is hardy to physical and chemical inactivation. ASF in domestic pigs most commonly appears in the acute form as haemorrhagic fever which kills domestic pigs within 7-10 days post infection (Radostits *et al.*, 2000). However, sub acute and chronic forms of the disease exists (FAO Report, 2000). Mortalities in domestic pigs usually range from 85% to 100% and pigs of all ages are affected. Pigs that survive the disease are resistant to the same virus isolates but they do not produce classical neutralizing antibody (Takamatsu *et al.*, 1999). Pigs develop antibodies against ASF virus from 7 to 10 days post infection and these antibodies persist for a long period of time in those pigs that survive the disease. Thus antibody detection is a rational approach to the diagnosis of the sub acute and chronic forms of the disease (Radostits *et al.*, 2000).

African swine fever virus often infects domestic pigs through parental and oral or respiratory routes. Oro-nasal infection is followed by penetration of the virus into the pharyngeal mucosa and the virus is drained through lymphatics to the mandibular or retropharyngeal lymph nodes from where viral replication occurs. It then spreads through circulatory system to other organs and tissues of the body (Sandra *et al.*, 2012).

The virus infect and replicate in cells of the mononuclear phagocytic system (MPS), and the ability of the virus to replicate in MPS plays a critical role in the pathogenicity of the disease. The infected animals present with marked leucopaenia and severe impairment of lymphoid organs, characterized by apoptosis of lymphocytes and significant cellular depletion in spleen, lymph nodes and other lymphoid tissues (Ramino-Ibanez *et al.*, 1997). Infected monocytes and macrophages secrete a large range of soluble mediators, including pro-inflammatory cytokines such as interleukin - 1 (IL- 1), IL- 6 and tumour necrosis factor alpha (TNF α) (Salquero *et al.*, 2002).

The pathogenic effect of ASF and the resultant shock and haemorrhages are majorly

as a result of platelets aggregation and their release. The virus directly infects and lyses monocytes, macrophages and endothelial cells and alters the release of the products of the cyclo – oxygenase cleavage of arachidonic acid (Penrith *et al.*, 2004).

There is impairment of the release of anti – aggregatory prostaglandin, prostacyclin (PGI₂). Changes in the endothelia expose the sub-endothelial tissues and collagen to cytokines that leads to stimulation of the intrinsic coagulation pathway, disseminated intravascular coagulation and infarction. Immune mediated thrombocytopenia occurs during the period of antigen excess (immune complex formation) stages. The release of adenosine triphosphate (ADP) from the erythrocyte also contributes to thrombocytopenia (Edwards 1983). Several proteins are involved in fibrinolysis and morphological changes in blood vessel walls of pigs infected with virulent strains of ASF viral isolates. The infected pigs develop an increased fibrinolytic activity in the blood circulation due to the high plasma levels of tissue plasminogen activator. This correlates with an increased activation of interstitial capillary endothelial cells and high levels of fibrin monomers in the circulation. This contributes to increased bleeding tendency and high mortalities observed in pigs infected with virulent strains of ASF virus (Villada *et al.*, 1995).

Infection in domestic pig results into peracute, acute and sub acute disease, clinically characterized by pyrexia, where body temperature rise to 40 – 42 °C, later the pigs become depressed, anorexic, and reluctant to move and huddled together. Other signs include; lameness, hyperaemia and cyanosis of the skin, localised necrosis of the skin and disseminated intravascular coagulation with multiple haemorrhages in several tissues and death occur within a few days once clinical signs appear (Gomez *et al.*, 1998, Sandra, Claudia and Martin 2012).

The major lesions due to ASF occur in the lymphoid tissues, renal and circulatory system. The lymph nodes are enlarged and haemorrhagic with extensive necrosis of the lymphoid follicles. The spleen is markedly congested and enlarged while haemorrhages often observed in kidneys. Circulating

lymphocytes are depleted and leucopenia occur. Mononuclear cells infiltrate stroma of various tissues including brain heart, liver and lungs. Oedema accompanies the vascular lesions and affects the lungs, wall of gall bladder and subcutaneous tissues (Sandra *et al.*, 2012,).

Several studies on the pathogenicity of ASFV have been done, however limited literature is available on the effects of the virus on biochemical parameters, especially organ function tests. Furthermore pathogenic effects of different ASFV isolates on domestic pigs have not been studied extensively and the possibility of using biochemical parameters as markers for ASF diagnosis has not been evaluated.

In this study we demonstrated the changes in body temperature, leukocytes profiles and changes in the levels of selected enzymes in serum during clinical phase of ASF using Ugandan isolates of ASF virus.

Materials and Methods

Ethics statement

Full ethical clearance was obtained from the Uganda National Council for Science and Technology (UNCST) and the College of Veterinary Medicine, Animal Resources and Biosecurity, of Makerere University under reference number VAB/REC/11/110. Animal welfare and care was ensured in accordance with the international Guideline on Animal Welfare and Euthanasia. Any experimental animal in pain or moribund was immediately euthanized to relieve it from further suffering. Clean water and commercial feed were provided *ad libitum* to all pigs during study period.

Study design

Experimental animals' selection and viral inoculation

A total of eighteen, four months old, healthy cross bred Cambrough – Large White cross bred pigs that weighed an average 40kgs each were selected for this study. Twelve pigs were used as test animals and grouped into three (four per group) while six uninfected pigs were used as negative control. Each member of the test group was infected per os with 2mls of ASF viral culture solution containing 1×10^8 haemadsorbing units per millimetre (1×10^8

H.A.D.U/ml) of ASF virus. Each sub group of the experimental groups was infected per os with different ASF virus isolates from Uganda, i.e. genotype 2 sub types 1, 2, 3 respectively as characterised by Atuhaire *et al.*, (2013). Pigs in experimental Group One were infected with ASFV from Cluster one (Tororo, Kumi, Kanyanya and Moyo 2 Isolates); experimental group Two were given isolate two (Kansanga Isolate); and Experimental group three were infected with ASFV belonging to cluster 3 (Isolates from; Mpiigi, Wakiso, Lira, & Kabale). Each pig in the negative control group was given per-os 2 mls of uninfected porcine alveolar macrophages culture.

Evaluation of Clinical Parameters

The experimental animals were observed and examined daily during the study period and any changes in the clinical parameters noted. Body temperatures were recorded daily while blood samples were taken just before infection and at intervals of four days post infection for haematological and organs functions tests.

Leucocytes count and evaluation

Blood samples were obtained from the anterior vena cava and thin blood smears were prepared from fresh blood samples and stained using modified Giemsa solution based on manufacturers protocol (Sigma Aldrich® USA, 2012). Total white blood cells were recorded and percentages of each leucocyte type in samples taken at different stages of infection calculated. Light microscope was used to examine and evaluate leukocytes in random sequence. White blood cells were evaluated based on their morphologic characteristics and the percentage population. The data obtained on blood cell counts was evaluated by pair wise comparison and multivariate statistical analysis (MANOVA) as described by Abdelmonen *et al.*, (2012). Differences within and between groups were considered significant when $p < 0.05$ at 95% confidence interval. The results were expressed as percentage of each cell with confidential intervals.

Biochemical tests

Blood samples for biochemical tests

were collected in silconised vacutainers, left to settle at room temperature, centrifuged and serum extracted. The serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatine kinase (CK), alkaline phosphatase (ALKP), gamma-glutamyl transferase (GGT) and albumin were determined using an automated chemistry analyser, also known as vet test analyser from IDEXX Vet Labs® USA. Level of each enzyme in the blood samples was tested thrice, thus just before inoculation, in the fourth and eighth day post infection.

Challenges and Limitations of the study

The study used only Ugandan Isolate of ASF virus, therefore we were unable to compare effects of ASF virus isolates from other regions of the world with the local isolates.

This study was limited to acute, virulent ASF because we did not have isolates of ASF virus that cause sub acute or chronic disease. Samples for viral isolation were collected from pigs that died of the disease hence subclinical cases were missed out.

The clinical course of ASF in this study was short; hence there was little time available for repeated sample collection during the clinical phase of the disease.

Results

Experimental Group One, (Cluster 1:- Tororo, Kumi, Kanyanya/Kampala & Moyo 2 Isolates)

Daily Temperature Variation with Disease progress

In the first five days post infection, the temperature of all pigs remained within the normal range of 38 to 39.5 °C. However, by the seventh day post infection, 25% (n = 3) of the test pigs developed fever. By the 12th day PI, 33.3% (n = 4) of the pigs had succumbed to the disease and all of the infected pigs developed fever; the highest temperature recorded during the period of study was 41.8°C in two pigs. All the six pigs in the negative control group had body temperatures maintained within the normal range of 38 to 39.5 °C throughout the study period. The daily rectal temperature of the pigs was as presented in figure 1, 2 and 3

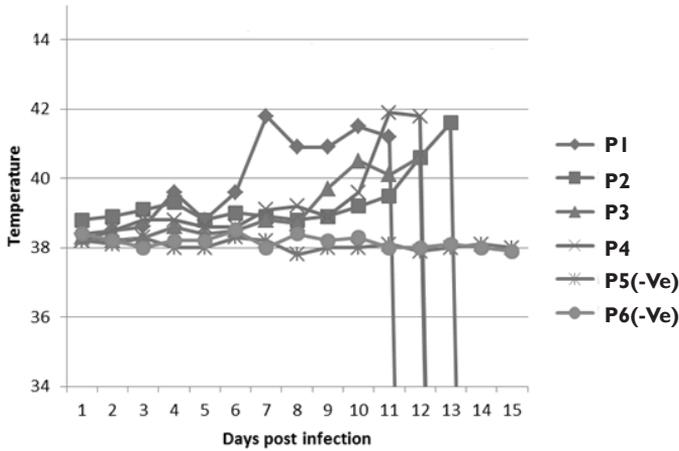


Figure 1: Group 1 infected with ASFV of Cluster 1; (Isolates from Tororo, Kumi, Kanyanya (Kampala I) & Moyo Districts). Fever was first detected at the sixth day post infection (PI) and by 13th day PI all infected pigs had succumbed to the disease while body temperature for negative control remained within 38 – 38.5.

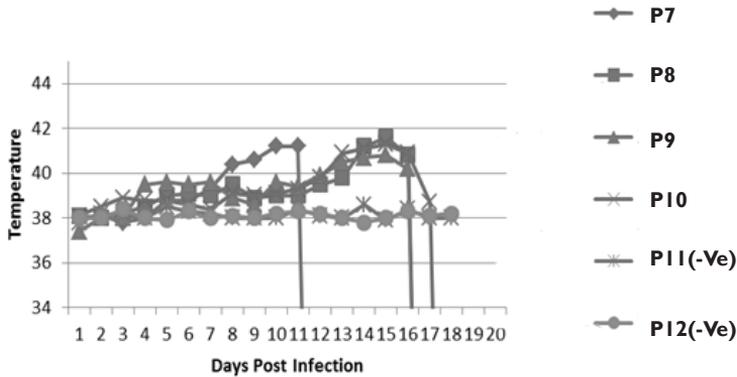


Figure 2: Group 2 infected with ASFV of Cluster 2; (Isolates from Kansanga/Kampala District). Fever was first detected eight days post infection and by 13th day PI all infected pigs had clinical disease while body temperature for negative control remained within 38 – 38.5.

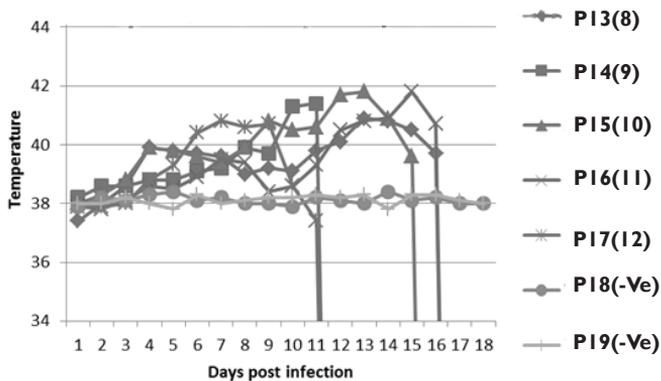


Figure 3: Group 3 infected with ASFV of Cluster 3; (Isolates from; Mpigi, Wakiso, Lira, & Kabale Districts). Fever was first detected six days post infection and by 12th day PI all infected pigs had clinical disease while body temperature for uninfected pigs remained within 38 – 38.5 °C.

Table 1: Descriptive Statistics showing changes in total leucocytes counts and absolute differential counts in ASFV infected and uninfected pigs as the disease progressed

GRP	Mean Leucocytes counts in pig blood samples Post infection/Inoculation in Different Groups																				
	Mean WBC Count (X 103) cells/µl		Lymphocytes (%)		Segmental neutrophils (%)		Band Neutrophils (%)		Eosinophils (%)		Monocytes (%)		Basophils (%)								
	D1	D4	D8	D1	D4	D8	D1	D4	D8	D1	D4	D8	D1	D4	D8						
1	19	12.8	8.8	59	48.6	51	1.8	5.8	9	30.3	34.5	27.8	4.5	5.3	3.3	4.3	5.8	9	.03	0	0
2	17.5	16.1	11.3	60.8	56.5	43.8	1	1.5	3.3	22.3	34	41.5	3	2.8	3.3	4.3	5.3	8.4	0	0	0
3	17.8	14.5	9.1	59.8	58	43.3	.5	2.3	8	33.3	29	29.8	3	2.8	3.3	3.5	8	13	0	0	0
Neg. Ctrl	17.9	18.4	18.1	62	63.8	62	1	.7	1.3	28.3	27.3	29.3	3.7	3	3.8	3.8	4.7	3.7	0	0	0

for the different experimental groups.

Apart from fever other clinical signs noted during the period of study included, reduced appetite, general weakness, wobbling, difficult respiration which was first noticed 10 days post inoculation in four pigs. At later stages of the disease, all pigs had dyspnea and hyperaemia of the skin of ears, legs and belly before death or euthanasia. The clinical course of the disease due to Ugandan isolate of ASFV used in this study ranged from two to seven days and the incubation period was between 7 - 15 days as shown in the Figures 1, 2 and 3.

Changes in leucocytes population

Changes in white blood cell (WBC) counts were first registered at day four post infection. In early stages of infection, there was increased number of immature forms of leukocytes, especially lymphoblasts, monoblasts and band neutrophil. Descriptive Statistics showed that there was a decreasing trend of mean total WBC count post infections over time and the mean leucocytes count eight days post infection was 9.73×10^3 cells/µl compared to 18.06×10^3 cells/µl before infection. The output from the Greenhouse-Geisser adjustment was highly significant, which indicated that there was a significant decrease in the leucocytes population as ASF progressed ($p = 0.00$). The magnitude of the strength of the “within-subject effect (i.e time)”, eta squared was 0.52; therefore 52% of the variance in Total WBC could be explained by the time effect. The mean leucocyte count in the uninfected group throughout the study period was 18.13×10^3 cells/µl. The multivariate test result indicated a significant decrease in lymphocytes count over time ($p = 0.001$) and output, eta squared was 0.493, hence 49.3% of the variance in mean Lymphocytes count as a percentage of Total WBC could be explained by the time effect. However the variation in the basophils count as the disease progressed was not significant ($p = 0.30$). Results further showed that there was a decreasing trend of mean Eosinophils as a percentage of Total WBC post infections over time. However the changes in the Eosinophils population was neither significantly linear nor quadratic ($p = 0.31$).

On the other hand Band Neutrophils progressively increased in number as the

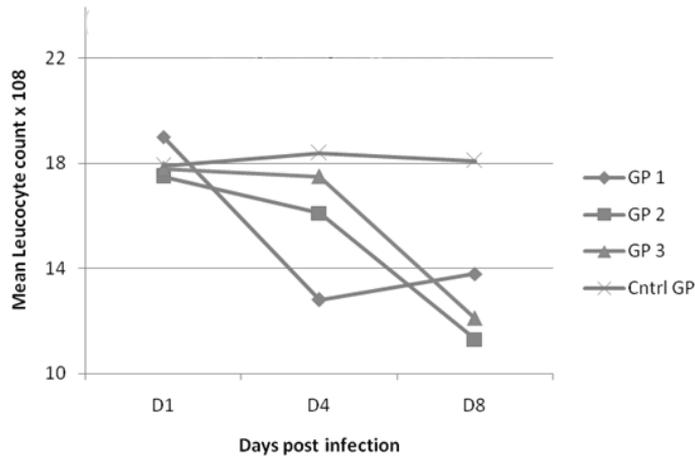


Figure 4: shows a decreasing trend of mean WBC counts, and MANOVA showed a significant decrease in the leucocyte count over time ($p = 0.00$) in all the infected groups (GP 1, GP 2, GP 3) but changes in leucocyte count in control group (Cntrl GP) was insignificant.

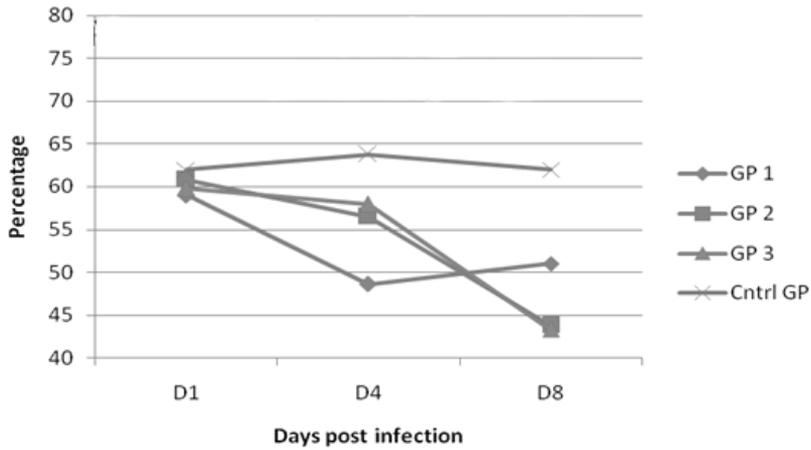


Figure 5: shows a decreasing trend of mean Lymphocytes as a percentage of Total WBC post infections over time in all the infected groups and the decrease was significant in the infected groups (GP 1, GP 2, GP 3, $p = 0.001$) but percentage changes in lymphocytes count in uninfected group (Cntrl GP) was insignificant ($p = 0.96$).

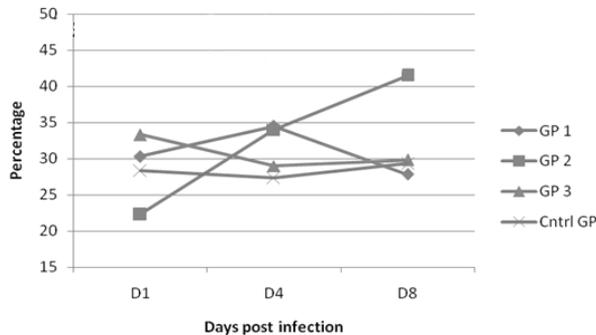


Figure 6: Shows an increasing trend of mean Band Neutrophils as a percentage of Total WBC post infections over time. MANOVA indicated that although not significantly linear nor quadratic ($p = 0.52$), there was an increase in the percentage Band neutrophils counts in the ASFV infected pigs over time.

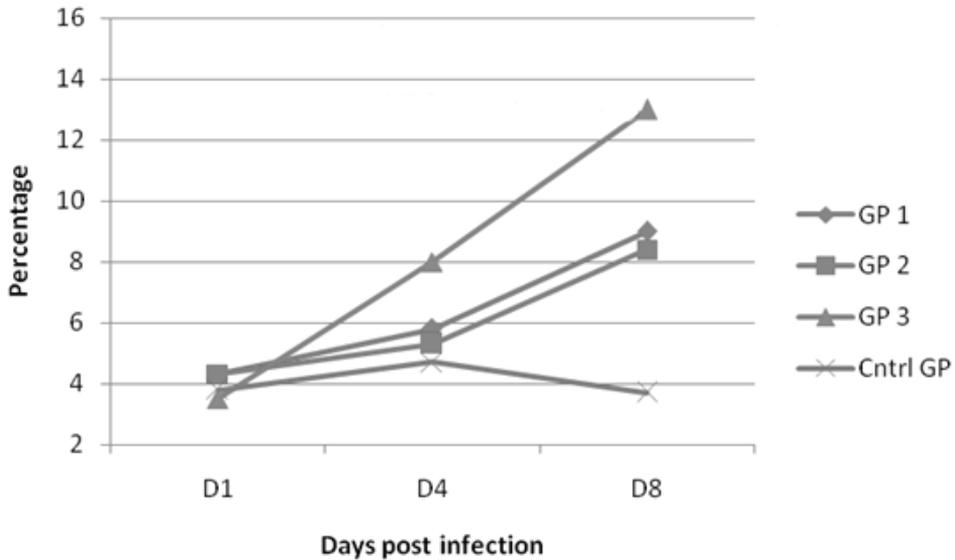


Figure 7: Shows an increasing trend of mean Monocytes counts, and MANOVA showed a significant increase in the percentage Monocytes count over time ($p = 0.01$) in all the infected groups (GP 1, GP 2, GP 3) unlike in the control group.

disease progressed. Nevertheless the changes in mean Band Neutrophils as a percentage of Total WBC was not statistically significant ($p = 0.52$). On the other hand mean monocytes count significantly increased in the infected groups during the study period ($p = 0.01$).

The results showing changes in leucocytes counts and absolute differential counts over time in the different groups are as shown in table I and figures 4, 5, 6, and 7 that follow. Table I: Descriptive Statistics showing changes in total leucocytes counts and absolute differential counts in ASFV infected and uninfected pigs as the disease progressed

Results shown in table I indicate that the mean Total WBC and lymphocytes counts decreased in all infected groups but remained within the normal average (18.13×10^3) in uninfected (Cntrl GP) group post inoculation. The mean percentage Band Neutrophils and monocytes counts increased in infected groups and remained within the normal range of 0- 5%, and 2 – 10% respectively, in the control group (Nemi 1993).

Biochemistry Analysis

Majority of the pigs, 83.3% ($n = 10$) in the infected groups had elevated levels of gamma-glutamyl transferase (GGT) in their

blood above the normal range of 16 – 50 μ /l (Rodostits *et al.*, 2000, IDEXX Vet Test® USA laboratories operator’s manual 2009). The highest concentration of GGT detected was 68 μ /l; in pig number 12. Generally in all infected pigs the serum GGT levels were elevated although in one pig (pig 6 of group 2, infected with Kansanga/Kampala isolate) the serum GGT levels remained within the normal range throughout the period of the study. Multivariate test results showed that there was a statistically significant increase in the GGT levels in infected pigs by the eighth day PI ($p = 0.00$). The mean GGT level in the infected groups before infection was 24 μ /L while after 8 days PI, it was 44.4 μ /L. Pairwise comparison showed that there were no statistically significant differences in GGT levels between the three infected groups ($p = 0.878, 0.975, 0.902$ when levels in infected groups 1, 2 and 3 were compared). When the mean levels of GGT 8 days PI in uninfected and infected groups were compared, it was higher in infected pigs (44.4) than in uninfected group (23.0) and the difference was statistically significant ($p = 0.000$).

Levels of alanine aminotransferase (ALT) 8 days post infection was elevated beyond the normal range of 9 – 43 μ /L in all infected pigs in the three groups. The highest

Table 2: Mean of serum enzyme levels in ASFV infected and uninfected pigs over time.

Grp	Mean Serum Enzyme levels Post infection/Innoculation in Different Groups													
	GGT (μ/L)		CK (μ/L)		CREA (μmol/L)		ALKP (μ/L)		ALT (μ/L)		AST (μ/L)		ALB (g/l)	
	Day 4	Day 8	Day 4	Day 8	Day 4	Day 8	Day 4	Day 8	Day 4	Day 8	Day 4	Day 8	Day 4	Day 8
1	24.0	45.0	369.3	458.0	117.0	128.8	211.3	210.8	31.6	76.8	30.8	22.8	22.6	32.2
2	23.9	43.2	433.3	533.3	123.8	158.8	143.8	162.8	32.6	85.8	46.3	23.0	23.2	33.2
3	24.0	45.0	236.8	384.3	115.3	186.6	209.5	209.0	28.2	76.8	26.8	19.5	21.2	31.6
Neg. Ctrl	23.7	23.0	362.0	377.7	114.0	115.3	180.7	183.0	32.3	30.6	20.8	23.0	22.4	24.0

Note: Pigs in group one were infected with Ugandan ASF virus strain IX belonging to cluster I (Tororo, kumi, Kanyanya & Moyo Isolates), group two with strain IX cluster 2 (Kansanga Isolate) and group three received strain IX cluster 3 (Mpigi, Wakiso, Lira, & Kabale Isolates). Negative control group were given uninfected porcine macrophages culture suspension.

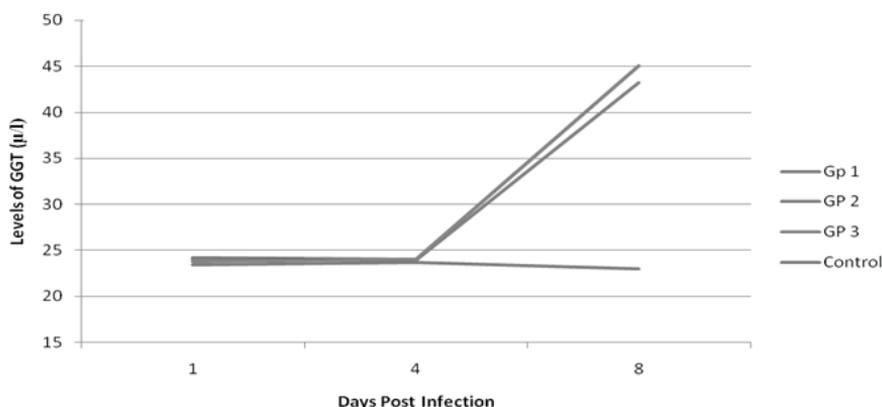


Figure 8: Shows increasing trend of GGT in all infected groups, multivariate analysis (MANOVA) revealed a statistically significant increase in the mean GGT level by the 8th day PI in all infected groups compared to control group ($p = 0.00$). Within and between the infected groups, changes in GGT over time was not significant ($p = 0.92$).

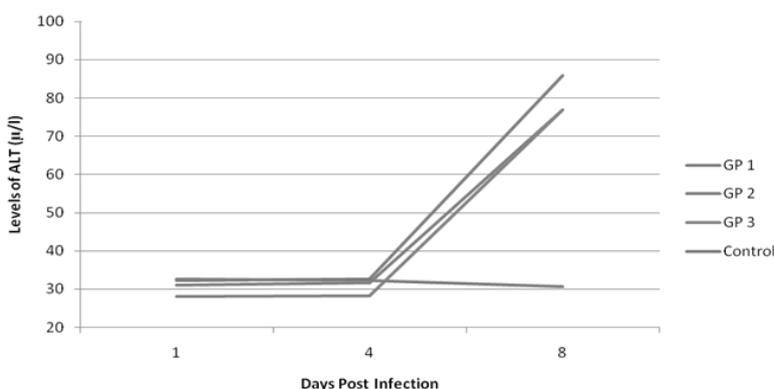


Figure 9: Shows an increasing trend of ALT in all infected groups but it remained within the normal range in uninfected group (Cntrl GP). The increase in ALT over time was very similar in Group 1 and 3 but higher (although not statistically significant) in Group 2. Pair wise comparisons of the mean ALT levels between the infected and uninfected groups 8 days PI was statistically significant ($p = 0.000$)

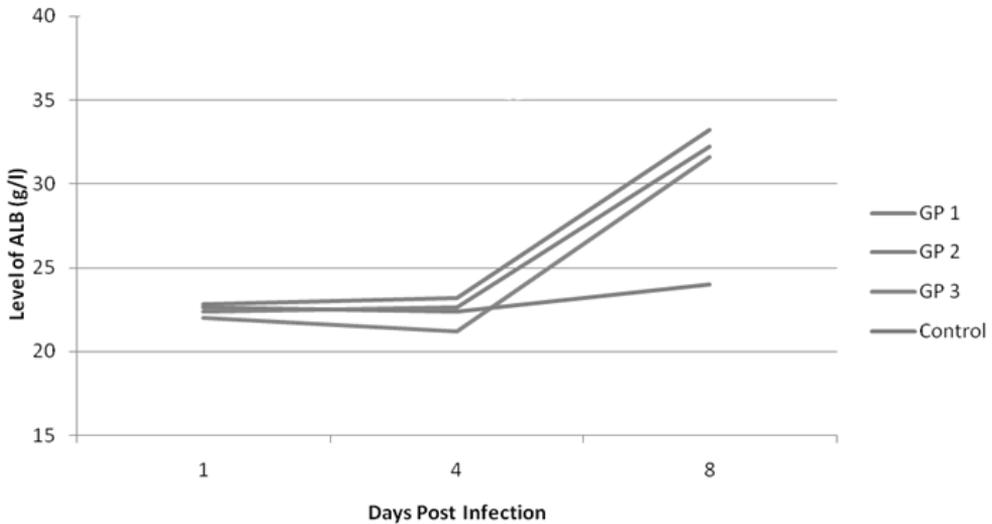


Figure 10: Shows an increasing trend of mean ALB in all infected groups which was significant ($p = 0.001$) 8 days post infection, while in control group the change was insignificant ($p = 0.90$). Group 2 experienced highest mean ALT followed by Group 1 and then Group 3.

level was $151 \mu\text{l}$ in pig number 12 and the lowest was $76 \mu\text{l}$; in three pigs i.e. number; 3, 7 and 10 respectively. Multivariate analysis showed that there was a statistically significant increase in ALT in infected pigs by the eighth day PI ($p = 0.00$). The mean ALT level in serum before infection was $30.8 \mu\text{L}$ while the mean by the eighth day PI was $79.8 \mu\text{L}$. Pairwise comparison showed that there were no statistically significant differences in ALT levels between the three infected groups ($p = 0.707$, 0.898 , 0.616 when the mean levels of ALT in serum of groups 1, 2 and 3 respectively were compared).

In 66.7% ($n = 8$) infected pigs, ALB levels were elevated in the serum samples above the normal range of $18 - 33 \text{ g/l}$ (Rodostits *et al.*, 2000, Peter and peter 2002, IDEXX Vet Test® USA laboratories operator's manual 2009) and the highest level of ALB recorded in serum samples was 39 g/l in pig number 3. Although in 33.3% ($n = 4$) infected pigs the level of ALB remained within the normal range, there was apparent increase in the levels of ALB in all pigs in the test groups. The level of ALB in the uninfected pigs (negative control group) varied from 23 g/l to 29 g/l these values were within the normal range of $18 - 33 \text{ g/l}$ published in IDEXX Vet Test® USA laboratories operator's manual 2009 and by Rodostits *et al.*, 2000).

Multivariate analysis showed that there was a statistically significant increase in the level of ALB by the eighth day post infection in the infected group ($p = 0.001$). The mean ALB level before infection was 22.3 while the mean after 8 days was 32.2 . Pairwise comparison showed that there were no statistically significant differences in ALT levels within and between the three infected groups ($p = 0.701$, 0.566 , 0.344 when groups 1, 2 and 3 respectively were compared). When the mean serum levels of ALB in uninfected and infected groups were compared, it was statistically significant ($p = 0.001$).

In 20% ($n = 3$) pigs the level of AST dropped below the normal range of $25 \mu\text{l} - 57 \mu\text{l}$ (Nemic 1986, Rodostits *et al.*, 2000, IDEXX Vet Test® USA laboratories operator's manual 2009). Other biochemical levels in the serum samples such as creatine kinase (CK), creatinine (CREA), and alkaline phosphatase (ALKP) remained within the normal range ($50 - 3531 \mu\text{L}$, $44 - 186 \mu\text{mol/L}$, $92 - 294 \mu\text{L}$, respectively). The results are summarised in table 2 that follows

Discussion

This is the first experimental study in Uganda on the effects of ASFV on the

biochemical and clinical pathology of domestic pigs infected with ASF viral isolates from this country under local (Ugandan) conditions other than those which have involved studies elsewhere.

African swine fever occurs as acute, sub acute or chronic forms depending on the virulence of the strain of the ASF virus. Severe acute form of the disease is characterised by fever in appetite, general body weakness and depression (www.cfsph.iawastaeu.edu. [africanswinefever](http://www.africanswinefever.org) the centre for food security and Public health Iowa State University). These clinical signs and symptom were also observed in this study.

In this study fever was the first clinical sign observed in the infected pigs and coincided with increased counts of monocytes and band neutrophils. This has been attributed to viraemia and release of pro-inflammatory cytokines during early stages of infection. Salquero *et al.* (2002) stated that increased production of proinflammatory cytokines such as TNF, IL-1 and IL-6 by activated monocytes and macrophages often coincides with fever, endothelial damage and lymphopenia. Initial viremia often occurs in the third day post infection in pigs infected intranasally with ASFV. Four days after infection all infected pigs may developed fever in which the temperature increased to 40 – 42°C and at this stage the viral titre in the blood is at its maximum value (Edwarda *et al.*, 1985). However in this study, fever was first detected in the seventh day post infection only in three pigs, the remaining nine infected pigs developed febrile condition later. The difference in the time of response to infection could be due to the fact that in the previous study, intra-nasal route of infection was used as opposed to the oral route employed in this study. Intranasal infection could have enhanced viral penetration and infection faster than the oral route. Alternatively within the same ASFV genotype IX found in Uganda (Atuhaire *et al.*, 2013), some isolates probably cause ASF with longer incubation period. More still individual host response to pathogens could have contributed to the range of incubation periods.

In this study we further report a significant decrease in the leucocytes population as ASF progressed ($p = 0.00$) and

52% of the variance in total WBC which could be explained by the time effect. This is in agreement with the findings reported by Blood *et al.*, (1975) which also noted a drop in total leukocyte count of approximately 40 – 50% of the normal range by the fourth day of clinical ASF. The leucopaenia that occurs in ASF is associated with extensive necrosis of lymphocytes in the lymphoid tissues (Gomez-Villamandos *et al.*, 1995).

Lymphocyte counts significantly reduced in all experimental groups ($p = 0.00$). Decreased percentage of lymphocytes observed in this study in all infected pigs were probably due to ASFV induced apoptosis and necrosis. This is in agreement with previous studies which found that pigs suffering from ASF generally had lymphopaenia that was attributed to apoptosis of lymphocytes induced by cytokines produced by ASFV infected monocytes and macrophages (Gomez-Villamandos *et al.* 2003, Oura *et al.*, 1998).

Lymphopaenia is usually pronounced while the population of band neutrophils tend to increase. Hence the findings in this study were similar to that reported by Gomez-Villamandos *et al.* (2003) and Oura *et al.*, (1998).

In all infected pigs, the population of the immature leucocytes, especially band neutrophils, and lymphoblast increased. This was probably due to the activation of leukocytes stem cells in the bone marrow in response to depletion of mature lymphocytes in the lymphoid tissues as a result of viral induced apoptosis and necrosis. The uninfected group had their leucocytes population maintained within the normal range; thus band neutrophil 0 – 4%, lymphocytes, 39 – 62%, monocytes 2 – 10% (Blood *et al.*, 1995). This implied that the changes in leukocytes population were likely due to effects of ASF viral infection.

The main target cells of ASFV are the monocytes and macrophages lineage (Sanchez-Torres *et al.*, 2003, Malmquist and Hay 1960). Sander *et al.*, (2012) reported that dendritic cells are also infected by the ASF virus and after 30 hours post infection ASFV become widely distributed in tissues of almost all organs. At the stage of viraemia haemadsorbing ASFV isolates associate with erythrocytes, lymphocytes and

neutrophils (Wardley and Wilkinson 1997). Chronic form of the disease is associated with autoimmune reactions leading to deposition of immune complexes in tissues such as renal plural and cutaneous tissues (Sander and Claudia and Martin 2012). Although a lot of scientific research has been done, many of pathogenic mechanisms of ASF are not yet fully understood (Sander and Claudia and Martin 2012). Wardley and Wilkinson (1977) reported that ASF is characterised by leucopaenia, however, the erythrocytes count remains within the normal range. Lymphocyte numbers in circulation are reduced while the band neutrophil count tends to increase. Sander *et al.*, (2012) first noted leucopenia after seven days post infection in pigs infected with highly virulent E70 isolates of ASFV. This was similar to our finding where leucopaenia was observed in all infected pigs ($n = 12$) by the eighth day post infection.

The apparent increase in monocytes count in infected pigs was probably in response to the increased stimulation of the production of monocytes. Ramiro-Ibanez *et al.* (1997) showed that ASF leads to increased monocytes and macrophages population within the first week post infection. The peak monocytes, macrophages count coincides with viraemia, onset of fever and detectable serum levels of antibodies. The team further reported an increased count of CD4+ and CD8+ T cells and peripheral monocytes in the second week post infection. Sanchez-Torres *et al.* (2003) showed that mature mononuclear cells that expressed higher levels of macrophages specific markers including SLA II antigens were most susceptible to ASFV infection. Expression of acute regulatory receptor, CD 163 and porcine CD 107a (surface antigen 4E9) is related to susceptibility to ASFV infection (Sanchez-Torres *et al.* 2003). CD 163+ monocytes are known to produce more TNF; they express high levels of adhesive molecules and are better antigen presenters than CD 163 – monocytes (Chamorro *et al.*, 2005). However this study looked at the variation in the counts of the different types of leucocytes in pigs post infection with virulent ASFV without characterising the individual cell types based on the specific cell markers. Ramiro- Ibanez *et al.*, 1995a, showed that monocytes in peripheral

circulation of pigs infected with virulent strains of ASFV had viral protein p30 antigens 5 days post infection and in 7 – 9 days post infection in those pigs infected with attenuated strain. Detectable levels of cytokines in tissues of infected pigs usually correlate with the presence of viral protein p73 antigen in the mononuclear phagocytic cells.

Carrasco *et al.* (1996) confirms that ASFV infect neutrophils and reported that both mature and immature neutrophils harbour ASFV. Comez – Villamandos *et al.* (1995) further pointed out that band neutrophils are transport vehicles for ASFV, hence aid the spread of the virus. Therefore the increased band neutrophils count reported in this study in the infected groups were probably in response to ASFV induced neutrophil proliferation. Increased band neutrophil and monocytes counts are indicators of active inflammatory response (Fred *et al.*, 2004).

The elevated level of GGT in the serum samples of majority of the infected pigs 8 days post inoculation could be associated with severe damage to kidneys and hepatobiliary tissues of the infected pigs. GGT is cell membrane bound enzyme found in high concentration in renal cortex and medulla. Small amounts of GGT occur in mucosa of intestines and in liver, especially in the bile canaliculi (IDEXX Vet Test® USA, laboratories operator's manual 2009)

Virulent ASF virus isolates cause severe damage to multiple organs including renal tissues characterised by wide spread haemorrhages and necrosis with mononuclear cells infiltrations. Extensive haemorrhages also occur in hepatobiliary system. In the kidney, acute ASF causes necrotising glomerulonephritis and the wide spread haemorrhages observed in various organs are attributed to the damage and dysfunction of endothelia as a result of viral replication in the endothelial cells (Gomez – Villamandos *et al.* 1995, Blome, *et al.*, 2012). Although the available literature shows that, renal diseases are note usually associated with increased levels of GGT (Nemic 1986), probably this depends on the extent of damage to the renal tissues.

This therefore calls for further study to determine the extent of renal tissue damage that can lead to elevated levels of GGT in

circulation.

Published reports pointed out that, chronic hepatobiliary disease usually result in increased levels of GGT in circulation (www.bloodwork.com/gamma-glutamyl-transpeptidase-ggt-test, December 2013). However, in this study, the course of the disease was acute in all the twelve infected pigs. The average incubation period for the isolates used was seven days and clinical course of the disease was in average five days, hence the Ugandan ASF viral isolates used in this study caused acute disease in domestic pigs. This therefore implies that acute severe multiple organ damage including hepatobiliary system also leads to elevated levels of GGT in circulation of the affected pigs. Contrary to the elevated levels of GGT in the majority of the ASFV infected pigs (91.7%, n = 11), the serum level of GGT in one pig (# 13 of group 2) remained within the normal range throughout the study period. This could be due to individual un-explained variation which needs to be investigated further.

In all infected pigs, the levels of alanine aminotransferase (ALT) 8 days post infection in the serum samples were elevated. ALT is one of the enzymes found in cell cytoplasm and mitochondria. The elevated levels of ALT in serum occur during hepatic necrosis and hepatitis. Similar elevated levels of ALT in serum were reported in dogs suffering from acute hepatic necrosis caused by infectious canine hepatitis (Cynthia and Scott 2005). Ramiro- Ibanez et.al. (1997) further used immunohistochemistry to demonstrated progress of ASFV infection in different cell types. He showed that monocytes and macrophages in lymphoid tissues are the cell types first infected by ASFV, later the virus spread to other organs and cells such as hepatocytes, renal tissue, endothelia, megakaryocytes. Infection of kuffer cells is followed by their activation, haemadsorption, phagocytosis of erythrocytes and lymphocytes attachment to the activated cells (Gomez –Villamondos et al.,1997). Sanchez- Cordon et.al. (2008) observed that the number of kupffer and sinusoid cells in circulation increased significantly. Secretion and expression of cytokines especially IL-1, IL-6 and TNF by kupffer cells also increased (Gomez – Villamondos et.al 1995, Blome,et. al., 2012). This

implies that ASFV infection causes damage to liver tissues that probably results into elevated levels of ALT in serum of infected pigs.

The elevated level of serum albumin in 66.7% of the infected pigs (n =8) was probably due to the excessive fluid loss from blood circulation into body cavities and subcutaneous tissues in form of oedema, hence increased haemoconcentration and albumin in serum. Cynthia and Scott (2005) reported dehydration as a major cause of elevated levels of protein (albumin) in animal serum. In animals, hyperalbuminemia is rarely seen, nevertheless in acute dehydration and shock it does occur (Coles 1986). Acute ASF causes significantly elevated levels of prostaglandin E2 that increases vascular permeability, hence aid in the development of oedema, circulatory collapse and shock (Anderson 1986, Penrith et al., 2004b). This could lead to the elevated level of albumin as noted in the study.

The value of other biochemical tests such as creatinin kinase, urea, and alkaline phosphatase remained within the normal range of 50- 3531 μ /L, 44 - 186 μ mol/L and 92 - 294 μ /L (IDEXX Vet Test® laboratories operator's manual 2009). Serum creatine levels often increases when there is renal dysfunction, blocked urethra and ruptured bladder. However in serum of all infected and uninfected groups the levels of these biochemicals did not vary significantly.

Conclusion

This is the first study in Uganda that attempts to describe the effect of African swine fever virus on the biochemical and clinical parameters including leukocytes count in domestic pigs using Ugandan isolates of ASFV. The experimental infection in domestic pigs was characterised by a significant decrease in leucocytes counts, lymphopenia, increased counts of band neutrophils and monocytes in circulation. There were also increased levels of GGT, ALT and ALB in blood circulation during clinical phase of the disease. Evaluation of the levels of biochemical parameters and leucocytes counts in pigs could therefore be used to complement screening assays for ASF in case of suspected outbreak of the disease. All

ASFv field isolates used caused acute disease in domestic pigs with similar clinical presentation and biochemical changes in the serum samples of the all infected pigs.

A study should be done to understand the mechanism through which ASF induce the observed clinical changes in the biochemical parameters of infected pigs. There is also need to compare changes in the levels of GGT, ALT and ALB in pigs suffering from chronic ASF with that of acute disease. This could probably be useful in diagnosis of subclinical ASF.

We recommend an in-depth study to understand how ASFv infection induces changes in the levels of GGT, ALT and ALB in the serum of infected pigs.

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EFFET DU *PEDIOCOCCUS ACIDILACTICI* SUR LE BILAN LIPIDIQUE SANGUIN DU POULET DE CHAIR

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Résumé

Pour évaluer l'effet du probiotique *Pediococcus acidilactici*, nous avons utilisé mille sept cent soixante-trois poussins d'un jour de l'espèce *Gallus domesticus*, appartenant à la souche Hubbard F15, de sexes mélangés et d'un poids homogène, provenant d'un même couvoir, mis en place dans le même bâtiment pour être élevés dans les mêmes conditions d'élevage durant une période de 52 jours. Ces animaux ont été répartis en deux lots. Les animaux du lot "expérimental" recevaient une eau de boisson exempte de tout additif et un aliment additionné de lyophilisat de *Pediococcus acidilactici* (CNCM MA18/5M ; Bactocell®, France) à raison de 100 ppm (concentration de 10^9 UFC.g⁻¹) jusqu'au 42^{ème} jour d'élevage. Ceux du lot "témoin" recevaient le même aliment, sans probiotiques, mais additionné d'un anticoccidien chimique (Cycostat) à raison de 0,5 kg par tonne d'aliment ainsi qu'une eau additionnée d'antibiotiques durant toute la période d'élevage. Les résultats relatifs aux performances zootechniques ont montré que l'addition du probiotique a amélioré significativement le gain de poids pendant la phase de croissance se traduisant par un indice de consommation meilleur. Les dosages du cholestérol total, des triglycérides, du HDL et du LDL ont été déterminés à la fin de chaque phase d'élevage (28^{ème}, 42^{ème} et 52^{ème} jours). Les moyennes des 4 paramètres (cholestérol total, triglycérides, HDL et LDL) étaient comparables à 28 jours pour les lots témoin et « Probiotiques ». Ces moyennes sont restées comparables à 42 jours, à l'exception du cholestérol total significativement plus bas dans le lot « Probiotiques » (1.10 ± 0.07 vs 1.52 ± 0.12 g.L⁻¹ ; $p=0.03$). Les moyennes des 4 paramètres étaient comparables à 52 jours entre les lots « témoin » et « Probiotiques ». Les probiotiques ont prouvé leur efficacité dans la diminution significative du cholestérol total au 42^{ème} jour.

Mots-clés: cholestérol, HDL, LDL, performances zootechniques, poulets, probiotiques.

PEDIOCOCCUS ACIDILACTICI EFFECT ON BROILER BLOOD LIPID PROFILE TITLE TRANSLATED

Abstract

To evaluate the effect of probiotic *Pediococcus acidilactici*, we used one thousand seven hundred and sixty three chicks of *Gallus domesticus* belonging to the Hubbard strain F15 mixed sexes and a uniform weight, derived from the same hatchery and, installed in the same building to be raised under the same conditions for a period of 52 days. These animals were divided into two experimental groups. Animals in the "experimental" lot received a free drinking water without additives and added anticoccidial the anticoccidial compatible with lactic flora and the lyophilised *Pediococcus acidilactici* CNCM MA18/5M (Bactocell®, France) at 100 ppm (concentration of 10^9 CFU.g⁻¹) until the 42nd day of livestock. The "control" lot received the same food, but without probiotic or anticoccidial natural extract but supplemented with a chemical anticoccidial (Cycostat) at 0.5 kg per ton of feed, as well as water containing antibiotics throughout the rearing period. The results for growth performance showed that the addition of probiotic improved significantly weight gain during the growth phase, resulting in an index of better consumption. Total cholesterol, triglycerides, HDL and LDL were determined at the end of each breeding phase (28th, 42nd and 52nd days). The average of the 4 parameters (total cholesterol, triglycerides, HDL and LDL) were comparable at 28 days for "Control" and "Probiotics" groups. These averages were comparable at 42 days except for total cholesterol which was significantly lower in the "Probiotics" group, 1.10 ± 0.07 against 1.52 ± 0.12 g.L⁻¹ ($p = 0.03$). The average of the four parameters were comparable at 52 days between "Control" and "Probiotics" lots. Probiotics have

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been proven effective in a significant lowering of total cholesterol at 42nd day.

Keywords: chicken, cholesterol, HDL, LDL, probiotics, zootechnical performance.

Introduction

Les antibiotiques ont été largement utilisés pour la production animale à travers le monde. Ces produits sont distribués dans l'aliment des volailles dès le premier jour d'âge des oiseaux, agissent préventivement en contrôlant le développement des coccidies dans le tube digestif et améliorent le potentiel de croissance. Mais devant l'apparition de bactéries résistantes aux antibiotiques utilisés pour traiter des infections humaines ou animales, la Commission Européenne a fini par interdire leur utilisation comme additifs alimentaires depuis 2006 (CE n° 1831/2003).

En Algérie, malgré l'interdiction de leur incorporation dans l'alimentation animale par la réglementation depuis 2007 (décision ministérielle du 24 Décembre 2006), l'usage des antibiotiques reste de pratique courante.

Face à cette situation, plusieurs voies alternatives de recherche ont été explorées en vue de leur substitution, en l'occurrence, l'emploi des probiotiques (Lactobacilles). Ces derniers sont capables de contrôler le portage et la dissémination d'agents pathogènes et zoonotiques et peuvent également contribuer à potentialiser l'aliment et donc la rentabilité de l'élevage (Trufanov *et al.*, 2008 ; Niderkorn *et al.*, 2009). De nombreux travaux ont montré, en plus leur efficacité zootechnique (Simon *et al.*, 2001, Vitorrio *et al.*, 2005), leurs effets bénéfiques sur la santé des volailles (Awaad *et al.*, 2005, Vandeplass *et al.*, 2009 et Higgins *et al.*, 2010) et par conséquent sur la santé humaine. Les aliments, enrichis en probiotiques, devraient assurer un meilleur équilibre alimentaire et une assimilation plus performante des nutriments essentiels et renforceraient aussi l'organisation du système immunitaire de la muqueuse intestinale et contribueraient à favoriser les défenses naturelles vis-à-vis d'agents infectieux (Favre, 2004).

Nos récents travaux (Djeddar *et al.*, 2012) ont certes montré que l'utilisation de *Pediococcus acidilactici* (Bactocell, souche

MA 18/5M) permettait une amélioration des performances zootechniques mais son efficacité demeure limitée à cause du problème de la coccidiose, pathologie majeure et récurrente dans nos élevages.

Le présent travail, portant sur l'intérêt d'une supplémentation alimentaire en *Pediococcus acidilactici*, au cours d'un cycle complet d'élevage du poulet de chair dans nos conditions locales d'élevage, vise à l'évaluation de l'impact de ce probiotique aussi bien sur la croissance, la mortalité que sur les paramètres sériques du bilan lipidique.

Materiel et Methodes

Animaux et aliments :

Trois mille cinq cent vingt six (3526) poussins d'un jour de souche Hubbard F15, de sexes mélangés, provenant d'un même couvoir ont été pesés et divisés en deux (2) lots de même taille (n=1763), d'un poids homogène (48.3 g). Ils ont été mis en place, fin janvier 2010 pour une durée de 52 jours, dans un bâtiment de type traditionnel, cloisonné de façon à offrir deux aires de vie de 180 m² chacune et subissant les mêmes conditions d'ambiance.

Un aliment de type farineux était distribué *ad libitum* durant les trois phases d'élevage : démarrage (J₁-J₂₈), croissance (J₂₉-J₄₂) et finition (J₄₃-J₅₈). Les animaux du lot "expérimental" recevaient l'aliment additionné de Bactocell® (*Pediococcus acidilactici* MA18/5M, LALLEMAND SAS France] à raison de 100 ppm (10⁹ UFC.kg⁻¹), depuis le premier jour (J₁) et une eau exempte d'additifs, particulièrement, les antibiotiques. Ceux du lot "témoin" recevaient le même aliment exempt de probiotiques mais une eau additionnée d'antibiotiques, traitements les plus fréquemment administrés sur le terrain.

Les sujets des deux lots ont été vaccinés contre la maladie de Newcastle UNI L CEVA® à J₆ et rappel avec NEW L CEVA® à J₁₉ et aussi contre la maladie de Gumboro IBD L CEVA® à J₁₅.

Méthodes

Le poids vif moyen, l'indice de consommation et le taux de mortalité ont été déterminés à la fin de chaque phase d'élevage (J_{28} , J_{42} et J_{52}). Nous n'avons pas comptabilisé les cas de mortalité enregistrés lors des trois premiers jours à cause du stress dû au transport.

Un à deux millilitres de sang, prélevés de la veine alaire selon la technique décrite par Boussarie *et al.*, (2002) sont récupérés dans un tube sous vide et sans anticoagulant, centrifugés à 3000 rpm pendant 10 min puis aliquotés et stockés sous froid (-20°C). Le sérum ainsi obtenu a servi aux dosages, par les méthodes colorimétriques enzymatiques, du cholestérol total, des triglycérides, du HDL et du LDL (la concentration du LDL est calculée à base de la concentration du cholestérol total, de la concentration du HDL cholestérol et de la concentration des triglycérides qui ont été déterminés à la fin de chaque phase d'élevage).

Analyse statistique

Des tests de comparaison des poids moyens entre lots "Expérimental" et "Témoin" ont été réalisés en se basant sur le calcul du rapport critique RC. Par ailleurs, les comparaisons du cholestérol total, des triglycérides, du HDL et du LDL ont été établies entre lot expérimental et témoin pour les trois périodes de l'expérimentation grâce à des tests de Student ou de Mann-Whitney (lors d'écarts importants à la normalité ou d'hétérogénéité des variances). Les différences ont été considérées significatives à $p < 0,05$.

L'analyse a été établie sur SAS ou Statistica 10 de Statsoft Inc., USA, les moyennes sont données sous forme moyenne \pm SE.

Résultats et discussion

Paramètres zootechniques

Les paramètres zootechniques obtenus chez les sujets des deux lots sont rapportés dans le tableau 1.

Les résultats des paramètres zootechniques obtenus en fin d'élevage ont montré un écart de poids entre les sujets des lots "Témoin" et "Expérimental" (2788g vs 2701g), mais statistiquement sans

différence significative ($\alpha=5\%$). Néanmoins, le traitement statistique des données par période (démarrage, croissance et finition) a donné un RC de 0,016 ; 4,16 et 1,71, respectivement. Seul l'écart de poids enregistré entre les deux lots ("Expérimental" et "Témoin") pour la période de croissance est significatif.

Les meilleurs indices de consommation, réalisés par les sujets du lot "Expérimental" par rapport à ceux du lot "Témoin" pour les trois phases d'élevages, pourraient trouver une explication par l'effet positif des bactéries lactiques sur l'efficacité alimentaire qui a été rapporté par Jin *et al.*, (1998) et Simon *et al.*, (2001).

Le taux de mortalité élevé enregistré pour le lot "Expérimental" par rapport à celui du lot "Témoin" (8,1% vs 4,1%), est conséquent aux deux épisodes pathologiques de coccidiose survenues au cours desquels nous avons dénombré plus de la moitié de la mortalité totale (74/142 sujets). Le faible taux de mortalité du lot "Témoin" semble être la conséquence d'une couverture médicamenteuse efficace.

Les performances zootechniques réalisées par les sujets du lot "Expérimental" s'avèrent aussi probants, voir meilleurs que ceux réalisés par les sujets du lot "Témoin".

L'amélioration des performances zootechniques des poulets ainsi que l'effet positif du probiotique "*Pediococcus acidilactici*" sur l'équilibre de la flore intestinale ont été rapportés par (Vittorio *et al.*, 2005 ; Jin *et al.*, 1998).

Paramètres sériques du bilan lipidique

Les paramètres sériques du bilan lipidique, obtenus par phase d'élevage, sont rapportés dans le tableau 2.

Les résultats montrent que le cholestérol total a significativement baissé à J_{42} chez les sujets supplémentés avec *P. acidilactici* par rapport à ceux du lot témoin [$1,10 \pm 0,07$ vs $1,52 \pm 0,12$ g.L⁻¹ ($p=0,03$)]. Bien que n'étant pas significativement différents ($p > 0,05$), les niveaux des triglycérides et du LDL dans le sérum baissent à J_{42} puis remontent à J_{52} chez les sujets complémentés par rapport à ceux du lot témoin alors que celui du HDL augmente à J_{42} et J_{52} .

Tableau 1 : Paramètres zootechniques (Djezzar *et al.*, 2012)

Lots		J ₂₈	J ₄₂	J ₅₂
Expérimental (n = 1763)	Poids vif moyen/sujet (g)	1007±11,2	1771±9,8	2701±7,4
	Indice de consommation	1,35	1,51	2,41
	Taux de mortalité (%)	2,9	5,7	8,1
Témoin (n = 1763)	Poids vif moyen/sujet (g)	1006±13,8	1872±11,7	2788±7,9
	Indice de consommation	1,50	1,68	2,86
	Taux de mortalité (%)	1,9	2,2	4,1

Tableau 2: Paramètres biochimiques enregistrés dans les 2 lots durant la période d'élevage.

Para- mètres	Témoin	J ₂₈	p	Témoin	J ₄₂	p	Témoin	J ₅₂	p
		Expéri- mental			Expéri- mental			Expéri- mental	
Ch T (g.L ⁻¹)	1.71±0.16	1.57±0.18	0.61	1.52±0.12	1.10±0.07	0.03	1.17±0.05	1.25±0.17	0.68
Trig (g.L ⁻¹)	0.87±0.060	0.82±0.09	0.68	0.76±0.06	0.64±0.04	0.14	0.76±0.06	0.80±0.05	0.63
HDL (g.L ⁻¹)	0.29±0.083	0.26±0.09	0.86	0.35±0.05	0.41±0.05	0.49	0.33±0.05	0.45±0.15	0.50
LDL (g.L ⁻¹)	1.25±0.23	1.15±0.22	0.77	1.02±0.18	0.57±0.06	0.08	0.69±0.05	0.88±0.30	0.58

Ch T: cholestérol total, Trig: triglycérides.

Les résultats des paramètres biochimiques, montrent que la supplémentation en probiotique *Pediococcus acidilactici* dans l'alimentation des poulets de chair peut réduire la teneur du cholestérol sérique de façon significative. Ces résultats sont en accord avec ceux enregistrés par Chafai *et al.*, (2007) qui rapportent les mêmes résultats en utilisant la même souche probiotique (*Pediococcus acidilactici*). Ces auteurs ont noté une diminution de taux du cholestérol chez les poulets durant toutes les phases d'élevage.

Par ailleurs, Mohan *et al.*, (1996) ont montré aussi que les *Lactobacillus acidophilus* et *Lactobacillus casei* abaissent le taux du cholestérol chez le poulet par comparaison au lot témoin.

De la même façon Jin *et al.*, (1998) ont conclu que la supplémentation de *Lactobacillus* dans le régime alimentaire entraîne une réduction significative du cholestérol sanguin et Navid Hosseini (2010) détermine l'effet hypocholestérolémiant de la supplémentation alimentaire avec *Lactobacillus acidophilus* et *Lactobacillus casei*, seul ou en combinaison avec de l'eau durant les phases de démarrage

et de croissance d'élevage.

Ainsi Ignatova *et al.*, (2009) signalent que l'addition des souches probiotiques, *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus reuterii*, *Bifidobacterium bifidum*, *Bifidobacterium animalis*, *Bifidobacterium infantis* réduit significativement le taux de cholestérol sérique ($p < 0,01$).

Dans une récente étude menée par Mayahi *et al.*, (2010) ; la supplémentation en probiotiques *Enterococcus faecium* et *Bifidobacterium genera* dans l'alimentation des poulets provoque le même effet sur le cholestérol sanguin que les autres probiotiques étudiés.

Les mécanismes possibles par lesquels les probiotiques pourraient agir sur la concentration en cholestérol dans le sang sont les suivants :

1. L'ingestion de probiotiques provoque une augmentation du contenu bactérien dans l'intestin à l'origine de la fermentation des glucides non absorbés qui produisent les acides gras à chaîne courte (AGCC) dans le côlon (Wong *et al.*, 2006). Les AGCC sont partiellement absorbés dans le sang

- et peuvent modifier les concentrations circulantes de cholestérol en empêchant la synthèse hépatique de cholestérol ou en redistribuant le cholestérol du plasma au foie (St-Onge *et al.*, 2000).
2. L'activité bactérienne accrue dans le côlon résulte d'un renforcement du catabolisme des acides biliaires (Chikai *et al.*, 1987). Ces derniers ne sont pas bien absorbés par la muqueuse intestinale et sont éliminés sous forme de résidus. Le cholestérol, précurseur des acides biliaires, est utilisé *de novo* pour la synthèse des acides biliaires (St-Onge *et al.*, 2000). Par conséquent, les bactéries facilitent l'élimination du cholestérol sous forme de résidus d'acides biliaires (Chiu *et al.*, 2006).
 3. Les bactéries empêchent l'absorption du cholestérol intestinal en le fixant. Ce cholestérol assimilé est incorporé dans les membranes ou les parois cellulaires des bactéries pour augmenter leur résistance à un environnement hostile (Tahri *et al.*, 1997).

Par contre, Kanashiro *et al.*, (2001) ont montré dans leur expérience que l'addition d'un probiotique composé de mélanges de différentes souches: *Lactobacillus* Sp, *bacillus* sp, *Enterococcus faecium* M-74 et *Rhodopseudomonas* dans l'alimentation n'affecte pas le taux de cholestérol chez les poulets durant toute les phases d'élevage. Cette observation a été constatée aussi par Djouvinov *et al.*, (2005) en utilisant un mélange composé de *Streptococcus thermophilus*, *Enterococcus faecium* et *Lactobacillus*.

La baisse en teneur des triglycérides n'était pas significative. Alors que Abdulrahim (1996); Jin *et al.*, (1998) ; Kalavathy (2003) ont pour leur part démontré que l'addition des *Lactobacillus* aux régimes alimentaires des poulets (poussins) induit une diminution significative du taux de triglycérides sanguin par rapport aux témoins. Arun *et al.*, (2006) affirment que le taux de triglycérides diminue en employant une souche bactérienne *Lactobacillus sporogenes*. Ainsi Navid Hosseini (2010) et Ignatova *et al.*, (2009) signalent que les souches probiotiques, *Lactobacillus acidophilus*, *Lactobacillus casei*, ont un effet

hypotriglycéridémiant, ainsi que les souches *Lactobacillus reuterii*, *Bifidobacterium bifidum*, *Bifidobacterium animalis*, *Bifidobacterium infantis* ont le même effet sur les triglycérides.

Par ailleurs, il a été rapporté que la consommation de lait fermenté avec *Lactobacillus* diminuait largement le risque de maladie cardiovasculaire chez les habitants Massai en Afrique de l'Est malgré leur alimentation athérogène (Mann, 1974). Des diminutions de cholestérol sanguin par l'administration de yaourt ou de lait fermenté avec des bactéries lactiques ont été observées chez la poule (Tortuero et Brenes Riopérez, 1975), l'homme (Hepner *et al.*, 1979; Taranto *et al.*, 1998), le lapin (Thakur et Jha, 1981) et le rat (Grunewald, 1982). Ainsi la supplémentation de *L. reuteri* a introduit une réduction de 40% des triglycérides et une augmentation de 20% sur le ratio de HDL_{ch} / LDL_{ch} . Les auteurs ont conclu que l'administration de probiotiques contribuait à la normalisation du cholestérol sanguin.

Les poulets nourris avec des régimes supplémentés aux probiotiques ont une teneur en cholestérol sérique inférieure à celle du témoin. La capacité des probiotiques à faire baisser le cholestérol sérique a été rapporté chez les poulets et les rats (Mohan *et al.*, 1996 ; Grunewald, 1982). Cependant, ces résultats sont en contradiction avec d'autres études qui ont montré que la supplémentation en probiotiques n'a eu aucun effet bénéfique sur les performances de poulets de chair (Pelicano *et al.*, 2004).

Conclusion

Les propriétés probiotiques permettent aujourd'hui d'envisager des stratégies de prévention, de contrôle, de maîtrise sanitaire et de rentabilité, à travers l'ensemble de la chaîne alimentaire.

Le probiotique *Pediococcus acidilactici* a montré des effets positifs sur l'équilibre de la microflore intestinale en donnant le meilleur rendement en poids vif et a agi sur le taux de lipides plasmatiques.

L'un des défis que doit relever l'industrie de la volaille est lié à la teneur en cholestérol des poulets. Il est donc nécessaire d'explorer

les avantages potentiels des préparations microbiennes comme une conduite pour la réduction du cholestérol des produits carnés.

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ASSESSMENT OF FETAL WASTAGE IN CATTLE, GOAT AND SHEEP SLAUGHTERED AT TAMALE ABATTOIR, NORTHERN REGION, GHANA

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Abstract

Information on the level of fetal wastages in slaughtered cattle, sheep and goat is scanty in Ghana. This obvious missing gap in information do adversely affects the strategic planning and decision-making on animal food security. Thus, the need for an assessment of fetal wastage in slaughtered cattle and small ruminants in the Northern region of Ghana which provides about 30% of livestock population is imperative. Data on the total number of cattle, sheep and goat slaughtered, pregnant animals and condemned fetuses were obtained from the abattoir records, analysed on a daily basis while percentage of pregnant female animals and other descriptive statistics were computed. The average of 6.8% pregnant cow, 19.5% pregnant sheep and 18.3% pregnant goats were slaughtered in the study period. The ratio of fetal loss to slaughtered cattle, sheep and goat are 1:15, 1:5 and 1:6 respectively. This showed that the fetal wastage is quite alarming and effort should be geared towards instituting routine veterinary checks including pregnancy diagnosis at cattle control posts and abattoirs.

Keywords: Abattoir, fetal loss, Reproductive wastage, Ruminants

ÉVALUATION DES PERTES DE VAUX, AGNEAUX ET CHEVREAUX PAR ABATTAGE DES FEMELLES GRAVIDES À L'ABATTOIR DE TAMALE, DANS LA RÉGION NORD DU GHANA

Résumé

Les informations sur pertes de vaux, agneaux et chevreaux par abattage des femelles gravides sont rares au Ghana. Ce manque d'informations affecte négativement la planification stratégique et la prise de décisions sur la sécurité alimentaire animale. Les données sur le nombre total de bovins, ovins et caprins abattus, d'animaux gravides et de fœtus morts ont été tirées des dossiers de l'abattoir et analysées sur une base quotidienne, et le pourcentage de femelles gestantes et d'autres statistiques descriptives ont été calculés. En moyenne, 6,8% de bovins gravides, 19,5% d'ovins gravides et 18,3% de caprins gravides ont été abattus pendant la période de l'étude. Les ratios pertes de vaux/bovins, agneaux/ovins et chevreaux/caprins sont respectivement de 1:15, 1:5 et 1:6. Ceci montre que le niveau de pertes est assez alarmant, et des efforts devraient être déployés pour instituer des contrôles vétérinaires de routine, y compris le diagnostic de gestation aux postes de contrôle et abattoirs de bovins.

Mots-clés : Abattoir, Perte, vaux, agneaux chevreaux femelles, Ruminants

Introduction

Livestock are important assets in Africa, contributing to improved nutritional status and the economic growth of the populace. The livestock subsector of food and agriculture make up 6.1% of the GDP in Ghana (SRID 2011). Most recent census puts Ghana livestock population at 58.4 million; cattle- 2.5%, sheep- 6.4% and goats- 8.3%, and the Northern Region which occupied 29.5% of land area, with a human population of about 2.5 million (GSS 2008) provides about 30% of these livestock (SRID, 2011).

Fetal wastage through slaughter of pregnant animals have received little or no attention over the years in Northern Ghana and worst still, the empirical evidence of the effect of this practice on meat production is limited in most west African states (Salami *et al.*, 2010) especially Ghana. The greatest problems resulting from slaughtering pregnant animals' is the fact that enormous potential economic and protein values are wasted (Garba *et al.*, 1992). Slaughtering of pregnant animals for meat purposes had been described to be unethical and contrary to rules of slaughter for the provision of wholesome meat (Wosu 1988).

This act frustrates efforts in livestock production and it remains a drain of imminent breeding animals, thus widening the gap between animal protein availability and the increasing human population of Ghana. Various reports on the level of fetal wastages in slaughtered cattle exist in states from Nigeria, a few other countries and one from Kumasi abattoir (Atawalna *et al.*, 2013) with very little information on small ruminants. This missing gap in information across West Africa will adversely affects the strategic planning and decision-making on animal food security in the sub region. Thus, the need for an assessment of fetal wastage in slaughtered cattle and small ruminants especially in the Northern region of Ghana which provides about 30% of livestock population (SRID, 2011).

Materials and Method

Description of study area: The study was carried out in Tamale abattoir in the

Northern Region Ghana between 2012 and 2013. Tamale municipal abattoir is located in Shishegu a suburb of Tamale Metropolis. The metropolis lies between latitudes 9°.15' and 9°.30'N of the equator and between longitudes 0°.45' and 10°W of the Greenwich meridian, at an altitude of 183.3 m above sea level. Shishegu lies along the main road linking Tamale and Tolon, the district capital of Tolon-Kumbungu. The abattoir, started operation on 30th September, 2005 and has a total land area of about 14000 m². It is about 3 km from Tamale (Weobong & Enyonam 2011). An average number of 42 cattle, 12 sheep and 16 goats are usually slaughtered every other day.

Data collection and analysis: data on the total number of cattle, sheep and goat slaughtered, pregnant animals and condemned fetuses were collected from the abattoir records. Data were analysed on a daily basis while percentage of pregnant female animals and other descriptive statistics were computed.

Results and Discussion

The analysed data from animals slaughtered is presented in Tab 1 and 2. The result of this investigation shows that a percentage of pregnant cow, sheep and goat; 6.8, 19.5 and 18.3 respectively were averagely slaughtered. The ratio of fetal loss to slaughtered cattle, sheep and goat are cattle- 1:15, sheep- 1:5 and goat- 1:6

The abattoir system in the developed world focused on providing services that are geared towards meat quality while those in the developing countries, including Tamale abattoir often focus on quantity of meat without due consideration of meat quality, food safety and protection of personnel as opined by Frimpong *et al.*, (2011). The ratio of inspecting personnel to butchers is very small; there is 55% morbidity in slaughtered animals (sheep and goat). The average percentage of pregnant animal slaughtered in Tamale Abattoir includes 6.8% for cow, 19.5% for sheep and 18.3% for does.

The findings in this study showed that more than one pregnant cow, sheep or goat is slaughtered daily. We recorded a slaughtering prevalence of pregnant cows as 6.8%, sheep-

Table 1: Distribution of slaughter for the year 2012 at Tamale abattoir

Month	Total Slaughtered			Percentage of the slaughtered animals pregnant			Total condemned fetuses		
	Cattle	Sheep	Goat	Cow %	Sheep %	Doe %	Calf	Lamb	Kid
Jan	619	81	89	5.8	26.1	32.5	35	21	27
Feb	721	105	146	5.8	23.9	21.5	42	25	31
Mar	813	112	122	6.6	16.3	14.2	56	18	17
Apr	645	79	116	6.1	17.8	15.0	38	14	17
May	719	84	91	6.8	17.0	15.8	47	15	14
June	762	93	107	6.6	14.2	18.9	51	13	20
July	678	104	128	7.7	21.3	17.0	50	22	22
Aug	744	86	92	6.2	22.4	16.5	39	19	15
Sept	827	95	141	6.2	22.2	13.1	49	21	18
Oct	763	106	129	8.3	16.0	16.5	62	17	21
Nov	697	96	88	8.6	16.1	21.8	59	15	19
Dec	741	76	138	6.7	19.5	19.1	48	15	26
Total	8729	1117	1387	6.6	19.2	17.8	576	215	247

Note: Goat and sheep could have more than one foetus.

Table 2: Distribution of slaughter for the year 2013 at Tamale abattoir

Month	Total Slaughtered			Percentage pregnant %			Total condemned		
	Cattle	Sheep	Goat	Cow	Sheep	Doe	Calf	Lamb	Kid
Jan	792	87	136	5.4	16.3	19.2	41	14	26
Feb	694	113	128	7.8	19.1	17.9	52	22	23
Mar	812	98	133	8.9	19.5	22.0	68	19	29
Apr	674	76	99	6.5	21.0	17.2	46	16	17
May	781	83	115	5.1	15.8	18.1	39	13	21
June	754	67	124	7.7	19.7	14.5	57	13	18
July	639	75	124	7.4	21.0	20.7	48	15	26
Aug	861	111	130	7.6	22.6	18.8	63	25	24
Sept	739	73	145	9.2	19.3	22.2	67	14	32
Oct	646	68	112	6.4	21.2	15.5	42	16	19
Total	7392	1651	1246	7.1	19.6	18.9	523	167	256

Note: sheep and goat could have more than one foetus.

19.5% and goats- 18.3%. Sheep has the highest prevalence of fetal loss. The percentage of pregnant cows slaughtered in this study (6.5%), is slightly less than 7.88% by reported Oyekunle *et al.*, (1992), 8.1% by Fayemi *et al.*, (2008), and 7.73% reported by Ndi *et al.*, (1993), and 9.7 to 11.4 reported by Oduguwa *et al.*, (2013) from states in Nigeria. The prevalence is also lower than that obtained in Cameroon by Ndi *et al.*, (2003) and quite lower when compared with the report of Atawalna *et al.*, (2013) in

Kumasi, Ghana. Our study also showed that one fetus was wasted for every fifteen (15) cattle slaughtered, one fetus for every five (5) sheep slaughtered and one fetus for every six (6) goats slaughtered. This ratio is similar to findings of Oduguwa *et al.*, (2013) {1:7} but lower than the findings of Muhammad *et al.*, (2008) {1:33} at Gombe abattoir, Sanusi *et al.*, (2006) {1:15} at Jos abattoir, Fayemi *et al.*, (2008) {1:14}, and Nwakpu *et al.*, (2007) {1:11} from Nigeria.

Overall, the relative high fetal wastage in this study may be due to the emergence of dry season and while the highest in sheep confirms preference for its household rearing. Also, the increasing fetal wastage in small ruminants underscores economic loss of rural wealth especially as it tends to impoverishment of the rural women who are more involved in small ruminant production. Toulmin (1986) reported that during extreme dry periods, herders increased the sales of aged cows and less productive females in order to meet household needs.

This showed that the fetal wastage is quite alarming and effort should be geared towards instituting routine veterinary checks including pregnancy diagnosis at cattle control posts and abattoirs. In addition adequate infrastructural facilities that will ensure adequate pregnancy diagnosis should be provided to aid quick detection of pregnant animals during inspection. Drastic efforts should be to increase future domestic meat supply in order to reduce or halt the incidence of slaughtering pregnant ruminants as opined by Atawalna *et al.*, (2013).

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Impact:

The investigation was able to show the level of fetal wastage in ruminants slaughtered in Tamale, which reflects the level of loss of potential breeding stocks and it bring to bear the need for further study on a national scale to determine the level of fetal loss in slaughtered ruminants which will in turn call for an emergency approach to develop livestock production in Ghana.

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SURVEY OF RABIES VIRUS ANTIBODIES IN CONFINED, HUNTING AND ROAMING DOGS IN OGUN AND OYO STATES, NIGERIA

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Abstract

In view of the recent upsurge in adoption of exotic and local dogs as pets in Nigeria associated with increased contact between the dogs and their owners, and the traditional close relationship between hunters and their dogs, there is a need for studies to determine the level of protection of these dogs against rabies. In this study, a community-based approach involving administration of questionnaires to dog owners was adopted to screen for rabies virus (RABV) antibodies in apparently healthy confined, hunting and roaming dogs. Sera from 230 (80 confined, 92 hunting and 58 roaming) dogs in some urban and peri-urban communities in Ogun and Oyo states, southwestern Nigeria were screened for RABV antibodies using the indirect ELISA method. Analysis of administered questionnaires showed that of 80 confined dog owners, 37 were aware of anti-rabies vaccination (i.e. they were informed) while 17 were negligent and 26 uninformed. Of the 230 sera tested, only 13 (5.7%) from vaccinated confined dogs in Oyo state were positive i.e. had optimal RABV antibody titres (mean 0.54; 95% CI: 0.42 – 0.67) while all confined dog sera in Ogun state were negative. Eleven (12.0%) and 14 (24.1%) of the hunting and roaming dogs respectively had sub-optimal RABV antibody titres while the rest were negative. Evidently, these groups of dogs are a totally unprotected and susceptible dog population that can serve as potential reservoirs of RABV in the study area. Moreover, the presence of sub-optimal RABV antibody levels in unvaccinated hunting and roaming dogs suggests they have had field exposure to rabies or rabies-related viruses. Responsible pet ownership, vaccination of hunting and roaming dogs, integration of veterinary and public health services in implementing mass dog vaccination programmes and community-based active rabies surveillance are therefore advocated in Nigeria.

Keywords: Rabies virus, antibodies, confined, hunting and roaming dogs, southwestern Nigeria.

ETUDE DES ANTICORPS DU VIRUS DE LA RAGE CHEZ LES CHIENS ENFERMES, CHIENS DE CHASSE ET CHIENS ERRANTS DANS LES ETATS D'OGUN ET D'OYO AU NIGERIA

Résumé

Compte tenu de la récente augmentation rapide de l'adoption de chiens exotiques et locaux comme animaux de compagnie au Nigeria qui conduit à l'accroissement des contacts entre les chiens et leurs propriétaires, et l'étroite relation traditionnelle entre les chasseurs et leurs chiens, il est nécessaire de mener des études destinées à déterminer le niveau de protection de ces chiens contre la rage. Dans la présente étude, une approche communautaire impliquant l'administration de questionnaires aux propriétaires de chiens a été adoptée pour rechercher les anticorps du virus de la rage (RABV) chez des chiens enfermés, de chasse et errants apparemment en bonne santé. Des sérums de 230 chiens (80 enfermés, 92 de chasse et 58 errants) dans certaines communautés urbaines et péri-urbaines des États Ogun et Oyo, dans le sud-ouest du Nigeria, ont été examinés pour rechercher la présence des anticorps RABV en utilisant la méthode ELISA indirecte. L'analyse des questionnaires administrés a révélé que des 80 propriétaires de chiens enfermés, 37 étaient au courant de la vaccination antirabique (c'est à dire qu'ils en étaient informés) tandis que 17 étaient négligents et 26 non informés. Des 230 sérums testés, seulement 13 (5,7%) des chiens enfermés vaccinés dans l'État d'Oyo ont donné des résultats positifs, c.-à-d. qu'ils avaient un pourcentage optimal de titres d'anticorps RABV (moyenne de 0,54 ; IC 95% : 0,42 - 0,67), alors que tous les sérums de chiens enfermés dans l'État d'Ogun ont donné des résultats négatifs. Onze chiens de chasse (12,0%) et 14 chiens errants (24,1%) avaient respectivement des pourcentages sous-optimaux

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de titres d'anticorps RABV tandis que le reste était négatif. Évidemment, ces groupes de chiens constituent une population canine non protégée et sensible, susceptible de servir de réservoir potentiel de RABV dans la zone d'étude. En outre, la présence de concentrations d'anticorps RABV sous-optimales chez les chiens de chasse et les chiens errants non vaccinés permet de penser qu'ils ont été exposés directement à la rage ou aux virus apparentés à la rage sur le terrain. La responsabilisation des propriétaires d'animaux de compagnie, la vaccination de chiens de chasse et de chiens errants, l'intégration des services de santé publique vétérinaire dans l'exécution des programmes de vaccination de chiens en masse et la surveillance active de la rage au niveau communautaire sont donc préconisées au Nigeria.

Mots-clés : Virus de la rage, anticorps, chiens enfermés, de chasse et errants, sud-Ouest du Nigeria

Introduction

Rabies is a fatal encephalitis caused by the classical rabies virus (RABV) which is a neurotropic virus commonly transmitted through the bite of an infected animal (Arai *et al.*, 2003). The virus has a single-stranded, negative-sense RNA genome, and belongs to genotype 1 of the genus *Lyssavirus* within the family *Rhabdoviridae*. Other lyssaviruses circulating in Africa include Lagos bat virus (genotype 2), Mokola virus (genotype 3) and Duvenhage virus (genotype 4) (Swanepoel *et al.*, 1993). As one of the most dreaded zoonoses, rabies satisfies all the World Health Organization (WHO) criteria for diseases that are a priority for control. The disease has traditionally been associated with dogs more than any other animal and, in parts of the world where domestic animal control and vaccination programmes are limited, dogs are the most important reservoir of the disease (WHO, 2002). Although safe and effective animal and human vaccines are widely available for its prevention and control, rabies remains a neglected disease that is poorly controlled throughout much of the developing world, particularly Africa and Asia, where most deaths from human rabies occur (Rutebarika *et al.*, 2000; WHO, 2002). According to the World Health Organisation (WHO, 2013), the annual number of human rabies deaths globally is about 61,000 with the vast majority (84%) occurring in rural areas. In Africa, rabies incidence is reported to be underestimated by more than 100-fold because most deaths occur in communities rather than in hospitals (Cleaveland *et al.*, 2002; Hampson *et al.*, 2008).

Recent increases in human rabies deaths in parts of Africa, Asia and Latin America suggest that the disease is re-emerging as a serious public health issue (WHO, 2005).

With rising urbanization and an increase in the presence of traditional pets in households, licking of humans by dogs that are rabid or suspected to be rabid poses a major risk to human health (Overgaauw *et al.*, 2009; Chomel and Sun, 2011). The rise in pet ownership is reflected in the population of pet dogs in developed and developing nations. According to one estimate, the current world population of domestic dogs may be as high as 500 million, of which a substantial proportion is poorly supervised or free-roaming (Matter and Daniels, 2000). Free-roaming dogs have long been considered to be a problem in many countries and regions mainly because dogs are likely to form packs and threaten, injure or kill children or adults, apt to chase or prey on livestock and are of primary importance in rabies control in about half the countries of the world (WHO, 1998; Slater, 2001).

Rabies has been reported in owned confined and free-roaming dogs in several countries (Kitala *et al.*, 2000; Kayali *et al.*, 2003; Ehizibolo *et al.*, 2008). In Nigeria, there have been reports of clinical rabies (Adeyanju and Addo, 1977; Okoh, 1983; Taiwo *et al.*, 1998) with the burden of the disease being worsened by atypical cases which present without specific signs or symptoms of rabies (Ogunkoya *et al.*, 2003). However, most of these reports were based on records of animals that visited private and government-owned veterinary clinics and hospitals (Adeyemi and Zessin, 2000; Olore *et al.*, 2007) with paucity of information on immune status of hunting dogs. In view of the recent upsurge in adoption of exotic and local dogs as pets in Nigeria accompanied by increased contact and bonding between the dogs and their owners (Oluwayelu *et al.*, 2011), and the traditional close association between hunters and their dogs, there is a need for

studies to determine the level of protection of these dogs against rabies. In the present study, we have adopted a community-based approach to investigate the presence of RABV antibodies in apparently healthy confined, hunting and roaming dogs in Ogun and Oyo states, southwestern Nigeria. These two states are noted for influx of people and dogs from all regions in Nigeria since dog trade across the country is common and unregulated (Adeyemi and Zessin, 2000).

Materials and Methods

Study locations

The study was carried out in Abeokuta, Sagamu and Odeda in Ogun state and in Ibadan, the capital of Oyo state. The two states are located in southwestern Nigeria at 7°00'N 3°35'E with estimated population of 4.1 million people and 8°00'N 4°00'E with estimated population of 5.6 million people, respectively (National Population Commission, 2006).

Sample population and specimen collection

A total of 230 apparently healthy dogs of both sexes (77 males and 153 females) were used for this study. They comprised 80 confined dogs presented for routine clinical examination or vaccination at some major government-owned veterinary clinics in Ogun and Oyo states, 92 hunting dogs from Odeda farm (hunting) settlement in Ogun state and 58 roaming dogs from urban and peri-urban areas of Ibadan (including dogs captured by traders and sold to the public for consumption as delicacies). Questionnaires were administered to collect pertinent demographic data including age, sex and breed of dog, ownership type, purpose for keeping the dog, type of management, anti-rabies vaccination history and educational status of owner.

Using sterile syringes and needles, about 2.5ml of blood was collected from each dog through the cephalic vein into plain sample bottles. The blood was allowed to clot at room temperature for about five to six hours and sera obtained were stored in appropriately labelled Eppendorf tubes at -20°C until tested.

Detection of rabies virus antibodies using ELISA procedure

The indirect ELISA technique used was as described (Aghomo *et al.*, 1986). Optimal working dilutions obtained following chequerboard titration were antigen 1:500, sera 1:100 and rabbit anti-dog IgG horse radish peroxidase (Sigma, USA) 1:1000. The cut-off Sample to Positive (SP) ratio was calculated to be 0.25, which corresponded to twice the optical density (OD) value of the negative control serum. Results were read using the Top-Read Microplate ELISA reader (Axiom, Germany) and were considered valid when the difference between the mean OD of the positive and negative control sera was greater than 0.2 and the mean OD of the negative control serum was less than or equal to 0.25. Samples with SP ratio greater than the cut-off value of 0.25 were considered to have optimal RABV antibody levels (positive), those with SP ratio lower than the cut-off had sub-optimal antibody levels while serologically negative samples were those with zero SP ratio. Data obtained were subjected to one-way ANOVA to determine statistical significance of the findings.

Results

The demographic data of the confined dogs in Ogun and Oyo states including breed and vaccination status as well as educational status of their owners are shown in Table 1. Interviews conducted among the dog owners showed that 46.3% (37/80) of them were aware of anti-rabies vaccination and got their dogs vaccinated (i.e. they were informed), 21.3% (17/80) of them were aware of anti-rabies vaccination but failed to vaccinate their dogs (i.e. they were negligent), while the remaining 26 (32.5%) were uninformed i.e. they had no knowledge of anti-rabies vaccination (Figure 1). The prevalence of RABV antibodies in confined dogs in the two states is shown in Table 2 with none of the vaccinated dogs from Ogun state being positive for RABV antibodies while 54.2% (13/24) from Oyo state were positive. None of the 92 hunting dogs, which were all unvaccinated, was positive for RABV antibodies but 11 (12.0%) had sub-optimal

Table 1: Vaccination status of confined dogs and educational status of owners in Ogun and Oyo states

Dog breed	Number	Educated owners	Uneducated owners	Vaccinated dogs	Unvaccinated dogs
OGUN					
Alsatian	19	14	5	10	9
Rottweiler	5	5	0	1	4
Mongrel	8	4	4	2	6
Others	2	2	0	1	1
Total	34	25 (73.5%)	9 (26.5%)	14 (41.2%)	20 (58.8%)
OYO					
Alsatian	24	18	6	14	10
Rottweiler	7	4	3	4	3
Mongrel	8	4	4	2	6
Others	7	7	0	4	3
Total	46	33 (71.7%)	13 (28.3%)	24 (52.2%)	22 (47.8%)

Table 2: Prevalence of rabies virus antibodies in confined dogs in Ogun and Oyo states

	No. of dogs		No. positive (%)		No. suboptimal (%)		No. negative (%)	
	Ogun	Oyo	Ogun	Oyo	Ogun	Oyo	Ogun	Oyo
Vaccinated	13	24	0(0.0)	13(54.2)	3(23.1)	9(37.5)	10(76.9)	2(8.3)
Unvaccinated	21	22	0(0.0)	0(0.0)	3(14.3)	9(40.9)	18(85.7)	13(59.1)
Total	34	46	0(0.0)	13 (28.3)	6(17.6)	18(39.1)	28(82.4)	15(32.6)

Table 3: Overall prevalence of RABV antibodies in confined, hunting and roaming dogs in the study locations

	Number	Positive (optimal)	Sub-optimal	Negative
Confined	80	13 (16.3%)	24 (30.0%)	43 (53.8%)
Hunting	92	0 (0.0%)	11 (12.0%)	81 (88.0%)
Roaming	58	0 (0.0%)	14 (24.1%)	44 (75.9%)
Total	230	13 (5.7%)	49 (21.3%)	168 (73.0%)

antibody levels while 81 (88.0%) were negative. Similarly, none of the 58 unvaccinated roaming dogs was positive for RABV antibodies but 14 (24.1%) and 44 (75.9%) had sub-optimal and no antibody levels respectively. Overall, the prevalence of RABV antibodies in confined, hunting and roaming dogs was 16.3%, 0% and 0% respectively (Table 3). In addition, all the hunting and roaming dogs and some confined dogs were mongrels.

Discussion

Rabies is endemic in settings where large groups of unvaccinated and unconfined domestic dogs are present (Institute of

Medicine, 2011) and the most cost-effective strategy for preventing the disease in people is by eliminating it in dogs through vaccination. Indeed, transmission of rabies has been reported to effectively stop when over 70% of the canine population is immuned (Coleman and Dye, 1995). In the present study, we provide the rabies antibody profile of confined, hunting and roaming dogs in two states in southwestern Nigeria. Compared to previous studies which reported higher rabies antibody prevalence rates of 71.4% and 42.6% in confined dogs in Ibadan, Oyo state and Ilorin, Kwara state respectively, the overall low (5.7%) rabies seroprevalence rate obtained for confined, hunting and roaming dogs in this study

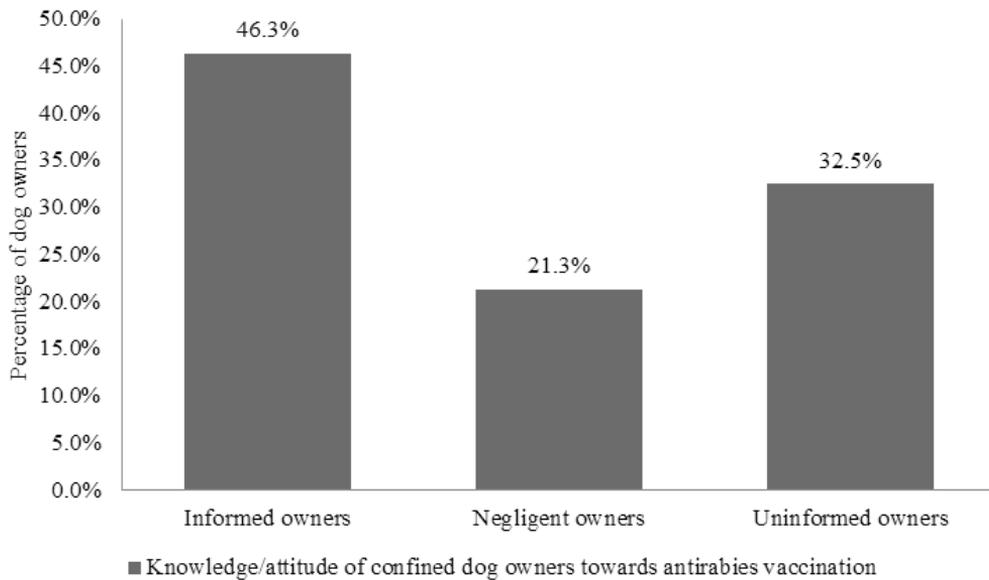


Figure 1: Attitude of confined dog owners to anti-rabies vaccination

suggests that only a small proportion of the dog population in the study locations received anti-rabies vaccination. This observation suggests a poor attitude/response of the dog owners to anti-rabies vaccination and is corroborated by the results of questionnaire analysis which showed that a substantial proportion of dog owners in this study did not vaccinate their dogs against rabies, even when they were educated. Thus, the low rabies seroprevalence rate obtained could partly be attributed to the fact that the dog owners were either negligent or totally uninformed about dog rabies vaccination (Table 1).

The non-detection of RABV antibodies in some vaccinated confined dogs suggests poor vaccination responses or waned rabies antibody levels as at the time of blood sample collection. These poor vaccination responses observed may be due to factors such as administration of incorrect or suboptimal doses of vaccine, failure of some dogs to seroconvert even with repeated doses of vaccine (Coleman and Dye, 1995), antigenic differences between the rabies virus strain used for vaccine production and the circulating wild rabies virus strains (Wiktor and Koprowski, 1980), and poor immunogenicity of the vaccine, probably as a result of fluctuation in storage temperature. In such circumstances, although dogs may have been correctly vaccinated, their immune

response and the quality of immunity may be inadequate. In tropical countries like Nigeria, vaccine virus titres may rapidly wane if proper cold storage is not maintained. Moreover, the fact that a large proportion (83.8%) of confined dogs studied was unprotected against rabies indicates that they are a highly susceptible dog population which constitutes a threat to public health.

It is noteworthy that all hunting and roaming dogs screened did not possess protective rabies antibody levels. These dogs, which are mongrels, were all unvaccinated. This finding corroborates the reports of Awoyomi *et al.*, (2007) who noted that local dogs have a high probability of being unvaccinated and Adeyemi *et al.*, (2005) who observed that there is inadequate rabies vaccination coverage in Nigeria, which increases public health risk. It is known that behavioral factors such as mating in these hunting and roaming dogs is indiscriminate and uncontrolled, and predisposes them to occasional fights over mating partners. Therefore, this unprotected group stands a great risk of rabies exposure that may occur when saliva of infected animal gains entry into the host through any skin abrasion or fresh wound (Ogunkoya *et al.*, 2003), or through virus splashing into the conjunctiva (Nottidge, 1994).

The detection of sub-optimal rabies antibody levels in unvaccinated hunting and roaming dogs suggests they may have had field exposure to rabies or rabies-related viruses. These hunting and roaming dogs could therefore serve as canid reservoirs which harbour inapparent infection and shed virus in their saliva in the absence of clinical disease (Aghomo *et al.*, 1986). Thus, they constitute a potential high risk dog population that may serve as exposure points for rabies in the community. Furthermore, there is possibility of exposure of the unprotected hunting dogs to sylvatic rabies during hunting expeditions. For instance, Radostits *et al.*, (1994) noted that it is likely that outbreaks occurring naturally amongst carnivores may originate through eating of bats infected with rabies virus. Also, Badrane and Tordo (2001) reported that all rabies virus variants of terrestrial carnivores are believed to have their origin from variants of rabies virus associated with bats. The occasional cross-species transmission (i.e. spillover) of rabies virus variants from a reservoir host to a secondary species in which the original variant subsequently becomes adapted could lead to its emergence as a novel or unique sub-variant of rabies (Smith *et al.*, 1995). Therefore, the detection of sub-optimal RABV antibody levels in unvaccinated hunting dogs in this study suggests that they might have had subclinical exposure to rabies or rabies-related viruses from wildlife. Consequently, they pose a threat to public health since they may serve as reservoirs of both urban and sylvatic rabies and ultimately, as vectors transmitting rabies to humans and other animals (McQuiston *et al.*, 2001; Messenger *et al.*, 2002).

Impact

This study revealed considerable apathy to routine vaccination of confined dogs even among educated dog owners, and showed that hunting and roaming dogs in the study areas are not protected against rabies. These categories of domestic dogs thus constitute a public health threat. There is therefore a need for promotion of responsible pet ownership in Nigeria through extensive public education. Community-based active surveillance for

rabies, inclusion of hunting and roaming dogs in future rabies vaccination campaigns, and integration of veterinary and public health services in implementing mass dog vaccination programmes are also recommended.

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SOME DISEASES ASSOCIATED WITH CARCASS CONDEMNATION IN TAMALE ABATTOIR, NORTHERN GHANA

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Abstract

There is dearth of information on occurrence of various diseases associated with carcass condemnation in countries other than Nigeria within West Africa. Hence, this study was conducted to identify the common disease conditions that results in carcass condemnation of food animals in Tamale, northern Ghana and the hub of cattle production of Ghana. Data obtained from abattoir records of diseases and lesions observed in food animals slaughtered in Tamale abattoir for a period of two years were analyzed. The disease conditions observed include Tuberculosis 14.3%, Contagious Bovine Pleuropneumonia 27.5%, Contagious Caprine Pleuropneumonia 1.6%, Lumpy Skin Disease 1.3%, Mastitis 1.1% and others. This showed that zoonotic and pneumonia associated transboundary diseases are the most important diseases in food animals in Ghana. Hence proper meat inspections should be conducted in abattoirs and good record keeping of disease conditions of food animals should be maintained for adequate surveillance of livestock diseases. Sustainable disease control planning should be enhanced in Ghana.

Keywords: Abattoir record, Animal disease, Lesion, Tamale, Ghana

QUELQUES MALADIES ASSOCIÉES À LA CONDAMNATION DE CARCASSES À L'ABATTOIR DE TAMALE DANS LE NORD DU GHANA

Resume

Il existe un vide d'informations sur l'apparition de diverses maladies associées à la condamnation de carcasses dans les pays autres que le Nigeria en Afrique de l'Ouest. Ainsi, cette étude a été menée dans le but d'identifier les maladies communes qui conduisent à la condamnation de carcasses d'animaux de boucherie à Tamale dans le nord du Ghana, qui est le centre de la production bovine ghanéenne. Les données extraites des dossiers de l'abattoir sur les maladies et les lésions observées chez les animaux abattus à Tamale sur une période de deux ans ont été analysées. Les maladies observées comprennent la tuberculose 14,3%, la pleuropneumonie contagieuse bovine 27,5%, la pleuropneumonie contagieuse caprine 1,6%, la dermatose nodulaire contagieuse 1,3%, la mastite 1,1%, et autres. Ceci montre que les zoonoses et les maladies transfrontalières associées à la pneumonie sont les plus importantes chez les animaux de boucherie au Ghana. Ainsi, des inspections appropriées des viandes devraient être effectuées dans les abattoirs ; des registres des maladies observées chez les animaux de boucherie doivent être convenablement maintenus en vue d'une surveillance adéquate des maladies du bétail, et une planification durable d'un contrôle des maladies devrait être renforcée au Ghana.

Mots-clés : Registre d'abattoir, maladie animale, lésion, Tamale, Ghana

Introduction

In most developing countries like Ghana, there is an acute shortage of protein of animal origin for human consumption. The average daily consumption per head is sub-optimal and insufficient to meet the minimum requirement for normal growth and development (FAO 1995). Though the country is endowed with abundant livestock resources having over 58.4 million livestock population (MOFA, 2010), there is still shortage of meat meant for human consumption.

Three quarter of the livestock resources are distributed in the northern part of the country and the productivity of the livestock industry remains low due to myriads of constraints. Some of the constraints range from management, nutrition, climate, veterinary services and diseases (Alton *et al.*, 2010). Abattoirs inspection is meant to ensure wholesome meat and meat products as well as providing abattoir by-products for livestock-based industries (Vanlontestijn, 1993). More importantly, abattoirs are used for the purpose of surveillance against animal and zoonotic diseases with a view to protect both man and animals from these diseases (WHO 2006, Swai and Schoonman 2012). With the promulgation of the veterinary law in Ghana in 1992, the ministry of food and Agriculture has been saddled with the vision to create an animal health system which provides quality animal health services to enhance livestock production and productivity (MOFA, 2011) hence it would be essential to have information on occurrence of various diseases and their direct economic losses from different parts of Ghana especially the northern region where 50% of the total estimated livestock population are found in order to afford for analysis of prevalence rate and planning strategy for the control of diseases of livestock.

Materials and Methods

Description of study area

The study was carried out in Tamale abattoir, northern region Ghana between 2012 and 2013. Tamale municipal abattoir is located in Shishegu a suburb of Tamale Metropolis. The

metropolis lies between latitudes 9°.15' and 9°.30'N of the equator and between longitudes 0°.45' and 10°W of the Greenwich meridian, at an altitude of 183.3 m above sea level.

Data obtained

Two (2) year retrospective data of the abattoir meat inspection records were obtained according to species of animals, organs affected or condemned. Identification of cases was based on criteria for diagnosing the diseases as used by meat inspectors and as presented in meat inspection records (FAO 1995).

The abattoir was visited for a week during which some important diseases were recorded and pictures of lesions observed during meat inspections were taken.

Data analysis

The data retrieved were analyzed and summarized in tables using descriptive statistics. The occurrence of different diseases diagnosed was calculated using percentages.

Results

The total number of food animals; cattle, sheep and goats, slaughtered in the study period are presented in table 1.

A total of 386 animals were partly or wholly condemned. Those from cattle was 95% (367) while sheep and goat made up 5% (19). The number and percentages of condemned carcasses from the varying diseases were presented in table 2.

The most common causes of carcass condemnation in sheep and goat include pneumonia (cranio-ventral), skin diseases and parasitic cysts, while causes of carcass condemnation in cattle include contagious bovine pleuropneumonia (27.5%) and Tuberculosis (14.3%).

Discussion

This study showed that varying disease conditions limit the quantity of protein available for human consumption as they lead to carcass condemnation. From the study, bacterial diseases account for over 60% of carcass

condemnation which is similar to findings of Edelstenet *et al.*, (1990) and that of Desta (2000) who reported herd prevalence of 34% for TB in Eritrea, Chizonda (1988) in a review of secondary data found estimates that ranged between 0.1% and 80% in Malawi. Bakunama (1994) reports that in Tanzania, detection of TB lesions at slaughterhouses ranged between 0 to 15%, but the majority had low levels of <1%. However, the detection was observed to be poor while the levels of disease were higher. This view was confirmed by Kazwala (1996) who tested 6,383 cattle in the Usangu plains and found that 14.3% were positive. In this study however, of the total 17,705 cattle slaughtered in the years under review 55 had TB lesions.

In this study hydatids cyts accounted for 0.3% as compared to that reported in Niger delta area of Nigeria where examination of the liver, lungs, spleen, heart and kidneys of slaughtered animals revealed hydatid cysts in 42.2% of goats, 24.4% of sheep and 31.6% of cattle (Arene, 1985). Antia and Alonge (1982) also reported that 45% of carcass condemnations in goats and 60% in sheep had hydatid cysts in Southern Nigeria, while a prevalence of 8.29% was recorded for goats in Bangladesh (Islam, 1980) and 56.29% for sheep in the Mymensingh District of Bangladesh (Islam, 1979). The possible reason for the low

prevalence recorded in this study may be connected with the indiscriminate slaughter of sheep and goats by owners and ineffective monitoring and recording of the slaughtering.

The high prevalence of CBPP and that of CAPP suggests that symptomless carriers of the disease may play active role in the epidemiology of the disease (Litamoi *et al.*, 1990) as observed in Ethiopia, where the morbidity rate was nearly 100% and the mortality rate was approximately 60% (Roeder *et al.*, 1994).

The prevalence rate observed could have been more if small ruminants were slaughtered at authorized places where appropriate veterinary inspections were carried out and lesions recorded. To avert this menace of non-compliance, the livestock owners should be encouraged to slaughter their animals in the abattoirs and Government should ensure payment of compensations for the parts or organs condemned during inspections.

Since the diseases of food animals are expected to be controlled through information obtained from abattoirs, the result of this investigation will assist field Veterinarians in their effort to control reportable diseases such as Tuberculosis and Contagious Pleuropneumonia (CBPP). Further studies are being carried to know regional based prevalence of the

Table 1: Number of food animals slaughtered for year 2012 and 2013 at Tamale abattoir

Month	Total Slaughtered 2012			Total Slaughtered 2013		
	Cattle	Sheep	Goat	Cattle	Sheep	Goat
Jan	619	81	89	792	87	136
Feb	721	105	146	694	113	128
Mar	813	112	122	812	98	133
Apr	645	79	116	674	76	99
May	719	84	91	781	83	115
June	762	93	107	754	67	124
July	678	104	128	639	75	124
Aug	744	86	92	861	111	130
Sept	827	95	141	739	73	145
Oct	763	106	129	646	68	112
Nov	697	96	88	763	72	126
Dec	741	76	138	821	83	135
Total	8729	1117	1387	8976	1006	1381

Table 2: The number and percentages of condemned carcasses due to disease conditions

Disease conditions	Number affected	Percentage
Tuberculosis	55	14.3%
CBPP	106	27.5%
CCPP	6	1.6%
Condemned fetus	87	22.5%
Lumpy skin disease	5	1.3%
Mastitis	4	1.1%
Traumatic pericarditis	4	1.1%
Multiple abscesses	18	4.7%
Black leg	1	0.3%
Hemorrhagic septicemia	6	1.6%
Liver cirrhosis	3	0.8%
Pneumonia	14	3.6%
Lung cancer	2	0.5%
Dermatophilosis	5	1.3%
Fracture	13	3.4%
Enteritis	3	0.8%
Hepatitis	2	0.5%
Hydatid cyst	3	0.8%
Mange	6	1.6%
Arthritis	3	0.8%
Jaundice	1	0.3%
Nephritis	2	0.5%
Orchitis	1	0.3%
Snake bite	2	0.5%
Hydronephrosis	1	0.3%
Splenic rupture	1	0.3%
Miscellaneous	33	8.6%
Total	386	100%



Figures: 1- Liver with numerous TB nodules, 2- Patchy pneumonia in goat, 3- Fetal wastage from pregnant cows.

forementioned zoonotic and transboundary diseases for eventual National control.

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Impact

The investigation was able to show that reportable diseases such as Tuberculosis and Contagious Pleuropneumonia (CBPP) are the major diseases often associated with carcass condemnation in Tamale Abattoir. It also highlighted the need to study the national prevalence of the aforementioned zoonotic and transboundary diseases for eventual control in Ghana.

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IN VITRO ACTIVITY OF AZADIRACHTA INDICA EXTRACTS ON SOME PATHOGENIC FUNGI

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Abstract

The objective of this study was to evaluate the activity of *Azadirachta indica* extracts on some pathogenic fungi. Hexane, methanol and aqueous extracts of *A. indica* seeds and leaves were screened for their antifungal activity against *T. mentagrophytes*, *T. soudanense*, *M. audouinii*, *M. canis*, *A. flavus*, *Curvularia lunata* and *C. albicans* using agar diffusion method. The obtained results indicated that both methanol and hexane extracts revealed antimicrobial activity against tested fungi at different concentrations used. The alcohol extracts were found most effective than the aqueous extract.

Among the pathogenic fungi tested, Dermatophyte species were found to be most sensitive to the extracts than *A. flavus*. *A. indica* extracts did not inhibit the growth of *A. flavus* but affect its sporulation while *C. albicans* was found insensitive to the extracts. Thus the microbial inhibition potential of *A. indica* leaf and seed extracts which was observed in this study opened the way for perspective use of *A. indica* to treat dermatophyte infection. Hence, the use of *A. indica* is highly recommended for treatment of infectious diseases caused by the test pathogens.

Keywords: *Azadirachta indica*, in vitro activity, pathogenic fungi, natural products

ACTIVITÉ INVITRO DES EXTRAITS D'AZADIRACHTA INDICA SUR CERTAINS CHAMPIGNONS PATHOGÈNES

Résumé

L'objectif de cette étude était d'évaluer l'activité des extraits d'*Azadirachta indica* sur certains champignons pathogènes. Des extraits d'hexane et de méthanol et des extraits aqueux de graines et feuilles d'*A. indica* ont été étudiés pour détecter leur activité antifongique contre *T. mentagrophytes*, *T. soudanense*, *M. Audouin*, *M. canis*, *A. flavus*, *Curvularia lunata* et *C. albicans* en utilisant la méthode de diffusion en gélose. Les résultats obtenus indiquent que les extraits de méthanol et d'hexane ont une activité antimicrobienne contre les champignons étudiés à différentes teneurs utilisées. Les extraits d'alcool se sont avérés plus efficaces que l'extrait aqueux.

Parmi les champignons pathogènes étudiés, les espèces dermatophytes se sont avérées plus sensibles aux extraits qu'*A. flavus*. Les extraits d'*A. indica* n'ont pas inhibé la croissance de *A. flavus* mais ont affecté sa sporulation, tandis que *C. albicans* s'est avéré insensible aux extraits. Ainsi, le potentiel d'inhibition microbienne des extraits de feuilles et graines d'*A. indica* observé dans cette étude a ouvert la voie à l'utilisation éventuelle d'*A. indica* pour traiter l'infection à dermatophytes. Par conséquent, l'utilisation d'*A. indica* est fortement recommandée pour le traitement de maladies infectieuses causées par les agents pathogènes étudiés.

Mots-clés : *Azadirachta indica*, activité in vitro, champignons pathogènes, produits naturels

Introduction

Azadirachta indica A. Juss is a tree in the Mahogany family Meliaceae. It is one of two species in the genus *Azadirachta*, and is native to India, Burma, Bangladesh, Sri Lanka, Malaysia and Pakistan, growing in tropical and semi-tropical regions. Locally in the Sudan it is known as neem tree. The Neem tree has enjoyed a revival, being rediscovered by the people of India where it is now the centre of a thriving industry, producing goods ranging from cosmetics and medicines to natural insecticides (Cole, 2002).

Tropical climate especially in the coastal regions of India creates the kind of humid hot house atmosphere that favors the growth of fungi. Traditionally, in Ayurveda, Neem seed oil, aqueous extracts of Neem leaf, Neem leaf powder, the smoke from burning dried Neem leaves, and Neem leaf pastes have been used for prevention and treatment of fungal conditions in India (Mishra *et al.*, 1995).

Athlete's foot, ringworm, and *Candida*, which causes vaginal yeast infections and thrush, are some of the more common fungi that attack humans ((Kannan *et al.*, 2006).

In Sudan, Muna *et al.*, (2003), studied the parasitic effect of seed kernel, oil and water extract of *A. indica* on *Argas persicus*. Decrease egg hatching and mortality of larvae were shown.

There are two medicinal compounds in the Neem leaf, gedunin and nimbidol, have been clinically proven to control these fungi. Jock itch, another fungal infection that attack humans, has been treated traditionally in India for thousands of years with Neem seed oil and leaf aqueous extracts. Creating medicinal smoke by burning dried Neem leaves is an ancient practice in Ayurveda for purifying the atmosphere around a seriously ill patient. A clinical study examining the efficacy of this ancient practice found that smoke from burning dried Neem leaves exerted an extreme suppression of fungal growth and germination.

Clinically, Neem is proven to be an effective analgesic; anti-inflammatory, anti arthritic; antipyretic; hypoglycaemic; antigastric ulcer; spermicidal; antifungal; antibacterial; diuretic; antimalarial; antitumour; and

immunomodulatory agent (Biswas *et al.*, 2002).

These qualities along with the above mentioned properties of Neem is what makes Neem so effective against serious skin conditions like eczema, psoriasis, acne, dermatitis, shingles, jock itch, athletes foot, ring worm, scabies, lice and *Candida* infection. Moreover, lotions and creams containing Neem oil and Neem leaf extracts are found effective externally while Neem leaf extract, capsules, and teas are useful internally because they effectively detoxify the blood from impurities which cause skin problems (Selvester, 1999)

Active ingredients of neem include: Nimbin (has an anti-inflammatory, anti-pyretic, anti-histamine, anti-fungal effect); Nimbidin (anti-bacterial, anti-ulcer, analgesic, anti-arrhythmic, anti-fungal); Ninidol (anti-tubercular, anti-protozoan, anti-pyretic); Gedunin (vasodilator, anti-malarial, anti-fungal) ; Sodium nimbinatate (diuretic, spermicide, anti-arthritic); Quercetin (anti-protozoal) ; Salannin(insect repellent) and Azadirachtin(insect repellent, anti-hormonal).

The highest concentrations of the active ingredients are found in the seed and oil, however the active ingredients are also found in lesser amounts in the bark and the leaves (Selvester, 1999).

Materials and Methods

Isolates of *A. flavus*, *C. albicans*, *T. mentagrophytes*, *T. soudanense* *M. audouinii*, *M. canis*, , *M. canis* and *Cu.rvularia lunata* were obtained from culture collection of Mycology Department at Central Veterinary Research Laboratories Centre (CVRLC), Khartoum, Sudan.

Neem seeds and leaves were collected from Elsrourab district located at North Omdurman, Khartoum state, Sudan. They were left to dry at room temperature (30oc) for 6 days and then ground into powder using mortar and pestle. The powdered plant parts were kept for further work.

Preparation of extracts:

Extraction of neem was carried out according to method described by (Harborne, 1984). Neem leaves and seeds were successively

extracted with n. hexane and methanol using soxhlet extractor apparatus for about 6h for hexane and 72h for methanol. Extraction was continued till the color of the solvent in the last siphoning returned colorless. Solvents were evaporated under reduced pressure using rotary evaporator apparatus and the yield percentages were calculated.

Water extract:

Water extract was prepared by adding 10ml of boiled distilled water to a sample of 10gm of coarsely powdered plant material in a beaker with occasional shaking for 4h at room temperature. The extract was then filtered and the precipitate was washed with small volume of distilled water and the filtrate was immediately used (Almagboul, 1992).

Susceptibility test of neem extract:

Preparation of fungal suspensions:

Tested fungi were sub cultured on Sabouraud's Dextrose agar (SDA) and incubated at 25oc for 4 days. Fungal growth was harvested and washed with 100 ml of sterile normal saline. Suspensions were stored in the refrigerator until used.

Agar diffusion test:

In vitro test:

Aspergillus, Candida and dermatophytes were maintained in pure cultures on SDA slant for evaluation of antifungal activity of A. indica leaves and seeds extracts. Concentrations of 50, 100, 200 mg /20 ml medium were prepared from neem's seeds and leaves extracts. The test samples and untreated control were inoculated with equal inoculum of the tested fungi. After 2 weeks when good growth in untreated control was obtained, the test was read (Elfadil et al., 2002).

Results

The yield percentage of neem methanolic and hexane extracts was calculated as follows:

Yield percentage = Weight of extract / weight of plant sample * 100

From 60 g of neem leaves and 100 g neem seeds obtained after extraction the yield is shown below (Table 1).

Agar diffusion test: In vitro test:

It was shown that the growth of the pathogenic fungi was inhibited with alcoholic extract of seeds and leaves of A. indica (Table 2). It was evident that the growth inhibition of fungi was more pronounced with methanol and hexane extracts of both leaves and seeds than aqueous extract.

Hexane extract of seeds:

The growth of *M. audouinii*, *M. canis*, *T. mentagrophytes* and *T. soudanense* was completely inhibited with 50 and 100 mg/ml of the hexane extract (Table 2). On the other hand, 100 mg/ml of neem seed was found to be fungistatic for *A. flavus* where white colony was obtained (fig. 3). A slide mounted in Lacto Phenol Cotton Blue (LPCB) revealed sterile hyphae without spores. Furthermore, both extracts affect the growth of *C. lunata*.

Methanol extract of seeds:

M. canis, *M. audouinii*, *T. mentagrophytes* and *T. soudanense* were found sensitive to 20% methanol extract. Also, 200 mg methanol extract was found to affect the growth and sporulation of *A. flavus*. A white colony with sterile hyphae was shown on LPCB slide mount.

Table 1: yield percentage of extracted leaves and seeds of A. indica.

Plant Solvent extract	Leaves		Seeds	
	Weight (g)	Yield %	Weight (g)	Yield %
Hexane	1.876	3.127%	22.976	22.976%
Methanol	8.6	14.333%	7.3	7.3%

Table 2: Activity of hexane and methanol extracts of *A. indica* seeds on dermatophyte species.

Extract	Concentration mg/ml	Microorganisms		Growth inhibition		T. soudanense
		<i>M.canis</i>	<i>M. audounii</i>	<i>T. mentagrophytes</i>		
Hexane	200	+	+	+	+	+
	100	"	"	"	"	"
Methanol	200	"	"	"	"	"
	100	"	"	"	"	"
Control	0.00	-	-	-	-	-

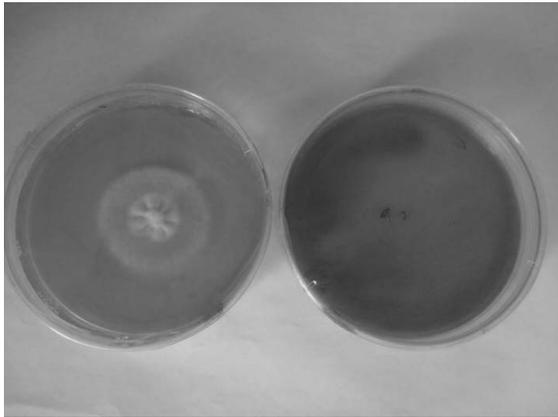


Figure 1: Control plate (left) showing growth of *M.canis* on SDA after 10 days of incubation at room temperature. Growth inhibition of *M.canis* in hexane extract of *A. indica* leaves at concentration of 100mg/ml after 10 days of incubation at room temperature ($\times 40$) (right).

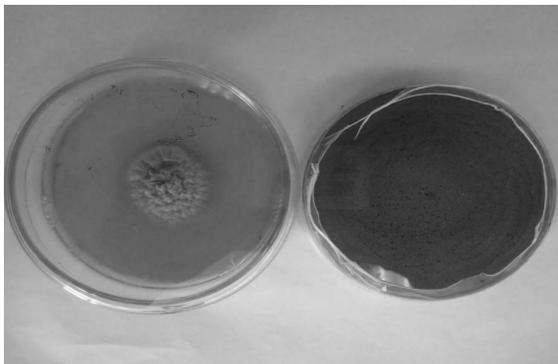


Figure 2: Control plate (left) showing growth of *T. soudanense* on SDA after 10 days of incubation at room temperature ($\times 40$). Growth inhibition of *T. soudanense* in hexane extract of *A. indica* leaves at concentration of 100mg/ml after 10 days of incubation at room temperature ($\times 40$) (right).

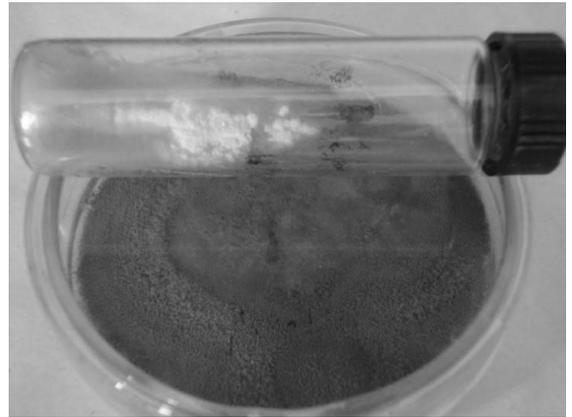


Figure 3: White colony of *A. flavus* on SDA at concentration of 100mg/ml Hexane extract of *A. indica* at concentration of 100mg/ml (vial) and green colony of the control (plate) after 7 days of incubation at room temperature ($\times 40$).

Hexane extract of leaves:

100 mg/ml of leaves hexane extract was found to affect the growth of *M. canis*, *T. mentagrophytes* while 50 mg/ml retard the growth of *A. flavus*. Sterile hyphae were seen on slide mounted in LPCB.

Methanol extract of leaves:

M.canis, *T. mentagrophytes*, *T. soudanense* and *M. audounii* were found susceptible to leaves methanol extract where complete inhibition of growth was observed (Table 3; fig 1&2).

Candida albicans was found to be resistant to all extracts at different concentrations used. Thus, *A. indica* extracts were found to have no effect on *C. albicans* when compared with the positive control (Nystatin).

Discussion

The activity of hexane and methanol extracts of *A.indica* against the growth of *Aspergillus flavus*, *Candida albicans* and some selected dermatophytes was tested in vitro. The use of n.hexane is also suggested by Liauw *et al.*, (2008).

The present results revealed inhibition of radial growth of *Aspergillus* and dermatophytes by alcoholic leaves and seeds extracts of *A. indica*, suggesting the presence of antifungal substances in the plant. This finding is in an agreement with Mondali *et al.*, (2009). This result shows that *A.indica* extract is well endowed with biologically active compounds that some dermatophytes were sensitive to it.

Inhibition of sporulation of *A.flavus* which observed in this study is similar to that reported by Joudo (2009) while resistance of *C.albicans* to *A.indica* extracts is not. This might be due to strain resistant or other factors such as age of leaves, solvent used for extraction and process of extraction.

Aqueous extracts of the plant was found to have no effect on fungi tested. This might be due to in solubility of active ingredients in water which have an antifungal action. The alcoholic extract was more potent than the water extract of *A.indica*. This finding is on line with Mondali *et al.*, (2009).

Conclusion

The effect of hexane, methanol and water extracts of *A. indica* against some pathogenic fungi was evaluated by agar diffusion method. The obtained results revealed that the alcoholic extract posses the stronger antifungal activity compared to water extract. Dermatophytes showed high sensitivity to alcoholic leaves and seeds extracts followed by *Aspergillus flavus*. Thus, this result confirms the efficacy of *A. indica* as a natural antimicrobial and suggests the possibility of employing it as a drug for treatment of infectious fungal infections.

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SUSTAINING N'DAMA CATTLE FOR THE RESOURCE-POOR FARMERS IN THE GAMBIA

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Abstract

N'Dama cattle which is endemic to West and Central African countries is a part of global livestock biodiversity that needs to be sustainably conserved in order not to lose its unique genetic characteristics which are important for meeting the challenges of insufficient animal protein production, food insecurity, rural poverty and climate change. This study assesses the genetic improvement, sustainable production, utilization and conservation of this breed of cattle in order to strengthen them through relevant technical strategies and policy measures. Review of relevant literature and policy documents, participatory group discussions were used while the information gathered was analysed through content analysis. Efforts to sustainably improve, utilize and conserve the adaptive traits of N'Dama cattle which are able to tolerate trypanosomosis and survive on low-quality feed will serve as impetus for the farmers. Particularly, strengthening of open nucleus breeding scheme, institutional support of the multipliers, financial and technical support of the extension services, and favourable policy environments are the packages that would maximize the potentials of N'Dama cattle in terms of food production and reliable income generation for the resource poor farmers in a country such as The Gambia.

Keywords: N'Dama; Production; Utilization; Conservation; Policy; Nucleus breeding.

PRÉSERVATION DE LA RACE BOVINE N'DAMA POUR LES ÉLEVEURS AUX RESSOURCES LIMITÉES EN GAMBIE

Résumé

La race bovine N'Dama, endémique dans les pays de l'Afrique de l'Ouest et de l'Afrique centrale, constitue une partie de la biodiversité mondiale du bétail qu'il faut conserver de manière durable afin de ne pas perdre ses caractéristiques génétiques uniques, qui sont importantes pour relever les défis posés par la production insuffisante de protéines animales, l'insécurité alimentaire, la pauvreté rurale et le changement climatique. La présente étude évalue l'amélioration génétique, la production, l'utilisation et la conservation durables de cette race bovine afin de la préserver par des stratégies techniques pertinentes et des mesures politiques. L'examen de la littérature et des documents stratégiques pertinents et les discussions de groupe participatives ont été utilisés, et les informations recueillies ont été soumises à une analyse de contenu. Les efforts visant à améliorer, utiliser et conserver de manière durable les caractéristiques d'adaptation des bovins N'Dama, qui sont trypanotolérants et capables de survivre avec une alimentation de faible qualité, donneront une impulsion aux éleveurs. En particulier, le renforcement du schéma de sélection de noyaux ouverts, l'appui institutionnel des multiplicateurs, le soutien financier et technique des services de vulgarisation, et les environnements politiques favorables sont des mesures qui permettraient de maximiser les potentialités des bovins N'Dama en termes de production alimentaire et de génération de revenus fiables pour les éleveurs pauvres en ressources dans un pays comme la Gambie.

Mots-clés : N'Dama ; Production ; Utilisation ; Conservation ; Politique ; Sélection de noyaux.

Introduction

The present day farm animals through the domestication processes that occurred several thousand years ago originated from more than one wild ancestor while the highest level of diversity is observed among the animals with long history of domestication and development (Diamond, 2002; Freeman *et al.*, 2006). Interestingly, archaeological findings and genetic marker analyses of indigenous cattle from the African continent indicated that the present-day humpless cattle which include the N'Dama breed was the earliest with an history dated as far back as 8000BC (Gifford-Gonzalez and Hanotte, 2011).

Following its initial domestication, N'Dama cattle through pure and crossbreeding programmes have been utilized in various production environments to meet nutritional demand, cultural, agricultural, and socio-economic needs of the resource poor farmers. Importantly, this breed because of its unique characteristics had been crossed with the New Jersey, Red Poll, Sahiwal, Fulani Zebu, Sokoto Gudali, and Simmental (Felius, 1995; Meyn, 2005) in order to maximize its disease resistant trait and also to increase its production potentials in the low-input production environments. Those mentioned crossbreeding activities were carried out within the African countries with a possible indication that the N'Dama genes may not have spread widely outside this region. Also, N'Dama cattle is a transboundary regional breed i.e. found in Africa which is one of the seven regions (Asia, Europe and the Caucasus, Latin America and the Caribbean, the Near and Middle East, Africa, North America, and the Southwest Pacific) defined for the State of the World Animal Genetic Resources (FAO, 2007a). However, there are evidences that this breed which originated from the Fouta-Djallon highland of Guinea had spread either in the live form or through gene transfer to over 20 countries thereby making it the 20th most widely spread cattle breed in the world (FAO, 2009).

As an important part of the global biodiversity, the N'Dama cattle is highly endemic to West and Central Africa. Within these sub-regions, N'Dama cattle is remarkably

productive under the moderate to high tsetse fly challenge areas (Fall *et al.*, 2003). Meanwhile, the relative high number of N'Dama cattle in the developing countries like The Gambia, Guinea, southern Senegal, Guinea-Bissau, Mali, Liberia, Sierra Leone, Congo and Gabon is related to its distinguishable disease tolerant abilities, relatively high adaptability to prevailing local climatic conditions, conservative nature of the farmers, traditional and socioeconomic importance attached to the animals. The importance of this cattle breed to smallholder farmers was also emphasized in a survey carried out in The Gambia during which 97% of the respondents signified their preference for this particular breed to any other trypanotolerant breed like West African Shorthorn cattle for draught purposes (CR-Gambia, 2003). Furthermore, its characteristic resistance to parasitic infections and its ability to survive on low quality feed when compared to the Zebu breed are among the factors for its high preference by the smallholder farmers. Despite those favourable adaptive traits resulting in food and agricultural utility values of the N'Dama cattle, the production, productivity and especially, the competitiveness of this breed still faces challenges in terms of seasonal shortages of feed and water, absence of measures for controlled mating, and gradual loss of natural habitats. In addition, the typical low-input system of animal husbandry, increased demographic pressure on available grazing land, inadequate veterinary services are among the threats confronting the resource-poor farmers in maximizing the potential of N'Dama cattle for draught power and animal protein production in the form of milk and meat.

Meanwhile, by considering the multipurpose utility functions of the N'Dama cattle, there is a need to adequately conserve this endemic animal that are domicile in the West and Central African countries in order to prevent gradual loss of its desirable genetic traits. In this regard and within the scope of this study, the technical, institutional and political supports were regarded as important incentives that would encourage the resource-poor farmers to continue the husbandry of this breed as a source of food, income, livelihood

and climate change adaptability strategies. Therefore, this study using The Gambia as a case study constructively examines past and present efforts taken towards sustainable genetic improvement, production, utilization and conservation of the N'Dama cattle. The choice of The Gambia among other countries is based on the high percentage (>90%) of N'Dama breed in the national cattle herd, its high preference and affordability by the resource-poor cattle husbandry men. Also, this study through careful assessment of the relevant Gambian national policy and regulatory frameworks offers useful suggestions for sustaining the husbandry of N'Dama cattle by resource-poor farmers and other stakeholders.

Material and Methods

Participatory group discussion

In order to extract information that is relevant to answer the research questions, the scientific articles, magazines, reports, conference proceedings, and websites were reviewed. The facts gathered from those literatures were used to describe the breeding and conservation strategies for the N'Dama cattle. This review section provided the clues and basis for the information in the semi-structured questionnaire used for the participatory group discussion about the on-going pure N'Dama breeding scheme of the International Trypanotolerance Centre (ITC), The Gambia. Also, this approach was used to assess the awareness and knowledge level of farmers concerning the relevant policies that are meant to enhance the overall management and production of N'Dama cattle. Meanwhile, the first set of questions during those discussions assessed the demographic information of the participants after which it was duly followed by series of questions focusing on the participants' general knowledge of N'Dama breed and then the Open Nucleus Breeding Scheme (ONBS) of ITC, The Gambia. The initial set of questions on local protocols and regulations served as warm-up for the participants. This activity gradually progressed into the discussions focused on the knowledge of the participants about relevant agricultural and natural resources (ANR) policies of

The Gambia which highlighted the needed steps to achieve a sustainable management of animal genetic resources (AnGR) including the N'Dama cattle.

The group discussions held at Keneba, Niamina and Nianija averagely consisted of 11 participants that were selected based on their good understanding of the subject matter. The specific choice of those sites was based on the relatively high number of N'Dama cattle breed and the existence of an integrated livestock development project called PROGEBE (Regional Project on Sustainable Management of Endemic Ruminant Livestock in West Africa). Also, gender, cultural and social factors that are embedded in the interpersonal relationships of farmers were considered in selecting the participants. However, 95% of the eventual participants were men because cattle ownership in The Gambia is rather associated more with men than women. The discussions were conducted in the local languages (Fula, Madinka, and Wollof) of the participants through translation of the original English script by the moderator while script writing was done simultaneously by the researcher and the attached livestock assistant who understood that local language of the interviewed farmers' group. During the discussions, group dynamics, motivation and curiosity was ensured in order to gather sufficient information from the respondents. The information collected through participatory group discussions were complemented by interview of specific stakeholders which included livestock project coordinators, scientists, livestock technicians, veterinary officers, literate farmers, and policy makers. This process enhanced a deeper insight into the group discussions because it enhanced further clarity of some contextual statements.

Content analysis

The documents, relevant literatures and reports were reviewed by searching for keywords including N'Dama cattle, production, policy, conservation, utilization, nucleus breeding that were in the research objectives. The paragraphs of the document that specifically contained those keywords were highlighted, contextually coded and reviewed in connection with the research objectives. Through this

process, the initially voluminous qualitative data were reduced to readable and descriptive texts. The relevant information was also summarised in a tabular format. Various responses from the participatory group discussions were written in paragraphs and collated on the basis of assigned keywords. With due attention to patterns and trends, the coded sentences and paragraphs were clustered into specific categories that could answer the research questions (Weber, 1990). The outcomes of this procedure were the descriptive sentences and paragraphs that could answer the research questions. This method was used to detect the extent and trends of activities over a particular period of time. It also enhanced identification of certain core consistencies and meanings from a volume of qualitative materials (Patton, 2002).

Results

Breeding and improvement of N'Dama cattle

In order to sustain an adaptive utilization and conservation of N'Dama cattle by farmers, efforts were previously made to genetically improve the desirable traits of this breed. Among the remarkable innovative steps taken was the design and implementation of a pure breeding programme called an Open Nucleus Breeding Scheme (ONBS). Concerning this ONBS that is operated by the International Trypanotolerance Centre (ITC),

it represents a scheme where due priorities are given to phenotypic selection, screening, breeding and genetic improvement of endemic ruminant species. The pictorial description of ONBS for N'Dama cattle is as shown in Fig. 1 with the arrows pointing in the different directions through which transfer of animals are carried out within the three tiers of this scheme. An important benefit of this ONBS is that the genetic gain of the animals in the nucleus herd is permanent, cumulative and can be disseminated to other farmers' herds.

Figure 1: An Open Nucleus Breeding Scheme (Dempfle and Jaitner, 2000)

In The Gambia, the breeding scheme for N'Dama cattle started in 1994 with the objective of improving milk and meat while maintaining the unique characteristics of the breed in terms of trypanotolerant and adaptive traits. To achieve those objectives, the nucleus animals have always been systematically selected based on the estimation of their breeding values using the Best Linear Unbiased Prediction (BLUP) method. To simulate the natural habitat of N'Dama cattle, the feeding and other management systems at the nucleus station are made similar as much as possible to the on-farm situations while sustainability and within-breed diversity in the ONBS is ensured through a mechanism that permits screening

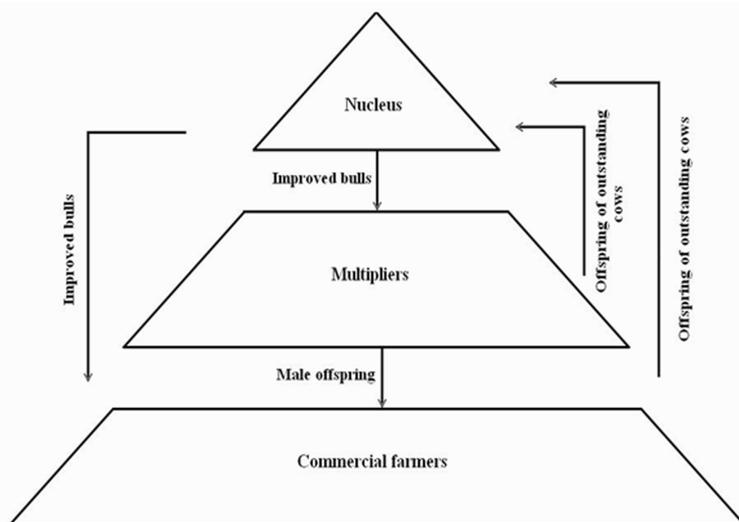


Figure 1: An open nucleus breeding scheme (Dempfle and Jaitner, 2000). The arrows indicate the directions for movement and exchange of animals within the scheme.

and introduction of outstanding offspring from farmers' herds into the nucleus. In addition, adequate involvement of multipliers and their association called Gambian Indigenous Livestock Multipliers' Association (GILMA) in the dissemination of improved bulls combined with training of livestock assistants, farmers and multipliers were planned and ensured until 2006. Unfortunately, trend of the described activities have changed due to limited funds, reduced number of nucleus animals and the collapse of multipliers' association. Also due to insufficient financial resources of ITC, and inadequate human capacity and extension services in the ONBS, the introduction of new animals from the outside herd into the nucleus has been suspended thereby resulting in a limited number of disseminated bulls and farmers' involvement in the whole breeding scheme. The following paragraphs therefore highlight some approaches that could be explored in order to solve the challenges of ensuring sustainable production and genetic improvement of the N'Dama cattle.

Sustaining the N'Dama cattle

Technical options

Given the challenges in meeting the farmers' needs for genetically improved elite bulls through ONBS, expanding the nucleus herd by introducing more bulls and increasing both the technical and financial resources were considered highly essential by the respondents in this study. However, this will require the commitments of government and other stakeholders that have interest in the sustainable utilization and conservation of endemic ruminant species. Such collaborative efforts in the form of a project (2008-2014) sponsored by the African Development Bank and the Global Environment Fund through the United Nations Development Programme which is favourably changing the landscape for endemic ruminant animal genetic improvement and management in the selected four West African countries (Gambia, Guinea, Senegal and Mali) was emphasized. This project through provision of infrastructures and capacity building of farmers and other actors is stimulating interests in the production of the three trypanotolerant ruminant species

(N'Dama cattle, West African Dwarf, and Djallonke sheep) and at the same time raising awareness about the need for their sustainable conservation.

As an alternative approach to the phenotypic selection in the ONBS, the more efficient technique called Marker Assisted Selection (MAS) and genomic selection can be explored because this will reduce generation interval and accelerate the rate of genetic gain in the ONBS. There is also a need to step up the initial approaches for dissemination the elite bulls by exploring new methods and latest advances in reproductive technology such as artificial insemination with the aim to fit this into the local context of farmers.

Concerning the dwindling institutional support for genetic improvement of N'Dama cattle breed, the International Trypanotolerance Centre which is regionally responsible for breeding of elite N'Dama bulls should be both financially and technically supported towards an enhanced fulfillment of its institutional mandates. Also, the Department of Livestock Service which is responsible for extension and training of livestock farmers should be duly supported in terms of human and technical capacity building. Empowerment of such institutions toward effective delivery of their mandates will motivate the farmers and other stakeholders thereby stimulating a multistakeholders' approach to the management of N'Dama cattle. Furthermore, organized marketing systems through private-public partnership and development of important marketing infrastructure will provide mediums for the farmer to easily link up with the potential input suppliers, livestock buyers and consumers. Training of farmers on alternative livestock feed production, processing, storage and supplementation will assist them in overcoming the challenge of seasonal food shortage. Also, adequate access to veterinary services can be facilitated through training of more livestock technicians and veterinarians towards early animal disease detection, prevention and control strategies.

Relevant policies options

Contextually, there are policy frameworks in place to guide various actors in

The Gambian livestock sector. Those policies were formulated on a periodical basis with each framework spanning from four to six years. The first of its kind is the framework called “The First Five Year Development Plan” that lasted from 1975 to 1980. Since then, other frameworks that recognised the important roles of endemic ruminant livestock in the production of animal protein and also in economic development through foreign exchange had been designed with the most recent one called the “Agriculture and Natural Resources (ANR) Policy 2009-2015”.

Specifically, analysis of the Gambian ANR Policy shows that improvement of livestock production and productivity to a level that surpasses any previous achievement is a key objective of the national government. Also included as the targets of this policy framework is the provision and enforcement of guidelines, regulations, and measures that will ensure a sustainable management of the country’s natural resource base. However, this policy that contains details of what the livestock sector should be in a future term is nonetheless silent on how sustainable conservation of animal genetic resources within the Gambian territory can be holistically achieved. To address this issue, the interviewed farmers and livestock production experts suggested that the government needs to involve livestock keepers and other actors along the livestock value chain in the review and design of an improved policy version in order to increase its recognition among these stakeholders. Also, proper and joint evaluation of N’Dama cattle in terms of their nutritional, cultural, economic, agricultural and environmental values should be prioritized in this exercise. This process will enhance effective policy dialogue among the relevant stakeholders and provide a platform for a wide recognition and implementation.

Perspectives on the impacts of existing policies

The farmers in the participatory group discussion and who are also the main custodians of N’Dama cattle breed were not explicitly aware of any national policy that concerns the N’Dama cattle production or conservation. The reason for this remark can be attributed to high illiteracy level of the

farmers which potentially impedes proper understanding and interpretation of relevant government initiatives. However, the combined approach to management of agricultural and natural resources which was the objective of The Gambian ANR policy was familiar to the interviewed farmers. Nevertheless, the farmers observed that the Gambian ANR policy have also restricted livestock herd movement as well as limited the size of available grazing area for the ruminant animals especially during the dry season. The Gambia National Agricultural Investment Plan (2011-2015) which was designed to provide opportunities for enhanced management of shared resources, pastoral infrastructure, control of transhumance, greater awareness and adherence to harmonized regulatory and health provisions is addressing some of these stakeholders’ concerns. In order to enhance awareness of ANR policy and other related protocols that entail sustainable production, utilization and conservation of N’Dama cattle, the farmers advocate for more representation and flexibility in the general policy making processes.

The review of The Gambian past and present AnGR related policies shows that N’Dama cattle is an essential asset to meet the national demand for milk, meat and draught power. As a result, increase in N’Dama cattle production has always been inherent objectives of different Gambian policy frameworks that target a sustainable AnGR management. However, some farmers and other stakeholders such as livestock technicians and scientists are not duly aware or appropriately informed of those policies. They claimed that policy documents are available to certain top government officials while accessibility to other field staff is occasionally restrained. A further elaboration of those policies with particular attentions on the aspects that relate to N’Dama cattle is shown in the table below. In Table 1, the sustainable management of N’Dama cattle for food production, income generation, employment opportunities and economic development have always been an integral part of different policy frameworks of The Gambia. Also, there are various policy options and strategies that are deployed to ensure optimal contributions of N’Dama cattle

to increased food security and better livelihood of the citizens. Those policies and frameworks were also means to operationalize some international agreements and conventions which The Gambia had ratified or consented. Table I: Summary of the relevant Gambian policies in relation to production and utilization of N'Dama cattle

Discussion

With a specific reference to the N'Dama cattle, it is particularly interesting that this breed is still a choice of many resource-poor farmers. Nonetheless, it is important to save this unique breed from threats like

indiscriminate crossbreeding and destruction of its natural habitats. The comparative performance of this breed especially in terms of lower quantity of daily milk production when compared to the Zebu and exotic breeds constitutes a factor that makes some farmers to carry out breed substitution and crossbreeding in certain production environments such as peri-urban areas. Drucker *et al.*, (2001) reported that AnGR erosion manifested in the form of animal breed replacement (substitution and crossbreeding) with a more suitable and highly productive breed is a significant factor in the global loss of farm animal biodiversity while limited access rights to grazing land and water resources was pointed out by

Table I: Summary of the relevant Gambian policies in relation to production and utilization of N'Dama cattle

Policy frameworks	Specific phrases relating to N'Dama cattle breed	Proposed strategies
1st 5-Year Development Plan (1975/76 - 1979/80)	Development of N'Dama cattle improvement programmes.	Use of artificial insemination involving crosses with exotic breeds.
2nd 5-Year Development Plan (1981/82 - 1985/86)	Facilitate the export of N'Dama cattle breeding stock to tsetse fly infested countries.	Enforcement of existing legislation and formulation of new ones if necessary.
Economic Recovery Programme - 1985	Handing over of the N'Dama nucleus in the DLS to the Livestock Marketing Board.	Commercialization of the livestock enterprise.
Programme for Sustained Development (1990 -1994)	Improvement of N'Dama production.	Advocacy for liberalized trade, private businesses and foreign investments.
Gambian Vision 2020	Increasing N'Dama cattle producers' off-take rate, access and coverage of livestock production services through private-public partnership.	Strengthening disease reporting, surveillance and monitoring systems; provision of quality feed, drinking points, and cattle tracks.
Poverty Reduction Strategy Paper I and II (2008-2011)	Improving animal health delivery and provision of AI services and feed concentrates for commercial dairy; intensification of fodder production.	Reorganize the livestock extension delivery system; mass vaccination campaigns; and training of technical advisers.
Agriculture and Natural Resources (ANR) policies (2009-2015)	Continuous exploitation of the highly productive and well adapted species of livestock.	National policies coordinated and harmonized with regional and international policies; Value chain approach to production, processing and marketing.
Gambia Agricultural Investment Plan (2011-2015)	Provision of specific incentives to e.g. N'Dama cattle farmers.	Improved rangeland infrastructure, feed availability and increased awareness.

Köhler-Rollefson (2005). Taking a look at the developing countries, it has been observed that the greatest risk of AnGR loss is found in these parts of the world for various reasons that could be summarized as socio-politically, economically, and agro-ecologically motivated (FAO, 2007a; Signorello and Pappalardo, 2003).

Concerning the ONBS for the N'Dama cattle, Fall *et al.*, (2003) also reported that proper evaluation and phenotypic selection based on those farmers' desired traits would improve the production, productivity and competitiveness of animals in a cumulative manner while Bosso (2006) stated that the sustainability of this breeding scheme in The Gambia is feasible and could serve as a good model for both low and medium livestock production systems in the West African subregion. Furthermore, nucleus herds/flocks are the basis of breeding and genetic improvement programmes in most of the developing countries including those of Africa (Kosgey *et al.*, 2002). As an alternative approach to dissemination of elite bulls to the farmers, Olaniyan and Hiemstra (2012) had previously explored the option of artificial insemination (AI) but the authors however identified financial constraint, low success rates, complex logistical requirement, and farmers' inadequate knowledge of keeping animal breeding records as some of the challenges that may be encountered in using this reproductive technology in The Gambia. In addition, careful considerations should be given to the undesirable effect like inbreeding depression that may result from an inaccurate or unguided use of AI (van Arendonk, 2011). Another realistic option to explore would be the reorganization and proper orientation of the defunct GILMA towards real ownership of the N'Dama cattle breed. This should be done in addition to provision of financial, institutional, political and operational supports by the relevant government agency and other stakeholders with interests in sustainable utilization and conservation of endemic ruminant species. Also, regular monitoring of the GILMA's activities by the relevant government institution with a view to offering them timely advice on statutory roles, financial management, independence and continuity would improve this organization's efficiency.

Interestingly, the ANR Policy and other related frameworks such as the National Trade Policy, Gambian Livestock Marketing Agency 2008 Act, and Poverty Reduction Strategy Paper I and II (2008-2011) have stimulated sustainable production and utilization of livestock species to a good extent. Meanwhile, it has been recognized that for a successful utilization and conservation of animal genetic resources (AnGR) which include the N'Dama cattle, there is a need for promotion of favourable public policies and legislations (FAO, 2007b; Hiemstra *et al.*, 2007). A comprehensive review of alternative policy instruments including the pros and cons for their implementation in the different live-stock-related domains was however proposed by Pica-Ciamarra *et al.*, (2010). To address the crosscutting issues and conflicting interests of stakeholders, transparency and clearly defined objectives are also required in policy design and implementation stages. Hiemstra *et al.*, (2007) also recommended an enabling environment for all relevant stakeholders as an option for strengthening policy and regulatory frameworks in sustainable AnGR utilization and conservation. For the policies that deal with how to sustain N'Dama cattle in The Gambia, all efforts should be made to embrace the principle of stakeholders' inclusiveness at every stage starting from the design to implementation.

There are other groups with stakes in ensuring N'Dama cattle production, utilization and conservation in The Gambia. These stakeholders according to Olaniyan and Hiemstra (2012) included N'Dama cattle owners, policymakers, scientists, veterinarians, agricultural officers and livestock technicians. However, there is a need to consider other actors along the livestock value chains in order to enhance an effective stakeholders' approach to sustainable management. Such political, financial, intellectual, human and technical resources of these stakeholders are highly essential in ensuring inclusiveness in policy making. By properly harnessing the capabilities, roles and experiences of these stakeholders in combination with the farmers' willingness to continue the husbandry of N'Dama cattle, a participatory or collaborative approach to

sustainable production and utilization of this cattle breed could be achieved. Moreover, the willingness of farmers to get informed on how to achieve improved production goals set for N'Dama cattle by the government should be capitalized upon as a stimulus for policy dialogue, design, awareness, and implementation. Effective and efficient means of communicating the objectives of relevant livestock policies through educational campaigns, orientation workshops, traditional communication media and importantly through local leaders or chiefs would be effective in this regard. Strengthening of available human capacity and institutional framework for transforming policies into projects is another relevant option. This same recommendation also put forward by FAO (2007b) and Hiemstra *et al.*, (2006) apart from making the stakeholders to be better informed will also encourage collaborative efforts and adaptive management in terms of sustainable utilization and conservation of local breeds. Involvement of different category of stakeholders at every stage of policy design, wide dissemination of the resulting policy documents, creating awareness about its objectives through sensitization, publications and wide distribution would contribute to effectiveness of such policies. Meanwhile, FAO (2007a) warned that there may be conflicting interests of some stakeholders and cumbersome logistical operations may be involved. In this regard, policy targeting the sustainable production and conservation of N'Dama cattle in The Gambia has to be in line with the country's agricultural objectives and should capitalize on the existing institutions or particular ad-hoc committees as vital instruments in overall policy making and implementation. This process in addition to being cost effective will also help in filling the knowledge and information gaps of the relevant stakeholders at the national level. Meanwhile, Olaniyan and Hiemstra (2012) reported that local protocols on forestry, wildlife and other environmental regulations of The Gambian government which were designed for natural resources management have indirectly stimulated cattle production and its conservation by the farmers and such model could be further explored.

Conclusions

Optimization of the open nucleus breeding scheme for N'Dama cattle as a means of providing genetically improved bulls for the farmers will enhance sustained genetic improvement, production and utilization of this endemic cattle breed. To ensure adequate supports for conservation of genetic traits in the N'Dama cattle, financial and technical capacity building of experts in the relevant institutions should be ensured. Also, transparency, inclusiveness and flexibility in terms of policy making and implementation as recommended by the relevant stakeholders should be considered. The N'Dama cattle breed can be adequately protected against any form of human activities that could lead to its endangerment in order to maximize its important traits that are needed for ensuring resource-poor farmers' access to animal protein, draught power and income generation.

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GENETIC DIVERSITY OF CAMEROON NATIVE GOAT POPULATIONS REVEALED BY CAPRINE MICROSATELLITES

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Abstract

A total of seventeen caprine microsatellite markers were used on 169 goats to investigate genetic diversity of eight Cameroon native goats and to assess genetic differentiation with the east African small goat. All microsatellites showed a high polymorphic content (PIC) of more than 0.5 in almost all ecotypes. Thus demonstrating that these microsatellite markers were useful for within and between ecotypes variability appraisal of domestic goat. Expected heterozygosity of all ecotypes was above 0.5, varying from 0.2 to 0.7. Only goats from Rain forest-east ecotype deviated from Hardy-Weinberg Equilibrium test ($P > 0.001$). Although geographic distribution was a good indication of differentiation there appeared a tendency of genetic exchange between various ecotypes in Cameroon native goats.

Key words : Characterization, biodiversity, goat, ecotypes, selection

DIVERSITÉ GÉNÉTIQUE DES CHÈVRES LOCALES DU CAMEROUN MISE EN ÉVIDENCE PAR DES MICROSATELLITES CAPRINS

Résumé

En vue d'évaluer la diversité génétique de 08 écotypes de chèvres locales du Cameroun et d'estimer la distance génétique avec la petite chèvre de l'Afrique orientale, 17 marqueurs microsatellites caprins ont été utilisés sur un échantillon de 169 chèvres. Tous les marqueurs microsatellites ont été hautement polymorphique, avec une valeur de l'indice de polymorphisme de plus de 0,5 dans tous les écotypes. Ces résultats révèlent que ces marqueurs microsatellites constituent un puissant outil d'investigation de la variabilité génétique caprine. L'hétérozygotie attendue moyenne était supérieure à 0,5, variant de 0,2 à 0,7 dans l'ensemble des échantillons de l'étude. Un seul écotype, celui de la région forestière de l'Est a dévié du test de l'équilibre de Hardy-Weinberg ($P > 0,001$). Cependant que la distribution géographique semble se corréliser avec une indication de différenciation génétique, il est apparu une tendance de mélange au sein des divers écotypes de chèvres locales du Cameroun.

Mots clés : Caractérisation, biodiversité, caprins, écotypes, sélection

Introduction

There is scarce documentation on animal genetic resources diversity in most of developing countries (Guimares *et al.*, 2007; FAO, 2008). Little attention has been given to small ruminant genetic resources management policies till last recently (Pica-Ciamarra, 2005). This situation has consequences in many cases of poor performance yields, random mating and loss of diversity (Kosgey *et al.*, 2006; Groeneveld *et al.*, 2010). Small ruminants sector in Cameroon provides for about 20% of present meat consumption (MINEPIA, 2010). Small ruminants are found in all agricultural systems, mainly made of smallholders in rural areas. They contribute to livelihoods, especially for the rural poor. Goats produce a variety of foods and goods, which are very useful for both urban and rural markets; more importantly, there are no religious taboos against their products (Tchouamo *et al.*, 2005). Cameroon indigenous goats diverse naming suggests some variability. They are invariably called in extant literature Cameroon Dwarf, West African Dwarf Goat, Djallonke Goat, Nigerian Goat, Pygmy Goat, Dwarf Goat, Fouta Djallon Goat and Kirdi (Epstein, 1953; Lauvergne *et al.*, 1993; Devendra and Burns, 2001). The earliest description of Cameroonian goats based on physical features was made in the late forties (Doutressoule, 1947). The primary nature of Cameroon goat populations was established in the northern part of the country using morphometric indices and coat color patterns (Lauvergne *et al.*, 1993). Physical features may be useful for conservation issues, but not enough for breeding for performance (Dekkers and Van Der, 2007). Only few sub-Saharan goats were sampled while designing microsatellites which have never been applied to Cameroon goats (Muema *et al.*, 2009). The objective of this study was to evaluate the native goat diversity of Cameroon using microsatellite markers (FAO, 2011). Our present work is therefore of scientific relevance. The aim is to evaluate the polymorphic information contents of caprine 17 microsatellites markers in Cameroon native goat populations. The findings could then result into a range of indications useful for a better goat populations' management.

Materials and Methods

Ecotypes studied and DNA extraction

A total of 169 adult and non related goats were sampled from Cameroon major ecological zones (figure 1 below) as follows Zone 1 (Sahelian, n =21; Soudanian, n = 24); Zone 2 (High guinean Savannah, n = 21); Zone 3 (Western Highlands-West, n=22; Western Highlands-North West, n = 25); Zone 4 (Coastal, n = 35) and Zone 5 (Forest-Centre, n = 9; Forest-East, n = 12). Geographical and morphometric patterns described earlier have been used to segregate ecotypes (Doutressoule, 1947; Lauvergne *et al.*, 1993). In order to evaluate the accuracy of genetic distance, 21 East African Small Goats (EASG) were considered in the final analysis. Blood samples were collected on Whatmann paper and genomic DNA isolated from dry blood spots with Invitrogen PureLink Genomic DNA kits (Invotrogen, 2010).

Microsatellite markers list, PCR conditions and genotyping

Seventeen caprine microsatellites selected from recommended list FAO (2011) were applied in the study. The microsatellites markers were: ILSTS029, SRCRSP3, ETH10, CSRD247, TCRVB6, SRCRSP7, SRCRSP9, McM527, ILSTS005, MAF209, OarFCB20, MAF065, DRBPI, INRA063, MAF70, BM6444, and SPS113. Primers were synthesized by BIONEER. The PCR conditions applied was derived from StepDown method, with a total volume reaction of 20µl in 30 cycles as shown in table I below.

Multiplex PCR was applied following indications given in previous molecular characterization (Mburu and Hanotte, 2005). Genotyping was performed under ABI 3730 (Applied Biosystems, 2010) and GeneMapper V.4.1 (Applied Biosystems, 2005).

Statistical analysis

Allele frequencies: allelic frequency was estimated based on genotypic frequencies and mean heterozygosity for each ecotype using GenAlex 6.0 according to Peakall and Smouse (2006) procedure.

Heterozygosity and gene diversity:

GenAlex 6.0 program was used to obtain estimates of observed heterozygosity (Hob) and expected heterozygosity (Het), using algorithm consistently used (Nei, 1987). Genetic distances and relationship: Neighbour Joining (NJ) dendrogram construction was done under PowerMarker V.3.25 to estimate Nei's DA genetic distances between pairs of goat ecotypes on the basis of the 17 microsatellites markers.

Polymorphic Information Content (PIC): the Polymorphic Information Content (PIC) for each locus was estimated from allele frequencies of non related individual in each ecotype. The PIC model is as follows:

$$PIC = 1 - \sum_{i=1}^n p_i^2 - \sum_{i=1}^{n-1} \times \sum_{j=i+1}^n 2p_i p_j^2$$

Where P_i is allele frequency of i th allele within the ecotype; P_j , the frequency of j th allele within the ecotype; and n , allele's number.

Molecular variance (AMOVA): AMOVA was estimated in Cameroon goat populations using GenAlex 6.0 and Arlequin 3.5.1.3 software according to classic procedure (Weir et Cockerham, 1984).

Principal Component Analysis (PCA): principal components for all ecotypes were calculated using alleles frequencies of 17 microsatellites markers. Principal Coordinates estimates were obtained with PowerMarker V.3.25 procedure.

Results

All 17 microsatellites markers in this study were polymorphic at their respective loci. A total of 148 alleles were scored in all the ecotypes as described by table 2 below.

Hardy-Weinberg Equilibrium probabilities (table 3 below) were very significant ($P < 0.001$) for 6 markers, but not in all ecotypes. Samples from forest ecological zones revealed the least positive response

HWE test.

The observed and expected heterozygosities were medium in a great majority of ecotypes as displayed in table 4 below. Allele's number was very low to low in goat from Forest regions.

Expected Het was lowest in Rain Forest-East ecotype and highest in East African Small Goat breed. The relatively low number of heterozygotes indicates excess of homozygotes which could be due to locus under selection, null alleles, inbreeding or

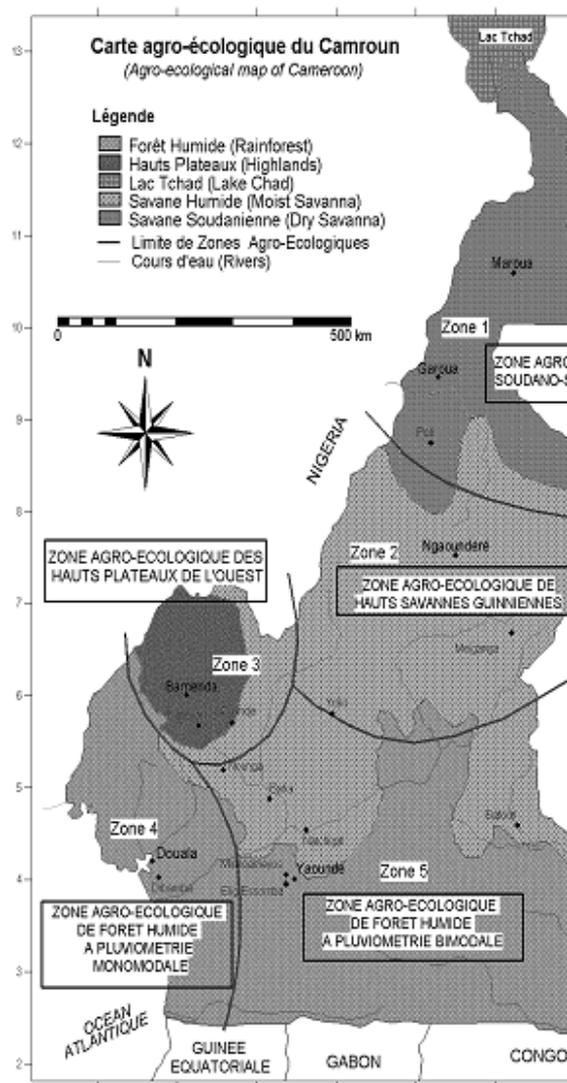


Table 1: PCR conditions for DNA amplification

Phase	1 Hold	3 Temp	5cycles	30 cycles	Extension	Hold		
Temp. (°C)	94	94	55	72	72	15		
Duration	30mn	15s	30s	1mn	15s	1mn	10mn	∞

Table 2: Allelic frequencies, allele's number and heterozygosity according to each marker

Marker	Maj.All.Frq	All.No	Hob	Het
BM6444	0.241	15	0.251	0.258
CSRD247	0.317	13	0.506	0.602
DRBPI	0.301	10	0.290	0.239
ETH10	0.357	5	0.506	0.561
ILSTS005	0.869	7	0.185	0.102
ILSTS029	0.237	12	0.771	0.793
INRA063	0.632	7	0.298	0.376
MAF065	0.383	10	0.434	0.453
MAF70	0.341	14	0.688	0.677
MAF209	0.424	5	0.492	0.529
MCM527	0.368	10	0.551	0.515
OarFCB20	0.237	10	0.536	0.555
SPS113	0.421	8	0.664	0.713
SRCPS3	0.513	6	0.485	0.557
SRCRSP7	0.286	5	0.436	0.443
SRCRSP9	0.340	12	0.526	0.597
TCRVB6	0.386	9	0.666	0.697
Mean	0.386	9.294		0.510

Maj.All.Frq: Major Alleles Frequencies; **All.No:** Alleles Number; **Hob:** Heterozygosity observed; **Het :** Heterozygosity expected

presence of population substructure (Wahlund effect).

All microsatellites markers used had rich polymorphic contents (table 5). Only ILSTS 005 had a low PIC (<0.3) while all markers demonstrated very high gene diversity in all ecotypes (>0.5). Approximately 50% of Cameroon goats ecotypes studied showed significant differentiation and diversity within themselves with a mean F_{st} estimate showing a great variability among ecotypes (F_{st} >0.15) when considering the total number of microsatellites markers.

Cameroon goats had a considerable variation (among and within) ecotypes based on analysis of molecular variance (figure 2 below). The intra and inter individual variability is very high, respectively 49 and 45%. The differentiation among ecotypes is moderately low.

The PCA (figure 3 below) and NJ tree (figure 4 below) classified the populations into four major clusters mainly along the geographical locations. The EASG stood out

distinctly different from the rest thus suggesting a different ancestry, while SHL are FM2 were the most distant among ecotypes variability. Cameroon goat ecotypes clustered roughly in 4 different subgroups depending on axis considered.

The NJ tree gave diverse relationship patterns among all ecotypes considered. Tendencies observed under PCA analysis was confirmed while clustering the various ecotypes, with genetic distances (expressed) in absolute frequencies. The genetic distances varied from 0 to 0.05, showing a variety of relationships among ecotypes.

Discussion

Genomic DNA from dry blood samples stored on FTA cards has been developed and give a high degree of satisfaction in genotyping (Mburu and Hanotte, 2005). The main issues stills their cost and procurement for less resources institutions.

Table 3: Hardy-Weinberg Equilibrium probabilities in Cameroon goat ecotypes

Markers	Ecotypes								
	FMI	FM2	SDN	CST	HGS	WH2	WHI	SHL	EASG
BM6444	0.157		0.002**	0.115	0.000***	0.002**	0.572	0.022*	0.023*
CSR247	0.112		0.565	0.120	0.003**	0.422	0.064	0.609	0.817
DRBPI	0.054	0.157	0.000***	0.000***	0.000***	0.000***	0.008**	0.000***	0.000***
ETH10	0.808		0.792	0.506	0.164	0.036*	0.006**	0.836	0.864
ILSTS005	0.029*	0.317	0.000***	0.000***		0.000***	0.000***	0.145	0.881
ILSTS029	0.498	0.637	0.973	0.333	0.990	0.290	0.627	0.647	0.971
INRA063	0.157		0.236	0.974	0.987	0.725	0.560	0.330	0.023*
MAF065	0.506		1.000	0.007**	0.018*	0.018*	0.587	0.729	0.031*
MAF70	0.320	0.572	0.831	0.087	0.588	0.855	0.302	0.297	0.802
MAF209	0.131		0.406	0.000***	0.739	0.000***	0.919	0.638	0.000***
MCM527	0.215	0.572	0.529	0.003**	0.000***	0.000***	0.002**	0.405	0.260
Oar-FCB20	0.431	0.637	0.000***	0.192	0.238	0.260	0.011*	0.465	0.655
SPS113	0.157	0.317	0.343	0.464	0.135	0.029*	0.741	0.285	0.354
SRCPS3	0.033*		0.200	0.003**	0.066	0.088	0.694	0.387	0.017*
SRCRSP7	0.386		0.217	0.058	0.044*	0.056	0.007**	0.655	0.281
SRCRSP9	0.245		0.001**	0.335	0.160	0.518	0.101	0.175	0.324
TCRVB6	0.682	0.157	0.720	0.526	0.564	0.773	0.333	0.042*	0.998

CST: Coastal; **FMI:** Forest (rain forest-centre); **FM2:** Forest (rain forest-east); **HGS:** High Guinean Savannah; **SDN:** Soudanian; **SHL:** Sahelian; **WHI:** Western Highlands-West; **WH2:** Western Highlands-North West; **EASG=** East African Small Goat. Significance : * P<0.05, ** P<0.01, *** P<0.001

Table 4: Hétérozygoties et nombre d'allèles en fonction des écotypes caprins

Ecotypes	N	Ho	Het	No. alleles
FMI	9	0.463	0.579	5.176
FM2	12	0.353	0.235	1.647
SDN	24	0.468	0.643	16.000
CST	35	0.495	0.644	15.353
HGS	21	0.405	0.642	15.941
WH2	25	0.550	0.630	17.529
WHI	22	0.504	0.633	13.000
SHL	21	0.514	0.644	11.412
EASG	21	0.615	0.706	18.529

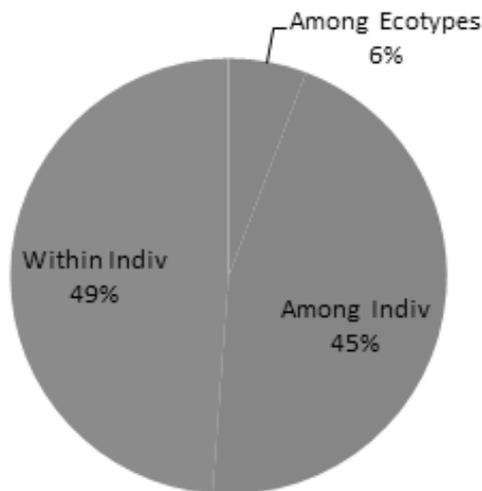
N: Size; **Ho:** Heterozygosity observed; **Het:** Heterozygosity expected; **No.alleles:** Number of Alleles

CST: Coastal; **FMI:** Forest (rain forest-centre); **FM2:** Forest (rain forest-east); **HGS:** High Guinean Savannah; **SDN:** Soudanian; **SHL:** Sahelian; **WHI:** Western Highlands-West; **WH2:** Western Highlands-North West.

Table 5: Polymorphic Information Contents, Genetic diversity and Fst estimates in Cameroon native goats

Marker	PIC	Gen.Div	Fst
BM6444	0.851	0.864	0.255
CSRD247	0.788	0.811	0.188
DRBPI	0.799	0.820	0.119
ETH10	0.608	0.677	0.171
ILSTS005	0.232	0.239	0.146
ILSTS029	0.836	0.853	0.118
INRA063	0.496	0.543	0.113
MAF065	0.758	0.782	0.238
MAF70	0.769	0.794	0.085
MAF209	0.615	0.677	0.127
MCM527	0.712	0.749	0.119
OarFCB20	0.798	0.821	0.128
SPS113	0.783	0.809	0.248
SRCPSP3	0.628	0.682	0.168
SRCRSP7	0.444	0.545	0.167
SRCRSP9	0.831	0.846	0.198
TCRVB6	0.743	0.776	0.132
Mean	0.688	0.723	0.160

Marker : Microsatellite Marker; **PIC** : Polymorphic Information Contents; **Gen.Div**: Genetic Diversity; **Fst** : F statistic estimate

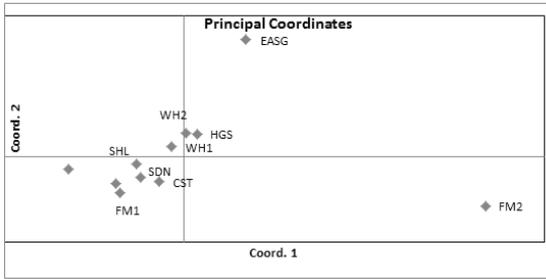
**Figure 2:** Percentages of AMOVA in Cameroon native goats

The number of alleles for Cameroon goats ranged from 5 to 18 which is lower than 4 to 23 reported in Ethiopian goats (Tesfaye, 2004), 4 to 21 in West African Dwarf goats (Missohou *et al.*, 2006), and 3 to 19 in Swiss goat

breeds (Saitbekova *et al.*, 1999), but greater than 5 to 7 obtained in Egyptian and Italian breeds (Agha *et al.*, 2008).

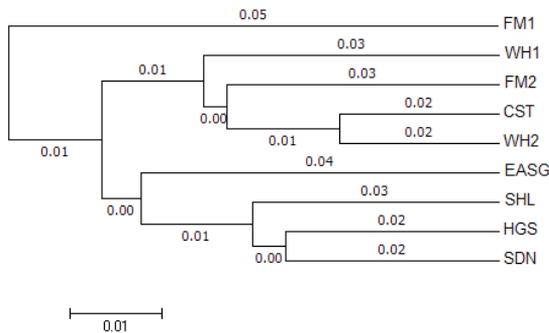
The total genetic diversity observed in this studied between populations was similar to other studies done on African goat populations namely West African goats (Missohou *et al.*, 2006). But populations outside Africa showed slightly higher values between population variation, 17% among Swiss goats (Saitbekova *et al.*, 1999), 11% among Italian goats (Ajmone-Marsan *et al.*, 2012) and 10.5% among Chinese goats (Li *et al.*, 2008). When a population is divided into isolated subpopulations, there is less heterozygosity than there would be if the population was undivided, like in Turkish context (Agaoglu and Ertugrul, 2012). Founder effects acting on different schemes generally lead to subpopulation with allele frequencies that are different from the larger population, particularly when generations are overlapping (Toro *et al.*, 2011).

The southern ecotypes showed a tendency of admixture to be confirmed under



CST: Coastal; **FMI:** Forest (rain forest-centre); **FM2:** Forest (rain forest-east); **HGS:** High Guinean Savannah; **SDN:** Soudanian; **SHL:** Sahelian; **WH1:** Western Highlands-West; **WH2:** Western Highlands- North West; **EASG=** East African Small Goat.

Figure 3: Principal coordinates of Cameroon native goat ecotypes



CST: Coastal; **FMI:** Forest (rain forest-centre); **FM2:** Forest (rain forest-east); **HGS:** High Guinean Savannah; **SDN:** Soudanian; **SHL:** Sahelian; **WH1:** Western Highlands-West; **WH2:** Western Highlands- North West; **EASG=** East African Small Goat.

Figure 4: Neighbour Joining tree with 1000 bootstraps showing relationships among Cameroon goats ecotypes

structure analysis, probably because of random mating (Agaoglu and Ertugrul, 2012) and trading systems linked to urbanization.

Single nucleotide polymorphisms (SNPs) are becoming now markers of choice in diversity studies (30) and could be applied in our future investigations.

Conclusion

Cameroon native goat ecotypes studied varied genetically. Very low number of alleles was detected at ILSTS005 locus. There was observed tendency of genetic mixing in southern ecotypes compared to northern

ecotypes, and EASG. The phylogenetic tree clustered the various groups accordingly to agro climatic patterns and husbandry systems. These results could be strengthened by application of larger number of microsatellites, mitochondrial DNA analysis and designing of markers targeting specific interesting trait like precocious and multiple birth in some of local subpopulation. The information obtained in this study will aid their rational development, utilization and conservation.

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PREVALENCE OF BRUCELLA ANTIBODIES IN SHEEP AND SPRINGBOK (*ANTIDORCAS MARSUPIALIS*) REARED TOGETHER IN THE KARAS REGION, NAMIBIA

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Abstract

An outbreak of brucellosis in sheep in 2009 on a farm in the adjacent Hardap Region of Namibia and the lack of information on the brucellosis status of springbok prompted a serological investigation of brucellosis in sheep and springbok in the Karas Region, Namibia as these two species are utilized for meat. The main aim of the study was to find out if springbok reared with sheep are infected with brucellosis. Sera collected from sexually mature naïve sheep (n=332) and from springbok (n=345) on 11 randomly selected commercial farms and from adult sheep (n=472) and springbok (n=9) on eight commercial farms identified as positive for *Brucella melitensis* between 2008 and 2010 tested negative for *Brucella* antibodies. However, 10% (95% CI: 2.78-26.0) of the tested sheep on one farm tested positive for *B. ovis* antibodies confirming the presence of this agent in the region. On the eight exposed farms, both sheep and springbok tested negative for *Brucella* antibodies, providing evidence that control measures that were implemented following the detection of the disease had been effective. It was concluded that sheep and springbok on the eleven farms had not been exposed to *Brucella melitensis* and *B. abortus* infections and that on previously positive farms the infection had been eliminated in sheep and had not spread to springbok.

Key words: springbok, sheep, *Brucella melitensis*, *Brucella abortus*, *Brucella ovis*, Namibia

PREVALENCE DES ANTICORPS DE BRUCELLA CHEZ LES MOUTONS ET LES SPRINGBOKS (*ANTIDORCAS MARSUPIALIS*) ELEVES ENSEMBLE DANS LA REGION DE KARAS EN NAMIBIE

Résumé

Un foyer de brucellose chez les ovins apparut en 2009 sur une ferme de la Région Hardap de la Namibie et le manque d'informations sur l'état de la brucellose des springboks ont entraîné une enquête sérologique de cette maladie chez les ovins et les springboks dans la région de Karas en Namibie, car ces deux espèces sont utilisées pour la viande. Le principal objectif de l'étude était de savoir si les springboks élevés avec des ovins peuvent attraper la brucellose. Les sérums prélevés sur des ovins naïfs sexuellement matures (n = 332) et sur des springboks (n = 345) dans 11 exploitations commerciales choisies de manière aléatoire et sur des ovins (n = 472) et springboks (n = 9) adultes dans huit fermes commerciales identifiées comme étant positives pour *Brucella melitensis* entre 2008 et 2010 ont donné des résultats négatifs pour les anticorps de *Brucella*. Cependant, 10% (IC 95% : 2,78 - 26,0) des ovins examinés sur une (1) ferme ont donné des résultats positifs pour les anticorps de *B. ovis*, confirmant ainsi la présence de cet agent pathogène dans la région. Sur les huit fermes exposées, les ovins et les springboks ont été déclarés négatifs pour les anticorps de *Brucella*, une preuve de l'efficacité des mesures de contrôle mises en œuvre suite à la détection de la maladie. Il a été conclu que les ovins et les springboks dans onze fermes n'avaient pas été exposés aux infections de *Brucella melitensis* et *B. abortus*, et que dans les fermes auparavant positives l'infection avait été éliminée chez les ovins et ne s'étaient pas étendues aux springboks.

Mots-clés : springbok, ovins, *Brucella melitensis*, *Brucella abortus*, *Brucella ovis*, Namibie

Introduction

The contribution of game species to Namibia's economy through trophy hunting, game meat exports, live game sales and as a tourist attraction is well documented (Weidlich, 2007). Although game species are confined to national parks, game reserves, conservancies, relatively large numbers exist on commercial farms, where they are reared together with domestic livestock. In the Karas Region of Namibia, sheep and springbok (*Antidorcas marsupialis*) are the major animal species reared on commercial farms. About 500 000 sheep are slaughtered annually through regional local and export abattoirs. During the hunting season (April-August), approximately 15 000 springbok are harvested by professional hunters for local meat consumption and for meat exports.

Brucellosis is an economically important zoonotic disease that is endemic in many African countries (Mangen *et al.*, 2002) and affects humans, domestic and wild animals. It is caused by Gram-negative bacteria of the genus *Brucella*.

In humans, *B. melitensis*, *B. abortus*, *B. canis* and *B. suis* are the species associated with the disease known as Mediterranean or undulant fever. Infection in humans is commonly acquired through occupational exposure to infected animals or through indirect contact with infected material such as aborted fetuses, the consumption of unpasteurised milk and dairy products (Godfroid *et al.*, 2010).

The main species of importance in domestic and wild ruminants are *B. melitensis*, *B. abortus* and *B. ovis*. *B. melitensis* was first isolated in Karakul sheep in Namibia in 1953 (Godfroid *et al.*, 2004) and is a common cause of brucellosis in sheep and goats (SANCO, 2001; Robinson, 2003). Sporadic cases of ovine and caprine brucellosis caused by *B. abortus* infections have been reported, but clinical disease is rare (McDermott *et al.*, 2002; FAO, 2003). Brucellosis has been reported in a wide variety of wild herbivores reared with domestic herbivores on ranches (McDermott *et al.*, 2002) and *Brucella* antibodies have been detected in a number of game species such as bushbuck (*Tragelaphus scriptus*), common

eland (*Taurotragus oryx*), impala (*Aepycros melampus*), greater kudu (*Tragelaphus strepsiceros*), common duiker (*Sylvicapra grimmia*), Thomson's gazelle (*Gazelle thomsonii*), Kafue lechwe (*Kobus lechwe kafuensis*), Oryx (*Oryx beisa*) and wildebeest (*Connochaetes taurinus*) (Paling *et al.*, 1988; Thorne, 2001; Godfroid, 2002; Muma *et al.*, 2007). Game species may acquire *Brucella* infections from livestock species (SANCO, 2001) and act as reservoir hosts of such infections (Godfroid *et al.*, 2010). In Namibia, *Brucella* antibodies have been reported in eleven unspecified antelope species (Depner, 1993).

The rearing of sheep and springbok together on commercial farms may result in the cross-infection of brucellosis between the species enabling the disease to enter the human population through the handling of livestock and game or their products (Böhm *et al.*, 2007). Cross-infection of brucellosis between springbok and sheep may complicate control measures for brucellosis, as game species may serve as reservoirs of infection (Muma *et al.*, 2007).

To meet the sanitary requirements of importing countries, the Directorate of Veterinary Services, Namibia embarked on an annual brucellosis testing of sheep farms to certify sheep flocks as free of the disease. An outbreak of brucellosis in sheep on a farm in a region adjacent to the Karas Region in 2009 (Magwedere *et al.*, 2011) and the lack of information on the brucellosis status of springbok despite the use of their carcasses for meat necessitated this study. It was decided to carry out a serological study of *Brucella* (*B. melitensis*, *B. abortus*, *B. ovis*) antibodies in sheep and springbok because brucellosis due to *B. melitensis* and *B. abortus* is zoonotic and *B. ovis* is endemic in sheep in the region. The aim of the study was to find out if *Brucella* infections occur in springbok reared with sheep.

Materials and Methods

Study Area

The Karas Region of Namibia is located at the southern end of Namibia and shares borders with South Africa to the south and

east, the Hardap Region to the north and the Atlantic Ocean to the west. The region is divided into four magisterial districts. This study was conducted in the Keetmanshoop, Karasburg and Bethanie districts which have farms that rear sheep and springbok together. The region has a hot and dry climate, with unpredictable average summer rainfalls (October to March) of between 142-152mm. In the hottest months, temperature can go above 40°C, whilst in winter temperatures frequently drop to below the freezing point at night (NMS, 2011).

Farm Selection and Sample Size Determination

A two stage sampling approach was used to select farms for the serological study. Seventeen farms approved for springbok harvesting by the Directorate of Veterinary Services in the Karas Region in 2009 for the purposes of exporting meat through the regional export abattoir were grouped into three magisterial districts. A total of 11 farms with no brucellosis vaccination history were selected by simple random sampling from each district.

The formula for determining disease prevalence as described by Martin *et al.*, (1987) was used to calculate the number of samples to be taken in sheep and springbok on each farm.

Sheep brucellosis testing results from 2008 to 2010 were used to identify eight previously positive or exposed farms for sampling in sheep and springbok to determine the effectiveness of control measures that were implemented following positive results - by checking for the absence or presence of the disease in both sheep and springbok. The sample size for this study was determined using the formula for detecting the absence or presence of disease in a population as described by Martin *et al.*, (1987), as the diseased sheep on positive farms had already been culled, but no action had been taken in regard to springbok sharing pastures with sheep.

Collection of Sera from Sheep and Springbok

The number of sera collected from sheep and springbok on the eleven farms are shown in Table 1. A total of 332 sera were collected from sheep. Each sheep sampled had 10ml of blood taken from the jugular vein using

individual sterile 20G needles and sterile plain vacuum tubes (BD Vacutainer Systems, Pre-Analytical Solutions, United Kingdom). A total of 345 blood samples were collected from the jugular vein of springbok immediately after shooting and killing using individual sterile 20G needles and sterile plain vacuum tubes (BD Vacutainer Systems, Pre-Analytical Solutions, United Kingdom). Sampling was done from springbok populations that were known to share pastures with sheep as confirmed by the farm owner/ manager. In addition, the number and species of game animals on each farms was collected at each sampling visit.

The number of sera collected from sheep and springbok on the eight previously positive farms is shown in Table 2. A total of 472 sera were collected from sheep and nine sera from springbok.

Identification, Packing and Dispatch of Sera

All collected blood tubes were identified with respect to the farm and animal species, date of sampling and given an individual identification number to prevent the mixing of samples from different animals and farms. The tubes were then placed in identified metal containers and packed in such a way as to prevent damage and leakage during transport to the regional laboratory. Ice packs were added to transport containers to preserve the samples during transportation from the

Table 1: Number of sheep and springbok sampled on the eleven commercial farms

Farm	Sheep sampled	Springbok sampled
1	30	28
2	31	31
3	30	35
4	30	32
5	31	30
6	30	31
7	30	30
8	30	29
9	30	34
10	30	32
11	30	33
Total	332	345

Table 2: Number of sheep and springbok samples collected from the exposed farms

District	Farms tested	Sheep sera sampled	Springbok sera sampled
Keetmanshoop	5	292	5
Karasburg	2	119	2
Bethanie	1	61	2
Total	8	472	9

Table 3: Number of domestic and game species on the 11 commercial farms

Farm Number	Farm size (ha)	Sheep		Goats		Cattle		Springbok	Other
		ewes	rams	does	bucks	cows	bulls		
1	9000	900	70	0	0	0	0	300	0
2	10324	2604	30	128	12	104	2	650	0
3	4812	890	15	25	2	25	1	435	0
4	6543	754	10	0	0	0	0	345	0
5	20600	2300	70	0	0	70	2	1000	60
6	7493	500	6	300	7	0	0	40	9
7	13347	643	20	0	0	76	3	300	70
8	12831	240	8	0	0	0	0	150	0
9	10365	1880	40	80	10	101	2	400	60
10	9561	1700	70	250	4	61	2	100	23
11	5237	740	48	281	32	52	22	350	0
Totals	110113	13151	387	1064	67	489	34	4070	222

Table 4: Serology results for sheep on the 11 commercial farms

Farm number	Sheep population	Number tested	Number positive for:		
			<i>B. melitensis</i> / <i>B. abortus</i> antibodies	<i>B. ovis</i> antibodies	% positive for <i>B. ovis</i>
1	970	30	0	0	0
2	2634	31	0	0	0
3	905	30	0	0	0
4	764	30	0	0	0
5	2370	31	0	0	0
6	506	30	0	0	0
7	663	30	0	0	0
8	248	30	0	0	0
9	1920	30	0	0	0
10	1770	30	0	0	0
11	788	30	0	3	10% (2.78-26.0)*
Totals	12386	332	0	3	

place of collection to the regional laboratory. At the regional laboratory, the blood was allowed to clot, serum separated and stored in a refrigerator. Cooled sera were dispatched in refrigerated containers (4°C) to the Central Veterinary Laboratory in Windhoek.

Serological Testing of Sheep and Springbok Sera

Testing for *Brucella* antibodies (*B. melitensis* or *B. abortus*) was done using the Rose Bengal Test (RBT) as a screening test and samples testing positive on the RBT were confirmed using the Complement Fixation Test (CFT) as described in the OIE Manual (2004). The antigenic suspensions used in the detection of *Brucella* antibodies (*B. abortus* and *B. melitensis*) in the RBT and CFT tests were obtained from *B. abortus* strain 99 as described in the OIE Manual (2004). Any visible agglutination was considered as test positive in the RBT test. *Brucella ovis* serology was done using the CFT as described in the OIE Manual (2004). In the CFT test, titres of 1:8 and above were recorded as positive based on the presence or absence of haemolysis.

Data Analyses

Data from the study was stored and processed using Microsoft Excel®, Microsoft Corporation 2007. To account for the clustering effect of sampling on farms, 95% confidence intervals around the mean prevalence were adjusted according to Reiczigel *et al.*, (2010).

Results

Animal Numbers and Species

The number of animal species reared on the eleven commercial farms is shown in Table 3. Sheep and springbok were the main species on the farms representing 69% and 21% of the total animal population respectively, but goats, cattle, blesbok and Oryx were recorded on some farms in variable numbers. The dorper breed was the most common breed of sheep on all the farms, but other breeds such as the karakul and merino were observed. The average size of the springbok herd per farm was 370 ± 269 (range: 40-1000). The sheep population on the farms was more than double the number of springbok on 10 farms (91%). The overall

stocking density for all animal species was between 0.03 animals/ha and 0.34 animals/ha.

Sheep and Springbok Serological Results

Serological results for sheep sera are shown in Table 4. *Brucella* antibodies (*B. melitensis* and *B. abortus*) were not detected in sheep on the 11 farms. However, on one farm, 10% (95% CI: 2.78%-26%) of the sampled sheep tested positive for *Brucella ovis* antibodies. *Brucella* antibodies (*B. melitensis*, *B. abortus* and *Brucella ovis*) were not detected in 345 springbok sera collected from a total population of 4070 springbok reared with sheep on eleven commercial farms.

Results for Previously Exposed Farms

All 472 sheep sera and 9 sera collected from the eight exposed farms (that tested positive for *Brucella* antibodies between 2008 and 2010) tested negative for *Brucella* (*B. melitensis*, *B. abortus*, *B. ovis*) antibodies.

Discussion

Results of this study confirm that sheep and springbok were the main species of animals on commercial farms and that the sheep population on the farms was more than double the size of the springbok population. Other species of livestock and game such as cattle and kudu were also present in low numbers on the farms.

Antibodies against *Brucella* were not detected in both sheep and springbok sera, although the sera were taken from sexually mature animals which are expected to harbor permanent *Brucella* infections (FAO, 2006; CFSPH, 2009). These results are consistent with the fact that the farms had no serological and clinical history of brucellosis as confirmed by the records at the regional state veterinary office. The extensive management of sheep and springbok in hot and dry conditions prevailing on the farms may have played a part in reducing the survival and transmission of *Brucella* bacteria on pasture (SANCO, 2001). As confirmed by this study, the stocking density of both species was very low and not favorable for the transmission of brucellosis. According to Godfroid (2002), the establishment and

sustainability of brucellosis in a species depends on infectious dose, host susceptibility, contact with infected animals, management and environmental factors. In this study, sheep and springbok were naïve and susceptible, but environmental and management factors were not ideal for the survival and spread of the agent even if the agent had been introduced from outside. Although *Brucella* antibody titers can fluctuate in infected adult sheep (FAO, 2010), it is unlikely that this could have been the reason for the failure to detect antibodies in sheep, because sampling was carried out over a period of nine months. The other species of livestock and game present on the farms were not considered for the serological study because they occurred in low numbers.

Sera collected from sheep and springbok on the eight previously exposed farms tested negative for *Brucella* antibodies, confirming the absence of brucellosis on these farms. Only nine sera were collected from springbok reared with sheep on exposed farms as a result of a lack of cooperation from the farmers. Although the nine sera tested negative for *Brucella* antibodies, the sample size was deemed too small to make inferences about the brucellosis status of springbok herds reared with brucellosis exposed sheep. These results confirm the effectiveness of the test-and-slaughter interventions implemented by the Directorate of Veterinary Services to control *B. melitensis* infections in sheep and goat populations. According to the protocol, when a flock tests positive for brucellosis, the farm is placed under quarantine and all sheep above six months of age are serologically tested for *Brucella* antibodies. All sheep that test positive on the CFT test are eliminated. Quarantine restrictions on the remaining sheep are removed after two consecutive negative CFT serological results at least three months apart. In addition, the protocol encourages farmers to keep a closed flock and purchase replacement stock from brucellosis-free flocks (DVS, 2009).

The results of this study are in agreement with the results of a study by Karesh *et al.*, (1997) in impala in Namibia in which no positive reactors were found. Although other wild ruminant species have tested positive for *Brucella* antibodies in Namibia (Depner,

1993; Karesh *et al.*, 1997) and in Southern Africa (McDermott *et al.*, 2002; Paling *et al.*, 1988; Thorne, 2001; Godfroid, 2002; Muma *et al.*, 2007), there is no record of springbok testing positive for *Brucella* antibodies in the literature. In the current study, the absence of reactors in springbok is a reflection of the status of the farms rather than the difficulty of transmission of the disease between springbok and sheep (Ferroglio *et al.*, 1998), as the later also tested negative. The role of springbok in the epidemiology of sheep brucellosis could not therefore be inferred due to the negative results recorded. The serological tests for *Brucella* antibodies in sheep were directly transposed onto springbok sera, but it is unlikely that these tests could have influenced the results as all sheep sera also tested negative. Positive reactors in sheep have been recorded on some commercial farms in Namibia. Therefore, the absence of reactors in sheep in this study may be attributed to lack of exposure to the agent on the tested farms.

B. ovis, a common cause of ram epididymitis, orchitis and infertility in Southern Africa (Blasco *et al.*, 2004), was detected in sheep, but not in springbok reared with serologically positive sheep. These results are consistent with the fact that *B. ovis* has not been reported in wild ruminants in Southern Africa (Blasco *et al.*, 2004). Close contact is necessary for the transmission of *B. ovis* (OIE Manual, 2004). It was unlikely that the degree of close contact necessary for the transmission of *B. ovis* between sheep and springbok could be achieved on the study farms because of the shy nature of game species. The detection of *B. ovis* antibodies in sera from three rams confirms that this agent is present in the region. The prevalence of *B. ovis* antibodies (10%) detected was high considering that the rams were not housed but extensively managed on pasture. Group penning of rams is known to facilitate the transmission of *B. ovis* infections (OIE Manual, 2004). The serological reactions observed in this study were due to active infections or past exposure to the agent because the rams were not vaccinated. The detection of *B. ovis* in sheep is of economic significance because of the associated reproductive losses in the affected flocks.

In conclusion, *B. ovis* was confirmed in rams, but no evidence of brucellosis (*B. melitensis* or *B. abortus*) was found in sheep and springbok reared together. These results indicate that the slaughter of sheep and the harvesting of springbok meat for local and export meat markets in the Karas Region may not present an occupational health risk. The measures implemented by the Directorate of Veterinary Services to control brucellosis on previously positive farms had been effective in eliminating the disease from the farms.

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PERFORMANCE AND CARCASS YIELD OF SEXED BROILER CHICKENS REARED ON TWO HOUSING TYPES

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Abstract

In spite of availability of specially formulated feeds and other aids to intensive poultry production, the provision of appropriate housing remains the most basic requirement for successful poultry production. This study thereby determined the performance, carcass yield and meat composition of 300 sexed Arbor Acre broiler chickens reared on deep-litter and deep-litter with a run housing types. The birds were brooded for 2 weeks, differentiated into male and female by feather sexing and balanced for weight. Thereafter, 150 male and female chicks each were confined separately in deep litter and deep litter with a run. Weekly live weights and physiological parameters were taken. At the end of the study, 2 birds which were similar to the average weight of each replicate were selected for carcass analysis. Serum cholesterol and calcium were also determined at the end of the experiment. The data obtained were arranged in a 2×2 factorial experimental layout in a Completely Randomized Design. Male birds had higher final weights, weight gain and cost of feed per day of 2208.33g/b, 44.41g/b/d and N21.96, respectively compared to female birds. Birds on deep litter had higher live weight and plucked weight of 2216.67 and 1985.00g, respectively. Female birds had highest percentage breast of 22.81. Serum cholesterol and calcium of birds on deep litter with run was higher. It was concluded that both male and female broiler chickens had higher carcass yield on deep litter housing type. However, for higher live weight gain female broiler chickens should be reared on deep litter while male broiler chickens could be reared conveniently on any of the housing types.

Keywords: Performance, carcass yield, female broiler, male broiler, serum cholesterol, calcium

PERFORMANCE ET RENDEMENT EN VIANDE DES POULETS DE CHAIR ÉLEVÉS SÉPARÉMENT PAR SEXES DANS DEUX TYPES DE LOGEMENT

Résumé

En dépit de la disponibilité d'aliments spécialement préparés et d'autres aides à la production intensive de volailles, la fourniture de logements adéquats reste la condition élémentaire pour une production de volailles réussie. Ainsi, la présente étude a déterminé la performance, le rendement en viande et la composition de viande de 300 poulets de chair Arbor Acre regroupés par sexe, élevés en système de litière accumulée et en système de litière accumulée avec une cour. Les oiseaux ont été couvés pendant 2 semaines, différenciés en mâles et femelles par sexage de plumes et équilibrés pour le poids. Par la suite, 150 poussins mâles et femelles ont été confinés séparément en stabulation à litière accumulée et en stabulation à litière accumulée avec une cour. Les poids vifs et les paramètres physiologiques ont été enregistrés sur une base hebdomadaire. A la fin de l'étude, 2 oiseaux dont le poids était similaire au poids moyen de chaque répétition ont été choisis pour l'analyse de carcasse. Le cholestérol sérique et le calcium ont également été déterminés à la fin de l'expérience. Les données obtenues ont été organisées dans un dispositif expérimental factoriel 2 × 2 selon un schéma complètement aléatoire. Les oiseaux mâles avaient un poids final, un gain de poids et un coût alimentaire par jour plus élevés, respectivement de 2208,33g/b ; 44,41g/b/d et N21,96, par rapport aux oiseaux femelles. Les oiseaux en stabulation avec litière accumulée

avaient un poids vif et un poids plumé plus élevés, respectivement de 2216,67 et 1985,00g. Les femelles avaient le plus haut rendement filet de 22,81. Le cholestérol sérique et le calcium des oiseaux sur litière accumulée avec cour étaient plus élevés. Il a été conclu que les poulets de chair mâles et femelles avaient un rendement en viande plus élevé lorsqu'ils étaient élevés en stabulation avec litière accumulée. Cependant, pour augmenter le gain de poids vif, les poulets de chair femelles devraient être élevés en système de litière accumulée tandis que les poulets de chair mâles peuvent être élevés facilement dans l'un ou l'autre système de logement.

Mots-clés : Performance, rendement en viande, poulet de chair femelle, poulet de chair mâle, cholestérol sérique, calcium

Introduction

Broiler birds have been a source of quality protein supplies and other nutrients needed for growth and development of man particularly in the tropical environment (Gwaza and Egahi, 2009). They are also potential means of income generation and employment either in a small scale or large scale production (Aphunu and Akpobasa, 2009). Besides, other considerations for broiler production bother on their short generation intervals, faster growth rate, lower age at maturity, high meat yield at slaughter and absence of cultural barrier or taboos to consumption (Ehebha et al., 2010).

There has evolved several different methods or systems in poultry production. Attempts are made to approach animal agriculture with the aim of creating integrated, humane, environmentally and economically sustainable housing types to produce acceptable livestock for human nutrition. The natural environment should thereby be enhanced or protected and product quality be maintained or enhanced in any housing type adopted. In the production chain, carcass yields provide useful information to guide farmers as to strain, sex, and slaughter age options that would supply consumers' demands. Consumers prefer chickens with high yield of economic parts, such as breast, drumsticks, and thighs. Female broilers have more flesh than the male of similar weights because the male have relatively bigger or heavier bones which could be attributed to hormonal differences between the two sexes. These differences may be related to growth and muscle development potential between males and females (Toldrá, 2003; Le Bihan-Duval, 2004). An adequate adjustment of the nutritional level for broiler chickens requires

the knowledge on body composition and bird growth potential for enhanced performance and profits. This on the other hands, may be related to the housing types which may mar or make the bird's ability to exhibit its natural behaviour as previously studied by Sogunle et al., (2013) for cockerel chickens. This study thereby explored the differences in the body conformation of female and male broiler chickens as influenced by housing types. The aim of this research is therefore to determine the effects of housing types (deep litter and deep litter with a run) on the performance and carcass yield of sexed (female and male) broiler chickens.

Materials and methods

Experimental Site

The experiment took place at the poultry unit of the Teaching and Research Farm Directorate, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria. This area is situated in the rainforest vegetation zone of South-western Nigeria on Latitude 7°13'49.46"N, Longitude 3°26'11.98" E and altitude of 98m above sea level. The climate is humid with a mean annual rainfall of 1003mm annual mean temperature and humidity ranges from 31.9 to 34.8°C and 79.7 to 90.1%, respectively.

Experimental animal and design

A total number of 300 Arbor Acre broiler breed of birds were purchased from a reputable hatchery and brooded for two weeks. Vaccination schedule and medications for broiler chicks were strictly adhered to. The birds were sexed after the second week of age using feather sexing and weights were balanced. The birds were subdivided into two sub-groups

of Housing type (deep-litter and deep-litter with a run) of 75 birds each thus making the study a 2x2 factorial experimental layout with four (4) treatment groups. Each sub-group was replicated three times with 25 birds each. The treatment groups were as follow; Male birds on deep-litter, Male birds on deep litter with run, Female birds on deep litter and Female on deep litter with run.

Housing

The birds were brooded on deep-litter for 2 weeks in confinement and fed ad-libitum on starter diet. Thereafter, 150 male and female chicks each were confined separately in deep-litter and deep-litter with a run (an outside run with a provision for perching). The deep litter housing system was a concrete floor with dwarf wooden wall of about (0.7m) from floor level with chicken mesh at the upper side for cross ventilation. The roof was made of corrugated zinc sheet. Birds on the deep litter were stocked at 0.08m²/bird. The same stoking density was used for the birds in deep-litter with a run but with an additional open space (outside run) of 0.16m²/bird. The birds were fed ad-libitum on both systems with the same quality and quantity of feed.

Experimental diet

The birds were fed commercial starter and finisher diets formulated to meet the nutrient requirement of the birds (NRC, 1994).

Data collection

Performance

Daily records of average body weight gain and mortality of birds at the different Housing types were taken using the following formulae:

Feed intake (g) = Total feed given (g) – feed left-over (g)

Average feed intake (g/bird/day) =

$$\left\{ \frac{\text{Feed intake}}{\text{Number of birds}} \right\} / \text{Number of days}$$

Weight gain (g) = Final weight (g) – Initial weight (g)

Average body weight gain (g/bird/day) =

$$\left\{ \frac{\text{Weight gain}}{\text{Number of birds}} \right\} / \text{Number of days}$$

Feed conversion ratio =

$$\frac{\text{Average feed intake (g)}}{\text{Average body weight gain (g)}}$$

Percentage mortality =

$$\left\{ \frac{\text{Number of birds that died}}{\text{Total number of stocked birds}} \right\} * 100$$

The birds were observed regularly to detect any occurrence of diseases such as foot pad lesion (i.e. a sore on the foot pad), breast blisters, skin burns and bruising. The daily body temperatures of the birds were taken via their rectum. The values were between 41-42 °C which fell within the range for optimum performance of birds in the tropical environment (Sogunle *et al.*, 2010, 2012).

Carcass Yield

At the end of the eighth week of the experiment, two birds which were of average live weight for each replicate were selected and sacrificed humanely by cervical dislocation. They were properly bled for two minutes and then scalded using water at temperature, 60 °C. After de-feathering, the plucked weight was recorded. The heads and shanks were removed and weighed.

After evisceration, the carcass yield was recorded. The weight of the cut-up parts (head, shanks, thighs, breast, neck, back, wings and drumsticks) and organs (heart, kidneys, lungs, livers, spleen, gizzard, proventriculus and spleen) and abdominal fat were determined.

Cost Benefit

The prevailing market costs at the time of study (N158.70 = 1\$) were used to calculate the total cost of feed consumed per bird (N/day) and the economy of feed per weight gain (N/g/bird).

The following were determined under the cost benefit:

- i. Cost of feed intake per bird per day = Cost of 1 kg feed * feed intake

$$\text{ii. Cost of feed intake per weight gain} = \frac{\text{Cost of feed intake per bird per day}}{\text{Weight gain per bird}}$$

Serum Cholesterol level determination

At the end of the experiment, 2 ml of blood samples were drawn from the wing (bronchial vein) of a bird per replicate group into a sample bottle. The cholesterol of the serum was determined using enzymatic endpoint method. The absorbance of the sample was measured against the blank reagent within 60 minutes with the reading taken at wavelength 520 nm.

Serum Calcium level determination

Also, at end of the experiment, 2 ml blood samples were drawn from the wing (bronchial vein) of a bird per replicate group into a sample bottle. Blood Calcium (Ca⁺) was determined by flame emission spectrometry as described by Cheesbrough (1991).

Statistical Design and Analysis

Data obtained were arranged in a 2×2 factorial experimental layout and then subjected to Completely Randomized Design. Significantly (P<0.05) different means were separated using Duncan's multiple range test as contained in Statistical Analysts Software (SAS, 2003) package. The model in the factorial experimental layout is shown below:

$$\gamma_{ijk} = \mu + A_i + B_j + (AB)_{ij} + \varepsilon_{ijk}$$

Where:

γ_{ijk} = individual observation

μ = general mean

A_i = effect of factor A (Sex; male and female)

B_j = effect of factor B (Housing type; deep litter and deep litter with run)

$(AB)_{ij}$ = effect of interaction AB (Sex*Housing type)

ε_{ijk} = experimental error

The data obtained for serum cholesterol and serum calcium were compared using Bar Charts.

Results

Effect of sex and housing type on growth performance and cost benefit of broiler chicken

The main effect of sex and housing type on growth performance and cost benefit of broiler chickens is presented in Table 1. Significant (P<0.05) differences were found between sexes in final weight, weight gain, feed intake and cost of feed intake with male birds recording higher values of 2208.33g/bird, 44.41, 120.48g/bird/day and N11.33/bird/day, respectively than those obtained in female birds. In the housing type, significant (P<0.05) differences were found in final weight, weight gain, mortality and cost of feed intake per weight gain. Birds managed on deep litter had higher final weight and weight gain than birds on deep litter with run while birds on deep litter with run recorded a higher (poorer) mortality and cost of feed intake per weight gain than those on deep litter.

Table 2 shows the effect of interaction between sex and housing type on growth performance and cost benefit of broiler chicken. There were significant (P<0.05) differences in final weight, weight gain, feed intake, feed conversion ratio, cost of feed intake and cost of feed intake per weight gain. Male birds on deep litter recorded the highest weight gain which was comparable to value obtained for male birds on deep litter with a run. This same trend was obtained in feed intake and cost of feed intake. The best feed conversion ratio (P<0.05) was recorded in female birds on deep litter and it was similar (P>0.05) to the values obtained in male birds on deep litter and deep litter with a run. Female birds on deep litter with a run recorded the poorest feed conversion ratio and cost of feed intake per weight gain.

Serum cholesterol and calcium of sexed broiler chickens on Deep Litter and Deep Litter with Run Housing types

Figure 1 shows the serum cholesterol of female and male broiler chickens on deep litter and deep litter with run housing systems at week 8. The serum cholesterol levels of male birds on deep litter and male birds on deep litter with run were numerically higher than those obtained for female birds on deep

litter and female birds on deep litter with run, respectively. It could be observed that in both sexes, birds on deep litter with a run housing type had increased serum cholesterol.

Figure 2 shows that the serum calcium of the male birds was higher than in female birds on both housing types. The birds (both male and female) on deep litter with run had

increased serum calcium than those on deep litter housing type.

Discussion

The higher final weight, weight gain and feed intake of the birds with respect to deep litter housing type was in agreement with the

Table 1: Main Effect of sex and housing type on growth performance and cost benefit of broiler chicken

Parameter	Sex		Housing type	
	Female	Male	DL	DLR
Initial weight (g/bird)	333.33 ±4.53	343.33 ±5.02	342.00 ±6.10	334.67 ±3.62
Final weight (g/bird)	1965.83±44.77 ^b	2208.33 ±25.62 ^a	2140.83±46.63 ^a	2033.33±72.19 ^b
Weight gain (g/bird/day)	38.87 ±1.11 ^b	44.41 ±0.58 ^a	42.83 ±1.02 ^a	40.44 ±1.74 ^b
Feed intake (g/bird/day)	108.92 ±0.52 ^b	120.48 ±0.75 ^a	114.36 ± 2.84	115.04 ±2.47
Feed conversion ratio	2.82 ±0.09	2.72 ±0.04	2.67 ±0.04	2.86 ±0.07
Mortality (%)	2.00 ±1.37	1.33 ±0.84	0.00 ±0.00 ^b	3.33 ±1.23 ^a
Cost of feed intake (N/bird/day)	10.24±0.07 ^b	11.33±0.13 ^a	10.75±0.48	10.81±0.42
Cost of feed intake per weight gain (N/g/bird)	0.26±0.02	0.26±0.01	0.25±0.01 ^b	0.27±0.01 ^a

^{a,b} Means in the same row by factor with different superscripts differ significantly (P<0.05)

DL – Deep litter,

DLR – Deep litter with run

Average cost of 1 kg feed = N94.00

Table 2: Effect of interaction between sex and housing type on growth performance and cost benefit of broiler chicken

Sex	Female		Male	
	DL	DLR	DL	DLR
Initial weight (g/bird)	331.33 ±8.17	335.67 ±5.61	352.67 ±2.40	334.00 ±5.77
Final weight (g/bird)	2048.33 ±11.67 ^b	1883.33 ±55.48 ^c	2233.33 ±46.67 ^a	2183.33 ±21.86 ^a
Weight gain (g/bird/day)	40.88 ±0.41 ^b	36.85 ±1.33 ^c	44.78 ±1.13 ^a	44.03 ±0.52 ^a
Feed intake (g/bird/day)	108.16 ±0.40 ^b	109.68 ±0.78 ^b	120.55 ±1.28 ^a	120.39 ±1.06 ^a
Feed conversion ratio	2.65 ±0.03 ^b	2.98 ±0.11 ^a	2.69 ±0.09 ^a	2.74 ±0.16 ^b
Mortality (%)	0.00 ±0.00	4.00 ±2.31	0.00 ±0.00	2.67 ±1.33
Cost of feed intake (N/bird/day)	10.16±0.05 ^b	10.30±0.12 ^b	11.33±0.24 ^a	11.32±0.16 ^a
Cost of feed intake per weigh gain (N/g/bird)	0.25±0.01 ^b	0.28±0.02 ^a	0.25±0.02 ^b	0.26±0.00 ^b

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

DL – Deep litter,

DLR – Deep litter with run

Average cost of 1 kg feed = N94.00

Table 3: Main effect of sex and housing type on carcass yield of broiler chicken

Parameter	Sex		Housing type	
	Female	Male	DL	DLR
Live weight (g)	2133.33 ± 33.89	2154.17 ± 40.57	2216.67 ± 38.59	2170.83 ± 18.93
Carcass yield				
Plucked weight (g)	1950.00 ± 41.74	1864.17 ± 67.05	1985.00 ± 59.01 ^a	1829.17 ± 44.58 ^b
Dressing percentage	83.36 ± 0.76	79.16 ± 1.86	81.43 ± 1.92	81.09 ± 1.07
Eviscerated weight (g)	1540.83 ± 31.42 ^a	1450.00 ± 28.87 ^b	1497.50 ± 32.43	1493.33 ± 33.81
Cut-up parts¹				
Head	2.16 ± 0.069 ^b	2.51 ± 0.07 ^a	2.23 ± 0.07 ^b	2.44 ± 0.09 ^a
Shanks	3.77 ± 0.074 ^b	4.92 ± 0.09 ^a	4.36 ± 0.18	4.34 ± 0.21
Thighs	10.48 ± 0.26	14.14 ± 4.04	10.30 ± 0.41	14.33 ± 4.02
Breast	22.81 ± 0.42 ^a	18.74 ± 0.40 ^b	20.92 ± 0.82	20.64 ± 0.64
Neck	4.05 ± 0.19	3.79 ± 0.17	3.67 ± 0.23	3.98 ± 0.14
Back	12.34 ± 0.41	13.56 ± 0.43	12.88 ± 0.53	13.02 ± 0.36
Wings	9.71 ± 0.25	9.98 ± 0.38	10.03 ± 0.38	9.66 ± 0.26
Drumsticks	10.31 ± 0.16	10.78 ± 0.22	10.52 ± 0.21	10.58 ± 0.20
Organs²				
Heart	0.38 ± 0.02	0.41 ± 0.02	0.39 ± 0.02	0.40 ± 0.02
Kidneys	0.41 ± 0.05	0.37 ± 0.03	0.37 ± 0.04	0.41 ± 0.03
Lungs	0.46 ± 0.04	0.51 ± 0.03	0.45 ± 0.04	0.53 ± 0.03
Liver	1.49 ± 0.08	1.49 ± 0.15	1.48 ± 0.12	1.50 ± 0.12
Gizzard	1.72 ± 0.06	1.71 ± 0.06	1.65 ± 0.05	1.78 ± 0.06
Proventriculus	0.34 ± 0.02	0.29 ± 0.01	0.31 ± 0.02	0.33 ± 0.02
Spleen	0.09 ± 0.01	0.09 ± 0.01	0.09 ± 0.01	0.09 ± 0.01
Abdominal fat	0.90 ± 0.13	0.73 ± 0.09	0.77 ± 0.12	0.86 ± 0.11

^{a,b} Means in the same row by factor with different superscripts differ significantly (P<0.05)

^{1,2}: Percentages of the live weight

DL – Deep litter, DLR – Deep litter with run

findings of Sogunle *et al.*, (2013) that chickens reared in confinement achieved considerably higher body weight compared to those on other housing type or management system. However, contrary to the submissions of Castellini *et al.*, (2008), the male birds on deep-litter with run housing type had the highest weight gain and feed intake in the interaction. The higher feed intake of the males group than females of similar ages had also been reported by Laseinde and Oluyemi (1996). This could be attributed to the higher activity of the males than the females. Fischer (1985) reported that male birds are behaviorally more active than the females; hence consume more feeds and gain weight faster than the females. The result

of this study showed that deep-litter with run significantly decreased body weight and feed intake of female broiler chickens compared to the deep-litter housing type. This is in agreement with Castellini *et al.*, (2002) which reported that outdoor rearing of birds reduced growth rate but with no particular reference to the sexes. This was expected because the chickens dispensed a lot of energy as they move freely on run. The housing types influenced the mortality rate as birds managed on deep litter with run had a higher mortality than those managed on deep litter. This further confirmed the findings of Olaniyi *et al.*, (2012) who reported higher mortality in birds managed on free range as an alternative to total confinement.

Table 4: Effect of interaction between sex and housing type on carcass yield of broiler chicken

Parameters	Female		Male	
	DL	DLR	DL	DLR
Live weight (g)	2200.00 ±21.64	2166.67 ±24.72	2233.33 ±31.46	2175.00 ±30.96
Carcass yield				
Plucked weight (g)	2025.00 ±64.23 ^a	1875.00 ±35.94 ^{ab}	1945.00 ±102.75 ^{ab}	1883.30 ±81.31 ^b
Dressing percentage	83.66 ±1.03	83.07 ±1.99	79.21 ±3.62	79.12 ±1.44
Eviscerated weight (g)	1536.67 ±44.09	1545.00 ±48.91	1458.33 ±45.49	1441.67 ±39.62
Cut-up parts¹				
Head	2.05 ±0.05 ^c	2.26 ±0.12 ^{bc}	2.41±0.06 ^{ab}	2.61 ±0.12 ^a
Shanks	3.83 ±0.10 ^b	3.71 ±0.11 ^b	4.89 ±0.14 ^a	4.96 ±0.13 ^a
Thighs	10.51 ±0.46	10.45 ±0.29	10.09 ±0.70	18.19 ±8.05
Breast	23.19 ±0.68 ^a	22.43 ±0.49 ^a	18.64 ±0.67 ^b	18.84 ±0.51 ^a
Neck	4.02 ±0.36	4.08 ±0.19	3.72 ±0.29	3.87 ±0.21
Back	12.38 ±0.70	12.29 ±0.48	13.37 ±0.79	13.74 ±0.38
Wings	9.64 ±0.44	9.77 ±0.29	10.42±0.61	9.54 ±0.45
Drumsticks	10.23 ±0.14	10.39 ±0.31	10.81 ±0.38	10.76 ±0.27
Organs²				
Heart	0.39 ±0.02	0.38 ±0.03	0.40 ±0.03	0.43 ±0.01
Kidneys	0.34 ±0.05	0.48 ±0.05	0.39 ±0.05	0.35 ±0.03
Lungs	0.41 ±0.05	0.52 ±0.06	0.49 ±0.05	0.53 ±0.03
Liver	1.56 ±0.12	1.41 ±0.12	1.39 ±0.21	1.59 ±0.21
Gizzard	1.71 ±0.07	1.72 ±0.10	1.59 ±0.08	1.84 ±0.05
Proventriculus	0.33 ±0.03	0.36 ±0.04	0.29 ±0.01	0.31 ±0.01
Spleen	0.10 ±0.01	0.09 ±0.01	0.09 ±0.01	0.09 ±0.01
Abdominal fat	0.94 ±0.20	0.86 ±0.17	0.59 ±0.10	0.85 ±0.15

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

^{1,2}: Percentages of the live weight

DL – Deep litter, DLR – Deep litter with run

Male broiler chicken on deep litter had the higher cost of feed intake per day than female broiler chicken which could be partly due to the higher feed intake recorded for the male broiler chickens. On the other hands, the female birds on deep-litter with run had the highest cost of feed intake per weight gain. The relatively reduced cost by female birds on deep litter is supported by the reports of Turkylmaz *et al.*, (2002) that broilers in floor management were more profitable than cage system.

The variations in the cut-up parts and organs of the broiler chickens due to different housing types agree with the earlier reports of Verapeen and Driver (2000). However, Swain *et al.*, (2002) reported no significant influence

for system of rearing on live weight gain, feed intake and carcass. The relatively bigger male's cut-up parts than obtainable in female's cut-up parts could be attributed to higher growth rate of the males. The higher weight of the head of the males than the females could be due to the presence of bigger comb and wattles in the males. Also the higher weight of the shank of the males is probably due to the greater activities of the male broiler chickens in the different housing types. The higher percentage breast found in the female broiler chicken was

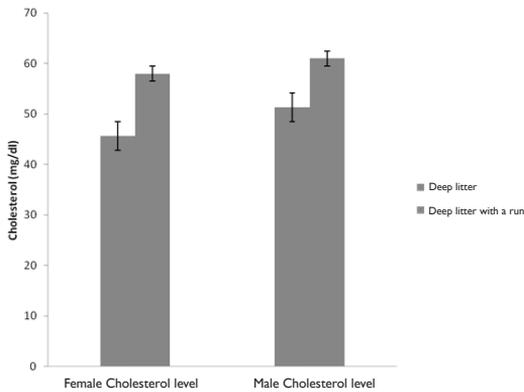


Figure 1: Serum Cholesterol of female and male broiler chickens on deep litter and deep litter with run Housing types at week 8

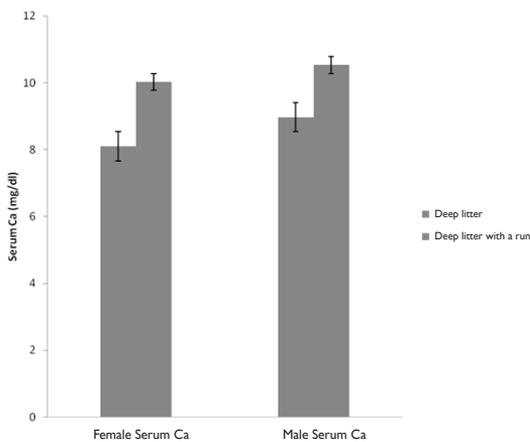


Figure 2: Serum Ca of female and male broiler chickens on deep litter and deep litter with run Housing types at week 8

in consonance with the reports of Merkley *et al.*, (1980) who found significant differences between sexes with heavier breast weight but smaller leg in female birds when compared to the male birds. However, this contrasted with the findings of Goliomytis *et al.*, (2003) who reported that chickens with heavier body weight produce a greater percentage of breast meat per carcass weight.

The serum cholesterol of male and female broiler chickens managed on deep litter with a run was higher than those managed on deep litter due largely to increased activity. On the contrary, Deshaies *et al.*, (1983) reported that increased activity did not affect serum total cholesterol values. In female broiler chickens, the values obtained was numerically lower than those recorded for the male broiler

chickens. This same trend was observed for the broiler chickens on the different housing types for serum calcium.

Conclusion

It could be concluded from this study that:

- Female broiler chickens on deep litter performed better than the males though the male broiler chickens consumed more feed than the females of similar age.
- Birds reared on deep litter had a higher carcass yield compared to those reared on deep litter with a run.
- The serum cholesterol and serum calcium of the broiler chickens were higher in the deep litter with a run housing type

Impact

The study was carried out for poultry farmers to further explore acceptable housing types/management systems that will engender profitable production of broiler chickens (sexed or unsexed) in order to bridge the ever widening protein consumption gap in developing countries. The study thereby confirmed profitable production of female broiler chickens in total confinement while the male broiler chickens could survive and attain the expected market weight of 2 kg in either of the housing types studied.

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SEROPREVALENCE OF MYCOBACTERIUM AVIUM SSP PARATUBERCULOSIS INFECTION IN ETHIOPIAN DAIRY FARMS

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Abstract

This study aimed to determine the seroprevalence of antibodies for *Mycobacterium avium* subspecies paratuberculosis (MAP) in dairy cattle in the Jimma zone of Ethiopia in 2011. A random sample of 29 herds was selected, and all mature cattle within these herds had a blood sample taken. Serum was tested in duplicate, as recommended by the manufacturer, for evidence of infection with MAP, using an antibody ELISA. A questionnaire was used to collect information from the owner or farm manager on cow and herd demographics and management to allow for comparisons of our results with other studies. Herd sizes ranged from 3 to 17 cows per herd, with 95% of cows being Holstein crosses, ranging in age from 3 to 15 years old (mean of 5.5 years). Milk production ranged from 1 to 20 kg/cow/day, with an average of 8.4 kg/cow/day. All farms used tie-stalls for their cows, 59% of farms allowed newborn calves to suckle their dams on the day of calving, and 54% of farms purchased cattle in the last 5 years. Of the 242 cattle tested, 5 cows (2.1% with a 95% confidence interval – 95%CI - of 0.2% to 3.9%) were seropositive. Due to the low test sensitivity, the true animal prevalence estimate was calculated to be 2.6% (95%CI: 0.6% to 4.6%). At least one animal tested positive in 3 of the 29 herds (10.3% - 95%CI: 0% to 21.7%). Adjusting for the low test sensitivity, the true herd prevalence estimate was calculated to be 32.6% (95%CI: 15.2% to 50.0%). This study provides the first immunological evidence of the prevalence of exposure of Ethiopian cattle to MAP, and at levels similar to other countries with small-scale dairy production. Corroboration of these prevalences with pathological, microbiological, and/or immunological MAP research in Ethiopia is warranted.

Keywords: Dairy Cattle, Johnhe's, Paratuberculosis, Seroprevalence, Western Ethiopia

SEROPREVALENCE DE L'INFECTION A MYCOBACTERIUM AVIUM SSP PARATUBERCULOSIS DANS LES FERMES LAITIÈRES EN ETHIOPIE

Résumé

Cette étude a été menée en 2011 dans le but de déterminer la séroprévalence des anticorps pour *Mycobacterium avium* sous-espèce paratuberculosis (MAP) chez les bovins laitiers de la zone Jimma en Éthiopie. Un échantillon aléatoire de 29 troupeaux a été choisi, et du sang a été prélevé sur tous les bovins adultes au sein de ces troupeaux. Le sérum a été analysé en double, tel que recommandé par le fabricant, en vue de rechercher la preuve d'une infection à MAP, en utilisant ELISA aux anticorps. Un questionnaire a été administré aux propriétaires ou aux gérants de fermes afin de recueillir des informations sur la démographie et la gestion des vaches et des troupeaux et permettre des comparaisons de nos résultats avec d'autres études. La taille des troupeaux variait entre 3 et 17 vaches par troupeau, 95% des vaches étant des croisées Holstein, âgées de 3 à 15 ans (moyenne de 5,5 ans). La production de lait allait de 1 à 20 kg / vache / jour, avec une moyenne de 8,4 kg / vache / jour. Toutes les exploitations utilisaient des stabulations entravées pour leurs vaches, 59% des exploitations agricoles laissaient les nouveau-nés téter leurs mères le jour de la mise-bas, et 54% des exploitations avaient acheté les bovins au cours des 5 dernières années. Sur les 242 bovins examinés, 5 vaches (2,1% avec un intervalle de confiance de 95% - IC à 95% - de 0,2% à 3,9%) étaient séropositives. En raison de la faible sensibilité du test, la vraie estimation de la prévalence chez les animaux a été établie à 2,6% (IC 95% : 0,6% à 4,6%). Au moins un animal a été déclaré séropositif

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dans 3 des 29 troupeaux (10,3% - 95% IC : 0% à 21,7%). Compte tenu de la faible sensibilité du test, la vraie estimation de la prévalence dans les troupeaux a été établie à 32,6% (IC à 95%: 15,2% à 50,0%). Cette étude fournit la première preuve immunologique de la prévalence de l'exposition de bovins éthiopiens à l'infection MAP, et à des taux similaires à d'autres pays ayant une petite production laitière. La corroboration de ces prévalences par des recherches pathologiques, microbiologiques et / ou immunologiques sur la MAP en Éthiopie est ainsi justifiée.

Mots-clés : Bovins laitiers, maladie de Johne, paratuberculose, séroprévalence, Éthiopie occidentale

Introduction

Mycobacterium avium subspecies paratuberculosis (MAP) is the cause of Johne's Disease (JD), a chronic infectious disease, ultimately causing diarrhea, that has been shown to have a substantial economic impact on milk production and culling in cattle, even at the subclinical stage of infection (Chi *et al.*, 2002; Tiwari *et al.*, 2005; Tiwari *et al.*, 2008). The primary methods for reducing MAP prevalence on infected farms are: 1) preventing exposure of calves to feces from shedding cows; and 2) testing and culling infected animals (McKenna *et al.*, 2006). Treatment and vaccination are of questionable value (Rossiter and Burhans, 1996).

Although JD is thought to occur world-wide, there is limited information on MAP prevalence for much of Africa (Okuni, 2013). There is recent anecdotal evidence from the pathology department at Jimma University, Ethiopia, confirming the presence of MAP in cattle in the area, as well as a report of a suspected case of paratuberculosis in a bull in Ethiopia in 1995 (Okuni, 2013). A study among mixed crop-livestock smallholder farmers in central Ethiopia found 6.8% of cattle and 100% of herds tested positive for bovine tuberculosis, but the authors also found 4.4% of cattle were positive for *Mycobacterium avium* complex (subspecies not determined), suggesting that paratuberculosis could have partially contributed to their tuberculosis prevalence (Tschopp *et al.*, 2011). However, in the African environment, humans and animals are exposed to a wide range of environmental *Mycobacteria* and related organisms (eg. *Nocardia*, *Corynebacteria*, *Rhodococcus*) (Kazwala *et al.*, 1998; Oloya *et al.*, 2007; Durnez *et al.*, 2009; Fetene *et al.*, 2011), which could also have been partially responsible for the

tuberculosis results encountered. In Ethiopia, cattle prevalence studies for MAP infection have not been conducted.

Our study objective was to estimate the seroprevalence of antibodies for MAP in dairy cattle in the Jimma zone of Ethiopia.

Materials and Methods

Study zone and study population

The study area, Jimma zone, is located about 355 km southwest of Addis Ababa, between 70 13° and 80 56° north latitudes, and 350 52° and 370 37° east longitudes, with an average altitude of 1700 m above sea level. The zone is characterized as having a humid tropical climate of heavy annual rainfall ranging from 1200 to 2000 mm, with 70% coming during the rainy season between May and September. Temperatures range from 7 °C (daily minimum in December) to 31 °C (daily maximum in May).

In this zone, there are approximately 75 small-scale dairy farms registered at Jimma town in which about 1200 cross-bred dairy cattle are kept. From this target population, a study population of 29 herds was randomly selected; not all herds could be tested due to logistical and funding limitations. With no previous report on seroprevalence of MAP in Ethiopia, a sample size calculation was difficult to determine with accuracy.

While on the farm, approximately 10 ml of blood was collected from all cows, and the serum was harvested and stored at -20 °C until all sera were ready for testing. A questionnaire was used to collect information from the owner or farm manager on cow and herd demographics and management to allow for comparisons of our results with other studies.

Laboratory analyses

At the National Animal Disease Diagnostic Laboratory in Addis, the sera were tested in duplicate, as recommended by the manufacturer, for evidence of infection with MAP, using an ELISA (IDEXX® Herdchek® ELISA kit; IDEXX Laboratories, Westbrook, Maine, USA). As with many diagnostic tests, this ELISA has not been validated in the Ethiopian environment.

Statistical analyses

Descriptive statistical analyses (means, medians, ranges) were calculated on the individual cow- and herd-level data. Apparent prevalences at the cow and herd levels (and their 95% confidence intervals) were calculated on the laboratory test results.

Because of the low sensitivity and imperfect specificity of the serological test for MAP, an estimate of the true prevalence (adjusted for test sensitivity and specificity) was calculated at the cow and herd levels, using the following equation (Rogan and Gladen, 1978):

$$p(\text{MAP}) = (\text{AP} + \text{SP} - 1) / (\text{SE} + \text{SP} - 1)$$

where $p(\text{MAP})$ was the estimated true MAP prevalence, AP was the apparent MAP prevalence from the test results, SE was the test sensitivity, and SP was the test specificity. Herd level sensitivity and specificity, and positive and negative predictive values at the cow and herd levels were also calculated (Dohoo et al., 2009).

Results

Cow-level results

A total of 242 animals were tested from 29 herds, for an average of 8 cows per herd, ranging from 3 to 17 cows per herd. The cows were 95% Holstein crossbreds, and ranged in age from 3 to 15 years old, with a mean of 5.5 years. The cows ranged in months-since-calving from 0 to 29 months, with a median of 6 months. Milk production ranged from 1 to 20 kg/cow/day, with an average of 8.4 kg/cow/day. Herd size ranged from 1 to 12 milking cows, with a median of 6 cows.

Overall, 5 of the 242 (2.1% with a 95% confidence interval – 95%CI - 0.2% to 3.9%)

sampled cows were seropositive (S/P ratio > 0.25). S/P ratios among the test-positive animals ranged from 0.54 to 1.74. The true animal prevalence estimate was calculated to be 2.6 % (95%CI - 0.6% to 4.6%). Based on this estimated true animal prevalence, predictive values for positive and negative test results at the animal level were calculated to be 53.4% and 98.5%, respectively.

Herd-level results

All herds used tie-stalls and were quite small in area (median farm area of 900 m², ranging from 200 to 3800 m²), despite the number of animals on the farms (mean of 14.6 animals), for a median density of 80.1 m²/animal (range of 11.1 to 357.1 m²/animal). Straw was used for bedding on all but 4 farms, which didn't provide any bedding. In the last year, the majority of farms had cattle that were either culled (64%) or died (57%), and 32% and 54% of farms purchased cattle in the last 1 and 5 years, respectively. Newborn calves were allowed to suck their dams on the day of calving on 59% of farms. Only 8% of farmers stored grass as silage for the dry season.

At the herd level, at least one animal tested positive for MAP infection in 3 herds (10.3% - 95%CI: 0% to 21.7%), while one herd had 3 test-positive animals. With a positive herd being defined as having at least one test-positive animal, herd level sensitivity and specificity were estimated to be 16% and 92%, primarily due to the low number of animals tested and low animal level sensitivity of the test. With this low herd level sensitivity, the true herd prevalence estimate was calculated to be 32.6 % (95%CI – 15.2% to 50.0%). Based on this estimated true herd prevalence, the herd-level positive and negative predictive values were calculated to be 49.2% and 69.4%, respectively.

Discussion

This is the first study estimating the prevalence of MAP infection at the cow and herd levels in Ethiopia, indicating exposure of Ethiopian cattle to MAP (or cross-reacting bacteria similar to MAP). The seroprevalence levels were similar to other countries with small-scale dairy production (Okuni et al., 2012;

Okuni, 2013). However, regional differences have been identified in North America; in Florida, Missouri and Eastern Canada, 17.1%, 8.0%, and 2.6% of tested cows were seropositive for MAP, respectively (Braun *et al.*, 1990; Thorne and Hardin, 1997; VanLeeuwen *et al.*, 2001). Therefore, additional research among other cattle populations in Ethiopia is necessary to determine if the study prevalences in Jimma are representative of other areas of Ethiopia.

Representativeness of the study population can be elucidated with demographic comparisons with other studies in Ethiopia, where possible. Cow median age and mean milk production in the present study were similar to 100 dairy herds studied for management practices associated with cattle health constraints near Addis (Mekonnen *et al.*, 2006), but median herd size was substantially larger among the cows of the present study. Therefore, the seroprevalence found in the Jimma zone may be an overestimate of the prevalence for smaller herds in the Addis zone or other parts of Ethiopia. Two herds of 70 and 14 cows located near Addis were recently tested for antibodies to MAP, with apparent seroprevalences of 0% and 14.3%, respectively (unpublished results).

The absorbed ELISA test kit that uses *Mycobacterium phlei* antigen to detect antibody against *M. paratuberculosis* (IDEXX Laboratories, Westbrook, Maine) is a commonly used antibody test. Although this kit is licensed by the United States Department of Agriculture, it suffers from low sensitivity, having a poor ability to detect subclinically infected cattle with *M. paratuberculosis* (Sockett *et al.*, 1992). The performance of an imperfect test varies depending on the population of cattle tested. For example, the ELISA correctly identified only 15% of subclinically infected cattle that were shedding only low numbers of bacteria (Sweeney *et al.*, 1995). This is probably the group of most interest during routine screening. It correctly identified 75% of subclinically infected cattle shedding large numbers of bacteria, and identified 87% of clinically affected cattle (Sweeney *et al.*, 1995). The impact of a low sensitivity is to underestimate the true prevalence of infected cattle.

The published specificity of the IDEXX

ELISA is 99%, which is quite good when the disease is not rare. If the disease is rare, or this specificity falls even to 98%, the false positive rate of a non-specific test increases substantially for individual cow tests, and therefore it should be noted that herds could falsely be declared infected with MAP, based on one false positive animal (Collins *et al.*, 1991; Sweeney *et al.*, 1995).

Conclusion

In support of the anecdotal evidence of the presence of MAP in Ethiopia, these initial estimates of infection prevalence give some indication of the magnitude of the JD problem in Ethiopia (which has the largest cattle population in Africa (Firdessa *et al.*, 2012)). However, further research is needed to corroborate these immunological results (eg. pathology, microbiology), and to provide better estimates of prevalence in larger populations, in order to develop and implement control programs suitable for the prevalence and economic impacts of JD in Ethiopia. Farmers should be made aware of this disease, its potential effects, and biosecurity methods of preventing it from entering a herd and spreading within a herd.

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SHEEP RESPONSE TO SUGARCANE TOPS SUPPLEMENTED WITH VARYING LEVELS OF LEUCAENA LEUCOCEPHALA FOLIAGE

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Abstract

This study was conducted to examine the response of West African Dwarf (WAD) sheep to sugarcane (*Saccharum officinarum*) tops supplemented with varying levels of *Leucaena leucocephala* foliage. Twenty WAD sheep averaging 10.14 kg were randomly divided into four groups of 5 replicates, and each group was fed sugarcane tops (SCT) supplemented with varying levels (0%, 25%, 50 and 75%) of *Leucaena leucocephala* foliage (LLF) in a completely randomized design. Results showed that sugarcane tops (SCT) supplementation with 25% LLF increased ($p < 0.05$) weight gain of sheep. Dry matter intake (DMI) declined with increase in LLF supplementation in the diets. Influence of LLF inclusion in the diets significantly ($P < 0.05$) increased serum biochemical profiles of experimental the animals. The inclusion of llf at 50% and 75% in diets III and IV respectively resulted in higher values serum glutamic oxaloacetic transaminase (93.15iu/l and 109.10iu/l) and serum glutamicpyruvate transaminase (25.25 and 39.85iu/l). However, supplementation of SCT with LLF at 25% seemed to be best as the animals had highest performance in term of DMI (530.81 g/day) and weight gain (11.83kg). This can be interpreted to mean that improved animal performance can be achieved in the dry season with the use of grass (sugarcane tops) supplemented with 25% LLF without any deleterious effect on health.

Keywords: Sheep, Performance, Nitrogen Utilization, blood, sugarcane tops and LLF.

RÉACTION DE MOUTONS À L'ALIMENTATION AUX POUSSES DE CANNE À SUCRE COMPLÉTÉE AVEC DIFFÉRENTES QUANTITÉS DE FEUILLES DE LEUCAENA LEUCOCEPHALA

Résumé

Cette étude a été menée dans le but d'examiner la réaction des moutons nains d'Afrique de l'Ouest (WAD : West African Dwarf) aux pousses de canne à sucre (*Saccharum officinarum*) complétées avec différentes quantités de feuilles de *Leucaena leucocephala*. Vingt moutons WAD pesant en moyenne 10,14 kg ont été répartis de manière aléatoire en quatre groupes de cinq répétitions, et chaque groupe a reçu une alimentation aux pousses de canne à sucre (SCT) complétée avec différentes quantités (0%, 25%, 50 et 75%) de feuilles de *Leucaena leucocephala* (LLF : *Leucaena leucocephala* foliage) dans un schéma complètement aléatoire. Les résultats ont montré que la supplémentation de pousses de canne à sucre (SCT) avec 25% LLF a augmenté ($p < 0,05$) le gain pondéral des moutons. L'ingestion de matière sèche (DMI) a diminué avec l'augmentation de la supplémentation LLF dans les régimes. L'influence de l'inclusion de LLF dans les régimes a augmenté significativement ($P < 0,05$) les profils biochimiques sériques des animaux soumis à l'expérience. L'inclusion de LLF à 50% et 75% dans les régimes III et IV a respectivement produit des valeurs plus élevées de transaminase glutamique oxalo-acétique sérique (93.15iu/l et 109.10iu/l) et de glutamicpyruvate transaminase sérique (25,25 et 39,85iu/l). Cependant, la supplémentation de SCT avec LLF à 25% semble être la meilleure puisque les animaux ont eu les meilleures performances en termes de DMI (530,81 g/jour) et de gain pondéral (11,83 kg). L'interprétation qu'on peut en faire est que l'amélioration de la performance des animaux peut être réalisée pendant la saison sèche en utilisant de l'herbe (pousses de canne à sucre) complétée avec 25% LLF, sans aucun effet néfaste sur la santé.

Mots-clés : moutons, performance, utilisation de l'azote, sang, pousses de canne à sucre et

Introduction

Dry season small ruminants feeding pose a great challenge to the small holder farmers that represent the greater percentage of livestock producers. This is because feeds become scarce as most grasses are dried, coarse, and generally very low in essential nutrients and are incapable of meeting the maintenance requirement of the animals let alone production (Ademosun, 1994). Consequently, animals normally lose considerable proportion of the weight they have gained in the proceeding wet season (Ummuna, 1981).

Though, forage legumes are noted to maintain their quality longer into the dry season than grasses, there is need for their combination with energy sources that are cheap and readily available during the dry season in the feeding of ruminants (Devendra and Burns, 1984). The combination is also necessary to obtain the required bulk.

Sugarcane top is a major by-product of the sugar industry which is readily available and often left in the field unutilized after harvesting. The sugarcane tops consists of 3 distinct parts: The green leaves (blades), the leaf sheath bundle and a variable amount of immature cane. The yield of tops varies considerably with variety, age at harvest, growing conditions and management practices. It accounts for 16 – 18% of the total biomass production or about 28% of the weight of the stalk (Nguyen *et al.*, 1996).

Leucaena leucocephala however, has been shown (NAS, 1984) to have great potential as a source of high quality roughage and live weight gain in large and small ruminants. This legume has in its leaves a high protein content (>20%CP) and is able to retain feeding quality than tropical grasses especially during dry periods (Clavero and Razz, 1997). The transportation of feed protein into body protein is an important process of nutrition and metabolism. This study therefore evaluates the potential of supplementing sugarcane tops with varying levels of LLF on sheep performance.

Materials and Methods

Site of Study

The experiment was carried out at the Small Ruminant Unit of the Teaching and Research Farm Directorate, University of Agriculture, Abeokuta, Ogun State, Nigeria. The site is located in the rain forest vegetation zone of South-Western Nigeria on Latitude 7° 13' 49.46" N, longitude 3° 26' 11.98" E and altitude 76m above the sea level. The climate is humid with a mean annual rainfall of 1037mm and mean temperature and humidity of 34.70C and 83%, respectively.

Experimental Animals and Management

Twenty growing West African Dwarf sheep with an average weight of 10.14kg and about a year old were used for the experiment. Sugarcane tops were obtained in large quantity from two farms located at Papalanto and Lafenwa in Ewekoro and Abeokuta North Local Government Area, Ogun State, Nigeria respectively. The tops were chopped to 5cm length while *Leucaena leucocephala* foliage which was obtained from various trees grown within the University community were harvested and sun dried for 5 days to reduce the level of anti-nutritional factors.

The basal diet, sugarcane tops was supplemented with varying levels of *Leucaena leucocephala* foliage at 0, 25, 50 and 75% representing diets I, II, III and IV respectively. In addition, all the diets were supplemented 100g of concentrate diet containing brewers dried grain 38.5%, cassava peels 44%, Oyster shell 0.5%, groundnut cake 15%, Salt 1% and premix 1%. The summary of the dietary treatments hereby given below:

Treatment I: 100 %SCT +0% *Leucaena leucocephala* foliage

Treatment II: 75 % SCT ± 25% *Leucaena leucocephala* foliage

Treatment III: 50% SCT + 50% *Leucaena leucocephala* foliage

Treatment IV: 25%SCT + 75% *Leucaena leucocephala* foliage

Data Collection

The twenty rams were randomly

grouped into four treatments of five (5) animals per treatment housed in individual pen. Each group was assigned to one of the experimental diets (I, II, III and IV) in a completely randomized design. The animals were allowed 14 days period of adjustment to their respective diets and environment before the collection of data. Animals were fed various diets in each treatment at 4 % of their body. The remnant were collected and weighed. The animals were given water ad libitum. They were fed twice daily at 0008hr and 0016hr. The animals were weighed once a week with spring balance in the morning before feeding throughout the 120 - day period.

In the last ten days of the experiment, the animals were transferred to the individual metabolic cage for separate collection of faeces and urine. The faeces and urine were collected in the morning before the feed and water were given. The total faeces and urine were weighed and aliquots (10%) of each days, faecal collection was dried in an oven at 75°C to a constant weight then grounded, bulked and stored in air tight polythene bags until required for chemical analysis.

Ten percent of the total daily urinary output was also collected and preserved with dilute sulphuric acid to prevent loss of Nitrogen and this also bulked for seven days for each animal in plastic bottle with lid and stored as separate samples in a refrigerator for each separate required for analysis.

Blood samples (5ml) from each of the animal was collected from the jugular vein puncture into heparin bottles for biochemical studies without anti - coagulants before commencement of the experiment and on the last week of the experiment.

Chemical Analysis

Samples of Sugarcane tops, *Leucaena leucocephala* leaves, concentrate diets, faeces and urine were analysed for their proximate composition (A.O.A.C., 1995). Energy content of the diet was determined using India ballistic bomb calorimeter method. The fibre fractions of SCT, *Leucaena leucocephala* foliage and supplementary diet were also determined (VanSoest, 1994).

Statistical Analysis

Data generated were subjected to analysis of variance ANOVA (SPSS, 2006). Mean were separated where applicable using Duncan's multiple range test (Duncan, 1955).

Results and Discussion

The result of the proximate composition (g/100gDM) of the diets is presented in Table I. The crude protein (CP) contents (%) ranged from 8.76 to 17.82 in the four diets. While mean dietary crude protein values increased with increasing levels of *Leucaena leucocephala* foliage supplementation. The values of crude protein reported in the study were for optimal ruminant nutrition as they were comparable to the level (11-12%CP) required for moderate levels of meat production (ARC 1980). However, the crude protein (CP) value of 8.76% of the sugarcane tops (diet I) higher than the limiting level of 6.8% which Minson (1982) and Forbes (1982) reported to be inadequate for production. Ether extract (EE); increased with increasing level of *Leucaena leucocephala* foliage inclusion. Neutral detergent fibre (NDF) and Acid detergent fibre (ADF) contents were higher but the NFE values increased with increasing level of *Leucaena leucocephala* foliage supplementation. Dry matter content is an important factor and is a critical determinant of energy intake and performance in ruminants (Devendra and Burns 1983), and the values of the dietary dry matter from this study across the treatments are 88.48 (Diet I), 88.53 diet II, 88.59 diet III and 88.64 diet (iv), were comparable. Similar results of DM were reported by Vaneys *et al.*, (1986) and Veereswara *et al.*, (1993) in the diets containing LLF and SCT. Slightly variations were however observed for gross energy content (Mcal/kg) of the diets with highest and lowest values of 4.03 and 3.80 were recorded for diets VI and I respectively. Dietary energy contents observed in this study were also adequate for growing sheep and similar to the recommended (ARC 1980) value of 10MJ/kg.

The summary of the performance characteristics of WAD sheep is shown in Table 2. The dry matter intake, DMI (g/day) of WAD sheep varied with the dietary treatments and

Table 1: Chemical Composition g/100gDM of the experimental diets fed the WAD sheep

Parameters	DIETS					CONC
	I	II	III	IV	LL	
	100%SCT	75%SCT +25%LLF	50%SCT +50%LLF	25%SCT +75%LLF		
Dry matter	88.48	88.53	88.59	88.64	88.69	90.06
Organic matter	76.72	79.62	82.51	85.41	82.30	3.22
Crude Protein	8.76	11.78	14.80	17.82	20.84	15.69
Crude Fibre	33.28	30.63	19.84	25.34	22.69	11.76
Ether Extract	2.34	3.08	3.81	4.51	5.28	4.97
Ash	11.76	10.42	9.08	7.73	6.39	6.84
Neutral detergent fibre	65.68	61.67	57.65	53.64	49.62	59.74
Acid detergent fibre	44.92	39.20	33.48	27.76	22.04	31.21
Acid detergent lignin	11.67	10.38	9.10	7.81	6.52	2.97
Gross Energy (Mcal/kg)	3.80	3.88	3.95	4.03	4.11	3.22

SCT = Sugar cane top

LL = *Leucaena leucocephala* foliage

CONC = Concentrate

Table 2: Performance Characteristics of growing West African Dwarf Sheep Fed sugarcane tops supplemented with varying levels of *Leucaena leucocephala* foliage

Parameters	DIETS				SEM
	I	II	III	IV	
	100%SCT	75%SCT +25%LLF	50%SCT +50%LLF	25%SCT +75%LLF	
Initial live weight (kg)	14.88	15.50	10.20	14.00	0.69
Final body weight (kg)	20.35 ^a	22.73 ^a	16.81 ^b	19.84 ^{ab}	0.69
Weight gain (kg)	5.47 ^b	7.23 ^a	6.61 ^b	5.84 ^b	1.52
Average daily gain (g/day)	45.58 ^b	60.25 ^a	55.08 ^b	48.67 ^b	5.02
Total DMI (g/day)	450.72 ^c	530.8 ^a	516.96 ^a	498.95 ^b	22.79
Total DMI (g/day/kgVW0.75)	97.82 ^c	110.59 ^a	108.41 ^b	105.50 ^b	5.39
Av DMI (SCT)	450.72 ^a	398.1 ^b	258.48 ^c	124.74 ^c	5.32
Av DMI (LLF)	0.00 ^d	132.70 ^c	258.48 ^b	374.21 ^a	2.43
Feed efficiency	9.89 ^a	8.81 ^b	9.39 ^b	10.25 ^a	1.80

^{abcd}means with different superscripts on the same row are significantly different (P<0.05)

SEM = Standard Error of Means

DMI = Dry matter intake

declined (P<0.05) with the increasing dietary levels of LLF supplementation. Though sheep on diet I (100%SCT) had a least DMI value of 450.70 probably which may be due to non-supplementation with llf also reported elsewhere (Osakwe; 2006). The highest value for DMI (530.81) was obtained in goats with 25% LLF supplementation. This indicates adequate level of combination (75%SCT and 25%LL) for optimal sheep protection.

Furthermore, the trend of DMI which declined with LLF inclusions in the diets can be interpreted to mean that excess of LLF beyond 25% might reduce acceptability of the diet which could be attributed to the increased dietary levels of anti-nutritional effect of mimosine. (D'Mello, 1992). Therefore, diet III and IV might not be too palatable to the experimental sheep which resulted in their lower DMI. However, the DM Intake per KgBW0.75 was similar to

the level recommended by AFRC (1998) for growing sheep (66gDMI/kg BW 0.75 daily).

In addition, the differences in the DMI of the animals might also be due to different stages of regrowth of both forages as they were obtained from different sources, which could have resulted to differences in nutritive values (Whiteman 1980). This probably made the forages to deposit more carbohydrates and oilier nutrients in their tissues as shown in the proximate composition.

The trend of the body weight changes of the WAD sheep is similar to that of DMI. It ($P < 0.05$) decreased with increase in LLF inclusion from 50% level (516.96 g/day) such that at 75% level the live weight gains was 5.84g/day. Live weight gains recorded was comparable to the reported values for tropical breed sheep by Devendra and Chenost (1993). Sheep on diet had significantly ($P < 0.05$) highest mean value for weight gain compared to others. Significantly different in live weight gains, may be attributed to increased crude protein intake, DM and digestibility. Kay and MacDearmid (1973) indicated that a dietary crude protein content of 11% was ideal for normal weight gain by sheep and goat. This agrees with the reports of Freer *et al.*, (1985) and Mafwererere and Mtenga (1998).

Nutrient digestibility (%) of the diets is summarized in the Table 3. Variations observed

for digestibility of DM, OM, CP and EE, values were not significant ($P > 0.05$). Except OM, the digestibility values (DM, CP, EE, Ash, NFE) increased with LLF supplementation up till 50% level (diet III) with highest values of 79.60, 81.46, 96.44, 83.21 and 79.21 respectively before declined in diet (IV). This indicates that equal level of combination of grass and legumes improved nutrient digestibility.

The crude protein digestibility ($P > 0.05$) increased with increasing level of LLF but least value of 79.65% was obtained for diet I at 0% level of LLF. This indicates that the productivity of animals given sugarcane tops without protein supplement (diet I) may be limited by inefficient utilization of Nitrogen and that could be enhanced by LLF supplementation which improves nitrogen utilization in the body (Getachew, 1994).

Fibre fractions digestibility was influenced ($P < 0.05$) by *Leucaena leucocephala* supplementation. In contrast with the nutrient (DM, OM, CP, EE) digestibilities (%) the digestibility values of fibre fractions declined with LLF supplementation. Highest values of 85.88, 90.10 and 86.27 were obtained for NDF, ADF, ADL respectively of the diet I that contained 0% LLF supplementation. This can be interpreted to mean that LLF supplementation had reduced digestibility of fibre fractions occasioned probably by the

Table 3: Nutrient Digestibility (%) of the experimental diets fed the WAD Sheep

Parameters	DIETS				SEM
	I	II	III	IV	
	100%SCT	75%SCT +25%LLF	50%SCT +50%LLF	25%SCT +75%LLF	
Dry matter	76.65	70.11	79.60	75.57	1.60
Organic matter	71.89	74.33	80.08	82.16	4.72
Crude Protein	78.18	78.20	81.46	81.19	1.64
Ether Extract	96.14	95.27	96.44	95.75	0.20
Ash	83.82 ^a	75.10 ^b	83.21 ^a	75.47 ^b	1.31
Nitrogen free extract	72.92 ^b	67.27 ^c	79.21 ^a	76.96 ^b	1.34
Neutral detergent fibre	85.88 ^a	73.08 ^b	71.50 ^b	71.58 ^b	3.57
Acid detergent fibre	90.10 ^a	74.75 ^c	80.58 ^b	70.83 ^d	0.27
Acid detergent lignin	86.27 ^a	79.33 ^b	84.08 ^a	82.17 ^{ab}	0.27

^{abcd} means with different superscripts on the same row are significantly different ($P < 0.05$)

SEM = Standard Error of Means

SCT = Sugarcane tops

LLF = *Leucaena leucocephala* foliage

Table 4: Summary of Nitrogen utilization of West African Dwarf Sheep fed Sugarcane tops supplemented with varying levels of *Leucaena leucocephala* foliage

Parameters	DIETS				SEM
	I	II	III	IV	
	100%SCT	75%SCT +25%LLF	50%SCT +50%LLF	25%SCT +75%LLF	
Initial intake (g/day)	19.47 ^c	23.2 ^b	24.1 ^b	27.99 ^a	0.85
Faecal N-output (g/day)	4.62 ^b	6.76 ^a	4.24 ^c	5.26 ^b	0.28
Urinary N-output (g/day)	0.09 ^d	0.31 ^b	0.21 ^c	0.16 ^a	0.06
Nitrogen absorbed (g/day)	14.84 ^c	16.44 ^c	19.86 ^b	22.5 ^a	0.89
N-retained (g/day)	14.75 ^b	16.31 ^c	19.74 ^a	22.39 ^a	0.83
N-retention (%)	75.75 ^b	70.30 ^b	81.91 ^a	79.99 ^a	7.52
N-intake (g/day/kgBW ^{0.75})	2.42 ^c	2.85 ^b	3.75 ^a	3.60 ^a	0.15
N-absorbed (g/day/kgBW ^{0.75})	7.55 ^c	8.16 ^c	9.41 ^b	10.35 ^a	0.30
N-retained (g/day/kgBW ^{0.75})	7.52 ^c	8.11 ^c	9.36 ^b	10.29 ^a	0.30
PER	0.56	0.68	0.43	0.43	0.06

^{abcd}means with different superscripts on the same row are significantly different ($P < 0.05$)

SEM = Standard Error of Means

PER = Protein efficiency ratio

highest fibre and anti-nutritional content of the diet. However, despite declining nature of the dietary fibre digestibility the values were high enough to have induced adequate dry matter consumption as observed in the experimental animals. One possible explanation for improved general nutrient digestibility could be that LLF supplementation was able to supply the required Nitrogen for microbial action in the rumen for the breakdown of the digesta, as a result of increased CP intake. Legume supplement have been shown to be more effective when fed with grasses containing less than 20% of Nitrogen /kg digestible organic matter, as evidenced by higher rumen ammonia concentration from increased availability of ruminally fermentable Nitrogen (Egon, 1986). Ademosun *et al.*, (1988) and Adejumo and Ademosun (1991) also observed improved digestibility of the diet and the total intake of digestible dry matter of the diet supplemented with graded levels of LLF and SCT.

The result of nitrogen 'N' utilization is presented in Table 4. The trend of N utilization (g/day) was such that nitrogen intake absorbed and retained ($p < 0.05$) increased with increased dietary protein contents occasioned by increased LL supplementation. Nitrogen intake

(g/day) increased ($p < 0.05$) with increased LLF supplementation with recorded least values of 19.47. However, mean nitrogen intake of sheep on diets II and III were similar. Dietary protein supplementation is known to improve intake by increasing nitrogen supply to the rumen microbes (VanSoest, 1982) and this can be attributed to the positive effects of increasing microbial population and efficiency to dietary intakes, thus enabling them to increase the rate of breakdown and passage-of digesta increases. This could also responsible for higher DMI of diets II and III sheep.

Nitrogen absorbed and released were significantly ($p < 0.05$) higher in sheep fed the LLF supplemented diets than those on control diet (Diet I). This justifies the need for supplementation grasses with feed resources of higher protein content. However, the LLF supplementation improved nitrogen retention in the animals with highest value (22.39) recorded for sheep on diet IV which contained highest crude protein value of (17.82 %). This indicates that the N- retention is a function of dietary protein content and quality. Sheep fed solely on SCT without legume supplementation had least value of 14.75 % which may limit efficient utilization of nitrogen by rumen

Table 5: Summary of Blood biochemical studies of West African Dwarf Sheep fed Sugarcane tops supplemented with varying levels of *Leucaena leucocephala* foliage

Parameters	DIETS				SEM
	I	II	III	IV	
	100%SCT	75%SCT +25%LLF	50%SCT +50%LLF	25%SCT +75%LLF	
Plasma urea (mg/l)	21.5 ^a	19.7 ^b	12.55 ^c	11.75 ^c	1.12
Serum glucose (g/dl)	70.55 ^a	65.75 ^b	60.55 ^c	56.45 ^c	1.50
Total protein (g/l)	8.05 ^a	7.55 ^{ab}	7.05 ^{bc}	6.75 ^c	1.06
Globulin (g/l)	4.80 ^a	4.50 ^{ab}	4.50 ^{ab}	4.05 ^b	1.00
Albumin (g/l)	3.25 ^a	3.05 ^{ab}	2.55 ^c	2.70 ^{bc}	0.9
Bilirubin (mg/dl)	0.05 ^a	0.13 ^b	0.11 ^c	0.20 ^d	0.15
SGOT (iu/l)	68.6 ^c	72.45 ^c	93.15 ^b	109.0 ^a	4.31
SGPT (iu/l)	11.45 ^d	12.00 ^c	25.25	39.85	2.32

^{abcd}means with different superscripts on the same row are significantly different ($P < 0.05$)

SEM = Standard Error of Means

microbes, consequently in low performance. Therefore, the least performance of animal on diet I with lowest ($p < 0.05$) N absorbed and retained values could be due to moderate levels of crude protein intake occasioned by non – supplementation of SCT leading to insufficient supply of nitrogen to ruminant microbes with resultant inefficient 'N' utilization as earlier reported (Vecresware *et al.*, 1993). The faecal nitrogen output is higher than the urine N-output in all the treatments. This may be due to the present of the anti-nutritional contents of the diets (Mould *et al.*, 1981) but increase can be compensated for by reduced urinary nitrogen loss.

The result of blood biochemical studies is presented in Table 5. Treatment effects on blood biochemical parameters were significant ($P < 0.05$). The plasma urea nitrogen of sheep on diet I (21.5) was significantly higher ($P < 0.05$) than the corresponding values obtained for other sheep which could indicate poor protein quality of sugarcane tops as suggested by Kaneko (1989) and Isah (2000). Also, these values of plasma fell within the normal range of (13.1-44mg/dl) reported for sheep (Pus, 1994).

Also, animal had sufficient dietary energy intake with serum glucose values of 56.45 - 70.55 g/dl which fall within the normal range (50-80 mg/dl) reported for sheep. Accordingly, animal with an increased glucose tolerance has been found to show a limited

rise in blood glucose while the animal with a decreased tolerance shows an excessive rise tolerance in original usage referred to the amount of glucose which could be ingested by animal without producing a glucosuria that is absence of glucose.

Apart from animals on diets III and IV which had higher serum glutamic pyruvic transaminase (SGPT) and serum glutamic oxala cetictranaminale (SGOT). Values of (93.15 and 109 in for SGPT) and (25.25 and 39.85 in for SGOT) which was higher than the normal values of 10-12 reported by (Puls 1994). Utilization of *Leucaena leucocephala* at 0-25% level did not pose a danger to the health of the animals. This is in agreement with the earlier reported by Onwukaet al., (1992) that supplementation of the diet with the *Leucaena leucocephala* should not exceed 30% to avert deleterious effect on animal health. Bilirubin and SGOT values obtained for all animals indicated that animals suffered no symptom of liver disease.

Conclusion

Sugarcane tops supplementation with *Leucaena leucocephala* foliage improves DM intake, weight gain, total nutrient intake and nitrogen retention and thus the performance of sheep improved with supplementation. The serum biochemical responses of the

sheep based on the different levels of the diet suggest that the animals were apparently in good condition but varied nutritional status. In addition, *Leucaena leucocephala* foliage can form a nutritive supplement to sugarcane tops thereby giving suitable diet during the dry season when grass availability is scanty.

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AFRICAN UNION - INTERAFRICAN BUREAU FOR ANIMAL RESOURCES (AU-IBAR)

Bulletin of Animal Health and Production in Africa
Guide for Preparation of Papers
Notes to Authors

The Editor in Chief
January 2013

Aims and scope

The Bulletin of Animal Health and Production in Africa (BAHPA) of the African Union Inter-African 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.

The Bulletin of Animal Health and Production publishes articles on original research on all aspects of animal health and production, biotechnology and socio-economic disciplines that may lead to the improvement of animal resources. Readers can expect a range of papers covering well-structured field studies, manipulative experiments, analytical and modeling studies of the animal resources industry in Africa and to better utilization of animal resources.

The BAHPA encourages submission of papers on all major themes of animal health and production, wildlife management and conservation, including:

- Veterinary microbiology, epidemiology
- Marketing, economics
- Infectious and non infectious disease
- Parasitology
- Genetic improvement and biotechnology
- Animal production, nutrition and welfare
- Science and policy in animal health and production
- Beekeeping and honey bees
- Ecology and climate change impacts on animal resources in Africa
- wildlife management
- Fisheries and aquaculture development
- Food safety and food hygiene
- One health
- Emerging and re-emerging issues in animal resources
- Biosecurity
- Animal resources trade and value chain
- Socio economics and economics of animal resources development

Language

The language of submission should be either in U.K. English or Standard French. The abstract is translated to the other three languages of the African Union (Arabic, English, French and Portuguese), by the editors, after acceptance. Full articles submitted in French will also be published in English.

Manuscripts Submission

Authors are invited to submit electronically their manuscripts via attachment only at bahpa@au-ibar.org in a secured PDF and word format. Manuscript can be sent by post in case of unavailability of internet services (authors should be aware that in this case it will take longer time to be published).

Authors submitting articles to the BAHPA must follow the guidelines in this document. Submissions that deviate from these guidelines will be returned to the corresponding authors for changes and compliance.

To be considered for publication in the BAHPA, any given manuscript must satisfy the following criteria:

- Originality. BAHPA does not accept manuscripts that have already been published elsewhere. However, studies that replicate results that are already in the literature may be considered for publication, as the independent confirmation of results can often be valuable, as can the presentation of a new dataset.
- Audience. Manuscripts submitted must be of broad interest to animal health and production professionals in general, they must capture and hold readers' attention.
- Usefulness. Manuscripts submitted must help researchers, trainers, educators and policy makers in all regions of Africa improve their effectiveness.
- Rigorous methodology. Manuscripts submitted must be based on valid and reliable information, documentation or sound concepts, empirically, logically and theoretically supported.
- Well written to ensure clear and effective presentation of the work and key findings. The BAHPA editorial staff does not copyedit the text of accepted manuscripts, it is therefore important for the work, as presented, to be intelligible. Perfect, stylish language is not essential but it must be clear and unambiguous. If the language of a paper is not clear, Academic Editors should recommend that authors seek independent editorial help before submission of a revision. Poor presentation and language is a justifiable reason for rejection.
- Experiments, statistics, and other analyses performed are described in sufficient detail. The research must have been performed to a technical standard to allow robust conclusions to be drawn from the data. Methods and reagents must also be described in sufficient detail so that another researcher is able to reproduce the experiments described.
- Conclusions are presented in an appropriate fashion and are supported by the data. The results must be interpreted appropriately, such that all conclusions are justified. However, authors may discuss possible explanations for their results as long as these are clearly identified as speculations or hypotheses, rather than as firm conclusions. Inappropriate interpretation of results is a justifiable reason for rejection.
- The research meets all applicable standards for the ethics of experimentation and research integrity. Research to be published must have been conducted to the highest ethical standards. A brief description of the most common of these is described in our Editorial and Publishing Policies.
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Letters to the Editor: the bulletin welcomes letters to the editor. The purpose of Letters to the Editor is to provide a forum for positive and constructive views on articles and matters published in the bulletin. Letters to the Editor must not exceed 300 words. Letters to the editors include technical reports from countries or projects.

Key notes and special calls: The editor will, from time to time, invite selected key figures in the field of animal health and production for key notes on specific topics. Book Reviews: are accepted and should provide an overview of the work's contents and a critique of the work's value. Book reviews should be limited to 1000 words.

Conference Proceedings: Special Issues of the bulletin may be dedicated to publication of proceedings of key meetings/conferences

Obituary articles to honor prominent African scientists that have made significant contribution to animal resources research and development

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Submission Guidelines

Full papers of original research

All manuscripts submitted to BAHPA should include the following features:

1. On cover page of the manuscript, the following should be clearly written/inserted: the corresponding author, name of the institution, title of the manuscript, names of the authors, the addresses of the authors and the e-mail address of the corresponding author. The corresponding author should ensure that all the other authors consent to their names being included. The consent should be sent directly by co-authors to the editor via email.
2. Each original article should be divided into Abstract and Keywords, Introduction, Materials and Methods, Results, Discussion, conclusion, Acknowledgments and References. A textbox containing a public brief on the study for the benefit of policy makers should also be provided. This textbox will not be included in the published article but will be compiled and published in a separate edition at the end of the year.
3. Title, which should be concise, preferably not more than 15 words long, followed by the author(s) name(s) and institution(s) to which work should be attributed and address for correspondence, if different.
4. The Abstract should not be longer than 300 words giving a synopsis of the work and should contain the objectives, briefs description of materials and methods, highlights of significant results, conclusions and recommendations. Up to six keywords should be provided.
5. The Introduction should contain the problem statement, the hypothesis and the objective of the work and cite recent important work undertaken by others.
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-

- repetitive way. Subheadings may be accepted.
8. Discussion of significance should be focused on in the interpretation of results. Subheadings are not accepted in this section.
9. Acknowledgements. Where necessary acknowledgements of grants and technical assistance should be included under this heading. Please also include any potential conflict of interests if appropriate. Suppliers of materials should be named and their location (town, state/county, country) included.
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Short Communications: Manuscripts should contain original data and be limited to 1500 words. The number of tables and figures are limited to two. A limited number of references should be included. Headings are not allowed in short communications.

Sequence of Preparation

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- *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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