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# FARMERS' KNOWLEDGE IN ANTIBIOTIC USAGE, ANTIBIOTIC RESIDUES, AND SUSCEPTIBILITY OF *SALMONELLA ENTERICA* IN BEEF SAMPLES FROM THE WA MUNICIPALITY, GHANA

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## Abstract

This study was conducted to determine the knowledge of livestock farmers in the Wa Municipality on antibiotic usage. It also determined the prevalence of antibiotic residues and resistant *Salmonella enterica* in beef samples in the Municipality. A semi-structured questionnaire was used to interview farmers on their knowledge on antibiotic usage. The Premi® Test kit (USA) was used to determine the presence of antibiotic residues. The procedure in the USA-FDA Bacteriological Analytical Manual was used to isolate *Salmonella enterica*. The disk diffusion method was used for antibiotic resistance tests. The farmers were mostly between 40-49 years of age (36%), had non-formal education (36.4%) and had 6-10 years of rearing experience (60.4%). The majority (63.6%) of the farmers had some knowledge in antibiotics administered to their animals. They acquired the knowledge from veterinary staff (51%), colleague farmers (29%) and extension officers (20%). The majority (51%) also relied on veterinary staff to administer drugs to their animals. The most commonly used antibiotic was ciprofloxacin (32%), while the least used were ceftriaxone (0.9%), tetracycline (0.9%) and chloramphenicol (0.9%). Overall, 29.17% of the beef samples were positive for antibiotic residues. The prevalence of antibiotic residues in the kidneys, liver and meat muscles were 43.75%, 37.50% and 6.25%, respectively. Of the 150 beef samples examined, 24% were positive for *Salmonella enterica*. Liver (32%), meat muscle (30%), and kidney (10%) samples were contaminated by *Salmonella enterica*. The total aerobic count was 3.57, 3.39 and 3.23 log cfu/cm$^2$ for meat muscle, liver and kidneys, respectively. The *Salmonella enterica* isolates exhibited a relatively high resistance to teicoplanin (97.62%) and azithromycin (30.95%). The presence of antibiotic residues and bacteria in beef samples signified lapses in the production and processing of animals, and a threat to public health. There is the need to educate livestock farmers on antibiotic usage.

**Keywords:** Antibiotics. Contamination. Farmers. Meat. Public health. Salmonella

# CONNAISSANCES DES ÉLEVEURS SUR L'USAGE D'ANTIBIOTIQUES, LES RÉSIDUS D'ANTIBIOTIQUES ET LA SENSIBILITÉ DE LA SALMONELLA ENTERICA DANS LES ÉCHANTILLONS DE BŒUF PRÉLEVÉS DANS LA MUNICIPALITÉ DE WA AU GHANA

## Résumé

La présente étude a été menée dans l'objectif de déterminer les connaissances des éleveurs sur l'usage d'antibiotiques dans la municipalité de Wa (Ghana). L'étude a également déterminé la prévalence des résidus d'antibiotiques et la résistance de la bactérie Salmonella enterica dans les échantillons de bœuf prélevés dans cette municipalité. Un questionnaire semi-structuré a été utilisé pour interroger les éleveurs à propos de leurs connaissances en matière d'utilisation des antibiotiques. L'étude a utilisé la trousse de dépistage PremiTest (USA) pour déterminer la présence de résidus d'antibiotiques. La procédure indiquée dans le Manuel d'analyse bactériologique américain / de la FDA a été utilisée pour isoler la bactérie Salmonella enterica. La méthode de diffusion sur disque a été utilisée pour les tests de résistance aux antibiotiques. Les éleveurs étaient majoritairement âgés de 40-49 ans (36 %), avaient reçu une formation non formelle (36,4

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%) et avaient 6-10 ans d'expérience (60,4 %) en matière d'élevage. La majorité (63,6 %) des éleveurs avaient une certaine connaissance des antibiotiques administrés à leurs animaux. Ils ont acquis cette connaissance du personnel vétérinaire (51 %), des autres éleveurs (29 %) et des agents de vulgarisation (20 %). De plus, la majorité (51 %) dépendait du personnel vétérinaire pour l'administration des médicaments à leurs animaux. L'antibiotique le plus couramment utilisé était le ciprofloxacine (32 %), tandis que les antibiotiques les moins utilisés étaient la ceftriaxone (0,9 %), la tétracycline (0,9 %) et le chloramphénicol (0,9 %). Dans l'ensemble, 29,17 % des échantillons de bœuf étaient positifs pour les résidus d'antibiotiques. La prévalence de résidus d'antibiotiques dans les reins, le foie et les muscles de la viande était respectivement de 43,75 %, de 37,50 % et de 6,25 %. Des 150 échantillons de bœuf examinés, 24 % étaient positifs pour Salmonella enterica. Les échantillons prélevés sur le foie (32 %), le muscle de la viande (30 %) et les reins (10 %) étaient contaminés par Salmonella enterica. La numération des microorganismes aérobies totaux était de 3,57 ; 3,39 ; et 3,23 log cfu/cm 2, respectivement pour les muscles, le foie et les reins. Les isolats de Salmonella enterica présentaient une résistance relativement élevée à la téicoplanine (97,62 %) et à l'azithromycine (30,95 %). La présence de résidus d'antibiotiques et de bactéries dans les échantillons de viande bovine est une indication de lacunes dans la production et le traitement des animaux, et constitue une menace pour la santé publique. Il est donc nécessaire de mener une action éducative auprès des éleveurs aux fins d'un usage prudent des antibiotiques.

**Mots-clés :** Antibiotiques. Contamination. Éleveurs. Viande. Santé publique. Salmonella

## Introduction

Antibiotics are chemicals produced naturally by living organisms or synthetically created in the laboratory that are capable of killing or inhibiting the growth of microorganisms (Aminov, 2010). The knowledge farmers have on the use of antibiotics determines the extent to which they use them for their farming activities. In livestock farming, the key uses of antibiotics are for the prevention, treatment and control of diseases and also as growth promoters (Draisci *et al*., 2001). Marazuela and Bogialli (2009) reported that the inappropriate application of antibiotics to farm animals poses the risk of leaving residues of the antibiotics in edible animal products such as meat (kidneys, liver, meat muscle), eggs and milk which can be dangerous to the health of the consumers.

This can be due to inappropriate knowledge on antibiotic dose, withdrawal period and the side effects when used without the knowledge of the veterinarian (Beyene, 2016). According to Tajick and Shohreh (2006), the main risk associated with antibiotic residues, is the body microflora becoming resistant to those antibiotics and this could cause severe problems when one is infested with microbes. In addition, Nisha (2008) reported that the consumption of food containing antimicrobial residues has the ability to cause cancer, mutation of cells, bone marrow toxicity (chloramphenicol) and allergy (penicillin) in humans.

It has been reported that the two main sources of microorganisms in meat are the hide of the animals or the abattoirs where the animals are slaughtered and processed (Adeyemo, 2002). Meat produced from different parts of Ghana have been shown to be contaminated by various microorganisms such as *Staphylococcus spp*., *Escherichia coli*, *Salmonella spp*., *Shigella spp*., and *Bacillus spp*. (Soyiri *et al*., 2008; Adzitey, 2015; Adzitey *et al*., 2015a; Anachinaba *et al*., 2015; Tay *et al*., 2019). Some of these organisms have been shown to be resistant to a number of antibiotics (Adzitey *et al*., 2015b; Guetaba, 2015; Saba, 2017; Republic of Ghana, 2017).

The Wa Municipality is located in the northern part of Ghana which dominates in livestock production (Adzitey, 2013). Thus, the Wa Municipality is a major contributor of livestock to the meat industry of Ghana. Nonetheless, the knowledge of livestock farmers in antibiotic usage has not been established. There is also limited information as to whether meat produced in this Municipality contains antibiotic residues and resistant bacterial species. Hence, this study aimed to determine the knowledge of livestock farmers

in the use of antibiotics during farming. The study also aimed to determine the prevalence of antibiotic residues and resistant *Salmonella enterica* in meat muscle, kidneys and liver of beef samples.

## Materials and methods

*Study location*

The study was conducted in Wa Municipality in the Upper West Region of Ghana (Figure 1). The Municipality has a land area of approximately 579.86 square kilometers, and lies within latitudes 1°40'N to 2°45'N and longitudes 9°32'W to 10°20'W (Ghana Statistical Services (GSS) 2014). The population is 81,348 which represent 11.6% of the regional population with 40,227 (49.5%) males and 41,121 (50.5%) females (GSS, 2004).

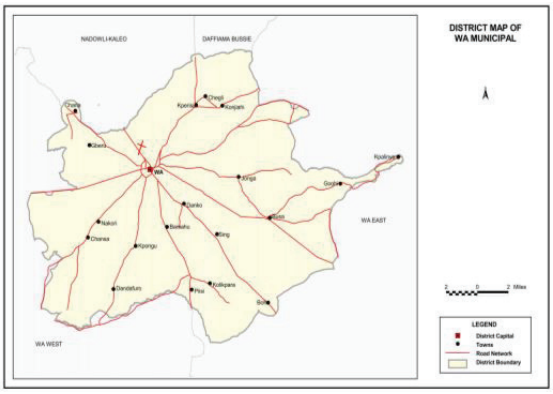


**Figure 1:** Map of Wa Municipality
*Source: GSS (2014)*

*Questionnaire administration*

Two hundred and fifty (250) farmers were interviewed on their knowledge and usage of antibiotics in livestock production using a semi-structured questionnaire (Supplementary data 1). Snowball sampling was used to select the farmers for this research.

*Antibiotic residues test*

Forty-eight (48) meat samples made up of kidney (n=16), liver (n=16) and meat muscle (n=16) were examined for antibiotic residues using the Premi®Test Kit (USA) by following the manufacturer's instructions. Approximately, 2cm$^2$ each of meat muscle, liver and kidney samples were frozen and thawed to obtain their extract/juice after which, 100µl of the extract was pipetted into ampoules and incubated at 37°C for 20 minutes for pre-diffusion. The extract was carefully flushed out by cleaning the test ampoules two-times with demineralized water. The test ampoules were covered with aluminum foil to avoid evaporation during incubation and incubated in a water bath at 64°C until the negative controls changed colour from purple to yellow. The absence of antibiotic residues caused the colour of the medium to change from purple to yellow; this was marked as "negative. The presence of antibiotic residues caused the medium to remain as purple; this was marked as "positive for antibiotic residues".

*Isolation of Salmonella enterica*

A total of 150 swab samples, made up of 50 meat muscles, 50 livers and 50 kidneys from slaughtered cattle were randomly collected from Wa Abattoir and examined for the presence of *Salmonella enterica*. Thirty (30) samples were collected per month from August to December, 2018 by swabbing approximately 10cm$^2$ of the beef surfaces. The isolation of *Salmonella enterica* was done by following the procedures in the USA-FDA Bacteriological Analytical Manual (Andrews *et al*., 2009).

*Enumeration of total viable counts*

Enumeration of total viable counts were done according to the method of Maturin and Peeler (2001). Briefly, the kidney, liver and meat muscle swabs were dipped in 9ml of 1% buffered peptone water (BPW) in universal bottles and serial dilutions of up to 104 were prepared from it. About 100µl aliquots were transferred from each of the dilutions and spread plated onto duplicate plate count agar plates. The plates were then incubated at 37°C for 24 hours after which the colonies were counted.

*Antibiotic resistance test*

Antimicrobial susceptibility tests of Salmonella isolates were done against the following antibiotics: amoxycillin/clavulanic acid

(30µg), azithromycin (15µg), ceftriaxone (30ug), chloramphenicol (30ug), ciprofloxacin (5ug), gentamicin (10ug), teicoplanin (30 µg), tetracycline (30ug) and suphamethoxazole/trimethoprim using the disc diffusion method (Bauer *et al*., 1966). The results were interpreted as sensitive, intermediate, or resistant according to Clinical and Laboratory Standards Institute guidelines (2008).

*Data analysis*

Results obtained from the laboratory tests and analyses of questionnaires administered were entered in Microsoft Excel for the calculation of simple percentages for the prevalence data. Survey data was analyzed using SPSS version 20 Copyright 2015. Data from total viable counts was analyzed using one-way ANOVA of GenStat 12.2 Release 12.1 (Copyright) 2009 and means were separated using standard error of means at 5% significance level. The results were presented in tables..

## Results

*Demographic characteristics of farmers*

Table 1 shows demographic characteristics of the livestock farmers in this study. From Table 1, the majority of the farmers were males (93.6%) aged between 40-49 years (36%). Most of them have been in the production of livestock for between 6-10years (60.4%), but, do not have formal education (36.4%).

**Table 1:** Demographic characteristics of respondents

| Variable | Frequency | Percentage (%) |
|---|---|---|
| **Gender** | | |
| Male | 234 | 93.6 |
| Female | 16 | 6.4 |
| Age | | |
| 20-29 | 30 | 12 |
| 30-39 | 46 | 18.4 |
| 40-49 | 90 | 36 |
| 50-60 | 66 | 26.4 |
| 61 and above | 18 | 7.2 |
| **Education Status** | | |
| Non Formal Education | 91 | 36.4 |
| Primary School | 34 | 13.6 |
| Junior High School | 69 | 27.6 |
| Senior High School | 20 | 8 |
| Tertiary Education | 10 | 4 |
| Others | 26 | 10.4 |
| **Work Experience** | | |
| 1-2 years | 26 | 10.4 |
| 3-5 years | 20 | 8 |
| 6-10years | 151 | 60.4 |
| Above 10 years | 53 | 21.2 |

*Type of antibiotics used by farmers in the Wa Municipality*

Table 2 shows the antibiotics used by the farmers in this study. The antibiotics were quinolones (ciprofloxacin) (32%), beta-lactams (penicillin-amoxicillin/clavulanic) (27%), sulfonamides (trimethoprim/sulfamethoxazole) (17.1%), macrolides (azithromycin) (5.4%), aminoglycosides (gentamicin) (1.8%), cephalosporin (ceftriaxone) (0.9%), chloramphenicol (0.9%), and tetracycline (0.9%). The farmers gave reasons such as

**Table 2:** Antibiotics used by farmers in Wa Municipality

| Antibiotic | Frequency/ percentage (%) |
|---|---|
| **Quinolones** | |
| *Ciprofloxacin* | |
| Yes | 71(32) |
| No | 151 (68) |
| **Beta-lactams (Penicillin)** | |
| *Amoxicillin/Clavulanic* | |
| Yes | 60 (27) |
| No | 162(73) |
| **Sulfonamides** | |
| *Trimethoprim/Sulfamethoxazole* | |
| Yes | 38 (17.1) |
| No | 174 (82.9) |
| **Macrolides** | |
| *Azithromycin* | |
| Yes | 12 (5.4) |
| No | 210 (94.6) |
| **Aminoglycosides** | |
| *Gentamicin* | |
| Yes | 4 (1.8) |
| No | 218 (98.2) |
| **Cephalosporin** | |
| *Ceftriaxone* | |
| Yes | 2 (0.9) |
| No | 220 (99.1) |
| **Chloramphenicol** | |
| Yes | 2 (0.9) |
| No | 220 (99.1) |
| **Tetracycline** | |
| Yes | 2 (0.9) |
| No | 220 (99.1) |
| **Others** | |
| Yes | 31 (14) |
| No | 191 (96) |

effectiveness of the antibiotic (84.6%), lower cost (3.7%), ease of administration (2.8%), colleagues' advice (6.1%) and others (2.8%) for their preference for a particular antibiotic.

*Farmers' source of knowledge and compliance to antibiotic usage*

All the farmers confirmed that they have encountered infections in their livestock (Table 3). The majority of farmers consulted veterinary officers (96%) and used antibiotics as medication (96.8%). This study also revealed that the majority (63.6%) of the farmers had some knowledge on the antibiotics they administered to their animals whereas a few farmers lacked knowledge about the antibiotics they used. The majority (73.2%) of the farmers did not know the correct withdrawal periods for the antibiotics they administered and some of the farmers administered the drugs by

**Table 3:** Farmers source of knowledge and compliance to antibiotic usage

| Variable | Frequency | Percentage (%) |
|---|---|---|
| **Encountered infection in the farm** | | |
| Yes | 250 | 100 |
| No | 0 | 0 |
| **Consult veterinary officers** | | |
| Yes | 240 | 96 |
| No | 10 | 4 |
| **Administer antibiotic medication** | | |
| Yes | 242 | 96.8 |
| No | 8 | 3.2 |
| **Knowledge on antibiotic usage** | | |
| Yes | 159 | 63.6 |
| No | 91 | 36.4 |
| **Source of antibiotics** | | |
| Extension officers | 32 | 20.1 |
| Colleague farmers | 46 | 28.9 |
| Veterinary staff | 81 | 50.9 |
| Total response | 159 | |
| **Who administers antibiotics** | | |
| Self | 43 | 17.8 |
| Veterinary staff | 123 | 50.8 |
| Both | 76 | 31.4 |
| Total response | 242 | |
| **Observation of safety and dosage instructions** | | |
| Yes | 105 | 88.2 |
| No | 14 | 11.8 |
| Total response | 119 | |
| **Observe withdrawal period** | | |
| Yes | 67 | 26.8 |
| No | 183 | 73.2 |

themselves (17.8%). For drug administration, most (88.2%) of the farmers who administered antibiotics by themselves indicated that they followed the manufacturers' safety instructions, but 11.8% did not observe any safety instructions before administering antibiotics to their animals.

*Prevalence of antibiotic residues and Salmonella enterica in beef (kidney, liver and meat muscle) samples*

Table 4, shows the prevalence of antibiotic residues in the kidney, liver and meat muscle of the beef samples. The prevalence of antibiotic residues in the kidneys, liver and meat muscle was 43.75%, 37.5% and 6.25%, respectively.

The overall prevalence of *Salmonella enterica* in beef samples collected from the Wa abattoir was 24% (Table 4). Liver samples (32%) were more contaminated with *Salmonella enterica* than meat muscle (30%) and kidney (10%) samples.

*Total aerobic plate count in beef (kidney, liver and meat muscle) samples*

The total aerobic plate count (microbial load) was highest in meat muscle (3.57 log cfu/cm$^2$), followed by the liver (3.39 log cfu/cm$^2$) as shown in Table 5. The least was found in the kidney (3.32 log cfu/cm$^2$). However, there were no significant differences (P>0.05) in the microbial load among the beef samples.

*Antibiotic resistance of Salmonella enterica isolated from beef samples*

The *Salmonella enterica* isolates from the beef samples in Wa Municipality were highly resistant to teicoplanin (97.62%). Resistance to azithromycin was 30.95% (Table 6). However, the *Salmonella enterica* isolates were highly susceptible to chloramphenicol (100%), ciprofloxacin (100%), tetracycline (100%), suphamethoxazole/trimethoprim (100%), ceftriaxone (95.24%), amoxycillin/clavulanic acid (90.48%) and gentamicin (78.57%).

**Table 4:** Prevalence of antibiotic residues and *Salmonella enterica* in kidney, liver and meat muscle of beef samples

| Sample | No. Examined | No. Positive | Prevalence (%) |
|---|---|---|---|
| **Antibiotic residues** | | | |
| Kidney | 16 | 7 | 43.75 |
| Liver | 16 | 6 | 37.5 |
| Meat muscle | 16 | 1 | 6.25 |
| Total (Mean) | 48 | 14 | (29.17) |
| **Isolation of** *Salmonella spp*. | | | |
| Kidney | 50 | 5 | 10 |
| Liver | 50 | 16 | 32 |
| Muscle | 50 | 15 | 30 |
| Total (Mean) | 150 | 36 | (24) |

**Table 5:** Total aerobic bacteria plate counts of kidney, liver and meat muscles

| Sample | Bacterial load (log cfu/cm$^2$) |
|---|---|
| Kidney | 3.32 |
| Liver | 3.39 |
| Meat muscle | 3.57 |
| Standard error of difference | 1.196 |
| P-value | 0.959 |

**Table 6:** Antimicrobial susceptibility of *Salmonella enterica* isolated from beef samples

| **Antibiotics** | **R (%)** | **I (%)** | **S (%)** |
|---|---|---|---|
| Amoxycillin/clavulanic acid 30µg (AMC) | 2.38 | 7.14 | 90.48 |
| Azithromycin 15µg (AZM) | 30.95 | 9.52 | 59.52 |
| Ceftriaxone 30ug (CRO) | 0.00 | 4.76 | 95.24 |
| Chloramphenicol 30ug (C) | 0.00 | 0.00 | 100.00 |
| Ciprofloxacin 5ug (CIP) | 0.00 | 0.00 | 100.00 |
| **Antibiotics** | **R (%)** | **I (%)** | **S (%)** |
| Gentamicin10ug (CN) | 14.29 | 7.14 | 78.57 |
| Teicoplanin 30 µg (TEC) | 97.62 | 0.00 | 2.38 |
| Tetracycline 30ug TE | 0.00 | 0.00 | 100.00 |
| Suphamethoxazole/trimethoprim (SXT) | 0.00 | 0.00 | 100.00 |

**n, number of resistant Salmonella; S, susceptible; I, Intermediate; R, resistant*

## Discussion

The majority of the livestock farmers in this study were males. This can be associated with the fact that in Ghana, males are normally the heads of the family and perform all the roles associated with their position, including taking possession of things owned by women. This agrees with a study by Rupa *et al*. (2018) who reported that males are directly more involved in livestock production than females due to their responsibility as family heads. This study indicated that livestock production is a male dominated job. This study also revealed that a higher portion of those involved in livestock production were middle aged. This corroborates the work by Olafadehan *et al*. (2014) who reported that 42.7% of ruminant farmers were within the ages of 40-49 years. A study conducted by Olafadehan *et al*. (2014) recorded that only 9.8% of farmers had no formal education, which was lower than the results obtained in the current study. The high percentage of non-formal education among farmers could have negative impacts on the adoption of new knowledge in antibiotic usage and livestock production as a whole.

Quinolone (ciprofloxacin) was the most widely used antibiotic by farmers in this study for the treatment of bacterial infections. This could have been due to its effectiveness as observed by the farmers. Er *et al*. (2013) reported that quinolones (ciprofloxacin, nalidixic acid etc.) have been used widely in animal production and veterinary medicine for the treatment and prevention of diseases while Cháfer-Pericás *et al*. (2010) indicated that ciprofloxacin and enrofloxacin are generally prescribed for treating and preventing infectious diseases in farm animals. Ciprofloxacin is very effective in combating microorganisms that are resistant to other antimicrobial agents such as aminoglycosides, tetracyclines, macrolides, and beta-lactams (Sultan, 2014). Beta-lactams (27%) were the second most frequently used antibiotics by farmers in Wa Municipality to treat bacterial infections in their animals. This result was lower than the 48.84% of beta-lactams usage by beef farmers recorded by Birhan and Mulugojjam (2018) in Ethiopia. However, this was higher than the findings of Ezenduka *et al*. (2011) who recorded 14% penicillin usage in Nigeria. The usage of antibiotics in this present study could be attributed to the treatment of bacterial infections (rather than as growth promoters) which are common in the study area especially during the wet season.

Birhan and Mulugojjam (2018) indicated that 86% of the farmers had no knowledge on antibiotic usage which resulted in their poor application. In addition to this, a survey conducted by Beyene *et al*. (2015) reported that 67.6% of beef farmers in Central Ethiopia did not have any knowledge on antibiotics that were

administered to their animals. The majority of the farmers sampled in the Wa Municipality had some knowledge on antibiotic usage and this could be attributed to the involvement of the veterinary and extension staff in educating the farmers in the area. The results also confirmed that farmers acquired their knowledge from veterinary staff, extension officers and colleague farmers. Most of the farmers did not know the correct withdrawal periods for the antibiotics they administered and some administered the drugs by themselves. This could lead to the wrong administration of the drugs and the development of antibiotic residues or pathogens that are resistant to these antibiotics. A study by Birhan and Mulugojjam (2018) reported that 100% of beef cattle farmers did not respect the drug withdrawal periods and 48.84% of beef farms used non-professionals to administer antibiotic drugs to their animals. The farmers who followed manufacturers' safety instructions may have been those that were trained by veterinary and extension staff while the few who did not observe safety instructions may have been those who could not read the manufacturers' instructions and were taught by their colleague farmers. Most of the farmers (73.2%) who claimed they had some knowledge of antibiotics usage had no formal education and did not observe the withdrawal periods. The non-observance of withdrawal periods can lead to the accumulation of antibiotic residues in the meat of animals.

The mean prevalence of antibiotic residues in the kidney, liver and meat muscle of beef samples examined in Wa Municipality was 29.17%, which was similar to a report by Donkor *et al*. (2011) who reported a prevalence of 30.8% of antibiotic residues in beef samples in Ghana. The results from the present study also agreed with those of Abavelim (2014) who reported that beef samples collected from selected markets in Kumasi, Ghana contained antibiotic residues. Babapour *et al*. (2012) screened 500 samples of beef and mutton collected from Iran for drug residues and reported a prevalence of 22.8% and 14% for beef and mutton, respectively with a higher prevalence of antibiotic residues in kidney and liver than in meat muscles. The liver and kidneys are the major organs involved in the metabolism and elimination of drugs in the body hence the higher prevalence of drug residues in these organs. The results of this experiment were in line with those of Morshdy *et al*. (2013) in Egypt who reported that kidneys and livers had higher antibiotic residues compared to muscles. The antibiotic residues found in the present study were much lower than those recorded by Ezenduka *et al*. (2011) in beef (54.44%) in Nigeria and Muriuki *et al*. (2001) in beef (45.6%) in Kenya. The presence of antimicrobial drugs/residues in the beef samples examined may be attributed to the non-observance of withdrawal periods to antibiotics, self-administration of drugs, and non-adherence to recommended dosage/safety instructions.

Adzitey (2015) reported an overall prevalence of *Salmonella spp*. of 31% in beef samples in the Tamale Metropolis of Ghana, which was higher than the 24% observed in this study. Floors of abattoirs are easily contaminated with contents of gastrointestinal tracts under faulty slaughtering conditions. Gastrointestinal tracts harbor various types of bacteria including *Salmonella enterica*. In this study, the contamination of liver, meat muscle and kidney by *Salmonella enterica* could have been due to the slaughtering conditions as it was observed during sampling that carcasses were placed on the slaughtering floor. The knives used for cutting the carcasses were not sterilized in between cuts and could have served as sources of transfer of *Salmonella enterica* to other cut parts. Ed and Frans (1990) reported Salmonella Dublin in beef liver (53%), kidney (33%) and meat muscle (27%). Biswas *et al*. (2011) indicated that instruments used in slaughtering and dressing animals such as knives, saws, cleavers etc. may act as sources of contamination.

Contamination of beef samples by bacteria happens when there are lapses during the slaughtering of cattle as the muscles of healthy cattle are essentially sterile. The average *Salmonella enterica* load for meat muscle, liver and kidney were below the acceptable limit

according to the Australian guideline (5 log cfu/g) (Australian Standard (AS), 2002). The results of this study agree with those of Raji (2006) who reported 3.5 log cfu/g microbial count in dried sliced beef, locally called "kilishi" in Nigeria, but, lower than 5.35 log cfu/g reported by Ahmad *et al.* (2013) in Tanzania, and 5.37 to 5.62 log cfu/g in fresh beef carcasses reported by Twum (2015) in Ghana. Koffi-Nevry *et al*. (2011) indicated that, "sneezing and coughing among butchers can introduce microbes to meat products". The contamination of meat by microbes may occur during slaughtering and processing (AS, 2002; Raji, 2006; Adzitey *et al*., 2011; Komba *et al*., 2012) and this poses challenges to the meat industry (Komba *et al*., 2012).

The antibiotic susceptibility results for ciprofloxacin, amoxycillin/clavulanic and suphamethoxazole/trimethoprim in Table 6 confirm the reasons for which these antibiotics were used by farmers in Wa municipality to treat their animals. Adzitey *et al*. (2015a) reported 100% susceptibility of *Salmonella spp*. (isolated from beef samples in Techiman) to ciprofloxacin. Danikuu (2004) also reported that *Salmonella spp*. isolated from farm animals in the Kumasi Metropolis in Ghana were all resistant to tetracycline but susceptible to ciprofloxacin, which agreed with the results of the current study. In Egypt, Moawad *et al.* (2017) reported that *Salmonella spp*. of meat origin were resistant to amoxycillin–clavulanic acid (53%), trimethoprim/sulphamethoxazole (53%), tetracycline (40%) and ceftriaxone (33%). The present study found lower or no resistance to these antibiotics. In Lebanon, *Salmonella spp*. isolated from meat-based fast foods showed 100% resistant to teicoplanin (Harakeh *et al*., 2005), which was similar to the present study. Some of the *Salmonella enterica* isolates exhibited intermediate resistance. Such isolates have the tendency to easily become resistant. Intermediate resistance was also found for Salmonella species isolated from meat samples (Adzitey *et al*., 2015a; Moawad *et al*., 2017). The increasing resistance of bacteria to antibiotics constitutes a potential source of transmission of resistant strains to humans and is of great public health concern.

## Conclusions

Livestock farmers in the Wa Municipality in Ghana are predominantly youthful men with some years of rearing experience but without formal education. The farmers had knowledge in antibiotic usage but did not observe withdrawal periods and the safe administration of antibiotics. Some beef samples contained antibiotic residues and were contaminated with *Salmonella enterica*. The *Salmonella enterica* isolates were generally susceptible to most of the antibiotics examined. The presence of antibiotic residues and bacteria in the beef samples signifies challenges in the production and processing of farm animals and is a threat to public health. It is recommended that farmers should be educated on the use of antibiotics and meat should be well cooked before consumption.

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# SELECTION OF BREEDING BULLS IN COMMUNITY-BASED CATTLE BREEDING PROGRAMS IN BURKINA FASO

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## Abstract

Community-based breeding programs (CBBP), are being implemented in the South-West of Burkina Faso. CBBP are participatory programs that involve all local stakeholders in all steps, from designing and planning of the breeding program, all the way to the selection of the breeding stock. The breeding programs are being implemented in three sites Bouroum-Bouroum, Loropéni and Kampti. Cattle genotypes used in the program were pure Lobi (Baoulé) cattle in Bouroum-Bouroum and Lobi x Zebu crossbreds in the other two. The programs aimed to meet the main interests of farmers of bigger animals which are tolerant to trypanosomosis. In the first round of selection, the body weights of young bulls, aged 3 to 5 years, were recorded twice, at an interval of 6 months. The project team grouped the bulls into three groups (top, medium and inferior) based on an index combining current body weight and growth. Selection committees consisting of male and female cattle owners made their choice of the best young bulls based on this information and their own criteria. Of the 10 best bulls selected by the committees 5 were from the top group based on the preliminary grouping of the project team. A decision was taken by the farmers to castrate or sell out the non-selected bulls, to avoid undesired mating. The joint use of bulls and bull exchange are still being negotiated by farmers, as it is not a customary practice in the region. Farmers were open and very much appreciated the concept of the joint selection of the best young bulls for the community. They understood that CBBP are long term and committed their efforts and participation in the program.

**Key words:** Breeding bull, cattle, Selection, Baoulé, Zebu, body size, trypanotolerance

# SÉLECTION DE TAUREAUX REPRODUCTEURS DANS LES PROGRAMMES D'ÉLEVAGE BOVIN À ASSISE COMMUNAUTAIRE AU BURKINA FASO

## Résumé

Des programmes d'élevage à assise communautaire (CBBP : Community-based breeding programs) sont actuellement mis en œuvre dans le Sud-Ouest du Burkina Faso. Les CCPB sont des programmes participatifs qui engagent tous les intervenants locaux dans toutes les étapes, depuis la conception et la planification du programme d'élevage jusqu'à la sélection d'animaux reproducteurs. Les programmes d'élevage sont mis en œuvre dans trois sites : Bouroum-Bouroum, Loropéni et Kampti. Les génotypes bovins utilisés dans les programmes étaient des purs Lobi (Baoulé) de Bouroum-Bouroum et des croisés

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Lobi x Zebu dans les deux autres. Les programmes visaient à répondre aux principaux intérêts des éleveurs de grands animaux trypanotolérants. Au premier tour de sélection, le poids corporel des jeunes taureaux, âgés de 3 à 5 ans, a été enregistré deux fois, à un intervalle de 6 mois. L'équipe du projet a réparti les taureaux en trois groupes (supérieur, moyen et inférieur) sur la base d'un indice combinant le poids et la croissance corporels actuels. Les comités de sélection composés de propriétaires (des deux sexes) de bovins ont fait leur choix parmi les meilleurs taureaux sur base de ces informations et de leurs propres critères. Des 10 meilleurs taureaux choisis par les comités, 5 étaient du groupe supérieur sur la base du groupement préliminaire fait par l'équipe du projet. Les éleveurs ont décidé de castrer ou de vendre les taureaux non sélectionnés pour éviter des accouplements indésirables. L'utilisation conjointe et l'échange de taureaux sont toujours négociés par les éleveurs, car ce n'est pas une pratique coutumière dans la région. Les éleveurs étaient intéressés et ont beaucoup apprécié le concept de sélection conjointe des meilleurs taurillons pour la communauté. Ils ont compris que les CBBP portent sur une longue durée et qu'ils avaient engagé leurs efforts et participation au programme.

**Mots-clés :** Taureau reproducteur, Bovins, Séléction, Baoulé, Zébu, Taille du corps, Trypanotolérance

## Introduction

In West Africa, cattle are one of the most important ruminant species reared in different production systems, providing different products and services (FAO, 2016). The cattle population consists mainly of indigenous humped Zebu and humpless taurine animals. In general, the Sahelian regions constitute the traditional habitat of Zebu cattle while taurine cattle are kept mostly in the Southern tsetse-infested Sudano-Guinea area, due to their tolerance to trypanosomosis (Soudré *et al*., 2013). These indigenous breeds are recognized to be adapted to the local environment characterized by feed shortages and the persistence of endemic diseases. They equally exhibit a high degree of hardiness across a wide range of temperatures and are tolerant and resilient to diseases (Santoze and Gicheha, 2019). Yet, the productivity per head of these indigenous breeds is very low. Due to the low productivity, keepers of African livestock struggle to meet the demand of the increasing human population which is expected to reach 2.5 billion by 2050 (FAO, 2019).

One of the reasons for the low productivity of indigenous cattle is the lack of suitable genetic improvement strategies in most of the West African countries. Until recently, cattle improvement in any country often consisted of the importation of semen of exotic breeds for crossbreeding in dairy production. Furthermore, crossbreeding local cattle with Zebu cattle was done to increase the body size of the local taurine population. Indeed, the interest of the farmers to increase the size of their taurine cattle has led to an increasing use of Sahelian Zebu cattle for reproduction in both the transitional Sudan-Sahel area and the Southern Sudano-Guinea area (Traoré *et al*., 2015). The crossbreeding is increasing with the recent migration of Sahelian nomadic people with their Zebu cattle across the different agro-climatic zones of West Africa and their settlement in the Southern zones traditionally known to be hostile for Zebu cattle (Traoré *et al*., 2015). However, the unsupervised crossbreeding leads to introgression of Zebu into West African taurine ( Soudré *et al*., 2019; Alvarez *et al*., 2014) and the possible dilution of their trypanotolerant ability (Alvarez *et al*., 2015; Traoré *et al*., 2015). Several investigations have identified uncontrolled crossbreeding, uncontrolled mating, lack of genetic evaluation and transhumance as threats to the genetic integrity of African indigenous breeds (Mwai *et al*., 2015). The case of Baoulé cattle (called Lobi locally) has been reported and ways of promoting breeding programs suitable to its in-situ conservation have also been suggested (Mopaté, 2015; Soro *et al*., 2015; Sokouri *et al*., 2007). The best way to conserve a local breed is to increase the interest of farmers in it. The implementation of conservation and selection strategies that aim to increase the productivity of native West African taurine breeds while avoiding loss of trypanotolerance is highly

advisable (Traoré *et al*., 2015).

The aim of this study was to select the best young bulls in a participatory manner, to be used as sires in three community-based breeding programmes in Burkina Faso, in order to address the objectives of farmers of obtaining bigger animals which are tolerant to trypanosomosis.

## Materials and Methods

*Study area*

This study was carried out in the South West of Burkina Faso located at 10° 19´ 00´´ N latitude and 3° 10´ 00´´ W longitudes. The climate is typical Sudanese with two distinct seasons: a dry season from October to April followed by a rainy season from May to September. The average annual rainfall varies between 900 to 1200 mm. The area is trypanosomosis challenged (Silbermayr *et al*., 2013; Soudré, 2011). Three localities were chosen, Bouroum-Bouroum, Loropéni and Kampti corresponding to the target sites of a large collaborative project termed LoCaBreed. The project is implementing three community-based breeding programs, one for pure Baoulé in Bouroum-Bouroum owned by the indegenous populations and two for Baoulé x Zebu with one owned by the indigenous people in Loropéni and the other by migrants in Kampti (see Figure 1).

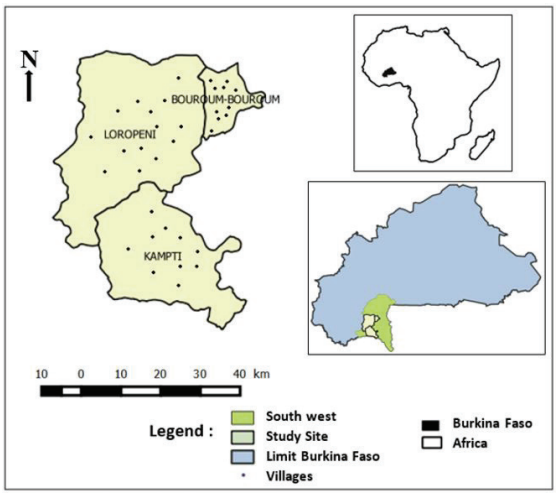


**Figure 1:** Map of Burkina Faso showing the study areas.

*Identification of animals and recording process*

The implementation of a breeding program requires the identification and the recording of animals´performances. In this study, the animals were identified using ear tags. Baseline data was collected on all the animals and pedigree information was recorded when available. Young bulls from 3 to 5 years of age were targeted as potential candidates for selection. The ages of the animals were determined through the examination of their teeth while their body weights determined using weigh-bands, were recorded twice, at an interval of six months. The height at the withers and body length were also measured. Blood samples were collected and ELISA tests on serum were done to assess the candidate bulls´ trypanosomosis infection status.

*Selection committees and selection process*

To allow the full participation of farmers in the selection process, selection committees consisting of local men (3), women (2) and one young person less than 25 years of age were set up in each locality. Bulls were placed in3 groups based on the selection index, putting equal emphasis on weight and weight gain, both corrected for age. Group 1 were bulls with high body weights and fast growth, group 2 were bulls with average body weights and growth and group 3 were bulls with low body weights and slow growth. Unfortunately, the trypanosomosis status was not available for many bulls at the time of selection and therefore could not be included in the ranking process. The selection committees were asked to choose across the 3 groups their 1st, 2nd and 3rd best bulls and give the reasons for their choices. Information on the grouping based on body weights and growth was provided to the committees before they made their choices and the committees were also previously informed that the best bulls would be awarded. To minimize conflict of interest, the committee members who owned bulls included among the candidates were asked to abstain from the choice.

Due to practical constraints like the accessibility of some villages and the difficulties

of grouping all the animals in the same place, each locality was subdivided in two or three locations for selection. The best bulls in each location were brought to a central place in the locality for the final selection of the 3 top bulls for the community.

*Data analysis*

The computer program "Statistical Analyses Software (SAS)", version 9.4 was used to analyze the data. A general linear model (GLM) was employed for body weight and weight gain considering the linear and quadratic effects of the covariate age. An index was calculated putting equal weights on the current body weight, corrected for age, and growth over the last six months. Animals were ranked based on the residual of the model with animals being heavier and or growing faster than others based on their age ranked higher.

## Results

The ranking of bulls by the linear model in the three areas is presented in Tables 1, 2 and 3. Of a total of 103 young bulls recorded, 63 were presented for selection. They consisted of 33 bulls in Bouroum-Bouroum, 15 in Loropéni and 15 in Kampti. Table 4 presents the results of the first round of selection by the committees in the three areas. The bulls presented were chosen as the best in each location based on the opinions of the committees and these qualified for the final selection in a central location. The committees chose predominantly (50%) animals from the top group according to the

**Table 1:** Bulls ranked based on their age, weight, gain and residuals in Bouroum-Bouroum

| Site | Group | ID | Age | Weight | Gain | Weight_R | Gain_R | Overall_R_std |
|---|---|---|---|---|---|---|---|---|
| **Bouroum I** | 1 | 11014 | 4.5 | 225 | 31 | 19.50 | 58.80 | 78.29 |
| | 1 | 11306 | 4.0 | 210 | 12 | 11.37 | 38.13 | 49.50 |
| | 1 | 11099 | 5.0 | 215 | 0 | 11.43 | 25.98 | 37.41 |
| | 1 | 11312 | 4.0 | 194 | 4 | -4.63 | 30.13 | 25.50 |
| | 1 | 11323 | 3.0 | 162 | 4 | 3.51 | 16.41 | 19.92 |
| | 2 | 11143 | 5.0 | 206 | -41 | 2.43 | -15.02 | -12.59 |
| | 2 | 11038 | 5.0 | 213 | -59 | 9.43 | -33.02 | -23.59 |
| | 3 | 11070 | 5.0 | 135 | 0 | -68.57 | 25.98 | -42.59 |
| | 3 | 11051 | 4.0 | 154 | -56 | -44.63 | -29.87 | -74.00 |
| | 3 | 11030 | 4.5 | 225 | -123 | 19.50 | -95.21 | -75.71 |
| | 3 | 11137 | 3.0 | 128 | -87 | -30.50 | -74.60 | -105.08 |
| **Bouroum II** | 1 | 11663 | 3.0 | 190 | 47 | 39 | 16.25 | 55.25 |
| | 1 | 765 | 5.0 | 248 | 38 | 30 | 14.00 | 44.00 |
| | 1 | 11719 | 3.0 | 174 | 46 | 23 | 15.25 | 38.25 |
| | 1 | 11852 | 3.0 | 174 | 34 | 23 | 3.25 | 26.25 |
| | 1 | 767 | 4.0 | 178 | 28 | 10 | 14.00 | 28.00 |
| | 2 | 11707 | 3.0 | 135 | 36 | -16 | -5.25 | -10.75 |
| | 2 | 726 | 3.0 | 154 | 14 | 3 | -16.75 | -13.25 |
| | 2 | 703 | 3 | 122 | 30 | -29 | -0.75 | -29.75 |
| | 2 | 11877 | 3 | 140 | 12 | -11 | -18.75 | -29.75 |
| | 2 | 757 | 3 | 119 | 27 | -32 | -3.75 | -35.75 |
| | 3 | 11876 | 5 | 188 | 10 | -30 | -14 | -44 |

| Site | Group | ID | Age | Weight | Gain | Weight_R | Gain_R | Overall_ R_std |
|---|---|---|---|---|---|---|---|---|
| **Bouroum III** | 1 | 11508 | 5.0 | 243 | 28 | 29.84 | 27.30 | 57.15 |
| | 1 | 11510 | 3.0 | 190 | 2 | 46.05 | 1.41 | 47.46 |
| | 1 | 11450 | 3.0 | 158 | 15 | 14.05 | 14.41 | 28.46 |
| | 1 | 11435 | 4.0 | 194 | 8 | 10.05 | 7.41 | 17.46 |
| | 1 | 11468 | 4.0 | 194 | 12 | 25.42 | -7.42 | 17.93 |
| | 1 | 11467 | 3.0 | 154 | 8 | 10.05 | 7.41 | 17.46 |
| | 2 | 11491 | 4.0 | 178 | 0 | 9.42 | 4.51 | 13.93 |
| | 2 | 11507 | 4.0 | 188 | -14 | 19.42 | -9.49 | 9.83 |
| | 2 | 11534 | 4.0 | 174 | 0 | 5.42 | 4.51 | 9.93 |

*ID= Identity of the bulls (ear tag), Age= age in years, Weight= Body weight, Gain= six months body weight gain, Weight_R= Residual of body weight, Gain_R= Residual of gain, Overall_R_Std= Overall residual standardized*

**Table 2:** Bulls ranked based on their age, weight, gain and residuals in Loropeni

| Site | Group | ID | Age | Weight | Gain | Weight_R | Gain_R | Overall_ R_std |
|---|---|---|---|---|---|---|---|---|
| **Loropéni I** | 1 | 11769 | 4 | 296 | 20 | 26.67 | 19.33 | 2.12 |
| | 1 | 11793 | 3 | 230 | 24 | 17.60 | 20.20 | 1.84 |
| | 1 | 11765 | 3 | 235 | 10 | 22.60 | 6.20 | 1.19 |
| | 2 | 11743 | 3 | 220 | 5 | 7.60 | 1.20 | 0.35 |
| | 2 | 11788 | 4 | 248 | 8 | -21.33 | 7.33 | -0.35 |
| | 3 | 11748 | 3 | 220 | -15 | 7.60 | -18.80 | -0.83 |
| | 3 | 11744 | 4 | 264 | -26 | -5.33 | -26.67 | -1.77 |
| | 3 | 11750 | 3 | 157 | -5 | -55.40 | -8.80 | -2.55 |
| **Loropéni II** | 1 | 3511 | 3 | 166 | 26 | 19.0 | 7.0 | 2.26 |
| | 2 | 3518 | 4 | 198 | 20 | -25.5 | 0.5 | -1.03 |
| | 3 | 3510 | 3 | 128 | 12 | -19.0 | -7.0 | -2.26 |
| **Loropéni III** | 1 | 252 | 4 | 290 | 50 | 20.5 | 18 | 2 |
| | 2 | 250 | 3 | 290 | 60 | 0.0 | 0 | 0 |
| | 3 | 274 | 4 | 249 | 14 | 20.5 | -18 | -2 |

*ID= Identity of the bulls (ear tag), Age= age in years, Weight= Body weight, Gain= six months body weight gain, Weight_R= Residual of body weight, Gain_R= Residual of gain, Overall_R_Std= Overall residual standardized*

**Table 3:** Bulls ranked based on their age, weight, gain and residuals in Kampti

| Site | Group | ID | Age | Weight | Gain | Weight_R | Gain_R | Overall_ R_std |
|---|---|---|---|---|---|---|---|---|
| **Kampti I** | 1 | 3257 | 3 | 272 | 45 | 24.4 | 14.2 | 2.88 |
| | 1 | 3658 | 3 | 260 | 35 | 12.4 | 4.2 | 1.13 |
| | 1 | 3231 | 5 | 320 | -10 | 0.0 | 0.0 | 0.00 |
| | 2 | 3358 | 3 | 248 | 31 | 0.4 | 0.2 | 0.04 |
| | 2 | 3355 | 4 | 247 | 13 | -0.0 | -0.0 | -0.00 |
| | 3 | 3256 | 3 | 246 | 29 | -1.6 | -1.8 | -0.28 |
| | 3 | 3659 | 3 | 312 | 14 | -35.6 | -16.8 | -3.77 |

| Site | Group | ID | Age | Weight | Gain | Weight_R | Gain_R | Overall_ R_std |
|---|---|---|---|---|---|---|---|---|
| **Kampti II** | 1 | 3798 | 3 | 276 | 51 | 29.11 | 181.86 | 2.33 |
| | 1 | 11393 | 3 | 257 | 87 | 10.11 | 208.73 | 1.95 |
| | 2 | 3828 | 3 | 272 | 42 | 25.11 | 115.19 | 1.68 |
| | 2 | 3247 | 3 | 296 | 19 | 49.11 | -48.64 | 1.15 |
| | 2 | 11416 | 4 | 330 | 5 | -0.00 | 0.00 | 0.00 |
| | 2 | 11375 | 5 | 330 | 40 | 0.00 | -0.00 | -0.00 |
| | 2 | 3251 | 3 | 240 | 18 | -6.89 | -56.39 | -0.66 |
| | 3 | 11398 | 3 | 182 | 37 | -64.89 | -23.83 | -2.21 |
| | 3 | 11367 | 3 | 220 | -10 | -26.89 | -242.44 | -2.73 |

*ID= Identity of the bulls (ear tag), Age= age in years, Weight= Body weight, Gain= six months body weight gain, Weight_R= Residual of body weight, Gain_R= Residual of gain, Overall_R_Std= Overall residual standardized*

**Table 4:** Bulls selected by the committees in each selection site in the three areas.

| Site | Group | ID | Age | Weight | Gain | Weight_R | Gain_R | Overall_ R_std |
|---|---|---|---|---|---|---|---|---|
| **Bouroum I** | 2 | 11038 | 5.0 | 213 | -59 | 9.43 | -33.02 | -23.59 |
| **Bouroum II** | 1 | 11852 | 3.0 | 174 | 34 | 23 | 3.25 | 26.25 |
| **Bouroum III** | 1 | 11435 | 4.0 | 194 | 8 | 10.05 | 7.41 | 17.46 |
| **Loropéni I** | 3 | 11748 | 3 | 220 | -15 | 7.6 | -18.80 | -0.829 |
| **Loropéni II** | 2 | 3518 | 4 | 198 | 20 | -25.50 | 0.50 | -1.03 |
| **Loropéni III** | 1 | 252 | 4 | 290 | 50 | 20.50 | 18 | 2 |
| **Kamti I** | 1 | 3257 | 3 | 272 | 45 | 24.4 | 14.2 | 2.88 |
| | 2 | 3358 | 3 | 248 | 31 | 0.4 | 0.2 | 0.04 |
| **Kampti II** | 1 | 11393 | 3 | 257 | 87 | 10.11 | 208.73 | 1.95 |
| | 2 | 11416 | 4 | 330 | 5 | -0.00 | 0.00 | 0.00 |

*ID= Identity of the bulls (ear tag), Age= age in years, Weight= Body weight, Gain= six months body weight gain, Weight_R= Residual of body weight, Gain_R= Residual of gain, Overall_R_Std= Overall residual standardized*

**Table 5:** Characteristics of the best bulls selected in the three areas

| Site | ID | Age | Weight | Reasons for choice |
|---|---|---|---|---|
| **Bouroum** | 11038 | 5 | 213 | Pure breed / Lobi<br>Head and Neck well developed<br>Beautiful coat color<br>Good for mating and ploughing |
| **Loropeni** | 252 | 4 | 290 | Body size and appearance<br>Best market value<br>Zebu |
| **Kampti** | 11416 | 4 | 330 | Body size and appearance<br>Development of hump |

*ID= Identity of the bulls (ear tag), Age= age in years, Weight= Body weight*

index, but also animals from the second (40%) and the third group (10%) were considered top according to committees' opinions.

Table 5 shows the results of the final ranking of bulls in the three sites. In Bouroum-Bouroum, a Baoulé bull of 5 years of age with a weight of 213 kg was selected. Blood analysis revealed that this bull was positive for trypanosomosis. In Loropéni, a crossbred bull of 4 years of age and weighing about 290 kg and negative for trypanosomosis was selected. In Kampti, an apparent Zebu bull of 4 years old and 330 kg weight but positive for trypanosomosis was ranked first.

The selection of the top bulls was followed by workshops with participating communities to discuss the use of the bulls for breeding in the communities. A decision was taken by the farmers on which bulls to keep for breeding and which ones to castrate or sell out, to avoid undesired mating.

## Discussion

This selection process showed some homogeneity in terms of the preference for bulls and cattle breeds in the study area. The reasons for ranking bulls as best were size, appearance and the potential growth of relatively young animals. This is in accordance with the interest of farmers for bigger animals. In addition to size, other body characteristics like coat pattern and the shape of horns were reported by farmers to be reasons for preference. Several studies investigated the preference and criteria of selecting cattle in Africa and reported a high preference for body size and appearance (Yakubu *et al*., 2019; Janssen-Tapken, 2009; Mapiye *et al*., 2009; Ndumu *et al*., 2008; Wurzinger *et al*., 2006). Growth as a criterion for selecting bulls for breeding purposes has also been reported by Ayantunde *et al*. (2007). In addition to productive traits, farmers are also interested in the beauty of an animal (Ndumu *et al*., 2008; Wurzinger *et al*., 2006). Furthermore in Eastern Africa, in addition to color and horns, the size of the hump has also been cited as a criterion for selecting breeding males (Janssen-Tapken, 2009). However, in the South West of Burkina Faso, farmers did not show preference for specific coat colors.

In addition to these common reasons, there was specific interest depending on the site and the specificity of the farmers. The populations in Bouroum-Bouroum and Loropéni were more oriented towards crop production and payed more attention to the ability of bulls for ploughing. Draft power and traction ability have been reported as preferred traits in male cattle selection in East and West Africa (Zewdu *et al*., 2018; Traoré *et al*., 2017; Ejlertsen *et al*., 2013; Janssen-Tapken, 2009). In Kampti, the migrant Fulani people emphasized some characteristics considered by them to be associated with fertility, like the testicle size of the bulls. Fertility of bull is also reported as a preferred trait in Eastern Africa (Janssen-Tapken, 2009).

Resistance to diseases plays an important role in the actual management of breeding herds. Farmers keeping Ankole cattle in Uganda considered resistance to East Coast Fever a very important trait (Ndumu *et al*., 2008). For the current bull selection, the results of trypanosomosis tests were only partly available at the time of bull selection and therefore this could not be included as an additional indicator for selection in this paper. However, this information is necessary for the final selection as well as information on the status of selected bulls for sexually transmitted pathologiesto avoid their spread through the sharing of bulls in the communities.

## Conclusion

The findings of this study show clearly that participatory selection for genetic improvement is possible in the low input cattle production system of the South West of Burkina Faso. The criteria established by farmer communities for the selection of the best bulls depend on the population and the types of animals reared. The farmers' preferred traits do not necessarily result in genetic gain for growth performance. In the study area, a good management system for the selected

bulls needs to be identified to help the different communities share their genetic resources and improve the performance of their animals. For this, it is necessary to diagnose the status of selected bulls in terms of sexual pathologies to avoid their spread through the sharing of bulls. Community management rules should be clearly defined about the use of selected bulls. The joint use of bulls and bull exchange are still being negotiated by farmers, as they are not customary practices in the region.

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# RECTAL TEMPERATURE AND RESPIRATORY RATE OF FOUR BROILER CHICKEN STRAINS SUBJECTED TO ACUTE HEAT STRESS CONDITIONS IN A TROPICAL CLIMATE

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## Abstract

Eight hundred (800) broiler chickens (200 each of Ross (RS), Arbor Acre (AA), Hubbard (HB) and Marshall (MS) strains) were used to study the effects of acute heat stress (AHS) on the rectal temperature (RT) and respiratory rate (RR) of broiler chickens. RT and RR of chickens were measured at room temperature (25±1°C) and considered as zero or no acute heat stress condition (0AHS), and during acute heat stress condition of 40°C ±1$^0$C at intervals of 30, 60 and 90 minutes at 28, 35, 42 and 49 days of age for three consecutive days. There were variations in RT (°C) and RR (breaths per minute, bpm) between the strains at 0, 30, 60 and 90 minutes of acute heat stress conditions. The RS strain had the highest RT and RR (40.11 to 48.88°C; 259.33 to 335.11bpm, respectively) during the experimental period compared to the other strains. RS strains frequently recorded higher RT that was greater than 43°C, followed by MS and AA, while HB had the least frequency of high RT throughout the experimental period. The RR obtained in this study for all the strains ranged from 159.15 to 335.11 bpm. This study revealed that the rectal temperature and respiratory rate values increased in broiler chickens with respect to time of exposure to acute heat stress conditions. The results also showed that rectal temperature and respiratory rate varied between strains of broiler chickens, and were directly proportional to the duration of the acute heat stress exposure.

**Keywords:** Broiler chickens, Heat Stress, Rectal Temperature, Respiratory Rate, Strains.

# TEMPÉRATURE RECTALE ET FRÉQUENCE RESPIRATOIRE DE QUATRE SOUCHES DE POULET À CHAIR SOUMISES À DES CONDITIONS DE STRESS THERMIQUE AIGU DANS UN CLIMAT TROPICAL

## Résumé

Huit cents (800) poulets de chair (200 chacun des souches Ross (RS), Arbor Acre (AA), Hubbard (HB) et Marshall (MS) ont été utilisés dans une étude des effets du stress thermique aigu (AHS : acute heat stress) sur la température rectale (RT : rectal temperature) et la fréquence respiratoire (RR : respiratory rate) des poulets de chair. La RT et la RR des poulets ont été mesurées à la température ambiante (25±1oC) et considérées comme condition de stress thermique zéro ou d'absence de stress thermique aigu (0AHS), et pendant l'état de stress thermique aigu de 40oC ±10C à des intervalles de 30, 60 et 90 minutes aux jours 28, 35, 42 et 49 pendant trois jours consécutifs. On a noté des variations de RT (oC) et de RR (respirations par minute - rpm) entre les souches à 0, 30, 60 et 90 minutes de stress thermique aigu. On a constaté que chez la souche RS, la RT et la RR étaient les plus élevées (respectivement 40,11 à 48,88oC et 259,33 à 335,11rpm) pendant la période expérimentale par rapport aux autres souches. La souche RS enregistrait souvent une RT plus élevée qui était supérieure à 43oC, suivie des souches MS et AA, tandis que la souche HB avait la plus faible fréquence de RT au cours de la période expérimentale. La RR obtenue dans cette étude pour toutes les souches variait de 159,15 à 335,11 rpm. Cette étude a révélé que les valeurs de température rectale et de fréquence respiratoire augmentaient chez les poulets de chair parallèlement au temps d'exposition aux conditions de stress thermique aigu. Les résultats ont également montré que la température rectale et la fréquence respiratoire variaient entre les différentes souches de poulets de chair

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et étaient directement proportionnels à la durée de l'exposition au stress thermique aigu.

**Mots-clés :** Poulets de chair, Stress thermique, Température rectale, Fréquence respiratoire, Souches.

## Introduction

Broiler chicken strains differ in their genetic make-up (Akporhuarho, 2011) and this could affect their performance characteristics and general response to varying management conditions. Yalcin *et al*. (1997) observed that the use of appropriate broiler strains in hot regions will result in better growth rate, increased body weight gain and reduced mortality. The optimum environmental temperature recommended for growing broilers effectively is reported to be between 18 and 22°C in South Western Nigeria (Charles, 2002) and any temperature above this could lead to heat stress according Niu *et al*. (2009). Lara and Rostagno (2013) identified heat stress as one of the most important environmental stressors challenging broiler chickens production worldwide while Van goor *et al*. (2015) reported that chronic heat stress can lead to changes in the physiology of poultry and affect their production negatively.

Emery (2004) observed that acute heat stress is caused by sudden exposure to extremely high ambient temperature (HAT) for a shorter period of time, while stated that Lin *et al*. (2010) when the thermal requirement of chickens is not satisfied, heat stress may occur, depending on the strain, feathering and nutrition. Normal rectal temperatures in chickens have been reported by Robertshaw (2004) to range from 40.6 to 43.0°C while Elson (1995) reported cloacal temperatures of 41 and 42°C for broilers', comfort conditions. Wiernusz and Teeter (1993) cited a core body temperature of 41.8$^0$C vs. 42.4°C and Respiratory rate (RR) of 129 vs. 160 breaths per minute (bpm) for normal vs. heat stressed fasted acclimated birds. Respiratory or breath rate in chickens is reported to range from 250 to 300 bpm by Detweiler and Erickson (2004) and 220 to 360 bpm by Audrey (2016). Resting respiratory rates for broilers during rearing periods were reported to range from 20 to 48 bpm by some authors (Swick, 1998; Marchini *et al*. 2007).

This study was designed to provide information that could be useful in addressing harsh environmental conditions and other issues related to broiler welfare during rearing under tropical environments through the evaluation of rectal temperatures and respiratory rates under acute heat stress in four strains of broiler chickens that are commonly reared in the North Central region of Nigeria.

## Materials and Methods

*Experimental Site*

The experiment was carried out on a private commercial farm (Fair and Firm Farm Limited, Ilorin, Kwara State Nigeria) under the ethical approval of the University of Ilorin, Ilorin, Kwara State, Nigeria, Ilorin is located in the North Central region of Nigeria on latitude 8030', and 80 50' N and longitude 40 20' and 40 35' E of the equator (KWSG, 2017).

The farm had all the necessary facilities for broiler production and a locally constructed climatic chamber that could supply regulated heat for the experimental purpose. The chamber was constructed to retain heat supplied for a minimum of one and half hours; it was well illuminated, with a hygrometer and a glass thermometer (Uniscope) suspended in the room for the measurement of humidity and regulation of the room temperature, respectively to the desired level. The experiment was carried during the cool season of the year (August to October) with an average environmental temperature range of 22 to 27°C and a humidity range of 75 to 80%.

*Experimental Animals and their Management*

Eight hundred (800) day-old broiler chicks of four different strains comprising of Ross (RR), Arbor Acre (AA), Hubbard (HB) and Marshall (MS) used for this study were purchased from a commercial hatchery in Ibadan, Oyo

state, Nigeria. Commercial feed for broiler chickens and drinkable water were provided ad libitum. All the necessary vaccinations were carried out as recommended (MVM, 2016). At 28 days of age, the chickens were moved to different deep litter pens according to their strains for rearing and experimental purposes.

*Experimental Design and Data collection*

The experiment was designed to use four broiler strains (RS, AA, HB, and MS) subjected to four rates of acute heat stress conditions for 0, 30, 60 and 90 minutes in a completely randomised design. The broiler chickens populations (200 per strain) were randomly distributed into sixteen pens at 28 days of age to house 50 broiler chickens each. The pens were labelled according to the broiler strain and heat stress conditions and the chickens were also wing tagged according to the experimental treatments.

Broiler chickens on zero (0) minutes of acute heat stress conditions were kept under normal atmospheric temperature of 25±1°C and a relative humidity of 70-75%. Measurements were taken on 25 (50%) identified birds of each population according to strain and were subjected to different rates of acute heat stress for the allotted experimental period at the appropriate ages in the locally constructed climatic chamber under a regulated temperature of 40 ± 1°C and a relative humidity range of 60-70%. At the 10th minute to the end of each exposure time, records were taken on each broiler chicken by two trained handlers. One trained handler carefully carried the birds at the breast region, allowed it to calm and measured the parameters; the values obtained were recorded by the second handler according to the tag number on the wing of the broiler chicken.

Rectal temperatures and respiratory rates were collected during 0, 30, 60, and 90 minutes of acute heat stress conditions (AHS) of each bird per strain at the age of 28, 35, 42 and 49 days for 3 consecutive days, within the hours of 8am to 12noon (GMT). The rectal temperatures (RT) of the birds were taken by inserting a digital thermometer into the cloaca of the chickens to a depth of 3-5cm and the value was read when the thermometer reading was stable for 15 seconds (Deeb and Cahaner, 1999), while the respiratory rates (RR) were determined by placing of the hand gently under the wing of the birds and counting the panting for 15 seconds using a stop watch as described by Nascimento *et al.* (2012); the values were multiplied by 4 and recorded as breaths per minute.

*Data Analysis*

The data collected from the study were subjected to the analysis of variance using the completely randomized design (CRD) of SPSS software program package (2017, version 17.0) to determine the effects of AHS on the rectal temperatures and respiratory rates of four strains of broilers (RS,AA, HB and MS) at 28, 35, 42 and 49 days of age using the model $Y_{ij} = \mu + a_i + b_j + e_{ij}$ ; where $Y_{ij}$ is the overall measurement, $\mu$ is the overall mean, $a_i$ is the strain effect, $b_j$ is the acute heat stress conditions and $e_{ij}$ is the random residual error. The frequencies of occurrence of the highest rectal temperature (above 43°C) and respiratory rate between 220 to 360bpm were determined using the Microsoft Excel (2007) programme.

## Results

The finding showed that broiler chickens strains respond differently to heat stress conditions as shown in their varying rectal temperatures (RT) and respiratory rates (RR). Table 1 showed significant ($P<0.05$) differences across the strains with respect to rectal temperatures at 0, 30, and 90 minutes AHS. The RS strain had the ($P<0.05$) highest rectal temperatures (41.61 to 44.46°C) compared to other strains whose rectal temperatures ranged from 41.07 to 43.05°C at 0, 30 and 90 minutes AHS. At 0 minutes AHS, the rectal temperatures of RS, AA and MS were similar ($P>0.05$) but significantly different ($P<0.05$) from the rectal temperatures of HB; while at 30 minutes AHS, the rectal temperatures of RS,AA and HB were also similar ($P>0.05$) but significantly different ($P<0.05$) from the rectal temperatures of MS. These results showed that at 60 minutes AHS,

all the rectal temperatures measured (42.56 to 42.87°C) were similar (P>0.05). Numerically within the strain, it was observed that the rectal temperatures measured increased with the times of exposure from 0 to 30 minutes AHS but had lower values at 60 minutes AHS. Increments in rectal temperature from 60 to 90 AHS minutes were 1.68°C in RS and 0.04°C in MS, while reductions of 0.23°C and 0.31°C were observed in the rectal temperature of AA and HB from 60 to 90 minutes AHS, respectively. Table 1 also showed significant (P<0.05) variation in the respiratory rates (RR) of the broiler strains studied. At 0, 30, 60 and 90 minutes acute heat stress condition, RS, AA and MS had similar (P>0.05) respiratory rates that were higher and differed (P<0.05) from the respiratory rates of HB (168.20 bpm to 188.60 bpm vs. 159.15 bpm to 173.90 bpm). It was also observed that the respiratory rates in all the strains were directly proportional to the time of exposure to the heat stress conditions..

At 35 days, there were significant differences (P<0.05) in the rectal temperatures of all the broilers studied and these ranged from 39.45°C in HB at 0 minutes AHS to 47.67°C in RS at 90 minutes AHS (Table 2). RS strains had significantly (P<0.05) higher rectal temperature at 60 and 90 minutes AHS. It was observed that the longer the time of exposure to acute heat stress conditions at 35 days of age, the higher that the value of the rectal temperatures recorded for the four strains of broilers studied when considered numerically. In Table 2, significant differences (P<0.05) occurred in the respiratory rates of all the broiler strains studied and this increased numerically with the time of exposure to acute heat stress conditions. At 0 minute AHS, the AA strain had significantly (P<0.05) the highest respiratory rate of 223.20 bpm, while at 60 minutes AHS, the MS strain had significantly (P<0.05) the highest respiratory rate of 299.20 bpm. At 90 minutes AHS there were no significant differences (P>0.05) in respiratory rates of the RS, AA and MS strains although they were significantly higher (P<0.05) than those of the HB strain (296.50bpm to 299.44bpm vs. 259.70 bpm).

Table 3 shows significantly different (P<0.05) rectal temperatures across the strains at 42 days of age in the broiler chickens during 0, 30 and 90 minutes of AHS. A similar (P>0.05) rectal temperature was recorded for all the strains studied at 60 minutes of AHS and it was observed that the HB strain had (P<0.05) higher rectal temperature than the other strains, while the RS and AA strains had similar (P>0.05) rectal temperatures that were significantly (P<0.05) higher than the rectal

**Table 1:** Rectal Temperatures and Respiratory Rates of four Broiler chicken strains Subjected to different conditions of AHS at 28 days of age

| | Rectal Temperature (°C) | | | | |
|---|---|---|---|---|---|
| | Broiler Strains | | | | |
| **Minutes of AHS** | **RS** | **AA** | **HB** | **MS** | **SEM** |
| 0 AHS | 41.61[a] | 41.58[a] | 41.07[b] | 41.65[a] | 0.117 |
| 30AHS | 43.05[a] | 43.05[a] | 42.79[a] | 42.33[b] | 0.130 |
| 60AHS | 42.78 | 42.62 | 42.56 | 42.87 | 0.106 |
| 90AHS | 44.46[a] | 42.39[b] | 42.25[b] | 42.91[b] | 1.074 |
| | Respiratory Rate (Bpm) | | | | |
| 0 AHS | 170.33[a] | 173.70[a] | 160.50[b] | 168.20[b] | 2.224 |
| 30AHS | 185.58[a] | 182.60[a] | 159.15[c] | 175.10[b] | 2.250 |
| 60AHS | 186.68[a] | 188.60[a] | 173.40[b] | 184.20[ab] | 2.080 |
| 90AHS | 195.67[a] | 190.20[a] | 173.90[c] | 180.60[b] | 2.218 |

*Means having different superscripts on the same row differed (P<0.05) significantly;*
*AHS: Acute Heat Stress conditions; BPM: Breaths per minute.*

**Table 2:** Rectal Temperatures and Respiratory Rates of four Broiler chicken strains Subjected to different conditions of AHS at 35 days of age

| | Rectal Temperature (°C) | | | | |
|---|---|---|---|---|---|
| | Broiler Strains | | | | |
| **Minutes of AHS** | **RS** | **AA** | **HB** | **MS** | **SEM** |
| 0 AHS | 40.11[a] | 40.58[a] | 39.45[b] | 40.60[a] | 0.147 |
| 30AHS | 43.06[a] | 42.08[b] | 42.69[a] | 43.08[b] | 0.149 |
| 60AHS | 43.24[a] | 42.15[c] | 42.88[b] | 43.57[a] | 0.145 |
| 90AHS | 47.57[a] | 42.70[b] | 42.65[b] | 43.91[b] | 1.002 |
| | Respiratory Rate (Bpm) | | | | |
| 0 AHS | 188.89[b] | 223.20[a] | 198.00[b] | 199.20[b] | 7.313 |
| 30AHS | 259.33[a] | 230.00[b] | 218.60[b] | 267.60[a] | 5.076 |
| 60AHS | 271.00[b] | 280.70[b] | 235.70[b] | 296.20[a] | 8.232 |
| 90AHS | 299.44[a] | 296.50[a] | 259.70[b] | 296.80[b] | 4.839 |

*Means having different superscripts on the same row differed (P<0.05) significantly; AHS; Acute Heat Stress Conditions; BPM: Breaths per minute.*

**Table 3:** Rectal Temperatures and Respiratory Rates of four Broiler chicken strains Subjected to different conditions of AHS at 42 days of age

| | Rectal Temperature (°C) | | | | |
|---|---|---|---|---|---|
| | Broiler Strains | | | | |
| **Minutes of AHS** | **RS** | **AA** | **HB** | **MS** | **SEM** |
| 0 AHS | 41.99[b] | 41.97[b] | 43.20[a] | 41.42[c] | 0.154 |
| 30AHS | 43.41[a] | 43.77[a] | 43.02[b] | 42.94[b] | 0.149 |
| 60AHS | 43.17 | 43.28 | 42.96 | 43.18 | 0.118 |
| 90AHS | 48.88[a] | 47.24[b] | 43.35[b] | 42.91[b] | 1.104 |
| | Respiratory Rate (Bpm) | | | | |
| 0 AHS | 221.68 | 213.78 | 213.90 | 224.60 | 3.924 |
| 30AHS | 274.42[a] | 280.56[a] | 237.70[b] | 268.90[a] | 6.949 |
| 60AHS | 291.01[a] | 282.72[a] | 274.30[b] | 287.40[a] | 1.771 |
| 90AHS | 320.44[a] | 302.38[b] | 284.10[c] | 300.60[b] | 4.062 |

*Means having different superscripts on the same row differed (P<0.05) significantly; AHS; Acute Heat Stress Conditions; BPM: Breaths per minute.*

temperatures of MS (41.99°C and 41.97°C vs. 41.42°C). At 30 and 90 minutes of AHS, the RS and AA strains had (P<0.05) higher rectal temperatures (43.77°C to 48.88°C) than the HB and MS strains (42.94°C to 43.35°C). Table 3 also shows that the respiratory rates were similar across the strains at 0 minutes AHS, but at 30 and 60 minutes AHS, the respiratory rates were (P<0.05) higher in RS, AA and MS than in the HB strain (268.90bpm to 291.01bpm vs. 237.70bpm to 274.30bpm). Significantly higher (P<0.05) RR were recorded at 90 minutes of AHS in the RS than in the other strains of broiler chickens studied. At 42 days, increases in the RT and RR were directly proportional to the increase in exposure time of the chickens to acute heat stress numerically in all the strains of broiler studied.

Table 4 shows that the rectal temperatures differed (P<0.05) significantly at 49 days across the strains. At 0 minutes of AHS, the RS and AA strains had significantly (P<0.05) higher rectal temperatures. The RT was also significantly (P<0.05) higher in the

**Table 4:** Rectal Temperatures and Respiratory Rates of four Broiler strains Subjected to different conditions of AHS at 49 days of age

| | Rectal Temperature (OC) | | | | |
|---|---|---|---|---|---|
| | Broiler Strains | | | | |
| **Minutes of AHS** | **RS** | **AA** | **HB** | **MS** | **SEM** |
| 0 AHS | 41.80[a] | 41.74[a] | 41.49[b] | 41.56[b] | 0.084 |
| 30AHS | 43.34[a] | 42.96[c] | 42.63[d] | 43.55[a] | 0.062 |
| 60AHS | 43.68[b] | 43.83[a] | 43.66[b] | 43.67[b] | 0.039 |
| 90AHS | 43.91[a] | 43.87[b] | 43.87[b] | 43.70[c] | 0.041 |
| | Respiratory Rate (Bpm) | | | | |
| 0 AHS | 231.26[a] | 211.60[b] | 201.20[b] | 228.40[b] | 4.123 |
| 30AHS | 310.21[a] | 360.00[a] | 267.40[b] | 308.10[a] | 3.907 |
| 60AHS | 312.95[a] | 308.20[ab] | 298.10[c] | 304.80[bc] | 2.806 |
| 90AHS | 335.11[a] | 317.20[a] | 303.40[c] | 316.20[b] | 3.443 |

*Means having different superscripts on the same row differed (P<0.05) significantly; AHS; Acute Heat Stress Conditions; BPM: Breaths per minute.*

RS and MS strains at 30 minutes of AHS, while at 60 minutes of AHS it was higher (P<0.05) in the AA, MS and HB strains than in the RS strain. At 90 minutes of AHS, the HB strain had significantly (P<0.05) higher RT compared to the RS and MS strains of broilers, but this value was not significantly (P>0.05) different from that of the AA strain. From Table 4, significant differences were observed in all the respiratory rates measured across the strains and at 0, 30, 60 and 90 minutes of AHS. It was observed that the respiratory rate values obtained for RS were significantly (P<0.05) higher for all the AHS conditions (231.26bpm to 335.11bpm) compared to the respiratory rates obtained for the other strains (201.20bpm to 317.20bpm). At 49 days of age, the increments in RT and RR measured were directly proportional to the increase in exposure time across the strains numerically.

Figures 1 and 2 show the frequencies of counts for rectal temperatures above 43oC and respiratory rates that fell within 220 to 360 beats per minute (bpm) as recorded for each strain of broiler throughout the experimental period. The results indicated that RS had the highest frequencies of RT that were higher than 43oC (68.7%), followed by MS, AA and HB with 43.75, 37.50, and 31.25%, respectively. Figure 2 shows that RS and MS had the highest

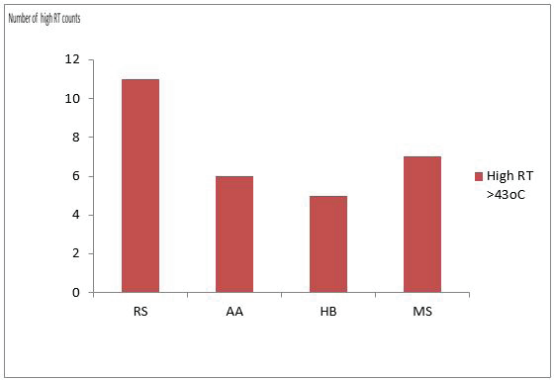


**Figure 1.** Frequencies of Rectal Temperature higher than 43°C in Strains of Broiler chickens studied

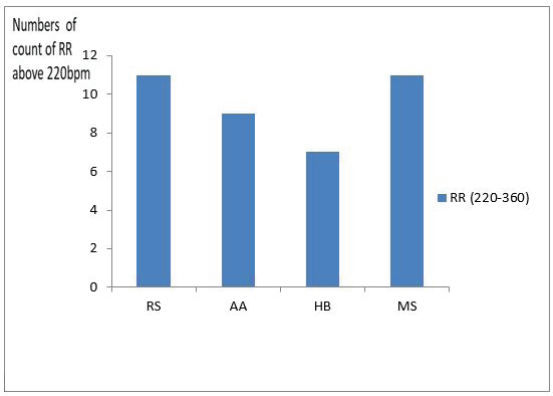


**Figure 2:** Frequencies of Respiratory rates within 220 to 360 BPM in Strains of Broiler chickens studie

frequencies (68.65%) of respiratory rates that fell within 220 to 360bpm, followed by AA with 56.25% and lastly HB with 43.75% of the total RR recorded during the experimental period.

## Discussion

The varying results obtained for rectal temperatures and respiratory rates in this study corroborate the fact that broiler chickens strains differ in their genetic make-up as stated by Akporhuarho (2011), and also with the report that physiological traits of broiler chickens changed in response to their environmental condition and age (Deeb and Cahaner, (2000). The rectal temperatures of the broiler chickens investigated in this study under no acute heat stress (0 minutes of AHS) at every age of observation fell within the range reported for normal rectal temperatures (39.45 to 41.99°C) except at 42 days of age when the average rectal temperature of HB under no heat stress conditions was recorded to be 43.20°C.

The rectal temperatures obtained from this study were in line with the fact that rectal temperature varies with respect to exposure time and corresponds with the findings of Lin *et al*. (2004) who observed that rectal temperatures exhibit oscillations at different exposure times during the period of acute heat exposure. The variations obtained for rectal temperatures and elevations of the values numerically at longer exposure times of acute heat stress conditions for the different strains of broiler chickens also corroborated the findings of Nascimento *et al*.(2012) where varying bands of respiratory rates and cloacal temperatures for different broiler strains were reported and these were suggested to be as a result of differences on the rate of heat exchange on the surface of the broiler chickens' body which might not be sufficient enough to the maintain the thermal equilibrium experienced by the chicken depending on the strain. Similar observations were made by Wiernusz and Teeter (1993); (1996); Zhou *et al*. (1998); Deeb and Cahaner (1999), Patra *et al*. (2002) and later by Lin *et al*. (2016). The rectal temperatures recorded for broiler chicken strains under different conditions of acute heat stress were higher than the recommended comfort zone rectal temperatures of 41°C and 42°C reported by Elson (1995) and the 40.6°C to 43°C reported for normal conditions by Robertshaw (2004), but fell within the value of 46°C reported for broiler thermal stress conditions at 42 days by Silva *et al*. (2007).

The numerical increments in respiratory rates and rectal temperatures with respect to the age of the birds reported in this study were in line with the fact that the values are dependent on the age of the birds, ambient temperature and exposure to heat periods and correspond with the results reported by Jaquie and Tony (2013) where the respiratory rate in chickens increased dramatically during hot weather (greater than 150bppm) and an average of 356 bpm was obtained during light periods compared to 231bpm in dark periods. It was also in line with the report of Syatwan *et al*. (2011) where an increase in the respiratory rate of Turkeys at 20 weeks of age was reported to be 195 beat per minute at 25°C compared to 230 bpm obtained at 32°C and also that of Olanrewaju *et al*. (2010), where an increase in the respiratory rate was reported for heavy broiler chickens exposed to high ambient temperatures and light intensity. Increased values obtained for respiratory rates at longer periods of exposure to heat stress conditions and older ages of broilers in this study also corresponds with the outcome of the study by Silva *et al*. (2007) where a significant increase in respiratory rates under high temperatures and a value of 165 breaths per minute were reported for broilers at 42 days of age. The increased values of both the rectal temperatures and respiratory rates recorded in this study correspond with the findings of Yahav (2000) and De Basilio *et al*. (2003) where increased body temperatures and respiratory rates of broiler chickens raised under high ambient temperatures were reported and also with the assertion of Waibel and MacLeod (1995) for Turkeys raised under high ambient temperatures.

Bossy (2007) observed that the intensity and duration of the heat stress event affects broiler performance, thus the variations observed in the frequencies of highest rectal temperatures and respiratory rates across the strains in this study are justified and this suggested that the effects of exposure to acute heat stress conditions and the intensity differed based on the tolerance level of each broiler strain to the acute heat stress they were subjected to. This could affect their performance as posited by Yalcin *et al*. (1997), that the use of unsuitable strains in hot regions could result in decreased growth rates, reduced protein gain and high mortality.

## Conclusions

The broiler chickens studied had different rectal temperatures and respiratory rates at varying time of exposure to AHS across the strains. The RS strain had the highest counts of high rectal temperatures and respiratory rates and could be said to have less tolerance for acute heat stress and is thus not recommended for rearing during heat stress conditions. The MS and AA strains recorded moderate numbers of high rectal temperatures and respiratory rates, while the HB strain had the lowest count for high rectal temperatures and respiratory rates during the experimental period and would thus be more suitable for rearing during heat stress conditions.

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## Authors Contributions

FES, DII and OT did the farm work and data collection for this experiment, DII was responsible for statistical evaluation, FES designed the experiment, FES and DII wrote the paper.

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# EFFORT TO REDUCE METHANE EMISSION IN SHEEP PRODUCTION BY FEEDING DRIED BROWSE LEAVES

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## Abstract

Ruminants are major contributors to methane production in the atmosphere. Methane production is a result of digestion inefficiencies but diet composition can be manipulated to reduce methane output. This study evaluated the productive efficiency and methane emission of sheep fed dried browse leaves. Sixteen forest type ram lambs with an average weight of 14.38 kg±0.51 were fed *Albizzia lebbek*, *Moringa oleifera*, *Millettia thonningii* and urea treated straw (Control) for 12 weeks. The predicted enteric methane emission was measured using a model equation (Methane (MJ/day) = 8.25 + 0.07 x MEI). Faecal nitrogen and urinal nitrogen were evaluated by proximate analysis and faecal energy was assessed using a bomb calorimeter. Data obtained was analysed as completely randomised design and subjected to Analysis of Variance using GenStat. There were significant differences ($p<0.05$) for all the parameters measured. The range of values for dry matter intake, energy intake, digestible energy, average daily gain, feed conversion efficiency, predicted enteric methane emission, faecal nitrogen, urinal nitrogen and faecal energy were 457.15 - 644.81 g/d, 13.23 - 16.17 MJ/kg DM, 11.30 - 13.10 MJ/kg DM, 26.79 - 68.46 g/d, 9.23 - 26.26, 7.53 - 36.19 MJ/d, 50.98 - 81.16 g/animal/d, 6.80 - 15.53 g/animal/d and 1.50 - 4.80 MJ/kg DM respectively. Sheep fed *Albizzia lebbek*, *Moringa oleifera* and *Millettia thonningii* recorded 188 %, 256 % and 159 % more weight gain respectively, than those fed urea treated rice straw. Sheep fed dried browse leaves emitted 65.52 %, 70.6 % and 79.19 % less predicted enteric methane for *Albizzia lebbek*, *Moringa oleifera* and *Millettia thonningii* respectively than urea treated rice straw. This work balanced reduction in methane production and improved weight gain of the sheep to ensure the sustainability of sheep production and the environment. The lower methane emission may contribute to minimise the effects of climate change.

**Keywords:** Chemical composition, condensed tannins, energy, nitrogen losses, Weight gain

# EFFORT VISANT À RÉDUIRE LES ÉMISSIONS DE MÉTHANE DANS LA PRODUCTION OVINE PAR ALIMENTATION AUX FEUILLES D'HERBACÉES SÉCHÉS

## Résumé

Les ruminants sont des contributeurs majeurs à la production de méthane dans l'atmosphère. La production de méthane résulte de l'inefficacité de la digestion, mais la composition du régime peut être manipulée pour réduire la production de méthane. Cette étude a évalué l'efficacité productive et l'émission de méthane de moutons nourris aux feuilles d'herbacées séchées. Seize agneaux de type forêt d'un poids moyen de 14,38 kg ± 0,51 ont été nourris aux feuilles d'*Albizzia lebbek*, Moringa oleifera, Millettia thonningii et à la paille traitée à l'urée (témoin) pendant 12 semaines. L'émission prédite de méthane entérique a été mesurée à l'aide d'une équation modèle (Méthane (MJ/day) = 8,25 + 0,07 x MEI). L'azote fécal et l'azote urinaire ont été évalués par analyse proximale, et l'énergie fécale a été évaluée à l'aide d'un calorimètre à

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bombe. Les données obtenues ont été analysées selon un schéma complètement randomisé et soumises à l'analyse de variance en utilisant GenStat. Des différences significatives (p < 0,05) ont été notées pour tous les paramètres mesurés. L'éventail des valeurs pour la consommation de matière sèche, la consommation de calories, l'énergie digestible, le gain quotidien moyen, le taux de consommation alimentaire, les émissions prédites de méthane entérique, l'azote fécal, l'azote urinaire et l'énergie fécale étaient respectivement de 457.15 - 644.81 g/d, 13.23 - 16.17 MJ/kg DM, 11.30 - 13.10 MJ/kg DM, 26.79 - 68.46 g/d, 9.23 - 26.26, 7.53 - 36.19 MJ/d, 50.98 - 81.16 g/animal/d, 6.80 - 15.53 g/animal/d and 1.50 - 4.80 MJ/kg DM. Les ovins nourris à l'*Albizzia lebbek*, Moringa oleifera et Millettia thonningii ont enregistré respectivement 188 %, 256 % et 159 % de gain pondéral plus élevé que ceux recevant de la paille traitée à l'urée. Les ovins nourris aux feuilles d'herbacées séchées ont émis 65,52 %, 70,6 % et 79,19 % moins que le méthane entérique prédit respectivement pour *Albizzia lebbek*, Moringa oleifera et Millettia thonningii par rapport à la paille de riz traitée à l'urée. Ce travail a équilibré la réduction de la production de méthane et amélioré le gain pondéral des moutons pour assurer la durabilité de la production ovine et de l'environnement. La baisse des émissions de méthane peut contribuer à minimiser les effets du changement climatique.

**Mots-clés :** Composition chimique, tanins condensés, énergie, pertes d'azote, gain pondéral

## Introduction

Ruminants are the main contributors to the production of biogenic methane and it has been estimated that reducing methane from domesticated ruminants could stabilize the concentration of atmospheric methane (Johnson and Johnson, 1995). Methane emissions from ruminants contribute immensely to total anthropogenic greenhouse gas emissions (Patra, 2012). Production of enteric methane is a source of loss of energy that is due to digestion inefficiencies mainly in the rumen (Johnson and Johnson, 1995), which reduces Metabolizable Energy Intake (MEI) by the animal (Goel and Makkar, 2012) and could potentially be channelled towards the production of meat and milk (Eckard *et al*. 2010). The majority of the variations in enteric methane production from animals can be attributed to composition of the diet and intake of feed (Bell and Eckard, 2012).

Browse leaves have been suggested as a reliable alternative as a sole diet for feeding ruminants (Yisehak *et al*., 2014 & 2012). Browse leaves contain condensed tannins and saponins which have been reported to reduce enteric methane. Feeding of dried browse leaves has the potential to reduce methane production and also improve weight gain. This is desirable because an attempt to use pasture supplemented with grain to reduce methane production resulted in thirty percent reduction in methane emission but ten percent reduction in milk production (Grainger *et al*., 2009).

Feeding of condensed tannins (CT) forage diets to animals have resulted in the reduction in methane production compared to control diets that did not contain CT (Puchala *et al*., 2005). Anti-methanogenic actions of CT directly impede the growth of methane producing microbes through its influence on their functional proteins such as microbial enzymes in or at sites available on the methane producing microbes (Field and Lettinga, 1987 and Field *et al*., 1989; Patra and Saxena, 2010). This causes bactericidal or bacteriostatic effects (Tavendale *et al*., 2005) or indirectly, the defaunation of populations of protozoa associated with methane producing microbes (Patra *et al*., 2006; Animut *et al*., 2008; Bhatta *et al*., 2009). Weight gain and methane emission by feeding dried browse leaves as a complete diet have not been extensively studied. The objective of this study was to evaluate the productive efficiency and methane emission of sheep fed dried browse leaves.

## Materials and Methods

*Study Area*

The study was carried out at the Livestock and Poultry Research Centre (LIPREC), University of Ghana, Legon (5° 68'N, 0°10'W) situated in the coastal savannah zone of Ghana. The pattern of rainfall is bimodal with

the major rains in June while the minor rains are in September-October. The total rainfall is from 508 mm to 743 mm annually. Maximum and minimum temperatures vary between 32.90 °C and 24.30 °C. The relative humidity is normally between 65 to 95 %.

## Experimental Procedure

*Chemical Analysis*

Dry matter (DM), Crude protein (CP) and Ash were determined by A.O.A.C (2016) and fibre components by Goering and Van Soest (1970). Condensed tannins (CT) was evaluated using butanol-HCl method as described by Iqbal *et al*. (2011).

*Animal Management and Feeding*

The use of animals and all other procedures were approved by Noguchi Institutional Animal Care and Use Committee of University of Ghana, Legon (Protocol number 2017-02-2R). Sixteen forest type ram lambs of 14.38 kg±0.51 average weight were used to measure weight gains. The animals were kept in individual pens with concrete floors (2m x 1.5m per pen) which had asbestos roofing and the sides were made of metal rails. Before the experiment begun, the animals were treated to control ectoparasites and endoparasites. The sixteen ram lambs were put into four groups with four animals per group. Each group was randomly assigned a treatment. Treatment of rice straw was carried out as described by Fleischer *et al*. (2000). The browse leaves were harvested from the natural ecosystem within the coastal savannah zone and sun dried for 48 hours. After a 14 day acclimatisation period, the animals were fed three dried browse leaves [*Albizzia lebbek* (AL), *Moringa oleifera* (MO) and *Millettia thonningii* (MO)] and urea treated (UT) straw (Control) for twelve weeks in a completely randomised design. The common names of AL, MO and MT are woman's tongue, never die or horse-raddish tree and turburku fruit tree respectively. AL and MT belong to the Fabacaea family and MO belongs to Moringaceae family. Urea treated rice straw (UT) was used as a control because it is one of the recommended dry season feeding strategies for ruminant livestock (Fleischer *et al*., 2000). In order to convince farmers to use dried browse leaves as a first choice over UT, other benefits apart from weight gain were highlighted such as the multiple potential of the dried browse leaves to reduce methane production which may contribute to minimise the effects of climate change. In addition, feeding browse leaves to animals has other benefits such as medicinal values, healthiness of meat and the promotion of organic livestock farming. Therefore, UT was used as the baseline to calculate the percentage reduction in methane production. The use of UT as a control provided a basis to compare the methane output of a non CT diet to diets containing CT. Live weight was measured every two weeks after fasting for twelve hours. Water was provided ad libitum to all animals during the study. Animals were fitted with faecal bags for the collection of faeces for the estimation of metabolizable energy intake (MEI) and determination of faecal energy (FE) and nitrogen losses. Nitrogen losses (NL) were measured as reported by Yeboah *et al*. (2017).

*Estimation of Metabolizable Energy Intake (MEI) and Determination of Energy*

Samples of feed refusals, faecal outputs and feeds were analysed for DM by oven drying at 55°C. Organic matter (OM) was estimated as DM less the residual ash obtained after ashing at 550°C for 6 hours. OM in the feed and faeces, DMI and Digestible Organic Matter in Dry Matter (DOMD) were estimated. The values were fitted into the equation by Ministry of Agriculture, Food and Fishery (MAFF) (1984) to calculate MEI for each diet as follows:

$$MEI = DOMD \times 0.15 \times DMI$$

Energy was determined by using a bomb calorimeter (Parr 6100 Calorimeter, Wagtech International). Digestible energy (DE) was estimated by subtracting faecal energy (FE) from energy intake (EI) as described by Sun *et al*. (2012).

*Methane Emission Estimation*

Predicted enteric methane production from AL, MO, MT and UT feeding was measured using the model equation by Mills *et al*. (2003) which used metabolizable energy intake (MEI).

*Methane (MJ/day) = 8.25 + 0.07 x MEI (MJ/day).*

The model equation for methane estimation was developed based on a large amount of data collected from the respiratory chamber method which was considered as the standard for measuring methane. This was one of the model equations recommended by Powers *et al* (2014) in a United States Department of Agriculture technical bulletin.

*Statistical Analysis*

Data obtained was analysed as a completely randomised design and subjected to ANOVA using GenStat (2009) version 12.1 by employing the model below:

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

$Y_{ij}$ was the response variable such as Dry Matter Intake, Energy Intake, Digestible Energy, Average Daily Gain, Methane, Faecal Nitrogen, Urinal Nitrogen, Faecal Energy; μ was the overall mean; $T_i$ was the different browse leaves and UT; $E_{ij}$ was the residual error.

Significant differences of means were separated using the Student Newman Keuls Test. Relationships among parameters were determined by regression.

## Results

*Chemical Composition, Performance Indices and Methane Production of Sheep Fed Dried Browse Leaves and UT.*

The chemical Composition of diets used for feeding the experimental sheep is presented in Table 1. Dry matter intake (DMI) was highest ($p<0.05$) for sheep fed UT and the lowest ($p<0.05$) was MT which did not differ ($p>0.05$) from AL whilst MO was intermediate (Table 2). Energy intake (EI) was lowest ($p<0.05$) for sheep fed AL and highest ($p<0.05$) for UT (Table 2). Digestible energy (DE) ranged from 11.3 - 13.10 MJ/kg DM with sheep offered MO being the highest ($p<0.05$) and those fed UT the lowest ($p<0.05$) as shown in Table 2. Average daily gain (ADG) was lowest ($p<0.05$) for sheep offered UT and the highest ($p<0.05$) was MO whilst AL and MT were intermediate but not different ($p>0.05$) as shown in Table 2. Sheep fed MO were most ($p<0.05$) efficient in terms of feed usage for weight gains but did not differ ($p>0.05$) from AL and MT whilst UT was the least ($p<0.05$) efficient (Table 2). Sheep fed MT emitted the lowest ($p<0.05$) enteric methane and those fed UT the highest ($p<0.05$) whilst enteric methane from MO was lower ($p<0.05$) than AL as in Table 2.

**Table 1:** Chemical Composition of the Experimental diets

| **Parameters (g/kg DM)** | **Diets** | | | |
|---|---|---|---|---|
| | **AL** | **MO** | **MT** | **UT** |
| DM(g/kg) | 899 | 870 | 894 | 917 |
| Ash | 74.1 | 159 | 110 | 200 |
| CP | 288 | 326 | 234 | 99.8 |
| CT | 1.35 | 1.03 | 1.10 | - |
| ADF | 346 | 198 | 411 | 521 |
| NDF | 455 | 195 | 537 | 552 |
| Cellulose | 163 | 94.5 | 241 | 381 |
| Lignin | 186 | 105 | 128 | 191 |

*AL- Albizzia lebbek; MO- Moringa oleifera; MT- Millettia thonningii; UT- Urea Treated Rice Straw*

The dried browse leaves were more efficient than UT (Table 2). The results for feed conversion of sheep fed AL, MO and MT were 60.47 %, 64.85 % and 48.76 % more efficient respectively than those fed UT. Sheep fed dried browse leaves gained more weight than those fed UT (control) as shown in Table 2. Sheep fed AL, MO and MT recorded 188 %, 256 % and 159 % more weight gain respectively than those fed UT. Sheep fed AL, MO and MT emitted 65.52 %, 70.6 % and 79.19 % less enteric methane production respectively than UT.

The range of values for faecal nitrogen (FN), urinal nitrogen (UN) and faecal energy (FE) of sheep fed sole browse leaves were 50.98 - 81.16 g/animal/day, 6.80 - 15.53 g/animal/day and 1.40 - 4.80 MJ/kg DM respectively (Table 2). Sheep fed MO recorded the lowest ($p<0.05$) FN and UN whilst those fed MT had the highest ($p<0.05$) of these nitrogen losses (Table 2). The highest ($p<0.05$) FE was recorded for sheep that consumed UT and the lowest ($p<0.05$) observed in sheep fed MO (Table 2).

Trends of DMI, weight gain and methane production of sheep fed solely with three dried browse leaves and UT for twelve weeks are presented in Figures 1, 2 and 3 respectively. Sheep fed AL, MO and MT had almost the same trend of dry matter intake for the first four weeks (Figure 1). Thereafter, they were lower than MO (Figure 1). Dry matter intake of UT was the highest except at the seventh and ninth weeks when it was similar with MO and thereafter had a similar trend as MO (Figure 1).

**Table 2:** Performance indices and Methane Production ($CH_4$) of Sheep Fed Dried Browse Leaves.

| Species | DMI (g/d) | EI (MJ/kg DM) | DE (MJ/kg DM) | ADG (g/d) | FCE | UN (g/ani./d) | FN (g/ani./d) | FE (MJ/kg DM) | $CH_4$ (MJ/d) |
|---|---|---|---|---|---|---|---|---|---|
| AL | 474.09$^a$ ±22.02 | 13.23$^a$ ±1.17 | 11.67$^a$ ±0.19 | 50.59$^b$ ±5.16 | 10.38$^a$ ±0.91 | 11.7$^b$ ±0.87 | 62.7$^b$ ±1.17 | 1.56$^b$ ±0.12 | 12.48$^c$ ±0.23 |
| MO | 584.33$^b$ ±17.18 | 14.50$^b$ ±0.10 | 13.10$^b$ ±0.10 | 68.46$^c$ ±1.72 | 9.23$^a$ ±0.35 | 6.80$^a$ ±1.05 | 51.0$^a$ ±0.52 | 1.40$^a$ ±0.23 | 10.63$^b$ ±0.34 |
| MT | 457.15$^a$ ±16.60 | 13.30$^a$ ±0.10 | 11.80$^a$ ±0.15 | 42.65$^b$ ±9.95 | 13.66$^a$ ±3.60 | 15.5$^c$ ±0.30 | 81.16$^c$ ±4.27 | 1.50$^b$ ±0.21 | 7.53$^a$ ±0.28 |
| UT (Control) | 644.81$^c$ ±7.26 | 16.17$^c$ ±0.7 | 11.30$^a$ ±0.18 | 26.79$^a$ ±3.84 | 26.26$^b$ ±4.23 | 10.6$^b$ ±0.09 | 66.35$^b$ ±0.89 | 4.80$^c$ ±0.20 | 36.19$^d$ ±0.77 |

*Means in the same column with different letters are significant ($p<0.05$)*
*DMI- Dry Matter Intake; EI- Energy Intake; DE- Digestible Energy; ADG- Average Daily Gain; FCE- Feed Conversion Efficiency; UN- Urinal Nitrogen; FN- Faecal Nitrogen; FE- Faecal Energy; $CH_4$ – Methane*

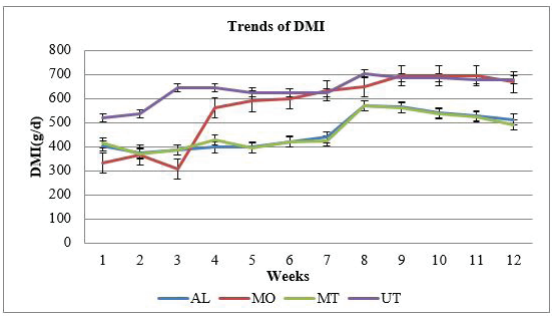


**Figure1:** Trends of Dry Matter Intake (DMI) of sheep fed dried *Albizzia lebbek* (AL), *Moringa oleifera* (MO), *Millettia thonningii* (MT) and urea treated rice straw (UT) for twelve weeks.

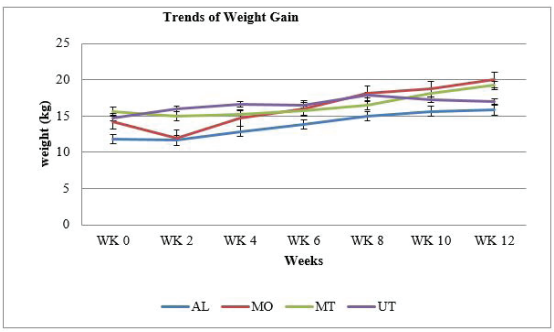


**Figure 2:** Trends of weight gain (kg) of sheep fed dried *Albizzia lebbek* (AL), *Moringa oleifera* (MO), *Millettia thonningii* (MT) and urea treated rice straw (UT) for twelve weeks.

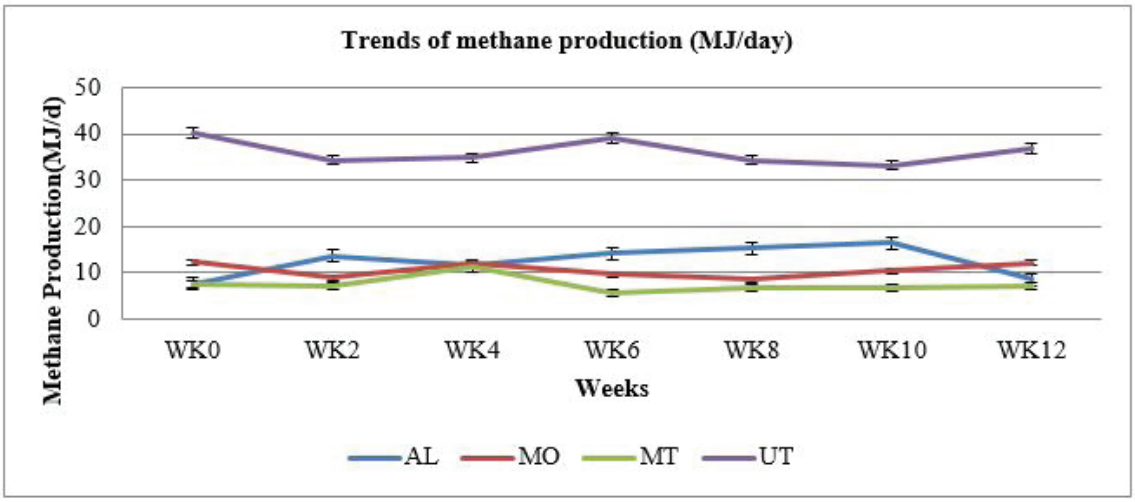


**Figure 3:** Trends of methane production from sheep fed dried *Albizia lebbek* (AL), *Moringa oleifera* (MO), *Millettia thonningii* (MT) and urea treated rice straw (UT) for twelve weeks.

**Table 3:** Relationships between Dry matter Intake (DMI), Digestible Energy (DE), Energy Intake (EI), Feed Conversion Efficiency (FCE), Average Daily Gain (ADG) and Methane Production for Individual Feeding

| Relationship | Equation | Regression |
|---|---|---|
| DMI &ADG for AL, MO, MT & UT. | Y= -0.05x+71.6 | 0.05 NS |
| DMI &ADG for AL,MO & MT | Y=0.19x- 41.2 | 0.97 * |
| DE & ADG for AL,MO, MT & UT | Y=20.5x- 198 | 0.84 * |
| DE & ADG for AL,MO & MT | Y=15.5x- 135 | 0.86* |
| EI & ADG for AL,MO, MT & UT | Y= -5.98x+133 | 0.22 NS |
| EI & ADG for AL,MO & MT | Y= 17.4x-184 | 0.88 * |
| FCE & ADG for AL,MO, MT & UT | Y= -2.01x+76.9 | 0.80 * |
| FCE & ADG for AL,MO & MT | Y= -5.08+110 | 0.78 * |
| DMI & Methane for AL,MO, MT & UT | Y=0.12x- 46.1 | 0.63 * |
| DMI & Methane for AL,MO & MT | Y=0.01x+ 5.37 | 0.07 NS |
| DE & Methane for AL,MO, MT & UT | Y= -9.27x+128 | 0.31 NS |
| DE & Methane for AL,MO & MT | Y=0.20x+ 7.81 | 0.0039 NS |
| EI & Methane for AL,MO, MT & UT | Y=8.61x- 106 | 0.81 * |
| EI & Methane for AL,MO & MT | Y= -9.27 x +128 | 0.31 NS |
| FCE &Methane for AL,MO, MT & UT | Y= 1.56x -6.55 | 0.86 * |
| FCE & Methane for AL,MO & MT | Y= -0.88x+76.9 | 0.65 * |
| ADG & Methane for AL,MO, MT & UT | Y= -0.55x+42.7 | 0.54 * |
| ADG & Methane for AL,MO & MT | Y=2.30x+30.4 | 0.19 NS |

** - Significant; NS- Not significant*

## Discussion

The current study recorded higher average daily gain (ADG) and feed conversion efficiency (FCE) in sheep fed *Albizzia lebbek* (AL) and *Moringa oleifera* (MO) than reported by Sarkwa (2008) when AL and MO were fed as supplements to a basal diet of rice straw. The weight gain of sheep (52.79 g/d) fed *Millettia thonningii* (MT) and sodium hydroxide treated rice straw reported by Fleischer *et al*. (2000) was higher than the value recorded (42.65 g/d) for MT in the present study. The variation between the value recorded in the present study and that reported by Fleischer *et al*. (2000) may have been due to a higher dry matter intake in the case of the latter study especially regarding the sodium hydroxide treated rice straw which was used as a basal diet. Yisehak *et al*. (2014) reported 14-35 g/d ADG when sheep were fed *Albizzia gummifera* (browse leaves) with or without polyethylene glycol which was lower than the 42.65-68.46 g/d recorded in the present study.

The predicted enteric methane production recorded in this study is in agreement with the report of Fan *et al*. (2006) that methane production from leguminous forages was lower than that from the grass Family. Factors that cause low methane production are modifications in the activity, density and population of protozoa and methane producing microbes (Animut *et al*., 2008; Mosoni *et al*., 2011). According to Bhatta *et al*. (2009), the combinations of condensed tannins (CT) and hydrolysable tannins have higher antiprotozoal effects than only hydrolysable tannins. The low methane production recorded in the present study may be due to the use of whole browse leaves in feeding as compared to the use of CT extract from browse leaves. This is because whole browse leaves may contain more than one substance such as CT, saponins and hydrolysable tannins that inhibits protozoa and methanogens activities in the rumen. The predicted enteric methane production from sheep fed UT was higher at the beginning of the study than towards the end of the 12 weeks which corresponded with an increase in DMI. This could be due to a subsequent increase in the intake (644.81 g/d) of UT by the sheep. This is in line with the findings that increased in intake caused a reduction in methane production (Hammond *et al*., 2011; Sun *et al*., 2012).

There was a negative relationship between digestibility and methane production (Sun *et al*., 2012) and this is confirmed by the current study regarding the relationship between DE and methane production for AL, MO, MT and UT feeds which was negative. However, the relationships between DE and methane production for AL, MO and MT were positive. The reason for the difference in the relationship involving all four feeds and only browse leaves is that methane production from sheep fed UT was higher than those fed solely on browse leaves and this influenced the relationship greatly. EI and methane production for AL, MO and MT had a negative relationship and this is in line with the report by Sun *et al*. (2012).

There were thus four observed desirable relationships. This is because ADG increased as DE increased, methane production decreased as DE increased, ADG increased as methane production decreased and FCE improved as methane production decreased. Regarding only browse leaves (AL, MO & MT), there were five desirable relationships. This is due to the fact that, DMI, DE and EI increased as ADG increased whilst EI and FCE increased as methane production reduced. These desirable relationships can be manipulated to improve weight gain and reduce methane production.

Assuming animals were fed UT and based on its methane production (36.19 MJ/d as recorded in the present study) as well as data from the Intergovernmental Panel on Climate Change (IPCC, 1996) on the percentage of methane produced by different animals, data from the Food and Agriculture Organisation of the United Nations (FAOSTAT Agricultural Data, 2013) and the country report on the population of ruminants in Ghana, it was estimated that the methane production of domestic ruminants per year in Ghana was 248,338,541,000 MJ/year with the following

breakdown: sheep (46,512,763,000 MJ/year), goats (58,063,018,000 MJ/year) and cattle (143,762,760,000 MJ/year).

Faecal nitrogen (FN) (3.84-8.37g/animal/day) and UN (3.16-6.17 g/animal/day) reported by Animut *et al*. (2008) were lower than the values of 50.98-88.16 g/animal/day for FN and 6.80-16.99 g/animal/day for UN recorded in the present study. However, the values of nitrogen losses recorded in the current study were lower than the values of 31.2-156 g/animal/day for FN and 191.2-349.9 g/animal/day for UN reported in sheep by Yeboah *et al*. (2017). It can be deduced that there were low to moderate nitrogen losses recorded in this study. The differences in the values of nitrogen losses by different authors may be due to the use of different kinds of ruminants and feed. The UN values were lower than the FN values and this may be due to the fact that the browse leaves fed contained condensed tannins which eliminated some protozoa and methanogens from the rumen and excreted them with the faeces. Acid detergent insoluble nitrogen (ADIN) was not determined. This could have given an indication of fibre-bound nitrogen because some heating of forages causes some of the nitrogen to become irreversibly bound in the fibre. It was expected by virtue of tannins in browse leaves, that excessive ammonia production in the rumen could be reduced thus decreasing UN. Hence, the physical and chemical characteristics of browse leaves may have contributed to the higher FN as compared to the UN. The range of FE recorded in this study (1.40-4.80 MJ/kg DM) was similar to the range of values reported by Sun *et al*. (2012).

## Conclusion

Sheep fed dried browse leaves emitted lower predicted enteric methane and had higher average daily gains (ADG) than sheep fed urea treated rice straw (UT). Sheep fed *Millettia thonningii* (MT) had the lowest predicted enteric methane emission and those fed *Moringa oleifera* (MO) had the highest ADG. Sheep fed *Albizzia lebbek* (AL), MO and MT emitted 65.52 %, 70.6 % and 79.19 % less predicted enteric methane than sheep fed UT. Sheep fed MO had the lowest faecal nitrogen (FN) and urinal nitrogen (UN) whilst those fed MT had the highest. Sheep fed dried browse leaves recorded lower faecal energy (FE) than sheep fed UT. The lower methane emission may contribute to minimise the effects of climate change.

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## Public Brief

Ruminants are major contributors of methane production in the atmosphere. Methane production is a result of digestion inefficiencies but diet composition can be manipulated to reduce methane output. This study seeks to evaluate intake, weight gain, digestible energy, energy and nitrogen losses and methane emission of sheep fed dried browse leaves. Some methane research works resulted in reduction in methane emission but also reduction in production. However, this work balanced reduction in methane production as well as improved weight gain of sheep to ensure sustainability of sheep production and the environment. The lower methane emission may contribute to minimise the effects of climate change. Therefore, dried leaves of *Albizzia lebbek*, *Moringa oleifera* and *Millettia thonningii* can be fed to ruminants to reduce methane emission and improve weight gain.

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# THE EFFECT OF SEASON AND DRYING ON THE CHEMICAL COMPOSITION OF FOUR BROWSE SPECIES.

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## Abstract

Condensed tannins are useful at certain concentrations but high levels are antinutritional. Drying has been shown to reduce the condensed tannin content in forages. The objective of this study was to determine the influence of six drying methods on the chemical composition and condensed tannin content of four browse leaves in the wet and dry seasons. Fresh leaves of *Albizzia lebbek* (AL), *Gliricidia sepium* (GS), *Moringa oleifera* (MO) and *Millettia thonningii* (MT) were collected monthly from the coastal savannah rangeland for three months in each season. The drying regimes were oven drying at 45, 60 and 65 ° C, shade drying for 72 hours and sun drying for 48 and 72 hours. The dry matter (DM), ash, crude protein (CP), fibre component and Condensed tannins (CT) were determined by standard methods. Data obtained were analysed as randomised complete block design and were subjected to analysis of variance using the GenStat statistical package. Interactions of species* season, species * drying methods, season * drying method and species * season* drying method were significant ($p<0.05$) for DM, ash, CP, CT, neutral detergent fibre (NDF), acid detergent fibre (ADF) and cellulose. The levels of DM, CT, ash, CP, NDF, ADF, cellulose and lignin were, 786.5 – 905.9 g/kg, 0.7-2.8 g/kg DM, 43.54-213.3 g/kg DM, 212.2-387.5g/kg DM, 76.9 - 593.3g/kg DM, 133.6-423.8g/kg DM, 67.4-266 g/kg DM and 41.9-229.6 g/kg DM respectively. The browse leaves were ranked based on the seasons, drying methods and the parameters were analysed as follows: first- MO; second-GS; third- AL; and fourth- MT. The differences between seasons in the parameters analysed were minimal and the nutritional quality did not fall short of the level for moderate production of ruminants. Therefore, these browse leaves can be used throughout the year in feeding ruminants.

**Key words:** Browse leaves, chemical composition, tannin, season, , Drying methods, Ruminants

# L'EFFET DE LA SAISON ET DU SÉCHAGE SUR LA COMPOSITION CHIMIQUE DE QUATRE ESPÈCES D'HERBACÉES

## Résumé

Les tanins condensés sont utiles à certaines concentrations, mais les niveaux élevés sont antinutritionnels. Il a été démontré que le séchage réduit le contenu en tanin condensé des fourrages. L'objectif de cette étude était de déterminer l'influence de six méthodes de séchage sur la composition chimique et le contenu en tanin condensé de quatre feuilles d'herbacées dans les saisons humides et sèches. Des feuilles fraîches d'*Albizzia lebbek* (AL), *Gliricidia sepium* (GS), *Moringa oleifera* (MO) et *Millettia thonningii* (MT) ont été récoltées chaque mois depuis la savane côtière pendant trois mois durant chaque saison. Les régimes de séchage étaient : le séchage au four à 45, 60 et 65° C, le séchage à l'ombre pendant 72 heures et le séchage solaire pendant 48 et 72 heures. Les matières sèches (DM : dry matter), les cendres, les protéines brutes (CP : crude protein), les composants de fibres et les tanins condensés (CT : condensed tannins) ont été déterminées au moyen de méthodes standard. Les données obtenues ont été analysées au moyen de

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dispositifs par blocs complets aléatoires et ont été soumises à une analyse de variance en utilisant le paquet statistique GenStat. Les interactions espèces * saison, espèces * méthodes de séchage, saison * méthode de séchage et espèces * saison* méthode de séchage ont été significatives ($p < 0,05$) pour les DM, les cendres, les CP, les CT, les fibres au détergent neutre (NDF), les fibres au détergent acide (ADF) et la cellulose. Les niveaux de DM, CT, cendres, CP, NDF, ADF, cellulose et lignine étaient respectivement de 786,5 – 905,9 g/kg, 0,7-2,8 g/kg DM, 43,54-213,3 g/kg DM, 212,2-387,5g/kg DM, 76,9 – 593,3g/kg DM, 133,6-423,8g/kg DM, 67,4-266 g/kg DM and 41,9-229,6 g/kg DM. Les feuilles d'herbacées ont été classées en fonction des saisons, des méthodes de séchage et des paramètres analysés comme suit : premier - MO ; deuxième - GS ; troisième - AL ; et quatrième - MT. Les différences entre les saisons dans les paramètres analysés étaient minimes et la qualité nutritionnelle n'était pas inférieure au niveau de production modérée de ruminants. En conséquence, ces feuilles d'herbacées peuvent être utilisées tout au long de l'année dans l'alimentation des ruminants.

**Mots-clés :** feuilles d'herbacées, composition chimique, tanin, saison, méthodes de séchage, ruminants

## Introduction

Browse leaves have the potential for solving feed shortages and poor nutrition experienced in the dry season (Sarkwa *et al.* 2011; Idan, 2014). However, the use of browse leaves is limited by deterring mechanisms related to the high content of tannin (Basha *et al.*, 2012). Ecological studies have indicated that the quality of browse leaves as feed for animals involve complex characteristics, which vary with season (Ukwanwoko *et al.*, 2013). Drying has been found to change the content of tannins and the fibre components in tanniniferous plants (Palmer *et al.*, 2000). Condensed tannin content varies with season (Scogings *et al.* 2013), plant species (Hattas and Julkenen-Titto, 2012), exposure to defoliation and environmental conditions (Kohi *et al.*, 2011) and developmental stage of tissue (Hattas *et al.*, 2011). Concentration of condensed tannin is a common measure in the study of condensed tannin-methane mitigation (Naumann *et al*, 2015).

The drying of browse leaves may improve the dry matter content, reduce condensed tannins levels and astringency thereby enhancing the potential of the browse leaves to be used as a sole diet. The drying also presents opportunities to conserve these browse leaves for use in unfavourable climatic conditions and in promoting intensive systems of keeping ruminants especially in urban areas at low or moderate cost. The objective of this study was to assess the chemical composition and condensed tannin levels in dried browse leaves and the influence of season on their levels.

## Materials and Methods

*Location*

The study was carried out at the Livestock and Poultry Research Centre (LIPREC), University of Ghana, Legon (5° 68'N, 0°10'W). The rainfall pattern is bimodal with the major rains in June while the minor rains are in September-October. The average annual rainfall is 881 mm (Adjorlolo *et al.*, 2014). Maximum and minimum temperatures vary between 32.90 °C and 24.30 °C (Sarkwa, 2008).

*Browse Species*

Fresh leaves of *Albizzia lebbek* (AL), *Gliricidia sepium* (GS), *Moringa oleifera* (MO), and *Millettia thonningii* (MT) were collected monthly for three months in the coastal savannah rangelands during the wet and dry seasons.

*Drying Methods*

Samples (12 kg) of each of the four browse species were collected monthly, divided into 6 parts (2 kg each) and subjected to six drying methods. The sample collections were done three times in each season. The samples were used to study the influence of drying on the levels of DM, CP, ash, fibre components and condensed tannins as described by Palmer *et al.* (2000). There were six treatments as follows: oven drying at 60 ° C (Control): normal drying

for analysis); oven drying at 45°C; oven drying at 65°C; shade drying for 72 hours; sun drying for 48 hours and sun drying for 72 hours. Proximate, fibre and condensed tannin analyses were carried out on the 144 samples (24 samples: 4 browse leaves X 6 drying methods; X 3 replicates/season: early, mid and late season; X 2 seasons). A thermohygrometer was used to measure the temperature and humidity during the drying of the samples.

*Chemical Analysis*

Dried browse leaves were ground to pass through a 1 mm sieve prior to chemical analysis. DM, Ash and CP were determined using the methods of A.O.A.C. (2016). Neutral detergent fibre (NDF), acid detergent fibre (ADF), cellulose and lignin analyses were carried out using the procedures of Goering and Van Soest (1970). Condensed tannins were analysed using the butanol-HCl method as described by Iqbal *et al.* (2011). All procedures and activities were approved by the Noguchi Institutional Animal Care and Use Committee of the University of Ghana, Legon (Protocol number 2017-02-2R).

*Statistical Analysis*

Data obtained was analysed as a randomised complete block design. The data was subjected to analysis of variance (ANOVA) using GenStat (2009) version 12.1 based on the model:

$$Yijkl = Si + Bj + Tk + SBTijk + eijkl$$

Where: Yijkl was the response variable such as DM, CT, Ash, CP, NDF, ADF, Cellulose and lignin; μ was the overall mean; Si was browse species; Bj was the seasonal effect (block); Tk was the different drying methods (Treatment); SBTijk was interactions; Eijkl was the residual error.

The effects of season and drying method were separated using Least Significant Difference.

## Results

The mean temperature and humidity recorded during the drying of the samples were 34.49°C and 73.73 % for the wet season and 32.22°C and 36.60 % for the dry season. Interactions of species*season, species*drying methods, season*drying method and species* season*drying method were significant ($p<0.05$) for DM, ash, CP, CT, neutral detergent fibre (NDF), acid detergent fibre (ADF) and cellulose. The range of DM, CT, ash, CP, NDF, ADF, cellulose and lignin were 786.5 – 905.9 g/kg (Table 1), 0.7-2.8 g/kg DM (Table 2), 43.54-213.3 g/kg DM (Table 3), 212.2-387.5g/kg DM (Table 4), 76.9 - 593.3g/kg DM (Table 5), 133.6-423.8g/kg DM (Table 6), 67.4-266 g/kg DM (Table 7) and 41.9-229.6 g/kg DM (Table 8) respectively. Wet season levels of CT were lower ($p<0.05$) than dry season values for both MO and MT (Table 2). Drying methods significantly influenced the composition of all components of AL harvested in the wet season (Table 2).

In general, sun drying treatments increased the ash content of the browse leaves (Table 3). Both the wet and dry season samples of MO decreased in CP content as the oven temperature increased (Table 4). Wet season samples of AL were higher ($p<0.05$) than dry season samples (Table 5). Wet season samples of AL and GS had majority of the values higher ($p<0.05$) than the dry season samples (Table 6). Increased oven drying temperatures increased the levels of cellulose for all the four browse leaves collected in the dry season (Table 7). Sun dried samples for AL and MT increased in the lignin content as the hours of sun drying increased whilst MO and GS had a contrary trend (Table 8).

Overall, the drying methods that recorded less nutritional qualities were oven drying at 65 °C and 72 hours sun drying. The best drying methods were oven drying at 45 °C, oven drying at 60 °C, 72 hours room drying and 48 hours sun drying. Browse leaves were ranked based on the seasons, drying methods and parameters that were analysed as follows: first- MO; second-GS; third- AL; and

**Table 1:** Seasonal Dry Matter Contents (g/kg) of Four Browse Species Subjected to Six Different Drying Methods

| **Drying Methods** | **Species** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *Albizzia lebbek* | | *Gliricidia sepium* | | *Moringa oleifera* | | *Millettia thonningii* | |
| | **Wet** | **Dry** | **Wet** | **Dry** | **Wet** | **Dry** | **Wet** | **Dry** |
| Oven Dried@45˚C | $905.3^{b}$ | $887.9^{a}$ | $882.1^{b}$ | $880.5^{a}$ | $786.5^{a}$ | $874.3^{b}$ | $893.9^{b}$ | $889.9^{a}$ |
| | E | C | F | E | A | C | E | E |
| Oven Dried @60˚C | $905.9^{b}$ | $889.3^{a}$ | $879.8^{b}$ | $876.8^{a}$ | $888.9^{b}$ | $875.8^{a}$ | $893.2^{b}$ | $891.9^{a}$ |
| | F | D | E | D | E | D | D | F |
| Oven Dried @65˚C | $902.0^{b}$ | $885.8^{a}$ | $878.0^{b}$ | $866.3^{a}$ | $894.8^{b}$ | $871.8^{a}$ | $892.1^{b}$ | $876^{a}$ |
| | D | B | D | C | F | B | C | B |
| 72 hours Sun Dried | $892.7^{b}$ | $867.5^{a}$ | $868.8^{b}$ | $819.1^{a}$ | $832.5^{a}$ | $879.9^{b}$ | $877.7^{a}$ | $884.1^{b}$ |
| | C | A | C | A | C | E | B | D |
| 48 hours Sun Dried | $872.1^{a}$ | $889.5^{b}$ | $834.2^{a}$ | $858.3^{b}$ | $838^{a}$ | $867.4^{b}$ | $876.9^{a}$ | $879.1^{b}$ |
| | B | D | B | B | D | A | B | C |
| Overall Mean of Species | $889.1^{d}$ | | $858.4^{a}$ | | $858.9^{b}$ | | $883^{c}$ | |

*Means with different capital letters in the same column are significantly different (p<0.05). Means with different superscripts under the same species within a column are significantly different (p<0.05). Means with different superscripts in the same row are significantly different (p<0.05).*

**Table 2:** Seasonal Condensed Tannin Contents (g/kg DM) of Four Browse Species Subjected to Six Different Drying Methods.

| **Drying Methods** | **Species** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *Albizzia lebbek* | | *Gliricidia sepium* | | *Moringa oleifera* | | *Millettia thonningii* | |
| | **Wet** | **Dry** | **Wet** | **Dry** | **Wet** | **Dry** | **Wet** | **Dry** |
| Oven Dried@45˚C | $1.3^{b}$ | $1.1^{a}$ | $0.9^{a}$ | $1.1^{b}$ | $1.0^{a}$ | $1.7^{b}$ | $0.9^{a}$ | $1.1^{b}$ |
| | C | C | C | C | B | C | C | A |
| Oven Dried @60˚C | $1.2^{b}$ | $1.1^{a}$ | $0.9^{a}$ | $0.9^{a}$ | $1.1^{a}$ | $1.4^{b}$ | $0.7^{a}$ | $1.1^{b}$ |
| | B | C | C | B | C | B | A | A |
| Oven Dried @65˚C | $1.5^{b}$ | $1.4^{a}$ | $0.8^{b}$ | $0.7^{a}$ | $1.1^{a}$ | $1.2^{b}$ | $0.8^{a}$ | $1.1^{b}$ |
| | D | D | B | A | C | A | B | A |
| 72 hours Sun Dried | $1.1^{a}$ | $1.5^{b}$ | $0.7^{a}$ | $0.9^{b}$ | $1.2^{a}$ | $2.8^{b}$ | $0.9^{a}$ | $1.3^{b}$ |
| | A | E | A | B | D | E | C | C |
| 48 hours Sun Dried | $1.9^{b}$ | $0.8^{a}$ | $1.0^{b}$ | $0.7^{a}$ | $0.9^{a}$ | $1.4^{b}$ | $1.1^{a}$ | $1.2^{b}$ |
| | E | A | D | A | A | B | D | B |
| 72 hours Room Dried | $2.5^{b}$ | $1.0^{a}$ | $1.0^{a}$ | $1.0^{a}$ | $1.0^{a}$ | $1.8^{b}$ | $0.8^{a}$ | $1.1^{b}$ |
| | F | B | D | C | B | D | B | A |
| Overall mean of species | $1.37^{a}$ | | $0.88^{a}$ | | $1.38^{d}$ | | $1.01^{b}$ | |

*Means with different capital letters in the same column are significantly different (p<0.05). Means with different superscripts under the same species within a column are significantly different (p<0.05). Means with different superscript in the same row are significantly different (p<0.05).*

**Table 3:** Seasonal Ash Contents (g/kg DM) of Four Browse Species Subjected to Six Different Drying Methods

| **Drying Methods** | **Species** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *Albizzia lebbek* | | *Gliricidia sepium* | | *Moringa oleifera* | | *Millettia thonningii* | |
| | **Wet** | **Dry** | **Wet** | **Dry** | **Wet** | **Dry** | **Wet** | **Dry** |
| Oven Dried@45˚C | $64.9^{b}$ | $45.6^{a}$ | $107.1^{b}$ | $99.0^{a}$ | $168.8^{b}$ | $137.9^{a}$ | $107.8^{a}$ | $109.9^{b}$ |
| | A | B | F | C | E | C | C | C |
| Oven Dried @60˚C | $64.9^{b}$ | $45.6^{a}$ | $107.1^{b}$ | $99.0^{a}$ | $168.8^{b}$ | $137.9^{a}$ | $107.8^{a}$ | $109.9^{b}$ |
| | B | B | B | B | D | B | D | B |
| Oven Dried @65˚C | $69.3^{b}$ | $43.5^{a}$ | $91.2^{a}$ | $95.7^{b}$ | $129.7^{a}$ | $133.9^{b}$ | $109.5^{b}$ | $105.2^{a}$ |
| | C | A | A | A | A | A | E | A |
| 72 hours Sun Dried | $109.7^{b}$ | $68.3^{a}$ | $117.9^{a}$ | $119.2^{b}$ | $170.9^{a}$ | $213.3^{b}$ | $120.5^{a}$ | $129.5^{b}$ |
| | F | E | E | F | E | F | E | E |
| 48 hours Sun Dried | $83.8^{b}$ | $65.5^{a}$ | $115.4^{b}$ | $112.0^{a}$ | $143.4^{a}$ | $171.4^{b}$ | $94.2^{a}$ | $125.0^{b}$ |
| | E | D | D | D | C | E | B | D |
| 72 hours Room Dried | $78.2^{b}$ | $55.9^{a}$ | $112.8^{a}$ | $115.5^{b}$ | $140.8^{a}$ | $153.3^{b}$ | $92.7^{a}$ | $124.8^{b}$ |
| | D | C | C | E | B | D | A | D |
| Overall mean of species | $65.4^{a}$ | | $109.1^{b}$ | | $15.7^{d}$ | | $111.2^{c}$ | |

*Means with different capital letters in the same column are significantly different (p<0.05). Means with different superscripts under the same species within a column are significantly different (p<0.05). Means with different superscript in the same row are significantly different (p<0.05).*

**Table 4:** Seasonal Crude Protein Contents (g/kg DM) of Four Browse Species Subjected to Six Different Drying Methods

| **Drying Methods** | **Species** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *Albizzia lebbek* | | *Gliricidia sepium* | | *Moringa oleifera* | | *Millettia thonningii* | |
| | **Wet** | **Dry** | **Wet** | **Dry** | **Wet** | **Dry** | **Wet** | **Dry** |
| Oven Dried@45˚C | $248.8^{a}$ | $251.6^{b}$ | $289.4^{b}$ | $233.0^{a}$ | $354.2^{b}$ | $294.8^{a}$ | $233.2^{b}$ | $217.4^{a}$ |
| | A | A | C | A | C | E | C | B |
| Oven Dried @60˚C | $255.5^{a}$ | $291.6^{b}$ | $282.7^{b}$ | $251.8^{a}$ | $322.8^{b}$ | $282.1^{a}$ | $225.1^{b}$ | $206.1^{a}$ |
| | B | D | B | C | B | C | A | A |
| Oven Dried @65˚C | $255.8^{a}$ | $291^{b}$ | $275^{b}$ | $270.5^{a}$ | $314.9^{b}$ | $212.2^{a}$ | $225.6^{b}$ | $217.9^{a}$ |
| | B | C | A | E | A | A | B | C |
| 72 hours Sun Dried | $291.8^{b}$ | $276.6^{a}$ | $351.2^{b}$ | $249.1^{a}$ | $368.6^{b}$ | $293.6^{a}$ | $237.6^{b}$ | $219.9^{a}$ |
| | E | B | E | B | E | D | D | E |
| 48 hours Sun Dried | $280.3^{a}$ | $293.1^{b}$ | $312.4^{b}$ | $264^{a}$ | $364.4^{b}$ | $294.9^{a}$ | $249.3^{b}$ | $218.5^{a}$ |
| | C | E | D | D | D | E | F | D |
| 72 hours Room Dried | $285.1^{a}$ | $299.4^{b}$ | $363.3^{b}$ | $273.2^{a}$ | $387.5^{b}$ | $260.7^{a}$ | $242.5^{b}$ | $225.1^{a}$ |
| | D | F | F | F | F | B | E | F |
| Overall mean of species | $276.8^{b}$ | | $284.3^{c}$ | | $312.6^{d}$ | | $226.6^{a}$ | |

*Means with different capital letters in the same column are significantly different (p<0.05). Means with different superscripts under the same species within a column are significantly different (p<0.05). Means with different superscript in the same row are significantly different (p<0.05).*

**Table 5:** Seasonal NDF Contents of Four Browse Species Subjected to Six Different Drying Methods (g/kg DM)

| **Drying Methods** | **Species** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *Albizzia lebbek* | | *Gliricidia sepium* | | *Moringa oleifera* | | *Millettia thonningii* | |
| | **Wet** | **Dry** | **Wet** | **Dry** | **Wet** | **Dry** | **Wet** | **Dry** |
| Oven Dried@45˚C | 502.1$^{b}$ | 476$^{a}$ | 341.3$^{a}$ | 360.5$^{b}$ | 76.9$^{a}$ | 125.5b | 548.6b | 515.2$^{a}$ |
| | E | F | D | E | A | A | D | B |
| Oven Dried @60˚C | 439.4$^{b}$ | 406.7$^{a}$ | 319.3$^{b}$ | 302.6$^{a}$ | 105.9$^{a}$ | 163.6b | 530b | 525$^{a}$ |
| | A | A | B | B | B | B | C | D |
| Oven Dried @65˚C | 467.3$^{b}$ | 440.6$^{a}$ | 302.3$^{a}$ | 410.4$^{b}$ | 130.1$^{a}$ | 293.3b | 508.9$^{a}$ | 585.8$^{b}$ |
| | C | D | A | F | C | E | B | F |
| 72 hours Sun Dried | 449$^{b}$ | 426.8$^{a}$ | 385.8$^{b}$ | 290.1a | 216.2$^{b}$ | 167.1$^{a}$ | 490.2$^{a}$ | 516$^{b}$ |
| | B | C | E | A | D | C | A | C |
| 48 hours Sun Dried | 482.5$^{b}$ | 423.8$^{a}$ | 336.5$^{b}$ | 307.1a | 237.2$^{b}$ | 163.6$^{a}$ | 555.1$^{b}$ | 508.9$^{a}$ |
| | D | B | C | C | E | B | E | A |
| 72 hours Room Dried | 509.3$^{b}$ | 470.5$^{a}$ | 494.2$^{b}$ | 333.6a | 305.3$^{b}$ | 186.1$^{a}$ | 593.3$^{b}$ | 556.3$^{a}$ |
| | F | E | F | D | F | D | D | E |
| Overall mean of species | 457.8$^{c}$ | | 348.6$^{b}$ | | 180.9$^{a}$ | | 536.1$^{d}$ | |

*Means with different capital letters in the same column are significantly different (p<0.05). Means with different superscripts under the same species within a column are significantly different (p<0.05). Means with different superscript in the same row are significantly different (p<0.05).*

**Table 6:** Seasonal ADF (g/kg DM) of Four Browse Species Subjected to Six Different Drying Methods

| **Drying Methods** | **Species** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *Albizzia lebbek* | | *Gliricidia sepium* | | *Moringa oleifera* | | *Millettia thonningii* | |
| | **Wet** | **Dry** | **Wet** | **Dry** | **Wet** | **Dry** | **Wet** | **Dry** |
| Oven Dried@45˚C | 334.6$^{b}$ | 307.1$^{a}$ | 244.1$^{b}$ | 238.2$^{a}$ | 171.8$^{a}$ | 176.2$^{b}$ | 362.5$^{a}$ | 383.8$^{b}$ |
| | A | A | A | A | B | B | A | A |
| Oven Dried @60˚C | 340.1$^{b}$ | 315.9$^{a}$ | 246.1$^{a}$ | 261.7$^{b}$ | 149.8$^{a}$ | 188.4$^{b}$ | 376.7$^{a}$ | 379.7$^{b}$ |
| | A | B | A | B | A | C | B | A |
| Oven Dried @65˚C | 386.2$^{b}$ | 343.4$^{a}$ | 266.1$^{b}$ | 264.1$^{a}$ | 148.8$^{a}$ | 219.7$^{b}$ | 397.6$^{b}$ | 383.2$^{a}$ |
| | D | C | B | B | A | D | C | A |
| 72 hours Sun Dried | 343.9$^{b}$ | 318.0$^{a}$ | 300.6$^{b}$ | 266.3$^{a}$ | 211.2$^{b}$ | 178.6$^{a}$ | 399.8$^{a}$ | 423.8$^{b}$ |
| | A | B | C | B | C | B | C | B |
| 48 hours Sun Dried | 351.7$^{a}$ | 354.7$^{b}$ | 262.6$^{b}$ | 255.8$^{a}$ | 209.3$^{b}$ | 193.8$^{a}$ | 407.1$^{b}$ | 389.5$^{a}$ |
| | B | E | B | B | C | C | D | A |
| 72 hours Room Dried | 361.4$^{b}$ | 353.4$^{a}$ | 298.2$^{a}$ | 300.2$^{b}$ | 176.5$^{b}$ | 133.6$^{a}$ | 410.6$^{b}$ | 395.3$^{a}$ |
| | C | D | C | C | B | A | D | A |
| Overall mean of species | 342.6$^{c}$ | | 264$^{b}$ | | 179.8$^{a}$ | | 392.5$^{d}$ | |

*Means with different capital letters in the same column are significantly different (p<0.05). Means with different superscripts under the same species within a column are significantly different (p<0.05). Means with different superscript in the same row are significantly different (p<0.05).*

**Table 7:** Seasonal Cellulose Contents (g/kg DM) of Four Browse Species Subjected to Six Different Drying Methods

| **Drying Methods** | **Species** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *Albizzia lebbek* | | *Gliricidia sepium* | | *Moringa oleifera* | | *Millettia thonningii* | |
| | **Wet** | **Dry** | **Wet** | **Dry** | **Wet** | **Dry** | **Wet** | **Dry** |
| Oven Dried@45˚C | $165.7^{b}$ | $148.6^{a}$ | $128.5^{b}$ | $105.6^{a}$ | $82^{a}$ | $90.2^{b}$ | $215.9^{b}$ | $176.6^{a}$ |
| | D | B | D | B | A | B | A | A |
| Oven Dried @60˚C | $116.9^{a}$ | $154.2^{b}$ | $104.8^{a}$ | $107^{a}$ | $105.3^{a}$ | $107.1^{b}$ | $227.8^{b}$ | $196.1^{a}$ |
| | A | B | B | B | C | C | B | B |
| Oven Dried @65˚C | $195.5^{b}$ | $174.8^{a}$ | $96.7^{a}$ | $130.3^{b}$ | $95.2^{a}$ | $146.9^{b}$ | $243.5^{b}$ | $221.9^{a}$ |
| | E | D | A | C | B | D | C | D |
| 72 hours Sun Dried | $145.1^{b}$ | $127.6^{a}$ | $183.1^{b}$ | $74.8^{a}$ | $132.1^{b}$ | $81.9^{a}$ | $244.2^{b}$ | $218.9^{a}$ |
| | C | A | F | A | D | B | C | D |
| 48 hours Sun Dried | $132.8^{a}$ | $179.7^{b}$ | $150.5^{b}$ | $80.1^{a}$ | $127.1^{b}$ | $67.4^{a}$ | $262.7^{b}$ | $206.5^{a}$ |
| | B | D | E | A | D | A | D | C |
| 72 hours Room Dried | $141.1^{a}$ | $166.5^{b}$ | $113.0^{b}$ | $101.8^{a}$ | $102.4^{a}$ | $146.7^{b}$ | $266.0^{b}$ | $199.1^{a}$ |
| | C | C | C | B | C | D | D | B |
| Overall mean of species | $154.1^{c}$ | | $114.6^{b}$ | | $107.1^{a}$ | | $223.3^{d}$ | |

*Means with different capital letters in the same column are significantly different (p<0.05). Means with different superscripts under the same species within a column are significantly different (p<0.05). Means with different superscript in the same row are significantly different (p<0.05).*

**Table 8:** Seasonal Lignin Contents (g/kg DM) of Four Browse Species Subjected to Six Different Drying Methods

| **Drying Methods** | **Species** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *Albizzia lebbek* | | *Gliricidia sepium* | | *Moringa oleifera* | | *Millettia thonningii* | |
| | **Wet** | **Dry** | **Wet** | **Dry** | **Wet** | **Dry** | **Wet** | **Dry** |
| Oven Dried@45˚C | $222.2^{b}$ | $179.7^{a}$ | $117.4^{a}$ | $138.1^{b}$ | $47.3^{a}$ | $73.4^{b}$ | $142.9^{a}$ | $142.9^{a}$ |
| | B | B | B | B | A | A | A | B |
| Oven Dried @60˚C | $227.4^{b}$ | $164.0^{a}$ | $144.4^{a}$ | $135.8^{a}$ | $41.9^{a}$ | $68.6^{b}$ | $150.0^{a}$ | $156.8^{b}$ |
| | B | A | C | B | A | A | A | B |
| Oven Dried @65˚C | $181.9^{b}$ | $177.9^{a}$ | $160.8^{b}$ | $132.3^{a}$ | $56.2^{a}$ | $68.8^{b}$ | $148.0^{a}$ | $161.8^{b}$ |
| | A | A | D | B | B | A | A | B |
| 72 hours Sun Dried | $177.1^{a}$ | $229.6^{b}$ | $143.8^{b}$ | $65.8^{a}$ | $62.5^{a}$ | $69.1^{b}$ | $149.3^{a}$ | $154.1^{b}$ |
| | A | C | C | A | B | A | A | B |
| 48 hours Sun Dried | $228.9^{b}$ | $153.5^{a}$ | $106.1^{a}$ | $172.7^{b}$ | $77.1^{a}$ | $132.6^{b}$ | $140.3^{b}$ | $135.0^{a}$ |
| | B | A | A | C | C | C | A | A |
| 72 hours Room Dried | $221.0^{b}$ | $158.5^{a}$ | $150.9^{b}$ | $131.1^{a}$ | $61.6^{a}$ | $121.0^{b}$ | $138.7^{a}$ | $147.3^{b}$ |
| | B | A | B | B | B | B | A | B |
| Overall mean of species | $193.5^{d}$ | | 133.3b | | 73.4a | | $147.3^{c}$ | |

*Means with different capital letters in the same column are significantly different (p<0.05). Means with different superscripts under the same species within a column are significantly different (p<0.05). Means with different superscript in the same row are significantly different (p<0.05).*

fourth- MT. Seasonal effects revealed that the dry season samples had superior nutritional qualities than the wet season samples in the case of GS and MT. Similar superior nutritional qualities were observed for AL and MO for both seasons. With regards to the effects of season on the parameters analysed, DM and CP had superior nutritional qualities during the wet season whilst the rest of the parameters were enhanced in the dry season with the exception of lignin which had similar qualities for both seasons.

## Discussion

With a moisture content of above 600 g/kg, there may be low dry matter intake and consequently reduced total nutrient supply and animal productivity (Crowder and Chheda, 1982). The dried browse leaves contained 94.10 – 213.5 g/kg moisture which is far less than 600 g/kg. The higher DM could imply that higher nutrients will be consumed by ruminants and may increase growth when consumed. According to Animut *et al.* (2008) methane emission could be reduced without any negative influence on protein digestion by feeding relatively low CT diets. Low CT will not have any adverse influence on digestion (Mueller-Harvey, 2006). The differences in the levels of CT obtained could be due to the age of browse species, physiological stage of the browse species at time of harvesting, postharvest procedures, environmental conditions before and after harvesting, assay techniques and chemical reactions employed in the methods. Different drying methods have been reported to influence the CT levels of legumes (Mahyuddin *et al.*, 1988) and this is in line with the results of this study. A condensed tannin content lower than 40 g/kg DM in the diet has the beneficial influence which is facilitated by forming complexes with protein and tannin in the rumen but the complex is broken down post-ruminally to enhance availability of protein in the feed to be used for production purposes (McSweeney *et al.*, 2001). The four browse leaves used in the current study may have the potential to lessen methane production in ruminants because they contain tannins. Additionally, when these browse leaves are fed to ruminants, they could supply by-pass protein in the rumen but in the small intestines the protein could be digested making amino acids available to the ruminant to enhance productivity. Low levels of CT have the potential to improve the synthesis of microbial protein by influencing fermentation in the rumen (Makkar, 2003). It is likely that the dried browse leaves when fed to ruminants may have the ability to increase microbial protein synthesis and there may be good digestibility because of their low levels of CT.

Wet season samples were in most cases higher in CP values than the dry season samples and this might be due to regrowth of new leaves during the wet season. The CP values were higher than the minimum level of 110 -120 g /kg DM considered as sufficient for moderate ruminants production (ARC, 1980). Also, the CP levels obtained were far higher than the threshold of 60 g/kg DM requirement for rumen microbes to support the host metabolic functions (Van Soest, 1994). Therefore, regardless of the season and the drying method used, the browse leaves used in the current study can be fed to ruminants throughout the year to obtain good weight gains. The high CP levels of the browse leaves used in this study may be an indication that they have high digestibility and hence the potential to lessen methane production and as supplements. The ash values obtained (43.54 - 213.3g/kg DM) exceeded the minimum reported by McDonald *et al.* (2002) as the recommended daily requirement of minerals (37.3 g/kg DM) for sheep. Hence, the dried browse leaves may satisfy the mineral requirements of sheep when fed.

The majority of the NDF values recorded for the wet season samples in this study were higher than the dry season values and this is similar to the findings by Arigbede *et al.* (2011). The range of NDF content recorded (76.9-593.3 g/kg DM) was lower than the 650 g/kg DM recommended as the threshold above which there will be a limitation on the intake of tropical forages by ruminants (Van Soest,

1994). Therefore, it is likely that there may be high intake of these dried browse leaves when fed to ruminants. Higher NDF levels result in increases in the production of methane by influencing short chain fatty acid proportions towards acetate formation which produces more hydrogen (Jayanegara *et al*., 2009). Forages containing tannins with low NDF levels between 200-350 g/kg DM have been shown to enhance high nutrients digestibility (Yisehak *et al*., 2014). High ADF and NDF causes low digestibility (Jin *et al*., 2012). Hence, the low to moderate ADF and NDF values recorded may imply that there may be moderate to high digestibility and low to moderate methane production when these dried browse leaves are fed to ruminants. Lignin prevents access of enzymes from microbes to the cell wall structural polysaccharides and is considered as a fraction that is not digested (Van Soest, 1994). The low lignin content of the dried browse leaves used in this study implied that, it may have high digestibility and low ability to inhibit the access of enzymes to the cell wall structural polysaccharides. Hence, feeding the dried browse leaves to ruminants may contribute to increased weight gain and result in low emission of methane.

## Conclusion

The best drying methods were oven drying at 45 and 60°C, 72 hours room drying and 48 hours sun drying. Overall, considering the parameters analysed, the effects of season and drying, MO, GS, AL and MT were ranked as first, second, third and fourth respectively. Dry season samples were superior to the wet season samples in nutritional quality. However, the difference between both seasons in the parameters analysed were minimal and the nutritional quality of both seasons did not fall short of the recommended levels for moderate production of ruminants. Therefore, these browse leaves may be used throughout the year in feeding ruminants. In general, the high DM, CP and ash levels, low CT levels and low to moderate fibre components recorded in this study may imply that regardless of season and drying method, feeding these dried browse leaves to ruminants may enhance digestibility and efficient protein utilisation which may contribute to weight gain and have the potential to reduce methane production.

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## Public Brief

Condensed tannins are useful at certain concentrations but high levels are antinutritional. Drying has shown to reduce condensed tannin content in forages. The objective of this study was to determine the influence of six drying methods on chemical composition and condensed tannin levels in four browse leaves in the wet and dry seasons. The browse leaves were ranked based on seasons, drying methods and parameters analysed as follows: first- *Moringa oleifera*; second-*Gliricidia sepium*; third- *Albizzia lebbek*; and fourth- *Millettia thonningii*. The difference between seasons in the parameters analysed were minimal and the nutritional quality did not fall short of the level for moderate production of ruminants. Therefore, these browse leaves can be used throughout the year in feeding ruminants. The drying of the browse leaves provides a way of conserving these leaves to be used during periods of bad climatic conditions and therefore, promoting intensive system of keeping small ruminants especially in urban areas.

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# SIGNATURES OF GENOMIC SELECTION AND PIG BREEDING - A REVIEW

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## Abstract

Domestication followed by selective breeding has resulted in hundreds of extant pig breeds worldwide. Traditional selection methods are based on phenotypic trait measurements and the breeding of animals which are adaptive and superior in desirable attributes. In terms of economically important production and reproduction traits, most local breeds fail to match the known commercial breeds, but they remain highly adapted to their environments in terms of disease resistance, survivability on poor nutrition and climate change resilience. These adaptations are valuable sources of genetic material for commercial breed improvement programmes. Globally, only a few selected breeds are used in commercial production systems, on account of their highly desirable productive attributes. As a result of the high demand for animal protein arising in part from the ever-growing human population, there is a need for strategic innovative technologies to sustain increased livestock production. Such innovative approaches beyond traditional selection include the use of genomic approaches to understand the unique alleles for adaptation, resilience and production and should ensure the sustainable use of animal genetic resources. This review brings to the fore some of the documented signatures of the selection of adaptive and productive traits which can be targeted in future breeding programmes. Such information is valuable in developing appropriate swine genetic resources for various production systems and agro-ecologies particularly in developing countries. It is hoped that this will also guide pig breeders to include resilience in their breeding goals in the face of changing climates and the attendant negative effects on the production, welfare and survivability of livestock breeds.

**Keywords:** adaptive traits· candidate genes·, fixation index·, genomics·, haplotype diversity

# EXAMEN DES SIGNATURES DE LA SÉLECTION GÉNOMIQUE ET L'ÉLEVAGE DE PORCS

## Résumé

La domestication suivie d'un élevage sélectif a donné lieu à des centaines de races de porcs qu'on retrouve partout dans le monde. Les méthodes traditionnelles de sélection sont basées sur des mesures de traits phénotypiques et l'élevage d'animaux ayant les attributs adaptatifs et supérieurs souhaitables. En termes de caractéristiques de production et de reproduction d'importance économique, la plupart des races locales ne correspondent pas aux races commerciales connues, mais elles restent très adaptées à leur environnement en termes de résistance aux maladies, de survie sur une mauvaise alimentation et de résilience au changement climatique. Ces adaptations sont de précieuses sources de matériel génétique pour les programmes d'amélioration des races commerciales. À l'échelle mondiale, seules quelques races sélectionnées sont utilisées dans les systèmes de production commerciale, en raison de leurs attributs de production hautement souhaitables. En raison de la forte demande de protéines animales provenant en partie de la population humaine toujours croissante, il est nécessaire de mettre en place des technologies stratégiques innovantes pour soutenir une production animale accrue. Ces approches novatrices au-delà de la sélection traditionnelle comprennent l'utilisation d'approches génomiques pour comprendre les allèles uniques pour l'adaptation, la résilience et la production et devraient assurer l'utilisation durable des ressources zoogénétiques. Cet examen met en évidence certaines signatures documentées de la sélection des caractères adaptatifs et productifs qui peuvent être ciblés dans les futurs programmes d'élevage. Ces informations sont précieuses car elles permettent de mettre au point des ressources génétiques appropriées

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pour divers systèmes de production et agro-écologies, en particulier dans les pays en développement. On espère que ces résultats guideront également les éleveurs de porcs à inclure la résilience dans leurs objectifs d'élevage face à l'évolution du climat et ses effets négatifs sur la production, le bien-être et la survie des races animales.

**Mots clés :** Caractéristiques d'adaptation · gènes candidats ·, indice de fixation ·, génomique ·, diversité des haplotypes

## Introduction

Changes in consumption patterns and demand for food (including meat and meat products) have been increasing as a result of high human population, rising income levels and rapid urbanization (Henchion *et al*., 2017). This is expected to worsen with the projected demand for animal protein expected to increase by 70% to feed an estimated human population of 9.6 billion in 2050 (UN, 2017). A greater proportion of this growth (about 8 billion) is expected in low to middle income countries: in sub-Saharan Africa alone, the growth rate is estimated to be at 1.2% per year (Capper, 2013). This situation calls for the strategic implementation of modern farming technologies to increase productivity and to ensure the future sustainability of animal production on the African continent (FAO, 2018). Fast-growing species with efficient feed conversion rates, such as poultry and pigs, will be good alternatives to cater for this high, estimated demand of animal protein (Rosegrant *et al*., 2009). These species are therefore likely to account for a major share in the growth of the livestock subsector (Mekuriaw and Asmare, 2014). The domestic pig is an important source of protein worldwide (Wilkinson *et al*., 2013) and pork is the most consumed meat worldwide accounting for over 36% of all global meat intake (Quan *et al*., 2017). Additionally, the pig has some unique advantages over other livestock, which makes it a suitable species for rapid multiplication and production. For instance, its relatively faster growth rate, superior prolificacy, efficiency of feed utilization, and high carcass quality are well known (Ogunniyi and Omoteso, 2011). Development of sustainable pig breeding programmes in the future will be greatly enhanced if identified selection signatures of economically relevant and adaptive traits are strategically explored. These will enhance the ability to target candidate genes for improvement in future progeny and guide pig improvement programmes in general. Therefore, the objective of this review was to document selection signatures of various traits of economic importance in pigs and posit them as important tools in future breeding initiatives.

## Molecular innovations in pig breeding

A common objective of animal breeding is to select superior animals to be parents of the next generation. Livestock breeds have over the millennia experienced intense selection pressures following their domestication for the development of desirable traits. This has led to a large diversity of livestock breeds that display variation in many production and reproduction traits (Wilkinson *et al*., 2013). In the pig industry, there are a number of traits that represent the most economically important phenotypes including meat quality, feed conversion rate, reproductive traits and disease resistance. Many of these have been improved through traditional selection over the years. Although most local pig breeds fail to match the best of the foreign breeds, they remain highly adapted to their environments in terms of disease resistance, survivability on poor nutrition and resilience to climate variability. These adaptations indicate that local pigs harbour very valuable genes which may be useful in future commercial breed improvement programmes. Globally, only a few selected breeds are in use by commercial breeding organisations, chosen for their highly desirable productive attributes. The ever-growing human population with its consequent demand for animal protein calls for the strategic implementation of modern

farming technologies to ensure sustainable use and conservation of local animal genetic resources worldwide (Schrijve *et al*., 2016).

The potential use of molecular techniques in pursuing this agenda is enormous. For instance, extensive application of genomic or whole-genome selection including gene screening has helped to control Porcine Stress Syndrome (PSS) from Blue Reese, Yorkshire sows in Taiwan as maternal line breeds and this has contributed to a high breeding rate and improvement in economic losses (VAHCL,2017). In North America, gene editing techniques have been used to control the porcine reproductive and respiratory syndrome in addition to identifying piglet genome contributions from each parent (Boyd *et al*., 2019). The introduction of marker assisted selection (MAS) has also been beneficial and helped in detecting genes responsible for genetic disorders, disease resistance and product quality, improvement in longevity, stress resistance, or the desired behavioural characteristics of the animals (Wakchaure *et al*., 2015). Unlike traditional breeding where progeny testing takes a very long time MAS reduces the time spent and the resources needed through early selection of potential breeding animals. Furthermore, MAS has enabled traits which have undergone genetic modification to be properly mapped and characterized.

Research in quantitative trait loci (QTLs) and the underlying causative mutations in pig breeding have improved over the last two decades. Genome-wide association studies (GWAS) have been widely used in the genetic dissection of complex traits (Blaj *et al*., 2018) in various livestock species. For example, it has been applied to investigate the genetic architecture of swine reproductive loss (Wu *et al*., 2019). These researchers used Genotype by Sequencing (GBS) data to identify genetic regions affecting the number of mummified (NM) and stillborn (NS) in Large White and Landrace pigs and indicated the candidate genes of interest (Table 1).

**Table 1:** Candidate genes responsible for NM and NS in pigs

| Trait (reproductive loss) | Candidate genes identified |
|---|---|
| NM | HMGB1, SOX5, KCNJ8, ABCC9, YY1 |
| NS | ASTN1 |

*Source: Wu et al. (2019)*

GWAS has been very useful in the discovery of candidate areas of the genome influencing traits of economic important whilst the individual genes and their polymorphic variants have been well studied. In commercial companies, once these alleles have been identified, the actual variants are genotyped and cross referenced to production data. Appropriate statistical analyses determine whether or not there are correlations, associations, and the contribution of each genotype to the trait. The Oestrogen Receptor (ESR) gene for instance, has been described as additive whilst other alleles such as those responsible for drip loss behave as recessive in a halothane test.

Unlike in traditional breeding where some of these traits were difficult, time-consuming or expensive to measure, the advent of whole-genome sequencing has made it possible to determine the mechanisms and contribution of each gene. In reproduction, genes responsible for traits such as litter size (Sell□Kubiak *et al*., 2015), number of teats (Verardo *et al*., 2016) and ovulation rate (He *et al*., 2017) have now been identified. Research on Landrace pigs has revealed that an allele at the myosin heavy chain 4 skeletal muscle gene (MYH4) may be a potential molecular marker related to meat quality traits (Cho *et al*., 2016). He *et al*. (2009) also reported that deficiency of the gene LPIN1 in pigs can lead to a remarkable reduction in adipose tissue mass. Ovulation rate has an impact on potential litter size whilst teat number is important for how many piglets the mother can suckle successfully. Other

candidate genes and Quantitative Trait Loci (QTLs) reported to be associated with traits of economic interest in the Sus species include candidate genes for growth (MC4R), disease resistance (FUT1, NRAMP, SLA), coat colour (MC1R), and litter size (RBP4, ESR, PRLR). Selection of alleles at the PLAG1 and NR6A1 loci specifically associated with the number of vertebrae in the backbone have been reported to have a significant effect on pig size and back elongation. For meat quality traits, genes such as insulin-like growth factor 2 (IGF2) ryanodine receptor 1 (RYR1) and melano-cortin 4 receptor (MC4R) (Oczkowicz *et al*., 2013) have significant effects on both meat quality and quantity. Most of this information has been used to improve commercial pig production through the application of Marker Assisted Selection (MAS) and other genomic approaches.

## Signatures of selection in pig breeding

In recent times innovative breeding strategies have employed genomic approaches to understand the unique alleles or polymorphisms that livestock breeds carry and the roles they play in ensuring their continuous survival for food security. The unique genetic patterns in the genome of individual animals/ breeds left behind after natural or artificial selection are known as signatures of selection. They are usually regions of the genome that harbour functionally important sequence variants. The detection of signatures of selection provides information that enables a better understanding of genome evolution and the mechanisms that influence specific traits that have been exposed to intensive selection either under natural or artificial selection.

Genome-wide detection of selection signatures has been applied to explore the genomes of sheep (Rochus *et al*., 2018), pigs (Wang *et al*., 2018), goats (Brito *et al*., 2017) and cattle (Cheruiyot *et al*., 2018). In Holstein cattle, a panel of genes including FABP3, CLPN3, SPERT, HTR2A5, ABCE1, BMP4 and PTGER2 have been detected and comprises some interesting candidate genes and QTL representing a broad range of economically important traits such as milk yield and composition as well as reproductive and behavioural traits (Qanbari *et al*., 2010). However, for pig breeds, the literature indicates an increasing trend towards searching for selection signatures based on comparison of the reference genome and de novo sequencing of animals from selected breeds. For instance, reports on genomic characterization studies from Kenya revealed genetic variation and breed compositions of two domestic pig populations providing a basis to explore possible genetic determinants underlying tolerance to infection by African Swine Fever (Mujibi *et al*., 2018). Furthermore, Single Nucleotide Polymorphisms (SNPs) discovery in pigs have been very active, driving the generation of a large database that can place these polymorphisms onto the reference genome. Wilkinson *et al*. (2013) found signatures of diversifying selection between pig breeds in genomic regions associated with traits related to coat colour and ear morphology as well as QTLs and genes associated with reproduction, growth and fat deposition. Other genes including ADAMTS12, SIM1 and NOS1 have been reported to show signatures of natural selection in Tibetan pigs and are important for genetic adaptation to high altitudes (Ai *et al*., 2013).

Advances in genotyping and sequencing techniques have helped identify changes in genomes that are guided by positive selection in populations with an accuracy and resolution that were previously difficult to achieve. The detection of signatures of selection has thus provided more accurate information to help dissect genomic evolution and the mechanisms underlying specific traits that have been exposed to intensive selection either under natural or artificial selection (Brito *et al*., 2017). These genomic approaches have the potential to elucidate the identities of genes, interpret the mutations associated with phenotypic traits, as well as understand the discovery and validation of genomic regions involved in the manifestation of economically important traits (Gouveia *et al*., 2017). Rubin *et al*. (2012) characterized and documented various genes which have been involved in the phenotypic

evolution in European pigs. Their discovery also illustrated how alleles in domestic animals may evolve by the accumulation of multiple causative mutations as a response to strong directional selection. Wang *et al*. (2018) detected selection signatures in Chinese Landrace and Yorkshire pigs using genotyping-by-sequencing data and reported a number of candidate genes influencing growth and development of normal differentiation and these included GHR, IGF1R and IGF2R gene. Fu *et al*. (2016) reported protein-coding genes that were associated with metabolic and developmental processes in Chinese pigs and outlined various signatures of selection and their specific genes (Table 2).

**Table 2:** Signatures of selection detected in genes involved in desirable traits in Chinese pigs

| Signatures of selection detected | Gene(s) |
|---|---|
| Body size and immunity | RPS10 and VASN |
| Lipid metabolism | GSK3 |
| Male fertility | INSL6 |
| Developmental processes | TBX19 |

*Source: Fu et al. (2016)*

Detecting selection signatures provides insight into the mechanism of artificial selection and also uncovers the causal genes related to phenotypic variation. There are several statistical tests for measuring the significance of identified selection signatures and the popular ones widely adopted in livestock improvement programmes include; the fixation index (FST), haplotype differentiation statistic (hapFLK), composite likelihood ratio (CLR) test and extended haplotype homozygozity (EHH) (Wright, 1949; Fariello *et al*., 2013). These methods are considered very robust to disentangle selective pressures from other effects such as admixture, migration and population bottlenecks (Onzima *et al*., 2018). Fixation index (FST) is normally used to detect the degree of differentiation between two populations by the allele frequency (Wright, 1950). If data is available for multiple populations, FST is highly recommended for detecting selection signatures as it detects highly differentiated alleles among different populations undergoing divergent selection. Low FST values indicate limited or no population differentiation or negative/neutral selection while high values of FST suggest strong breed differentiation or local positive adaptation (Brito *et al*., 2017). The hapFLK is used to measure differences of haplotype frequencies between multiple populations and accounts for the hierarchical structure of different populations and the varying effect of effective population sizes (Fariello *et al*., 2013). XP-EHH, a cross—population method was developed to detect selected sites on the basis of extended haplotype homozygozity used to compare different pig breeds on the genome-wide level (Li *et al*., 2016)

## Conclusions and Recommendations

Genomic selection has a huge impact on genetic progress of livestock including pigs. This becomes even more important in an era where resilience and climate change adaptation are needed in breeding goals. This indicates the gains that can be derived by adopting genomics in breed improvement programmes, particularly in the bid to meet the animal protein needs of an increasing human population. Several known candidate genes linked to economically important traits have been reported. Identification of selection signatures in pigs has made it possible for scientists to have better insight into various allelic variants of genes in different breeds and their roles. This review has provided insights on the genetic basis for phenotypic evolution in pigs as well as an understanding of the genetic mechanisms for production and reproductive traits which could have positive effects on pig

breeding programs.

Finally, the information provided here should motivate appropriate planning of breed improvement and conservation programmes for food security and sustainability. The development of resources and infrastructure that permits the application of these novel technologies is very critical. In many ways, low to middle income countries are perfectly placed to capitalize on the many useful research works done by developed countries. Therefore, researchers particularly in Africa need to be supported to take advantage of modern genomic approaches to improve upon the genetics of local pig breeds to ensure their sustainability and continued development for future breeding programmes. At the same time, the conservation of local pig breeds is very important and must be encouraged by every country considering such improvement programmes for the future.

Capacity building and application of the technology has the potential to bring huge advantages, but only if the local requirements and local genetic advantages are fully appreciated. Africa needs to improve on its human and institutional capacities to fully take advantage of the livestock genomic revolution.

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# BREEDING OBJECTIVES, TRAIT PREFERENCES AND INDIGENOUS KNOWLEDGE OF LOCAL CATTLE FARMERS IN SELECTED DISTRICTS OF THE GREATER ACCRA REGION OF GHANA

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## Abstract

Sustainable animal breeding programmes take into account the objectives and trait preferences of farmers as well as their indigenous knowledge and local circumstances. This study was conducted to document the breeding objectives, traits preferences and indigenous knowledge of local cattle farmers in selected areas of the Greater Accra Region of Ghana. This information is vital for the continuous development of more sustainable cattle improvement programmes. A survey was conducted between December, 2017 and January, 2018 among cattle keepers in Adentan Municipal, Ningo and Kpone-Katamanso Districts of the Greater Accra Region to identify their breeding objectives, traits preferences and indigenous knowledge. The results indicated that local cattle were mainly kept as a capital savings and security account and for income generation. The farmers' trait preferences were mainly fitness and type traits which play major roles in fulfilling multiple breeding objectives. These poor farmers with limited resources employ medicinal plants in the treatment of cattle diseases as they are cheaper, locally available, safer and efficient compared to orthodox veterinary medicines. The use of these medicinal plants if adequately harnessed could be an option for cattle farmers particularly those who cannot afford allopathic drugs in the treatment and control of cattle diseases. We recommended that local cattle be conserved and sustainably used through community-based breeding programmes whist strengthening the capacity of stakeholders and making grazing reserves and stock water points available.

**Keywords:** capital savings, community-based breeding programmes, fitness traits, multiple breeding objectives

# OBJECTIFS D'ELEVAGE, CARACTÉRISTIQUES PRÉFÉRÉES ET CONNAISSANCES INDIGÈNES DES ÉLEVEURS DE BOVINS LOCAUX DANS CERTAINS DISTRICTS DE LA RÉGION DU GRAND ACCRA AU GHANA

## Résumé

Les programmes d'élevage durable tiennent compte des objectifs, des caractéristiques préférées, et des connaissances autochtones des éleveurs et de leur situation locale. Cette étude a été menée pour documenter les objectifs d'élevage, les caractéristiques préférées et les connaissances autochtones des éleveurs de bovins locaux dans certaines régions de la région du Grand Accra au Ghana. Cette information est vitale pour l'élaboration continue de programmes plus durables d'amélioration de bovins. Une enquête a été menée entre décembre 2017 et janvier 2018 auprès des éleveurs des districts municipaux d'Adentan, Ningo et Kpone-Katamanso, dans la région du Grand Accra, afin d'identifier leurs objectifs d'élevage, leurs préférences et leurs connaissances autochtones. Les résultats indiquent que les bovins locaux sont principalement gardés comme économies en capital, compte de sécurité et pour la production de revenus. Les caractéristiques préférées par les éleveurs étaient principalement la santé physique et le type qui jouent un rôle majeur dans la réalisation de multiples objectifs d'élevage. Ces pauvres éleveurs disposant de ressources limitées emploient des plantes médicinales pour le traitement des maladies des bovins, car les plantes sont moins chères, sont disponibles localement, plus sûres et efficaces par rapport aux médicaments vétérinaires orthodoxes. L'utilisation de ces plantes médicinales - si elle est bien exploitée - pourrait être une option pour les éleveurs de bovins, en particulier ceux qui ne peuvent se permettre

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les médicaments allopathiques dans le traitement et le contrôle des maladies des bovins. Nous avons recommandé que les bovins locaux soient conservés et utilisés de façon durable à travers des programmes d'élevage communautaires qui renforcent la capacité des intervenants et rendent les réserves de pâturage et les points d'eau de bétail disponibles.

**Mots clés :** économies en capital, programmes d'élevage communautaires, caractéristiques de bonne santé physique, objectifs de reproduction multiples

## Introduction

Livestock production is a key component of Ghana's agriculture and a major source of livelihood for many rural households. It contributed, in direct products, between 1.8 percent and 8.8 percent of the national and agricultural Gross Domestic Products (GDP) respectively in 2012 and 2013.These increased by a rate of 5.2 percent in 2014 (MoFA, 2016). The main contribution of livestock to the national economy is food and nutritional security although secondary products such as manure, draught power, and transport are provided to the crop sector (Makina *et al*., 2014; MoFA, 2016). In recent decades, there has been a rapid shift in livestock productivity, particularly cattle breeds used in developing countries due to global influences such as an increasing demand for livestock products and market-oriented production (Traoré *et al*., 2017). Ghana's cattle population is largely concentrated in the former three northern regions which account for approximately 75 percent of the cattle produced in Ghana (MoFA, 2016). Generally, cattle production in Ghana is largely aimed towards commercial beef production since local breeds of cattle are poor milk producers (Aboagye, 2014); milk produced is mainly for household use (Adzitey, 2013; MoFA, 2016). The main cattle production system in Ghana is the extensive, sedentary system where cattle are led to graze by local herdsmen for hours during the day on natural range lands and returned to their stalls before nightfall (Oppong-Anane, 2013; MoFA, 2016). The semi-intensive system, however, is practiced by a few cattle farmers who provide cattle with additional feed from farm leftovers (Konlan *et al*., 2014). In Ghana, cattle farmers in rural communities share a vast knowledge resource which has been acquired over time, is aimed at preserving genetic traits of local cattle species and is built up through day-to-day experiences and is cheaper compared to modern practices (Konlan *et al*., 2014).

In spite of the importance of cattle production to Ghana's agriculture, information on breeding objectives, trait preferences, and indigenous knowledge in cattle farming in the country is not readily available. Documentation of farmers' breeding objectives, trait preferences and indigenous knowledge on local cattle production practices will enable their inclusion in the design and implementation of sustainable cattle breeding schemes to improve local cattle productivity. This study was therefore carried out to document breeding-related decision-making processes of local cattle farmers in selected districts of the Greater Accra Region of Ghana. Specifically, the breeding objectives, trait preferences and indigenous knowledge of local cattle farmers were documented. The findings could support community-based cattle breeding programmes in Ghana.

## Materials and Methods

*Location of Study*

This study was carried out in the Adentan municipal, Ningo-Prampram, and Kpone Katamanso districts districts of the Greater Accra Region of Ghana. A purposive sampling method was adopted to collect data from 48 cattle keeping households in the study area.

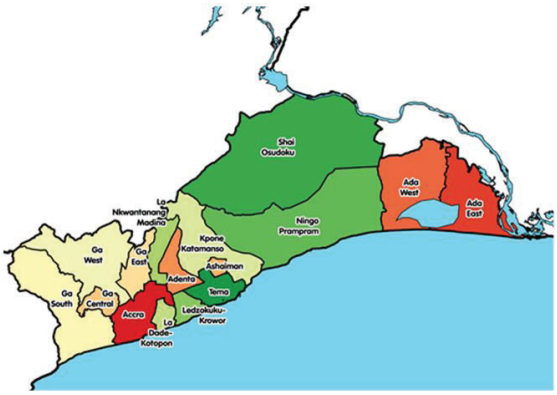


**Figure 1:** Map of the Greater Accra Region showing the districts sampled

*Data Collection*

A pretested structured questionnaire was administered to 48 cattle keeping households in the selected districts in December 2017 and January 2018. The data collected included general information on household characteristics of the farmers, such as number of persons in the household, religion, educational level, age and marital status, as well as cattle production objectives and cattle husbandry practices, production and trait preferences. Each respondent keeping cattle was asked to indicate the criteria used for the selection of breeding bulls and cows. The respondents also provided information on cattle breeds kept, husbandry practices, their challenges as well as ideas for improving their vocation.

*Data Analysis*

Data collected were compiled, coded and analysed using the Statistical Package for Social Sciences (SPSS, 2007). The results obtained were presented in tables and figures. Chi-square tests were used to determine the degree of association between socio-demographic factors and reasons for keeping cattle among others.

## Results

*Socio-Demographic Background of Cattle Farmers*

The age distribution of the respondents indicated that most of the cattle farmers (75%) were 40 years of age or older (Table 1).

All the respondents were males with over half (52%) of the cattle farmers having no formal education (Table 2). In addition, most of them (69%) were Muslims and married (96%).

Most of the sampled households (58%) had between 1 and 10 members with herd sizes mostly (65%) between 51 and 150 (Figure 2). Furthermore, most of the respondents (92%) did not provide supplementary feed for their animals and sold their cattle both at home (kraals) and at the market.

## Breeding Objectives and Traits Preferences of Cattle Farmers

The study showed that cattle farmers keep cattle for different purposes with the majority (60%) of these farmers reporting capital savings and security as the main reasons for keeping cattle (Table 3). The most preferred trait was fitness (being able to reproduce and survive in the environment) representing 56% of the farmers' trait preferences (Figure 3).

Most farmers (44%) obtained their breeding bulls through purchasing with 33% sometimes purchasing or using bulls from their own herds for breeding. The most common production system among the cattle farmers was the extensive/free range system (92%) with intensive and semi-intensive systems representing 6% and 2% respectively. The study showed that the majority of cattle farmers preferred bulls which were superior in body conformation (44%) and type traits (32%) for servicing their cows (Table 4). A similar pattern was observed in the trait preferences for breeding cows.

## Cattle Husbandry Practices

The majority of cattle farmers (83%) did not keep any formal records whilst a few (17%) had written down information on their animals. Most respondents (60.4%) did not castrate their bulls but for those that did, the main reason was for controlled breeding (19%). In addition, most cattle farmers culled unproductive stock and the most common reasons were poor body condition and old

**Table 1:** Age of Cattle Farmers

| Age Group | Frequency | Percent |
|---|---|---|
| ≤ 40 | 12 | 25.0 |
| > 40 | 36 | 75.0 |
| Total | 48 | 100.0 |

**Table 2:** Educational Background of Cattle Farmers

| Age Group | Frequency | Percent |
|---|---|---|
| No Schooling | 25 | 52.1 |
| Basic/Senior/Tertiary | 23 | 47.9 |
| Total | 48 | 100.0 |

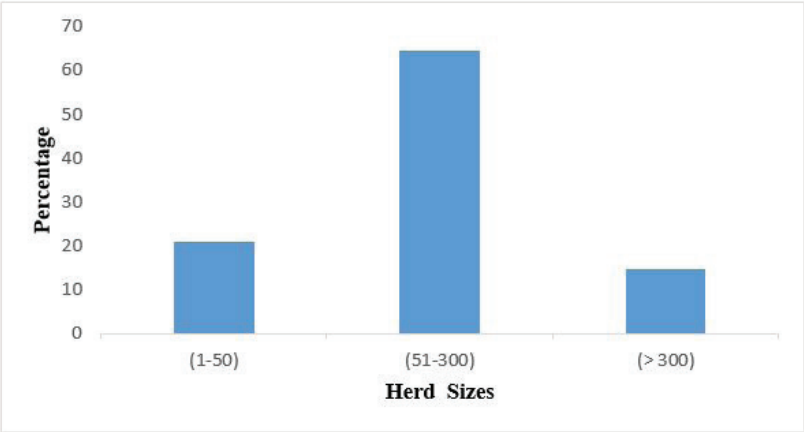


**Figure 2:** A Bar Chart Showing Cattle Herd Sizes

**Table 3:** Reasons for Keeping Cattle

| Reasons | Frequency | Percent |
|---|---|---|
| Capital Savings and Security | 29 | 60.4 |
| Income Generation | 13 | 27.1 |
| Socio-cultural reasons | 6 | 12.5 |
| Total | 48 | 100.0 |

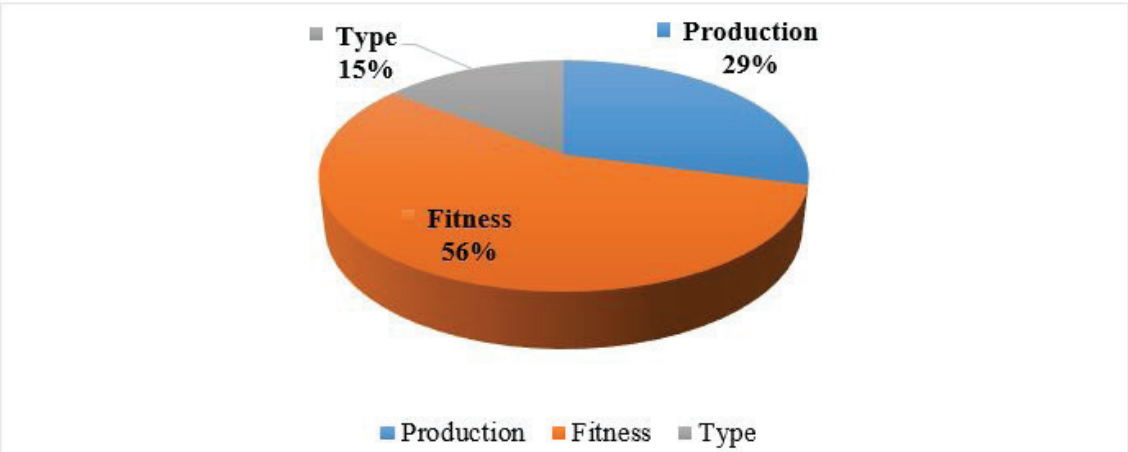


**Figure 3:** A Pie Chart Showing Traits Preferences of the Cattle Farmers

**Table 4:** Trait Preferences in Breeding Bulls

| Trait Preference | Frequency | Percent |
|---|---|---|
| Body conformation | 21 | 43.8 |
| Type | 15 | 31.8 |
| Production | 8 | 16.8 |
| Fitness | 4 | 8.4 |
| Total | 48 | 100.0 |

age. Most cattle farmers (81%) employed the services of veterinarians only for the treatment of diseases of cattle with 19% combining herbal and veterinary services in the treatment of cattle diseases. Most respondents (92%) learnt the skills of animal husbandry from their parents. The commonest breed of cattle kept by the respondents was the Ghana Shorthorn cattle (38%) with some (15%) keeping two or more breeds in their herds. The most common reason for keeping particular breeds was adaptation which accounted for 67% of the total respondents as shown in Figure 4.

In terms of the challenges they encounter in their cattle production, the respondents in general indicated feed and water problems, diseases, and encroachment of grazing areas which they expect to get worst given the impact of climate change and the increasing pressure on land resulting from human population growth.

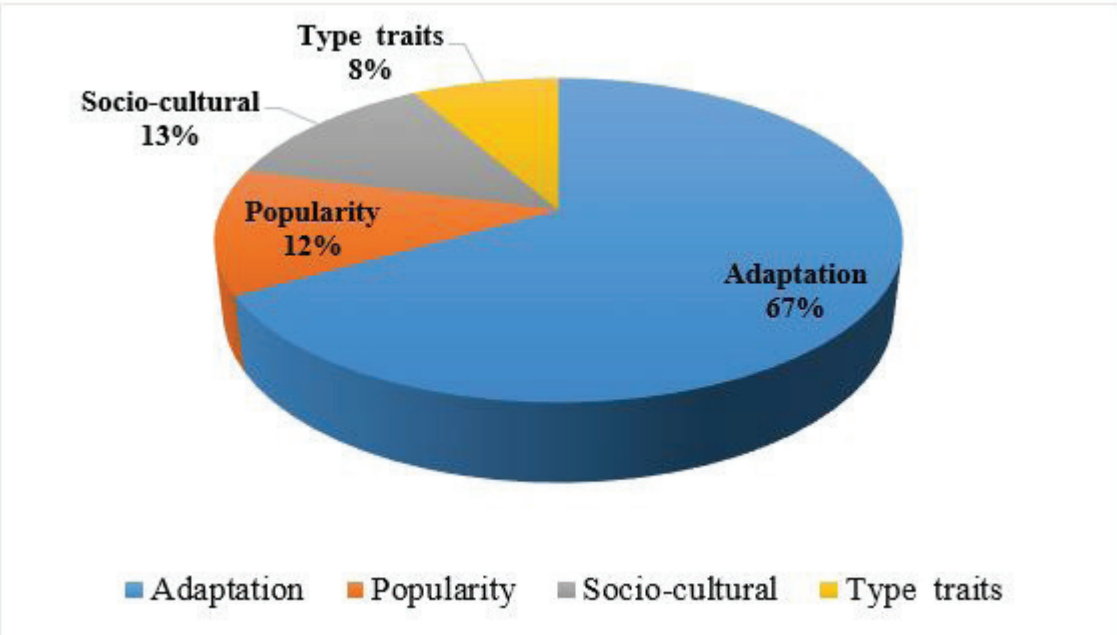


**Figure 4:** A Pie Chart Showing Reasons for the Choice of Breeds

**Table 5:** Ethno-veterinary Practices of Sampled Cattle Farmers

| Condition | Suggested Local Treatment of cattle |
|---|---|
| Diarrhoea and Calving difficulties | Leaves of Newbouldia laevis are ground, mixed with water and administered orally |
| Wound and Snake bite | Boil crushed fresh leaves of Newbouldia laevis and wash the wound with this warm concoction |
| Helminths infestation | Soak leaves or bark of Neem tree in water overnight and administer to affected cattle orally |
| Bloating | Mix a little lemon juice and potash with water and give orally to affected cattle |
| Poisoning | Administer palm oil orally to affected cattle |

## Discussion

*Breeding Objectives and Traits Preferences of Local Cattle Farmers*

Although there were no specialised breeding programmes in any of the study areas, a few cattle farmers in the peri-urban areas of Adenta were specialised in milk production. A similar trend was observed by Coffie (2014) who indicated that commercial cattle farming is almost entirely limited to the cities and towns. The cattle farmers in the study areas had various reasons for keeping cattle. The most common reason was for "capital savings and as a security account". Cattle are a source of meat and milk for the family, provide direct cash income, are capital assets, produce manure for use as fertilizers and fuel and may be a source of power for transport and cultivation. Elsewhere, cattle farmers indicated draught power and savings as the most important production objectives while productive traits were ranked highest (Traoré *et al*., 2019). Cattle are an inflation-free form of banking for resource-poor farmers and can be sold to meet household financial needs such as school fees, medical bills, and household expenses (Nyako *et al*., 2016). Rewe *et al*. (2006) and Traoré *et al*. (2017) reported that in pastoral systems, the main function of cattle is subsistence through their sale or the sale of their products. Since there are limited or no banks in rural communities, farmers' device their own means of saving through cattle production and when financial needs arise, they cater for those needs. Zewdu *et al*. (2018) reported the following trait preferences of dairy cattle farmers in the East Gojjam Zone, Ethiopia - higher milk yield, faster growth rate, adaptability to local environment (characterised by the shortage of feed resources and prevalence of diseases) and draught power. Furthermore, udder and teat size, and pelvic width were commonly considered to be of primary importance (Zewdu *et al*. 2018).

In the selected districts in this study, fitness traits were the most preferred traits of cattle. Farmers pay close attention to fitness because they do not want to lose their investment and also because they know the challenges cattle are exposed to in their respective environments such as drought and seasonal feed shortages. Animals are therefore chosen on the basis of their ability to survive the harsh environment and also being productive at the same time. According to Tano *et al*. (2003), in West Africa, farmers have high preference for fitness and production traits as opposed to type traits. In South Africa, the Nguni cattle breed is the most preferred by local herdsmen because it has low nutrient requirements for maintenance, excellent walking ability which enables it to walk long distances in search of pasture and water; these qualities enable it to survive under conditions that other breeds would find extremely difficult (Makina *et al*., 2014). In Somalia, pastoralists rank adaptation to harsh environmental conditions as the most important trait of cattle; particularly, resistance to droughts, the ability to walk far and to go for long without water (Marshall *et al*., 2016). On the other hand, a study conducted in Nigeria revealed that trait preference in breeding bulls and cows was not influenced by production system. In effect, disease resistance and walking

ability were seen as the least important traits of cattle among pastoral nomads; milk yield and fertility were most preferred for breeding cows whereas body conformation and temperament ranked high for breeding bulls (Ogah *et al*., 2017). Small scale dairy farmers in Tanzania preferred cows with high milk yields, good fertility, easy temperament, low feed requirement and enhanced tropical disease resistance (Chawala *et al*., 2019).

In the present study, most of the respondents considered body conformation and type traits as very important criteria for selecting breeding bulls and cows. This agrees with the findings of Irungu (2000) and Mirkena *et al*. (2010) that there is a general tendency for pastoralists to focus on observable attributes like body conformation, appearance (beauty), horn shape, ear size, body length, coat colour, tail length and body size for both bulls and cows to satisfy strong cultural inclinations for aesthetic value in all production systems. For instance, in Kenya, some local cattle farmers prefer dark-red coat-coloured animals which are slaughtered during traditional ceremonies (Ouma *et al*., 2005). This is despite the fact that such cattle suffer a high risk of being bitten by tsetse flies. Also, while market-oriented cattle production systems base market decisions on the measured performance and appearance of animals, visual appraisal of the form, size, appearance and in some cases relative performance of animals are used as the basis for making animal type choices and marketing decisions in the less market-oriented farming systems (Kassie *et al*., 2006). In the Gambia and Senegal, cattle farmers view body size and body conformation, growth rate and disease resistance as the most important traits during selection for breeding (Garcia, 2012). In another study on Sheko cattle keepers in Ethiopia, milk yield, body conformation, body size and coat colour were emphasized in the selection of cows while, body size, dairy character, draught character and coat colour traits were important traits for selection of bulls (Bayuo *et al*., 2018).

The most common breed kept by farmers in the present study was the Ghana Shorthorn, which is known for its adaptation to adverse environmental conditions. Others also kept the Sanga and the Sokoto Gudali. Although the local breeds of cattle in Ghana are known for their resistance to most endemic diseases, they are small in size, have low milk production and are late maturing (Hanotte *et al*., 2000; Ajmon-Marsan *et al*., 2010; Aboagye, 2014). They are therefore considered by some cattle farmers to be unproductive and this has led to cattle farmers in the study areas mixing their herds with Zebu breeds mainly as sire lines to improve body size and milk production. In a similar study, large body size was the most preferred trait, followed by fertility, draught ability and milk yield with crossbreds being the most favoured breed group (Traoré *et al*., 2019). Breed preferences were mostly explained by 'resistance to disease' for N'Dama cattle and 'high market price' for Fulani Zebu and crossbred cattle (Traoré *et al*., 2019). Production objectives, trait and breed preferences were mainly influenced by farmer groups (local farmers and settled transhumants). Local farmers put comparatively more emphasis on livestock functions linked to crop production such as draught power. They had a higher preference for traction ability as a selection trait and preferred the N'Dama over Fulani Zebu cattle (Traoré *et al*., 2017).

*Indigenous Knowledge of Cattle Husbandry Practices*

The cattle production system in the areas studied is the extensive system characterised by open grazing of cattle on natural pasture for hours during the day. This agrees with the observation made in Ghana (Oppong-Anane, 2013) and other African countries (FAO, 2002; FAO, 2004; Gabalebatse *et al*., 2013). The intensive system of cattle production requires high capital and skill in establishment and management to ensure its profitability and therefore dissuades resource-poor and uneducated farmers from adopting it. Not surprisingly most farmers keep their animals on the free-range system rather than in the intensive production system because of its relatively low production costs. The common

breeds found under the milk production system were foreign breeds mainly the Jersey, Friesian and their crosses.

The majority of cattle herd sizes per household fell within the range of 51-300 heads. Herd sizes were limited by feed constraints especially during the dry season as farmers depended on natural pasture to feed their animals. The majority of cattle farmers in this study did not keep written records on cattle. According to farmers they resist keeping written records because they do not like counting cattle since it could attract an evil eye on cattle which was a bad omen. However, they reported that they could tell the parentage of cattle up to the fourth and fifth generations. This was also observed by Mirkena *et al*. (2010) among the sheep breeding pastoralists of Kenya who memorised in great detail the ancestry of their animals up to seven generations. Similar practices have been observed among camel keeping pastoralists in India and the Maasai (Köhler-Rollefson, 2003). Cattle herders are rarely mistaken in recognizing their animals and almost all members of a family have the same ability. If a pastoralist loses one of his animals or his animal merges with another herd, he will, even after one or two years, be able to recognize the lost animal. For a pastoralist, having a good memory is of utmost practical and social importance, providing the herd owner with the esteem of his peers. The main reason for castrating animals is to control breeding. Most cattle farmers, however, resisted castrating their animals. Castrating requires some skill and practice which most farmers did not have due to their lack of education. Also, most farmers complained of low markets for castrated animals which dissuaded them from the practice. The main reason for the culling of animals was old age. The common method of culling was by selling of the animals. A few farmers did not cull animals at all. Old animals acted as leaders of herds and were more familiar with grazing routes. Farmers may also get attached to old animals because they may associate the presence of such animals in the herd with good luck. Additionally, farmers were concerned with increasing animal numbers in their herds and so were opposed to culling. Black coat colour was mostly unwanted by cattle farmers due to less market value and this agrees with Edea *et al*. (2012) who reported that certain coat colours have more market demand than others. Farmers had knowledge of colour inheritance in animals and tried to avoid black coat-coloured animals in their herds by using bulls that had desirable coat colours during breeding. Bright coat colours are preferred by the majority of cattle breeding communities to reduce the risk of attack by local endemic diseases, notably trypanosomiasis and acting as a natural control against them, while simultaneously reducing the incidence of heat stress as darker coat colours absorb more heat (Irungu, 2000; Msanga *et al*., 2012; Katiyatiya *et al*., 2014).

Only a few cattle farmers were willing to share their knowledge in herbal medicine which they had learned from their parents and friends. They usually try to keep it as a trade secret to themselves (Luseba and Tshisikhawe, 2012). Farmers' ethno-veterinary (herbal) knowledge involved the treatment and management of diseases such as diarrhoea, helminth infestations, bloating, calving difficulties, and poisoning. In many developing countries, farmers and herders combine indigenous ethno-veterinary knowledge and modern veterinary health care systems to treat their livestock (Luseba and Tshisikhawe, 2012; Nyako *et al*., 2016). However, the latter is often unavailable due to either staffing problems in agriculture extension services or because synthetic drugs are expensive (Abraha, 2016). This leads farmers to using traditional methods that are cheap and easily accessible (Mudzengi *et al*., 2014; Alhaji and Babalobi, 2015). The majority of farmers allowed inbreeding in their cattle herds and this may be attributed to their lack of knowledge on the negative impact of the practice. Farmers who did not allow inbreeding used different methods to prevent it including castration, selling young males before they reached crossing age, keeping bulls in the herd for at most 3-4 years, or purchasing bulls from markets or neighbours for breeding.

The main reason the farmers gave for

the choice of breed apart from its productivity is adaptation. The adaptability of an animal can be defined as the ability to survive and reproduce within a defined environment (Prayaga and Henshall, 2005) or the degree to which an organism, population or species can remain/become adapted to a wide range of environments by physiological or genetic means (Barker, 2009). Cattle farmers live in areas characterised by scarce feed and water resources and high disease pressure with large seasonal and annual variations and so keep animals that are adapted to their respective environments as is the case in low input pastoral systems (Mirkena *et al*., 2010). A few cattle farmers reared cattle due to socio-cultural reasons. Most farmers are religious and perceive cattle rearing as a cultural obligation to their fathers who raised cattle as a source of livelihood. Since these farmers had received cattle through inheritance, they felt the obligation to rear the animals and also pass it on to their children.

*Constraints of Cattle Production*

Generally, the productivity of cattle is limited by several constraints including natural uncontrolled and unorganized breeding practices, high prevalence of diseases, poor reproductive performance, limited feed availability, poor marketing and lack of recording systems (Zewdu *et al*. 2018). Challenges enumerated by the local cattle farmers in the present study include feed and water problems, diseases, and encroachment of grazing areas. The most important diseases hindering cattle productivity included, foot-and-mouth disease, bovine tuberculosis, contagious bovine pleuropneumonia (CBPP), and dermatophilosis. This observation could be due to the consequences of the free-range practice. MoFA (2016) reported that migrant herdsmen and their cattle constitute a potential means of introducing certain disease pathogens into indigenous livestock. Also, most kraals in the study areas had no roofs to protect cattle from inclement weather. Feed problems could be due to the negative impact of overgrazing by local herds of cattle as well as by transhumant cattle. Also, large portions of the natural range and forage resources are often destroyed by bush fires (Adzitey, 2013). Seasonal deficiencies in feed quality and quantity particularly during the dry season have been reported as a major threat to cattle production (Adzitey, 2013; MoFA, 2016; Abebe, 2017).

## Conclusions and Recommendation

The farmers' main motives for keeping cattle in the present study wereas 'capital savings, as a security for income generation. Adaptation was the main reason for the choice of cattle breeds while body conformation and type traits were the major criteria for the selection of breeding animals, both bulls and cows. Constraints to cattle production included seasonal feed and water problems, diseases, cattle rustling, inadequate veterinary services and encroachment of grazing areas. Additionally, the cattle farmers had unique indigenous knowledge on feeding, breeding and animal health.

Based on the findings of the study, the sustainable use and conservation of local cattle on account of their unique adaptive genetics is recommended. Additionally, community breeding programmes should incorporate breeding goals and trait preferences of the target cattle keepers. The use of community bulls if properly supervised would help farmers share and minimize costs as well as produce animal genotypes that meet their preferences. The documentation of ethno-veterinary knowledge would be a valuable source of information for cattle keepers who cannot afford the use of allopathic drugs in the treatment and control of diseases. Finally, the education of cattle keepers on record keeping, value of local breeds and feed conservation methods to protect against seasonal shortages during dry periods as well as the provision of dams in rural communities to water their animals should be explored.

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# OPTIONS FOR MAKING LIVESTOCK PRODUCTION IN WEST AFRICA "CLIMATE-SMART"

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## Abstract

Recent estimates of global emissions from ruminants suggest that developing countries contribute about two thirds of greenhouse gas emissions. This trend could be attributed to low animal productivity, poor animal health and low-quality feeds. The numbers suggest that there are opportunities for easy gains to be made in the livestock sector through reduction of emissions per unit of product. Although there are many research and analytical efforts by various actors, to minimize the impact of climate change on agriculture and livelihoods in Africa there is no coherent documented state of knowledge of livestock-based climate-smart-agricultural (CSA) practices in West Africa. Identifying and documenting successful livestock-based CSA practices has been a challenge. To this end, the study synthesized current knowledge on climate smart (CS) options related to livestock production in West Africa in terms of experiences, lessons learned and gaps in knowledge. Tis paper provides broad researchable areas to promote both adaptation and mitigation activities in the development of livestock projects. As climate smart practices in livestock production are highly context specific and may involve trade-offs between productivity, adaptation and mitigation, stakeholder consultation is important when deciding which climate smart practice to implement. Successful CSA strategies require investment in infrastructure that support smallholder farmers in understanding climate change, developing and refining strategies and evaluating CSA options. In addition, policy and institutional support are crucial for providing enabling environments for both researchers and farmers to implement livestock-related climate smart agriculture.

**Keywords:** Climate smart agriculture, livestock management, sustainable agriculture, West Africa.

# OPTIONS POUR RENDRE LA PRODUCTION ANIMALE DE L AFRIQUE DE L'OUEST « SENSIBLE AU CLIMAT»

## Résumé

Les estimations récentes des émissions mondiales provenant des ruminants portent à croire que les pays en développement contribuent pour près de deux tiers des émissions de gaz à effet de serre. Cette tendance pourrait être attribuée à une faible productivité animale, à une mauvaise santé animale et à des aliments de faible qualité. Les chiffres font penser qu'il existe des possibilités de gains faciles dans le secteur de l'élevage à travers la réduction des émissions par unité de produit. Bien qu'il y ait de nombreux efforts de recherche et d'analyse de la part de divers acteurs, visant à réduire au minimum l'impact des changements climatiques sur l'agriculture et les moyens de subsistance en Afrique, il n'existe pas de connaissances cohérentes documentées des pratiques agricoles sensibles au climat fondées sur l'élevage en Afrique de l'Ouest. L'identification et la documentation des pratiques agricoles sensibles au climat (SC) ciblant l'élevage constitue un défi. À cette fin, l'étude a synthétisé les connaissances actuelles sur les options SC liées à la production animale en Afrique de l'Ouest en termes d'expériences, de leçons apprises et de lacunes au niveau des connaissances. Le présent document fournit de vastes domaines pouvant faire l'objet de recherches pour promouvoir à la fois les activités d'adaptation et d'atténuation dans l'élaboration de projets d'élevage. Étant donné que les pratiques sensibles au climat dans la production animale sont très spécifiques au contexte et peuvent entraîner des compromis entre la productivité, l'adaptation et l'atténuation, la consultation des intervenants est importante lorsqu'il s'agit de décider de la pratique intelligente à appliquer. Les stratégies fructueuses d'élevage SC exigent des investissements dans

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les infrastructures qui aident les petits éleveurs à comprendre les changements climatiques, à élaborer et à perfectionner les stratégies et à évaluer les options d'élevage SC. En outre, les politiques et l'appui institutionnel sont essentiels pour permettre aux chercheurs et aux éleveurs de mettre en œuvre un élevage sensible au climat.

**Mots clés :** élevage sensible au climat, gestion de l'élevage, élevage durable, Afrique de l'Ouest.

## Introduction

Climate change is one of the greatest challenges to food security and sustainable development in general in recent history (Drolet, 2012; Mugambiwa and Tirivangasi, 2017). As a global phenomenon, developing countries are more exposed to the hazards of climate change as they are less resilient to them (Morton, 2007). Its negative impacts are more severely felt by poor people in developing countries who rely heavily on the natural resource base for their livelihoods. The livestock sector is one of the major contributors of GHG emissions in agriculture, contributing up to 18% by some estimates (Thornton and Herrero, 2010). A recent analysis by Herrero *et al.* (2013) suggests that developing world regions contribute about two thirds of the global emissions from ruminants, with sub-Saharan Africa a global hotspot for emission intensities, largely due to low animal productivity, poor animal health and low-quality feeds. These numbers suggest that there are opportunities for easy gains to be made in terms of mitigation in the livestock sector.

Thonton (2010) suggested that the biggest impacts of climate change would be seen in livestock in both pastoral and smallholder mixed crop and livestock systems in developing countries where people are already highly vulnerable. The impacts of climate change are expected to exacerbate the vulnerability of livestock systems and to reinforce existing factors that are simultaneously affecting livestock production systems such as rapid population and economic growth, increased demand for food (including livestock) and products, and increased conflict over natural resources (i.e. land, water etc.). One of the most important effects of climate change on livestock production is the change in the animal feed resources. This effect derives from poor regeneration of natural pasture as a result of delay in the onset of rains to include changes in species composition and dynamics as a result of changes in temperature, rainfall regime and carbon dioxide (McKeon *et al*., 2009; Izaurralde *et al*., 2011). All these culminate into changes in animal diets and possibly reduced nutrient availability for animals. The indirect effects on feed resources can have a significant impact on livestock productivity (Getu, 2014). Other effects include water shortage and heat stress in livestock (Abate, 2009; Digambar, 2011), increased mortality of livestock, vulnerability to diseases and physical deterioration due to long distance travel for water and pastures (Abate, 2009). The drect effects of climate change include high temperatures and changes in rainfall patterns, translating into increased spread of existing vector-borne diseases and macro parasites in animals as well as the emergence and spread of new diseases (FAO, 2008). For rural communities, losing livestock assets might push them into chronic poverty with long-term effects on their livelihoods (IFAD, 2011). Adapting livestock production systems to climate change is a must for the West African region, given the significant contribution of livestock to agricultural GDP of about 44% (SWAC-OECD/ECOWAS 2008). Identifying changes in agricultural practices especially in livestock production that result in effective adaption to site specific effects of climate change is essential to address challenges of food security in the region.

Although there are many research and analytical efforts by various actors to minimize the impact of climate change on agriculture and livelihoods in Africa, there is no coherent documented state of knowledge of livestock-based CSA practices in West Africa. This study is an attempt to synthesize current knowledge

on climate smart (CS) livestock production practices in West Africa and to identify gaps in knowledge. This paper presents some of the experiences and lessons learned regarding climate-smart agriculture in relation to livestock production in West Africa with a specific focus on Burkina Faso and Mali. Building on these, the paper provides broad researchable areas associated with smallholder farmers and pastoralists to promote both adaptation and mitigation activities in the livestock sector.

## Climate Smart Agriculture: concept and principles

Climate Smart Agriculture (CSA) is an approach that provides a conceptual basis for assessing the effectiveness of changes in agricultural practice to support food security under climate change. The aim of CSA is to integrate climate change in agriculture and make agriculture adapt to climate change and to reduce emissions (or mitigation) (FAO, 2012). Livestock production systems are considered to be "climate-smart" if they contribute to increasing food security, adaptation and mitigation in a sustainable way. Any livestock management practice that improves productivity or utilizes scares resources efficiently can be considered climate-smart because of the potential benefits with regards to food security, even if no direct measures are taken to counter detrimental climate effects (Ayantunde *et al*., 2015). CSA is not a single specific agricultural technology or practice that can be universally applied. It is an approach that requires site-specific assessments to identify suitable agricultural production technologies and practices. Its principles will be to develop the technical, policy and investment conditions for achieving sustainable agricultural development for food security under climate change. The CSA approach will have to identify and operationalize sustainable agricultural development within the explicit parameters of climate change.

According to FAO (2010) climate smart agriculture aims at sustainably increasing agricultural productivity and income, reducing climate change vulnerability (enhance adaptation), reducing emissions that cause climate change (mitigation), while protecting the environment against degradation and enhancing the food security and improved livelihood of a given society.

In addressing climate change adaptation for livestock-based livelihoods, Ayantunde *et al*. (2015) listed key questions to consider:

1. Which types of livestock management are suited to climate change and where?
2. Which animal species and breeds should be kept in which areas and what are the trade-offs?
3. Are there current livestock-based livelihood systems in the region that are best suited to climate change adaptation?
4. How can we add value to the existing livestock-based adaptation strategies?
5. Are there policy and institutional mechanisms to enhance adaptation of livestock production systems to climate change and variability?
6. How could the capacity of rural institutions be strengthened to use appropriate tools and strategies to cope better with consequences of climate change?
7. How could we balance the need for short-term adaptation, which is often reactive, with long-term climate change adaptation planning? At community level, climate change adaptation should be considered in the context of other significant drivers of change (demographic change, economic development, market opportunities).

## Methodology

Existing knowledge on livestock-related climate smart agricultural practices in West Africa

To explore the large body of literature, Google Scholar was used as the preferred search engine. This review also includes observational studies and farm surveys; on-farm and on-station experiments and the types of publications considered were scientific peer-reviewed articles, students' theses and research reports. Studies included were

located by searching through computer library databases (e.g. AGORA, FAO, Google Scholar, ICRAF and ILRI) and other databases such as CAB abstracts or Web of Science.

In West Africa, there is paucity of information on livestock-oriented climate smart practices, although, there are several crop-targeted projects based on CS principles. Most information at this stage is either on modelling, scoping of mitigation strategies or on resilience of livestock farmers. In other parts of Africa, East Africa for example (UNEP 2009 ; Wambugu *et al*., 2014) many livestock options for climate smart agriculture have been tested and documented while some are being scaled out (Kipkoech *et al*., 2015). Despite this, a few CS interventions related to sustainable intensification of livestock production in West Africa were identified within the current context and are described below. These interventions are grouped into three major types namely: Feed related interventions, livestock production management and environmental management.

## Results and discussion

The summaries of each intervention group and their related practices/technologies are presented. This eview further summarizes the management objectives under each listed practice citing the references and listed a few important knowledge gaps in relating to each practice underscoring why many of such practices are not fully adopted. Each practice was assessed by its contribution to the three major focus areas of climate smart interventions namely: increasing food security, adaptation and mitigation in a sustainable way. Each practice was scored either as low (+), medium (++) or high (+++) in terms of its contribution to the three major aims of climate smart interventions.

## Making livestock production practices more 'climate-smart' in West Africa.

Based on the above assessment of climate smartness of some livestock related interventions (Table 1), transforming animal agriculture in the region to be climate smart will require the following actions:

*Adaptive forage improvement*

Promoting adapted pasture species will play a major role in reducing the vulnerability of feed resources to climate change. This will improve preparedness for climate induced conditions thereby substantially raising survival rates and livestock performance due to availability of feed resources (Assan, 2014). Forage species with multiple traits to overcome a range of biotic and abiotic stresses induced by climate change are needed. Livestock keepers need to have better access to information regarding the variety of plants that are available to them, and the availability of seeds needs to be increased through market development or through extension projects. Livestock keepers can also benefit from learning different techniques for forage conservation and identifying solutions that fit with the production system, although the mobile herders who could benefit most from forage conservation may find the technique the least convenient.

*Improved rangeland and grazing management*

The starting point to improved rangeland management can be the allocation of land to pastoralists for grazing. This will encourage them to manage it and practice controlled grazing (Tumbo *et al*., 2011). Grazing lands can also be rehabilitated by planting improved grass and fodder trees but there have been few studies in Sub-Saharan Africa (SSA) on this aspect. Other adaptation actions include the conservation and storage of forage and keeping more animals of resilient species (Tumbo *et al*., 2011). Therefore, animal culling, improved pasture, improved breeds and ownership of land for grazing and pasture can be promoted widely among livestock keepers and contribute to achieve CSA.

Encouraging livestock farmers (through incentives) to match numbers of productive animals with the feed and water resources available within their sites will help to reduce

**Table 1:** Summary of feed-related livestock climate smart interventions in West Africa

| | Practices/ Technologies | Management objectives | Knowledge gaps | References | *Aggregate assessment | | |
|---|---|---|---|---|---|---|---|
| | | | | | Food security | Adaptive capacity | Mitigation of GHGs |
| Feed related interventions | Fodder cultivation | Dual purpose legumes; Forage grass and legume cultivation; Fodder bank | Regular availability of forage seed is often the main challenge to projects promoting planting of fodder species | Peters *et al*.,2012; Sanfo *et al*., 2015; Elbasha *et al*., 1999; Kassam *et al*., 2009; Sanon and Kanwe, 2004 | +++ | ++ | ++ |
| | Forage conservation | Harvesting and conservation of natural and cultivated pasture in form of silage and hay | Difficulty in impact assessment in the context of climate smart practices because interventions were not setup with the initial intention of climate change mitigation | Bayala *et al*., 2011; Kassam *et al*., 2009; | +++ | +++ | ++ |
| | Forage quality improvement | Supplementary feeding using concentrate and by-products; Urea treatment of crop residues | Problems of availability, health risks, costs, handling and additional labour. | Blümmel *et al*., 2009; Sanon, 2007 | + | ++ | +++ |
| | Forage integration | Forage legume incorporation into arable cropping | The use of forage legumes in many parts of the tropics is limited because they do not contribute directly to the human food supply | Ayarza *et al*., 2007; Umunna *et al*., 1995; Nandi and Haque, 1986; Amole *et al*., 2015; Nichols *et al*., 2007 | +++ | + | ++ |
| | Grazing management | Rotational grazing; Controlled grazing; Adjust stocking densities to feed availability | cultural barriers to grazing management. No credible information on stocking capacity of rangelands | Branca *et al*., 2011; Eagle *et al*., 2012; Achard and Chanono, 2006; Ayantunde *et al*., 2000; Schlecht *et al*., 2006; Badini *et al*., 2007 | ++ | +++ | +++ |
| | Crop-livestock-tree | Shade trees reduces heat stress on animals and contribute to improve productivity. Trees potentially reduce GHG due to the presence of various concentrations of phytochemicals. | It is labour intensive beside understanding how agroforestry will benefit transhumance pastoralists has not been addressed. Most feed related studies have failed to assess the amount of GHG reduction under in vivo condition as to basically identify best-fit species | Thornton and Herrero, 2010; Dharani *et al*., 2014; Bonkoungou *et al*., 2002; Zampaligre, 2012; Franzel *et al*., 2003; Cheikh *et al*., 2014; Noordwijk *et al*., 2011; Thorlakson *et al*., 2011 | +++ | +++ | +++ |

| | Practices/ Technologies | Management objectives | Knowledge gaps | References | *Aggregate assessment | | |
|---|---|---|---|---|---|---|---|
| | | | | | Food security | Adaptive capacity | Mitigation of GHGs |
| | Conservation agriculture | Improve soil condition and promote high yield and consequently crop residues | The question of trade-offs on crop residues as the main livestock feed on the semi-arid region of West Africa remained largely unanswered. | Corbeels *et al*., 2006; Scopel *et al*., 2004; Thierfelder and Wall 2010; Derpsch 2008 | ++ | + | + |

*Food security / adaptation / mitigation potential: + = low; ++ = medium; and +++= high*
**This general assessment may vary in different locations*

**Table 2:** Summary of livestock management and environmental management livestock climate smart interventions in West Africa

| | Practices/ Technologies | Management objectives | Knowledge gaps | References | *Aggregate assessment | | |
|---|---|---|---|---|---|---|---|
| | | | | | Food security | Adaptive capacity | Mitigation of GHGs |
| Livestock management intervention | Herd management | Species diversification | Shift from cattle to small ruminants will require overcoming a significant cultural barrier | Blench, 1999; Sanfo *et al*., 2015; Malonine, 2006; Fratkin, 2012 | +++ | +++ | ++ |
| | Breeding strategies | Alteration of animal species and breeds | Matching type of animal kept, and the nature of the livestock production systems remain a major problem most breeding intervention. Breeding intervention as an option within climate smart livestock production has to be disaggregated | Albuquerque *et al*., 2006.; Blench, 1999; Axford *et al*., 2000; FAO, 2007 | +++ | ++ | ++ |
| Environmental | Manure management | Anaerobic digesters for biogas and fertilizer. | Seasonal differences in the diet of grazing animals which also greatly influence manure output and its nutrient content. | Wambugu *et al*., 2014; Schlecht and Buerkert 2004; Harouna, 2002; | +++ | +++ | +++ |
| | | Composting, improved manure handling and storage, (e.g. Covering manure heaps) application techniques (e.g. Rapid incorporation) | Appropriate interventions need to focus on improving manure management to ensure that manure quality is not loss before applying it. | Bayala *et al*., 2011; Harouna 2002; Hassane 2002; Kanta 2002 | + | ++ | ++ |

*Food security / adaptation / mitigation potential: + = low; ++ = medium; and +++= high*
**This general assessment may vary in different locations*

overgrazing. The impacts from a combination of the various interventions can greatly reduce the total amount of GHG produced by livestock.

*Agro-silvo-pastoral practices*

Improved integration of trees into the crop-livestock systems does not only increase productivity of the farming system but also provides complementary feed resources to feed livestock, especially in rural communities where forage crops are rarely grown specifically for livestock as a source of protein for animals (Larbi *et al.* 2005) Excellent trees (Afzelia Africana, Khaya. senegalensis and Pterocarpus erinaceus) in an agro-silvo-pastoral system are accepted by herders who are facing new challenges to secure their livelihoods in increasingly reduced natural pastures. These trees are also among the most preferred by settled local farmers in agroforestry systems in the sub humid zone of Burkina Faso (Oue´draogo *et al.* 2000). Breeding priorities for fodder trees need to be identified through close interaction between breeders, farmers and climatologists.

*Selecting adaptive animal breeds*

For the optimal utilization of the adaptation traits harboured in all breeds, research in genetic characterization and understanding adaptation in stressful environments needs to be strengthened. Developing methods for characterizing adaptive traits relevant to climate-change adaptation (heat tolerance, disease resistance, adaptation to poor diet, etc.) and for the comprehensive evaluation of performance and use of animals in specific production environments will be attractive options. These will require coordinated efforts of both policy and institutional support.

*Improved manure management*

As animal production increases, measures to manage and recycle manure need to increase concurrently. Different manure treatments; dehydration, anaerobic digester and composting exist. The availability of low-cost, efficient and easy-to-adopt/adapt manure management technologies is expected to play a key role in stimulating a climate smart livestock production system. It should be noted that livestock are raised under different systems of production and this influences the manure management systems and the strategies that would be easily adopted in such systems. Low-cost implements, suitable for manure collection and spreading and appropriate institutional arrangements to strengthen complementary interactions between farmers and herders will improve manure utilization and consequently, mitigate methane emission, improve soil fertility and increase food security.

*Appropriate institutional support*

Strong institutional support is required to promote inclusivity in decision making, improve the dissemination of information, provide financial support and access to markets, provide insurance to cope with risks associated with climate shocks and the adoption of new practices, and support farmers' collaborative actions. Establishment of government livestock production areas including grazing areas, extension and support services, marketing and value addition platforms may be a useful

*Promoting a more conducive and inclusive policy*

Governments and policy makers should formulate country specific CSA policies that can help farmers especially livestock keepers to cope with the adverse effects of climate change. Farmers need policies that remove obstacles to implementing CS practices and create synergies with alternative technologies and practices. Policy makers should harmonize and bring together the various scattered CS practices, related policies, projects and programmes into one which is comprehensive and accessible to all stakeholders. Considerable policy support and capacity enhancement is needed for climate risk management including insurance and safety nets as well as improved access to weather information adapted to farmers' needs.

*Gender mainstreaming of CSA options*

The roles and rights of women livestock keepers are often overlooked and

development is frequently skewed towards the interest of men, yet women are responsible for the care of most sedentary livestock and play an active role in on-farm livestock duties and in the marketing of products (for example milk). Because of their key roles in livestock production, they are also key actors in The uptake of climate-smart practices. CS practices that will be more attractive to women should be considered. For example, in sub-Saharan Africa, insecure land tenure, workload, heavy tools, lack of capital and limited farm inputs posed major barriers to the adoption of conservation agriculture and agroforestry (often regarded as climate-smart) by women farmers (Nelson and Huyer, 2016). Efforts to build resilience in livestock keeping communities therefore requires particular emphasis on building the resilience of women by enhancing their capacity to adapt to climate change.

## Conclusion

Livestock remain a key component in the livelihood strategies of smallholder farmers in West Africa. They provide the much-needed inputs into crop production and provide key sources of income and household nutrition in most agro-pastoral communities. Mainstreaming livestock production practices that are climate smart should be the goal of any research for development intervention in West Africa. We therefore conclude that:

i. Livestock climate smart practices are highly context specific, and at times involve trade-offs between productivity, adaptation and mitigation. As such, stakeholder consultation is important when deciding which climate smart practice to implement, as factors such as labour availability and agro-ecological conditions may constrain outcomes.
ii. Successful CSA strategies will require investment in infrastructure that support smallholder farmers in understanding climate change, developing and refining strategies and evaluating CSA options.
iii. There is need to enhance farmers' access to and understanding of information, through improved communication approaches and better extension services.
iv. Policy and institutional support are crucial for providing an enabling environment for smallholder farmers to implement livestock-related climate smart agriculture.

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# FOOD SECURITY AND FOOD SAFETY ISSUES SURROUNDING THE DAIRY VALUE CHAIN IN TANZANIA

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## Abstract

There are increasing concerns with regards to quality and safety of milk and milk products obtained from the dairy value chain in Tanzania. Over 86 percent of the milk consumed in the country is obtained through informal distribution channels from animals with unknown health status and where good milking and handling practices are often not observed. This paper is compiled using part of the data from four research projects conducted between 2008 and 2015. The goals of the research projects were (1) to increase milk productivity among the smallholder farmers, and (2) to evaluate the prevalence, levels, and identify bacteria in common forms of milk marketed and consumed in the country. The data were obtained from surveys, systematic review of the literature and laboratory analyses. The major food security issues include production seasonality, lack of sufficient processing capacity, post harvest losses, and undernutrtion in some gender groups. With respect to milk safety, bacterial and coliform counts exceed the standard levels set by the Tanzania Bureau of Standards and the East African Community with total bacterial counts of 1.0×107 colony forming units per millilitre (cfu/ml) and total coliform counts of 1.1×107 cfu/ml. Rare species of bacteria including Pseudomonas aeruginosa, Listeria monocytogenes, Listeria innocua, Listeria ivanovii, and Klebsiella spp were isolated from raw and ready to consume milk. Additional challenges included adulteration and residues of antibiotics (tetracycline and sulfonamide). Furthermore, the sector has been shown to be dynamic and informal with a diversity of consumers who appreciate milk for its nutritional value but showing little concern for milk-borne hazards. These factors contribute to the contamination of milk with spoilage and pathogenic organisms leading to quality loss and infections contributing to food insecure commununities. It is recommended to work towards solutions that take an interdisciplinary and cross-sectoral approach to promote products of nutritional value that are safe to consume thereby improving nutritional security.

**Keywords:** Adulteration, Contamination, Dairy Sector, Informal markets, Nutritional Security, Pathogens

# PROBLÈMES DE SÉCURITÉ ALIMENTAIRE ET DE SÉCURITÉ SANITAIRE DES ALIMENTS AUTOUR DE LA FILIÈRE LAITIÈRE EN TANZANIE

## Résumé

La qualité et la sécurité du lait et des produits laitiers provenant de la filière laitière en Tanzanie sont de plus en plus une source de préoccupations. Plus de 86 % du lait consommé dans le pays provient de circuits de distribution informels et est produit par des animaux ayant un état de santé inconnu, dans un contexte où les bonnes pratiques de traite et de manipulation sont souvent inobservées. Ce document est compilé en utilisant une partie des données provenant de quatre projets de recherche menés entre 2008 et 2015. Les objectifs des projets de recherche étaient (1) d'accroître la productivité du lait chez les petits éleveurs et (2) d'évaluer la prévalence, les niveaux et d'identifier les bactéries dans les formes de lait communes commercialisées et consommées dans le pays. Les données ont été obtenues à partir d'enquêtes, d'examens systématiques de la littérature et d'analyses en laboratoire. Les principaux problèmes de sécurité alimentaire comprennent la saisonnalité de la production, le manque de capacités de transformation suffisantes, les pertes post-traite et la malnutrition dans certains groupes (des deux genres). En ce qui concerne la sécurité sanitaire du lait, les numérations de bactéries et de coliformes dépassent les niveaux standard fixés par le Bureau des normes de Tanzanie et la Communauté de l'Afrique de l'Est,

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les numérations bactériennes totales de 1,0 × 107 unités formant des colonies par millilitre (cfu/ml) et des dénombrements totaux coliformes de 1,1 × 107 cfu/ml. Les espèces rares de bactéries dont Pseudomonas aeruginosa, Listeria monocytogenes, Listeria harma, Listeria ivanovii et Klebsiella ont été isolées à partir de lait cru et prêt à consommer. Parmi les autres défis, mentionnons le frelatage et les résidus d'antibiotiques (tétracycline et sulfonamide). En outre, le secteur s'est montré dynamique et informel avec une diversité de consommateurs qui apprécient le lait pour sa valeur nutritionnelle, mais qui se soucient peu des dangers sanitaires liés au lait. Ces facteurs contribuent à la contamination du lait par des organismes de contamination et des pathogènes entraînant des pertes de qualité et des infections qui contribuent à l'insécurité alimentaire des collectivités. Il est recommandé de travailler à des solutions qui adoptent une approche interdisciplinaire et intersectorielle pour promouvoir les produits de valeur nutritionnelle qui sont sûrs pour la consommation, et améliorent ainsi la sécurité nutritionnelle.

**Mots clés :** frelatage, contamination, secteur laitier, marchés informels, sécurité nutritionnelle, agents pathogènes

## Introduction

The dairy value chain supports livelihoods of millions of rural and urban poor and acts as one of the pathways out of poverty, milk being the major product (Omamo *et al*., 2006; ILRI, 2011). Milk is a source of energy and provides important micro and macro nutrients which improve the nutritional status of individuals and populations (FAO, 2013). In developing countries where population is increasing rapidly, the economy is growing and urbanisation continues at a fast rate, the consumption of livestock products including milk was predicted to increase (Tschirley *et al*., 2014). In Tanzania milk production has increased several folds over the past two decades reaching over 2.2 billion litres per year in 2018 (MLF, 2018). The demand has been rising sharply, driven mainly by a human population that is growing fast at 3.3% per annum and a high economic growth rate of about 7% per annum over the same period (NBS, 2012). A larger contribution of the value chain to nutrition and the gross domestic product (GDP) has been predicted (Omamo *et al*., 2006; ILRI, 2011).

However, the dairy value chain in Tanzania is continuously reported to face enormous challenges which have been shown to hinder its full growth thus leading to its low contribution to the economy and nutrition (Msalya, 2017; Häsler *et al*., 2018, 2019). For example, a large proportion of milk and milk products are mainly sold through informal outlets including open markets, farm gate, door-to-door sales and small shops, where vendors or hawkers play a significant role in connecting producers to consumers. In many rural households, milk and milk products are produced on home farms or purchased from neighbours (Kurwijila *et al*., 2006; Msalya, 2017). The existence of these markets leads to at least 80% of the milk which is consumed off-farm to have a very short chain starting directly from the farmer to the consumer (Msalya, 2017). Also in these channels about two-thirds of the milk is obtained from the traditional cattle herd comprising of the Tanzania shorthorn zebu (TSZ) animals which are rarely inspected or vaccinated against diseases and therefore their health status is unkown (ILRI, 2011). It was recently shown that products from sick animals may also be consumed (Häsler *et al*., 2018). In such markets there is less formal inspection of the milk and consistent processing and value addition are lacking. Combined, these factors lead to potential burdens of food borne diseases and food (milk) quality loss. Fluactuation in milk quantity is another serious challenge in particular when processing is considered (Kurwijila *et al*., 2012) and poses potential business risks (Twine *et al*., 2018). Furthermore, this situation leads to the exclusion from consumption of milk and milk products for some gender groups, thus leading to nutritional insecurity.

The Sokoine University of Agriculture (SUA) in Tanzania in collaboration with the International Livestock Research Institute (ILRI)

based in Nairobi, Kenya, the Institute of Risk Assessment (BfR) of Germany and the Royal Veterinary College (RVC) in London teamed up to respond to the challenges and propose solutions which if are properly implemented can enhance the growth of the dairy value chain in the country. Under these partnerships, several projects were implemented in selected sites of Tanzania and significant results have been reported. Part of the results and success stories from these projects are presented in this paper.

*Implemented projects and approaches*

MoreMilk in Tanzania (MoreMilkiT) and in particular phase II was a four-year research-for-development (R4D) project targeted at improving rural based livelihoods through milk. The project's broad goal was to seek a better understanding of the policy environment and appropriate entry points for impacting the poor and marginalized; identify and consult a wide range of stakeholders; conduct a situational assessment nationally and value chain assessments (VCA) within the project sites to identify constraints and opportunities; and, initiate a process for strengthening the policy environment to better support pro-poor dairying. It was intended in the project to use the dairy market hubs (DMHs) approach to allow the marginalized groups to grow towards greater participation in the value chain.

*Rapid assessment of potential benefits to human health and nutrition from research on livestock and fish market chains in Asia and Africa*

The project was conducted in five countries in Africa and Asia. In Tanzania, the dairy value chain was assessed to determine the quality and safety of products produced and consumed in the informal markets. Specifically, the project assessed potential hazards associated with animal sourced-food (ASF) products in informal systems and which were likely to have the greatest risks to human health. Key constraints to the supply and demand of safe and nutritious foods and ways of overcoming risks and constraints were evaluated.

*Safe food, fair food: Improving food safety and market access in smallholder meat, milk and fish value chains in four African countries*

This project represents the second phase of the Safe Food, Fair Food project (SFFF), which started in 2008 with Tanzania being one of four target African countries. The overall goal of the project was to protect the health of poor consumers and safeguard livestock-based livelihoods of poor livestock keepers and other value chain actors. The purpose of the project was to improve food safety and market access throughout smallholder and informal meat, milk and fish value chains in sub-Saharan Africa. This was accomplished through food safety stakeholders' and value chain actors' practical application of risk analysis and social and economic analyses. In Tanzania, the project aimed to build capacity in risk analysis as a tool to assess and manage food safety in informal markets and participatory risk assessment of food safety was done in selected milk value chains and best bet interventions were suggested.

*Re-assessing diseases in smallholder dairying in Tanzania (What is killing my cow?)*

This was a major engagement in Tanzania to generate solutions and evidence for large scale and pro-poor development of smallholder dairy value chains. Activities from several donor-funded projects were integrated to characterize the current institutional context and technical challenges, evaluate options that were needed by dairy farmers and actors along informal milk marketing chains to improve their productivity and livelihoods and increase the supply of milk and the critical nutrients it contains to their communities and nearby urban centres. One area for immediate attention was cattle diseases which remain the major constraint for increasing dairy productivity. Diseases account for an overall mortality of between 12 and 14% in smallholder dairy cattle. Some of these diseases can be transmitted to humans, resulting in illness and even death. The purpose of the project was to conduct a broader assessment of cattle diseases and understand the links between

them and farm management practices.

## Methodology and approaches

*Project areas*

These projects were conducted in three regions of Tanzania namely Tanga, Morogoro, and Coast where farmers were requested to be available for surveys and where samples for laboratory tests were collected. Morogoro and Tanga are among the major milk-shed regions of Tanzania producing the largest volumes of milk from the smallholder dairy farming systems or SDS (Maleko *et al*., 2018b). There have been several interventions in the dairy sector in Coast region and for this reason, it was chosen and a few samples were collected and some surveys were conducted in the region. In each region, two districts representing the required farming system were selected. Villages (the smallest administration units) were selected within the districts for project implementatuion and sampling of milk. Participant farmers were randomly selected from among household heads who met the set criteria including possession of lactating cows and willingness to share any required information and provide milk samples. Milk samples were also obtained from milk vendors and milk collection centres in these sites.

*Participatory rural assessments of milk safety and nutrition risks*

The potential milk safety and nutrition risks and benefits within the dairy value chain were assessed using the rapid integrated assessment (RIA) toolkit (https://www.ilri.org/node/7007) that includes participatory rural appraisals (PRAs), producers and consumers' focus group discussions (FGDs) comprising 6 to 8 participants cross-sectional survey including a systematic literature review (Alonso *et al*., 2016a), value chain mapping (Sikira *et al*., 2013), producer and consumer surveys (Häsler *et al*., 2018) and biological sampling and laboratory testing (Joseph, 2013; Shija, 2013; Hyera, 2015), among other tools. The structure and contents of PRAs for producers and consumers were recently reported by Häsler *et al*. (2018, 2019). Data collection was done using structured questionnaires which included observation checklists. These instruments were applied at the production, bulking (wholesale or collection points), processing, retail, and consumption stages in the study sites. The FGDs were also conducted with mothers of young children, and included questions on food preparation practices, consumption of food products by different members of the community, possible associations between food and health problems, and the importance of food waste or animal feeds competing with food for humans. The ranking of constraints was converted to a scoring system (highest rank = highest score) to allow comparison between sites.

*Milk sampling*

Milk samples were collected from lactating cows and milk containers in the study sites within the rural to rural, or rural to urban production and marketing systems. About 50 mls of milk were collected in sterile containers which were purchased from the local markets. Care was taken to avoid contamination of the samples by washing and wiping the udder before milking. The sampling tube was held such that it could not come into contact with the udder and the samples were taken after a few first drops were discarded. For bulk milk, producers were encouraged to use clean containers from which samples were taken. The samples were immediately placed in a cool box with ice packs and were stored at -80°C before analyses (no sample stayed under this storage condition for more 30 days).

*Bacterial culture, coliform count, and initial procedures for pathogens identification*

The first step was to survey the total plate count (TBC) and total coliform count (TCC) which were estimated using conventional laboratory methods within 48 hours after sampling. Laboratory procedures to prepare for identification of pathogens were then carried out using the relevant protocols of the International Organization for Standardization (ISO) or DNA genotyping. Initial isolation procedures were performed

according to ISO 4833-1 protocols (ISO, 2013) and were followed by detection and confirmation of the target microorganisms based on colonial morphologies as well as confirmatory tests using the relevant protocols and commercial kits as for example the Listeria test kit (Oxoid® Ltd., Basingstoke, Hampshire, England) as well as the PCR protocols. To carry out bacterial culture and coliform counts, the pour plate technique (ISO 7218:2007(E)) was used. Culture solutions including 0.15mls of the milk sample and 1.35 mls of normal saline (BDH AnalaR®, BDH Limited Poole England, Prod 10241) diluted serially four times in a MacConkey agar (Oxoid LTD, England) was used in the coliform counts. From the solution, 1 ml was drawn and mixed thoroughly with melted media and the mixture. Incubation was done at 37°C for 24 hours after which the number of colonies in each dilution was counted. To ensure sterility, the samples were autoclaved for 15 minutes at 121°C and all laboratory experiments were carried out in a bio-safety cabinet.

*DNA extraction and polymerase chain reaction (PCR)*

From the same samples as those used in the bacterial culture and coliform counts, DNA was purified using the QIAamp Viral RNA Kit (QIAGEN Sciences, Maryland, USA) according to the manufacturer's instructions. Later the PCR was carried out targeting 726bp of the 16S-23S gene in Brucella abortus and 361bp of the hlyA gene in Escherichia coli (E. coli) using primers BRU-P5 TCGAGAATTGGAAAGAGGTC and BRU-P8 GCATAATGCGGCTTTAAGA (for 16S-23S) as well as 0157-3 GTAGGGAAGCGAACAGAG and 0157-4 AAGCTCCGTGTGCCTGAA (for hlyA) in a 25 µl reaction including 3µl of the DNA. The amplification conditions were 30 cycles of denaturation at 95°C (15 seconds), annealing at 55°C (30 seconds) and extension at 72°C (60 seconds) in a thermal cycler (StepOne PCR systems, Applied BioAsystems). The PCR products were separated on a 1.5% agarose gel and visualized with Ethidium bromide under ultra-violet light.

*Data analysis*

Data from surveys were presented as text or numbers in pre-defined charts, diagrams, or tables. Descriptive statistics (mean, frequencies and percentages) were used to describe the characteristics of the research samples and quantitative data. Qualitative data were summarised in a general narrative. The rnking of constraints was converted to a scoring system (highest rank = highest score) to allow for comparison between sites. The cleaned data was then copied into the STATA I/C 11 statistical package for further analysis. The relationships between practices as risk factors for microbial contamination in milk were computed against TBC and TCC.

*Ethics*

In Tanzania research permits are issued by the Commission for Science and Technology (COSTECH). For these projects research permits were issued by the SUA Vice Chancellor on behalf of COSTECH and projects were conducted in accordance to a Memorandum of Understanding (MoU) between SUA and ILRI. Before any attempt to work with the project participants, a verbal consent was obtained from each respondent after explaining the purpose and importance of the study. Furthermore, participation in the study was on a voluntary basis. All the information collected from the participants and the laboratory results obtained after milk samples analysis were kept under the custody of the researchers as confidential.

## Results

*Challenges facing the dairy value chain of Tanzania (Feedback: survey data from projects)*

The results of this study indicated very low access by farmers to feeding, breeding, animal health and credit services. The major constraits included limited technical know-how, a small number of improved dairy cows, diseases in the dairy herds, lack of investment, poor nutrition based on grazing in communal plots (the only means of ruminant nutrition in the traditional pastoral communities) and unreliable markets.

Among the suggested solutions, priorities were the creation of producers' associations and provision of subsdies and loans. In 2014 only 4% of poor livestock keepers had access to credit and only 1.4% of marketed milk was processed. The constraints were listed and ranked by the participating farmers and are summarized in Table 1. It was highlighted that the production of a marketable surplus remains a fundamental challenge and this reinforced the need for organizational change, such as adapted hub approaches to address the challenges. The identified entry points for piloting to 'grow' the existing informal system of milk production and marketing that the vast majority of cattle producers are part of, include dairy 'hub' constructs or approaches with emphasis on improving access to inputs and services.

**Table 1:** Production constraints as viewed by participating farmers

| Constraint | Ranking (All sites) | Importance measure |
|---|---|---|
| Lack of inadequate technical knowhow | 1 | 32 |
| Poor quality breeds | 2 | 21 |
| Cattle diseases | 7 | 15 |
| Lack of inadequate capital and less access to credit | 5 | 13 |
| Lack of inadequate pasture and/or water | 3 | 7 |
| Lack of or unreliable markets for livestock and milk | 8 | 6 |
| Poor feed quality | 6 | 5 |
| High input prices | 9 | 5 |
| Lack of grazing land | 4 | 1 |
| Lack of dip tanks | 10 | 0 |

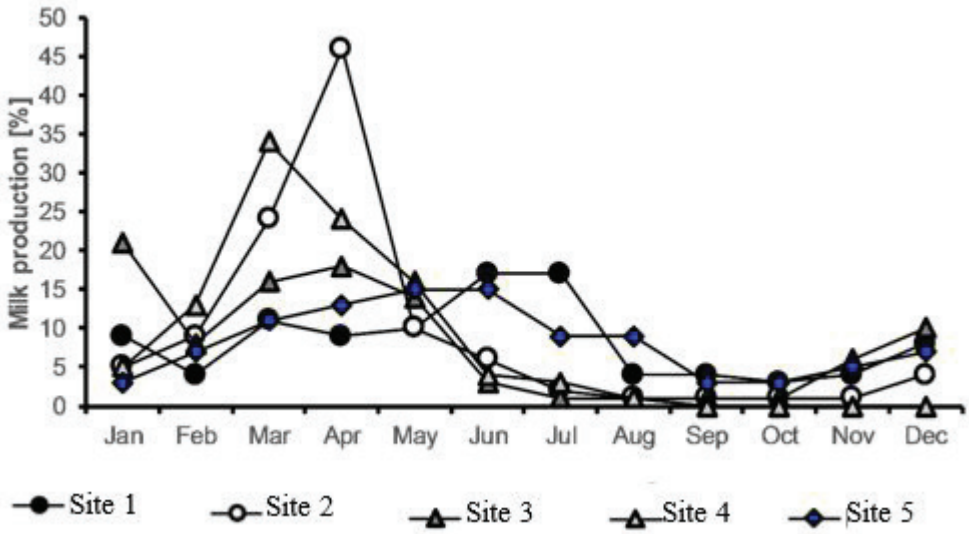


**Figure 1:** Trend of milk production in the study regions (average production) as reported by producers during the surveys (Reproduced with permission and modification from Häsler *et al.*, 2019)

*Milk production seasonality and rainfall patterns*

Regarding the seasonal calendar, it has been clearly shown that fluctuation in milk production in a year reflects rainfall patterns and the associated availability of pasture, water and by-products from crop production such as maize straws. The rainfall patterns have been described as long or intense rains falling between March and May in the project regions as well as the short or less intense rains falling between October and December. Milk production follows this trend whereby much milk is obtained during the long rainy season and the lowest milk production is in July to October (Fig. 1). In all the study sites, milk consumption was related to the availability of milk and purchasing power. The differences in consumption levels were also thought to be caused by different seasons of harvesting of horticultural crops (mainly fruits) in the sites.

*Diseases reported in the dairy value chain in Tanzania (human and livestock diseases)*

The dairy sector in Tanzania is challenged by many and varied diseases including East Coast fever (ECF) also known as theileriosis, black quarter (BQ) also called black leg, babesiosis, mastitis, and helminths. Other diseases which were mentioned during the surveys were tuberculosis (TB), lumpy skin disease (LSD), foot and mouth disease (FMD), contagious bovine pleuropneumonia (CBPP), milk fever, anaplasmosis, and diarrhoea. Some of the disease challenges were rare and diffcult to identify by farmers and they included brucellosis and query (Q) fever disease. It was also a concern that these diseases are not well known to the majority of farmers and consumers although they have been previously trained to recognise the clinical signs of some diseases and have indigenous knowledge of controling some of the diseases. Diseases were among the major causes of mortality which has been shown to be high ranging from 11 to 69%. Diseases were also a major cause for milk loss as some farmers discarded milk from sick animals (some producers consumed milk from sick animals).

*Nutrition aspects of milk in the study sites*

The consumption of milk was reported to be high in pastoralist systems where a child typically consumed up to 2 litres a day during time of the survey. The main dairy products were: fresh milk, boiled milk, ghee, fermented fresh milk, and fermented skim milk. A limited amount of commercial products were found in the farming communities but were consumed by very few. The main form of milk provided to children was boiled fresh milk. The use of fermented milk and raw milk for children was also observed, but was shown to be rare. In some sites, another practice was providing home-filtered and boiled milk or adding milk in porridge particularly for sick children. Generally, milk was treated and regarded as a very important food supporting growth and development as well as maintaining good health in children. In a few households, the consumption of milk was limited to children only due to the low production of their animals and low purchasing power of the households. There were some gender differences in consumption (for example pregnant women were less likely to drink fermented milk in some locations). Poor people were largely excluded from the consumption of dairy products. Other products consumed in the households were blood and ruminal juice. However, these were rare practices limited to a few pastoralist groups.

*Bacterial and Coliform counts*

A total of 169 milk samples from the study sites were cultured to re-evaluate the levels of total bacterial counts (TBC) and total coliform counts (TCC). There were high levels of bacteria and coliforms in different forms of milk obtained from the informal markets in the country. In Table 2, values of TBC ranging from $4.5 \times 10^4$ to $5.2 \times 10^6$ cfu/ml and TCC from $5.5 \times 10^4$ to $1.3 \times 10^7$ cfu/ml are shown.

*Identification of two pathogens using molecur techniques*

In the food safety study, two priority species were targeted. Molecular analyses were carried out to identify the prevalence of E. coli

O157:H7 and B. abortus in the milk samples. The contamination rate of 16S-23S (B. abortus specific sequence) was 30% of the total samples collected. All samples were negative for E. coli O157:H7 species-specific genes. Electrophoresis photos showed no bands at position 500 – 600 bp for E. coli, whereas these were clearly seen for B. abortus. These results are summarized in Table 3.

**Table 2.** Levels of bacteria and coliforms in milk samples collected from various places in the project sites

| Sample type (N = 169) | TBC (cfu/ml) | TCC (cfu/ml) |
|---|---|---|
| Mvomero District, Morogoro | $6.5x10^4$ | $2.9x10^6$ |
| Kilosa District, Morogoro | $1.9x10^6$ | $1.3x10^7$ |
| Collection centres | $5.2x10^6$ | $2.5x10^6$ |
| Households | $4.5x10^4$ | $6.7x10^4$ |
| Vendors | $5.0x10^5$ | $7.4x10^6$ |
| Kiosks | $1.2x10^5$ | $5.5x10^4$ |
| Pasteurized milk Mvomero | $6.5x10^4$ | $8.8x10^4$ |
| Pasteurized milk Kilosa | $1.9x10^6$ | $1.1x10^5$ |
| Lack of grazing land | 4 | 1 |
| Lack of dip tanks | 10 | 0 |

**Table 3.** Detection rate of 16S-23S (B. abortus) and E. coli O157:H7 genes in milk samples collected from Morogoro and Tanga regions

| Sample size (N = 169)/Genes | Positive Samples | Negative Samples |
|---|---|---|
| B. abortus | 51 (30%) | 118 (70%) |
| E. coli O157:H7 | 0 | 169 (100%) |

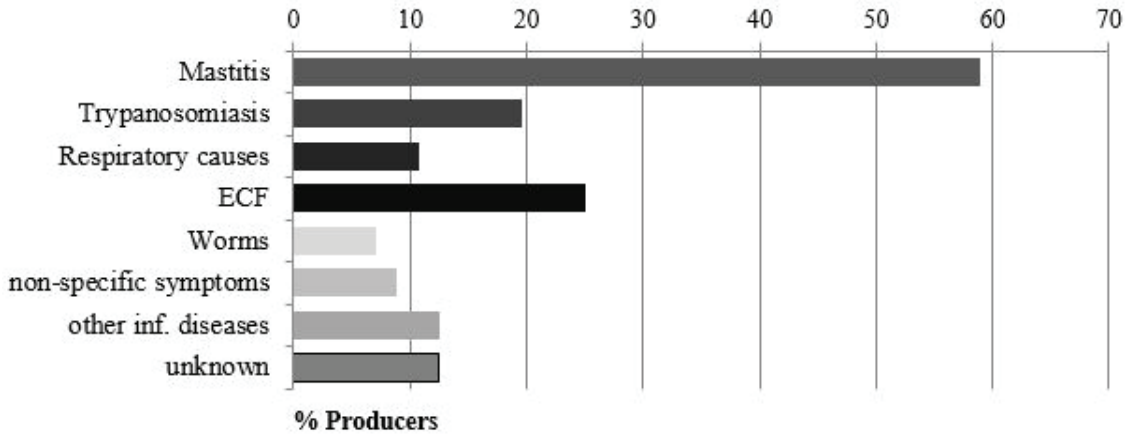


**Figure 2.** Diseases reported by participants in the study sites

*Quality and safety concerns*

Based on data collected during these surveys, it was evident that both food (milk) quality and safety in the study areas were not clearly known and therefore some forms of dairy products may not be safe to consume. The major challenges that have been previously highlighted includepoor milk quality, prevalence of antimicrobial residues in milk, occurrence of mastitis in the dairy herd, adulteration, poor composition of nutrients, and public health hazard if milk is adulterated with contaminated water (Kurwijila *et al*., 2006; Msalya, 2017). Poor milk quality is a major cause of diseases among consumers and the main health hazard was diarrhoea caused mainly by E. coli. For example, diarrhoea, vomiting, and/or skin itching was observed among allergic and non-allergic children soon after consumption of milk or milk products in the study sites. Other health problems caused by poor quality milk, poor handling of milk, and contamination included TB, brucellosis, anthrax, salmonellosis, and campylobacteriosis. Antimicrobial residues reduce the quality of milk and have been shown to jeopardise people's health in various ways. In addition, mastitis was described by farmers during the surveys as a disease contributing to large losses as well as reducing the quality of milk. The dairy products consumed by the majority of consumers were fresh and fermented milk some of which have been contaminated. The main risky practice was the consumption of raw milk and storage of boiled milk which is normally re-contaminated. These products can be detected by the farmers and consumers using sensory tests including smell, colour, observation of sick cows, and unattractive aroma. Although milk is regarded as an important food, poor milk quality and the fear of milk-borne diseases were among the causes of poor consumption of milk and milk products particulary among women and children.

*Suggestions for improving the quality and safety of milk*

To address these challenges various best bets were suggested aimed at reducing losses caused by the poor quality of milk, antimicrobial residues, and mastitis. It was suggested that there should be improved handling and storage of milk at the production (mainly household) level to enhance milk quality. Furthermore, the Tanzania Dairy Board (TDB) can collaborate with SUA and ILRI to (i) review the existing regulatory and quality assurance guidelines and integrate training and certification, and (ii) monitor and deliver training and certification schemes for milk quality. Milk safety/quality and success indicators could be monitored before and after implementation. To reduce the antimicrobials, the training of producers needs to be undertaken regularly followed by studies to compare the levels of drug residues in milk for trained and non trained farmers. Simple methods such as organoleptic tests (smell of antibiotics in milk and colour of milk) as well as laboratory (pH, acidity and inihibitor) tests can be taught to farmers. To address the mastitis problem, the responsibility has been given to the farmers to: (i) regularly screen and detect animals with clinical and sub clinical mastitis using the California mastitis test (CMT) and mastitis teat cups, (ii) treat all clinical cases of mastitis, (iii) report the disease signs in the herd to relevant authorities, and (iv) maintain closed herds. Moreover it was advised to sample milk and carry out periodic analyses to confirm the quality after interventions and share the results with the farmers.

## Discussion

The government of Tanzania is encouraging the processing of agricultural products and several investors are responding to this call. However research results from the projects recently implemented in the country indicate great concerns for the seasonality of milk production and poor quality and health safety issues (Msalya, 2017; Häsler *et al*., 2018, 2019). The Seasonality of milk production and the effects it causes have been described by various authors (Kurwijila *et al*., 2012; Twine *et al*., 2018). In this paper, the reality of seasonality, high levels of contamination in milk samples obtained from different points of the Tanzania

dairy value chain, and diseases affecting animals were elaborated. Additional information regarding the contamination of milk and milk products have been reported previously by various workers including Karimuribo *et al.* (2005); Joseph (2013); Shija (2013); Hyera (2015); Suleiman *et al.* (2017); Gwandu *et al.* (2018) and Mohammed *et al.* (2018). It has been noted that bacterial and coliform counts are high exceeding the levels recommended by the East African Community Standards (EAS, 2007). Challenges such as pathogens, antibiotic residues and antibiotic resistant bacteria are attributted to weaknesses in the value chain including the poor handling of milk and milk products, long transportation hours for raw milk, and lack of good storage facilities. Taken together, the status of the dairy value chain in the country limits the availability of sufficient quality milk as a raw material for industries. Integrated efforts are needed towards improving the capacity and performance of the dairy value chain in the country. The first step is increasing the stability of supply and secondly, increased processing will be needed in extending the shelf-life of milk. The major processing companies e.g. Tanga Fresh in Northern Tanzania, TanDairies in the business capital of Dar es Salaam, ASAS dairies and CEFA in the Southern highlands, and International Dairy Products in Arusha region among others have at some pointcomplained of not getting sufficient amounts of milk for their facilities and only operate at between 20 and 75% capacity (MLFD, 2011; Msalya, 2017). It is therefore of great importance to address these challenges, improve performance of the value chain and thereby increase the availability of raw materials for the processing plants and profit at various nodes of the chains and for the operators e.g. producers, businessmen etc.

Various ways of improving the situation (reducing the negative effects of seasonality and reducing contamination) have been suggested by various researchers (Maleko *et al.*, 2018a; Gershom and Ssemakula, 2017). Concerning safety, traditional processing methods such as fermentation are viewed by consumers and researchers as one among the ways of quality improvement, reduction of contamination, and reducing losses particularly in the poor communities, thus improving safety (Gershom and Ssemakula, 2017). However, this optiondoes not fully guarantee the availability of sufficient amounts of milk for the processing plants due to lack of cold storage at the level of the household. In addition, elements of food safety risks have been reported (Gershom and Ssemakula, 2017; Jans *et al.*, 2017). A reliable power supply will facilitate the availabiliy of cold chains for storage and may significantly improve the availability of milk and reduce losses. New technologies for cold storage such as the SimGas biogas milk chiller, biogas-powered milk cooling on-farm, and solar cooler have the potential to reduce microbial growth and spoilage and decrease post-harvest losses (Häsler *et al.*, 2019). Improving hygienic milking and handling at the farm and collection centres including timely delivery with better infrastructure and cooling systems may improve the quality and quantity of milk many folds. It has been suggested that if the collection centres are owned, empowered and operated by milk producer groups, associations or the private sector they can be profitable with effective management and governance (Moffat *et al.*, 2016). At such collection centres private standards including for example detection of adulteration, organoleptic checks, inhibitor tests to check whether the product is free from residues, and the Resazurin test can be implemented. These could be supported by incentives (e.g. higher prices for high quality milk) and disincentives (e.g. penalties for adulteration or residues). However, improved storage and processing would depend on a steady supply of milk.

The dairy sector and dairy value chain in Tanzania have been predicted to grow with better genetics, feed and health services, and policy support. With regard to seasonality of milk production, Maleko *et al.* (2018b) suggested the adoption of improved technologies for dairy cattle farming, promotion of on-farm research, and public-private partnerships and dairy farmers' cooperative associations. According to the Livestock Master Plan (LMP) these are the important elements for increasing production

and productivity (Michael *et al*., 2017). These authors also showed that increased investments are needed for a positive impact on the growth of the sector and increased productivity. These efforts need to go hand in hand with the control of diseases as well as the disease vectors and pathogens leading to the spoilage and contamination of milk and leading to the prevalence of TB, brucellosis and trypanosomiasis (Alonso *et al*., 2016b).

## Conclusion

Based on the information collected and the laboratory analyses, this study demonstrated that there are milk-borne zoonotic pathogens in the project sites. Though consumers have a clear understanding of quality and appreciate good quality, the use of raw or fermented milk was observed in all population groups resulting in potential exposure to milk-borne pathogens and development of disease. Further, there is a risk of exposure to zoonotic pathogens through direct contact with infected animals. It was shown here that there are some gender differences in consumption in particular related to pregnancy indicating that some groups are denied the right to consumption of some nutrients. Because poor people cannot afford to discard milk, they often drink it even if it is low quality. Therefore consumer choice was restricted in poor socio-economic groups. It is proposed to carry out more studies which show the extent of the bacterial problems and contamination. Of the most significance is improving performance of the dairy sector to increase both economic and nutrition benefits.

Declaration. I hereby declare to the Africa Union's Bulletin of Animal Health and Production that the procedure used in the survey and sample collection did neither harm humans nor animals and there is no conflict of interest for this article.

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# EFFECT OF AGE OF LAYERS AND HOUSING SYSTEMS ON EGG QUALITY OF TWO STRAINS OF LAYERS

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## Abstract

The current study was carried out to assess the effect of layer strain, housing systems and age of layers on egg quality. A total of three hundred and sixty (360) eggs were analysed for egg quality in a 2 X 2 X 3 factorial experiment involving two layer strains (Lohmann brown and white), two housing systems (deep litter and battery cage) and three layer age groups (24, 39 and 68 weeks into lay). Fresh eggs were randomly collected, labeled and analysed for egg quality within 24 hours of collection. External and internal egg characteristics such as weight of eggs, shell thickness, albumen height and pH, Haugh units, yolk colour and pH were examined. Three-way Analysis of Variance (ANOVA) with strain, housing system and layer age as fixed factors using the General Analysis of Variance procedure of GenStat, (Discovery edition) were used in the data analysis. Where differences in means existed, the means were separated using the least significant difference test at 5% level of significance. Data obtained showed no significant effects of housing system on these external egg qualities (egg weight, shell thickness and shell weight). There were however, significant strain effects on Haugh units with Lohmann white layers producing eggs with better Haugh units than their brown counterparts. The eggs produced by the two layer strains were not significantly different. The age at which eggs were produced did not significantly affect the weight of the egg but rather the freshness, which is determined by the Haugh unit.

**Keywords:** Age, Lohmann Brown, Lohmann White, Haugh unit, management system.

# EFFET DE L'ÂGE DES PONDEUSES ET DES SYSTÈMES DE LOGEMENT SUR LA QUALITÉ DES OEUFS DE DEUX SOUCHES DE PONDEUSES

## Résumé

La présente étude actuelle a été menée dans l'objectif d'évaluer l'effet de la souche des pondeuses, des systèmes de logement et de l'âge des pondeuses sur la qualité des œufs. Au total, trois cent soixante (360) œufs ont été analysés en vue de déterminer la qualité des œufs dans une expérience factorielle 2 X 2 X 3 impliquant deux souches de pondeuses (Lohmann Brown et White), deux systèmes de logement (litière profonde et cage en batterie) et trois groupes d'âge (24, 39 et 68 semaines en ponte). Des œufs frais ont été prélevés de manière aléatoire, étiquetés et analysés pour déterminer leur qualité dans les 24 heures suivant la collecte. On a examiné les caractéristiques externes et internes des œufs tels que le poids des œufs, l'épaisseur de la coquille, la hauteur de l'albumen, les unités Haugh, la couleur des jaunes et le pH. L'étude a eu recours à une analyse tri-directionnelle de variance (ANOVA) prenant la souche, le système de logement et l'âge de la pondeuse comme facteurs fixes utilisant le modèle d'analyse générale de variance GenStat (édition Discovery) dans l'analyse des données. Lorsque des différences de moyennes existaient, les moyennes ont été séparées en utilisant le test de différence le moins significatif à 5%. Les données obtenues n'ont montré aucun effet significatif du système de logement sur ces qualités externes des œufs (poids des œufs, épaisseur de la coquille et poids de la coquille). Cependant, on a noté des effets significatifs de la souche sur les unités Haugh, les pondeuses White produisant des œufs avec de meilleures unités Haugh par rapport aux pondeuses Brown. Les œufs produits par les souches des deux pondeuses n'étaient

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pas significativement différents. L'âge auquel les œufs ont été produits n'a pas affecté significativement le poids des œufs, mais la fraîcheur, qui est déterminée par l'unité Haugh, a été significativement influencée par l'âge de la pondeuse.

**Mots clés :** âge, Lohmann Brown, Lohmann White, unité Haugh, système de gestion.

## Introduction

Egg quality is on a higher scale when it comes to consumers' expectations, which signifies the need for ensuring high quality poultry products (Braun, 2018). Moraleco *et al*. (2019) defined egg quality as the physical or sensorial quality characteristics that are responsible for their acceptability in the market. Evidence abounds on the genetic influence of egg quality (Nassar *et al*. 2016; Sokołowicz *et al*. 2018 and Hagan and Eichie 2019). According to Hagan and Eichie, (2019) Lohmann brown layers produced eggs with better Haugh units (Haugh, 1937) as compared to those of Lohmann White layers. Scott and Silversides, (2000) also found that eggs from ISA-Brown hens were heavier than those from ISA-White hens and had more shell and albumen, but less yolk and albumen weight.

According to Sokołowicz and Krawczyk (2009) different housing systems adopted by farmers in the egg laying industry have generated significant debate among researchers, producers, environmentalists and consumers. What has become even more important is the effect of these systems on the health and general welfare of the birds. However, it is not easy to assess if a housing system provides for good health and the basic ethological and behavioural needs of birds. The type of housing system employed has been found to influence, directly or indirectly, not only the behaviour, productivity and health of birds, but also the quality of the eggs produced (Abrahamsson and Tauson, 1995: Tauson, 2005).

According to Johnston and Gous (2007) and Rakib *et al*. (2016) layer age is one of the main factors that influences egg quality indicators like weight, shape index and Haugh units. In general, egg weight significantly increases during the first three months of the laying period. The quality of the eggshell is also influenced by age. The eggshell gradually gets thinner with age, thus the percentage proportion of the eggshell decreases as well (Ledvinka and Klesalová, 2002). Eggs that are laid at the beginning of the laying period have higher Haugh units, albumen and yolk indexes (Bozkurt and Tekerli, 2009).

In Ghana, most farmers keep their layers beyond their productive period (beyond 72 weeks into lay) and later sell the birds as spent layers. The quality of eggs produced by old layers, according to Kraus and Zita (2019), could be compromised. There have been conflicting reports on the relationship between the age of layers and egg quality, with some showing that the weight of eggs, eggshell, yolk and albumen increased with increasing age of the hen. Other results also showed decline in egg quality with the increasing age of layers. According to Rakib *et al*. (2016) eggshell thickness and strength, egg shape index, albumen proportion, Haugh units, the albumen and the yolk indexes decreased sharply with advancing age of hens.

Previous work (Hagan *et al*., 2013 and Hagan and Eiche, 2019) examined either the effects of strain or the effects of age of layers or the influence of housing system on egg quality in Ghana and elsewhere, but not the effects of the combination of these on egg quality. The current study was therefore carried out to assess the quality of eggs produced by different layer strains of different ages reared under different housing systems.

## Material and Methods

*Location of the Experiment*

The study was carried out at the Teaching and Research Farm of the School of Agriculture, University of Cape Coast in Ghana. The average temperature of the Farm ranged between 19-340Cn with a humidity of 60-80%.

*Experimental Design*

Three hundred and sixty (360) eggs, 180 each from Lohmann Brown and Lohmann White layer strains (kept under the deep litter and battery cage systems) were collected at 24, 39 and 68 weeks into lay and analyzed for egg quality in a 2 X 2 X 3 factorial experiment. On each day of the examination, fresh eggs collected from the farm were labeled and examined for internal and external quality parameters within 24 hours of collection.

*Data Collection*

*Internal and External Egg quality analysis*

The internal and external egg qualities were examined. Egg weight in grams (g), shell weight (g) and thickness (mm) measurements were taken for external egg characteristics. Albumen weight (g), albumen height (mm), albumen pH, yolk height (mm), yolk weight (g), yolk pH and Haugh unit (%) were taken for internal egg quality parameters. An electronic balance (Mettler P 1210) with 0.01g precision was used to measure egg weight, albumen weight, yolk weight and eggshell weight. A digital Vernier calliper was used to measure egg shell thickness and albumen height. In measuring shell thickness, each shell was air-dried at room temperature for 24 hours after washing it with running tap water and cleaning it with tissue paper. Three pieces of shell consisting of the narrow end (the sharp region), the middle side (the equatorial region) and the broad end (blunt region) of each egg was taken after removing the shell membrane. This was done by breaking the egg with a knife close to the blunt region and the albumen carefully separated from the yolk using a yolk separator as described by (Nonga *et al*., 2010). The sharp region of the egg shell was separated from the equatorial region with the aid of the knife. The thickness of each shell piece was measured with the digital Vernier calliper and the average of the three measurements was computed as describe by Ehtesham and Chowdhury ( 2002). The digital Vernier calliper was then used to measure the height and diameter of the albumen and yolk. The albumen weight was computed by subtracting the yolk weight and the eggshell weight from the total egg weight as recommended by Parmar *et al*.(2006), that is

*Albumen weight = total weight - yolk weight - eggshell weight*

The yolk colour was determined with the aid of a Roche yolk colour fan with values ranging from 1-14 representing pale yellow to deep yellow colours. The albumen and yolk pH were determined using a pH meter (Hanna Inst., Woonsocker, RI 02895). The Haugh unit which is a measure of the freshness of the egg was computed from the values obtained from the albumen height and egg weight using the formula proposed by Haugh (1937)

*Haugh unit = 100log (albumen height - egg weight 0.37 + 7.57)*

*Data Analysis*

The data obtained were subjected to a three-way analysis of variance (ANOVA) with strain, housing system and age of layers as fixed factors using the General Analysis of Variance procedure of GenStat (Discovery edition). Where differences in means existed, the means were separated using the least significant difference (LSD) test at a 5% level of significance.

*The statistical model used was as follows:*

$$Y_{ijk} = \mu + S_i + H_j + A_k + (SH)_{ij} + (SA)_{ik} + (HA)_{jk} + (SHA)_{ijk} + e_{ijk}$$

Where:

$Y_{ijk}$ = observation on the ith strain, jth storage period and kth storage method

μ = overall mean

$S_i$ = effect of ith strain of layers

$H_j$ = effect of jth housing system

$A_k$ = effect of kth age of strain of layer

$(SXH)_{ij}$ = the interaction effects of the ith strain and jth housing system

$(SXA)_{ik}$ = the interaction effects of the ith strain and kth age of strain of layer

$(HXA)_{jk}$ = the interaction effects of the jth housing system and the kth age of strain of

layer
$(SXHXA)_{ijk}$ = the interaction effects of the ith strain of layer, jth housing system and kth age of layer
$e_{ijk}$ = random error

## Results

*Effects of strain of layer on the external and internal egg quality*

Table 1 shows the external and internal egg quality of the two layer strains (Lohmann Brown and Lohmann White). As shown in Table 1, there was no significant strain effect on egg weight but there were significant strain effects on the other egg quality traits examined. Eggs from Lohmann white strains were superior to the Lohmann brown in terms of the Haugh units.

*Effects of age of layer on the external and internal egg quality*

Table 2 shows the effects of layer age on the internal and external egg quality. The results obtained showed no significant age effects on egg weight, shell thickness, albumen weight, yolk weight and albumen pH. There were however significant age effects on the shell weight, albumen height, yolk colour and Haugh unit. The Haugh unit (which shows the freshness of the eggs) and the shell weights, reduced significantly ($p<0.05$) as the birds aged. The colour of the yolk was also significantly affected by the age of the birds with younger layers laying eggs with significantly more yellowish colour with the colour becoming pale as the birds aged.

*Effects of housing system on the external and internal egg quality*

Birds on the deep litter system laid significantly ($p<0.05$) bigger eggs than their counterparts which were kept under the battery cage system (Table 3). In addition, the birds on the deep litter system laid eggs with significantly better internal qualities compared to their counterparts on the battery cage system. However, there were no significant differences in the egg shell weight and yolk colour with the system of housing used.

*The interactive effects of strain, age of layer and housing system on the external and internal egg quality*

There were significant interaction effects of the age and housing system on the shell weight, albumen colour, albumen height and Haugh unit as shown in Table 4. There were also significant interaction effects of housing system and strain on egg weight, shell weight and albumen weight. Albumen height, yolk weight and Haugh unit were significantly affected by strain and age interaction.

**Table 1:** The effects of strain of layer on the external and internal egg quality

| Characteristics | Lohmann White | Lohmann Brown | SEM | P-value |
|---|---|---|---|---|
| Egg weight, g | 55.3 | 54.7 | 0.3 | 0.20 |
| Shell weight, g | $5.8^{b}$ | $6.1^{a}$ | 0.1 | 0.01 |
| Shell thickness, mm | $0.5^{a}$ | $0.4^{b}$ | 0.0 | <.01 |
| Albumen weight, g | $35.5^{a}$ | $33.9^{b}$ | 0.3 | <.01 |
| Albumen height, mm | $8.2^{a}$ | $7.3^{b}$ | 0.2 | <.01 |
| Yolk weight, g | $13.9^{b}$ | $14.7^{a}$ | 0.1 | <.01 |
| Yolk colour | $2.9^{b}$ | $3.0^{a}$ | 0.2 | 0.01 |
| Haugh unit, % | $90.7^{a}$ | $85.8^{b}$ | 0.8 | <.01 |
| Albumen pH | $8.1^{b}$ | $8.5^{a}$ | 0.0 | <.01 |

*[ab]: means in the same row with different superscripts are statistically different ($p<0.05$); SEM- Standard Error of Means*

**Table 2:** The effects of age of layer on the external and internal egg quality

| Characteristics | Age/weeks | | | SEM | p-value |
|---|---|---|---|---|---|
| | 24 | 34 | 68 | | |
| Egg weight, g | 55.7 | 54.9 | 54.5 | 0.5 | 0.09 |
| Shell weight, g | 6.3[a] | 5.9[b] | 5.8[c] | 0.1 | <.01 |
| Shell thickness, mm | 0.5 | 0.5 | 0.4 | 0.0 | 0.85 |
| Albumen weight, g | 34.8 | 34.7 | 34.6 | 0.3 | 0.96 |
| Albumen height, mm | 8.4[a] | 7.67[b] | 7.3[c] | 0.2 | <.01 |
| Yolk weight, g | 14.6 | 14.3 | 14.1 | 0.2 | 0.08 |
| Yolk colour | 4.4[a] | 2.6[b] | 1.7[c] | 0.2 | <.01 |
| Haugh unit, % | 91.2[a] | 87.4[b] | 86.1[c] | 1.0 | 0.01 |
| Albumen pH | 55.7 | 54.9 | 54.5 | 0.4 | 0.09 |

*[abc] : means in the same row with different superscripts are statistically different (p<0.05); SEM- Standard Error of Means*

**Table 3:** The effects of housing system on the external and internal egg quality

| Characteristics | Management System | | SEM | P-value |
|---|---|---|---|---|
| | Deep Litter | Battery Cage | | |
| Egg weight, g | 56.9[a] | 53.1[b] | 0.3 | <.01 |
| Shell weight, g | 6.0 | 6.0 | 0.1 | 0.92 |
| Shell thickness, mm | 0.4 | 0.5 | 0.01 | 0.02 |
| Albumen weight, g | 35.8[a] | 33.6[b] | 0.2 | <.01 |
| Albumen height, mm | 8.4[a] | 7.1[b] | 0.2 | <.01 |
| Yolk weight, g | 15.2[a] | 13.5[b] | 0.1 | <.01 |
| Yolk colour | 3.0 | 2.8 | 0.2 | 0.45 |
| Haugh unit, % | 91.5[a] | 85[b] | 0.8 | <.01 |
| Albumen pH | 8.5[a] | 8.1[b] | 0.04 | <.01 |

*[ab] : means in the same row with different superscripts are statistically different (p<0.05); SEM- Standard Error of Means*

**Table 4:** The interactive effects of strain, age of layer and housing system on the external and internal egg quality

| Characteristics | Significance Level | | | | | | |
|---|---|---|---|---|---|---|---|
| | S | A | H | S X A | S X H | A X H | S X A X H |
| Egg weight, g | 0.13 | 0.04 | <.01 | 0.14 | <.01 | 0.77 | 0.20 |
| Shell weight, g | <.01 | <.01 | 0.91 | 0.02 | <.01 | 0.03 | <.01 |
| Shell thickness, mm | <.01 | 0.84 | 0.02 | 0.02 | 0.07 | 0.23 | 0.50 |
| Albumen weight, g | <.01 | 1.0 | <.01 | 0.37 | <.01 | 0.9 | 0.50 |
| Albumen height, mm | <.01 | <.01 | <.01 | <.01 | 0.21 | 0.04 | <.01 |
| Yolk weight, g | <.01 | 0.03 | <.01 | 0.54 | 0.08 | 0.25 | 0.30 |
| Yolk colour | 0.7 | <.01 | 0.34 | <.01 | 0.01 | <.01 | <.01 |
| Haugh unit, % | <.01 | <.01 | <.01 | <.01 | 0.02 | 0.12 | <.01 |
| Albumen pH | <.01 | 0.03 | <.01 | 0.29 | <.01 | 0.21 | 0.30 |

**Significant at p<0.05, S = strain, A = age, H = housing, S X A = interaction between strain and age, S X H = interaction between strain and housing, A X H = interaction between age and housing, S X A X H = interaction between strain, age and housing system.*

## Discussion

*Effect of strain of layers on egg quality*

The results from the present study agreed with those of a previous study by Hagan and Eichie (2019) that the two Lohmann strains (brown and white) produced eggs of similar weights. However there were differences with earlier results by Hagan *et al*. (2013), Hanusova *et al*. (2015) and Khatun *et al* (2016) who found significant strain effects on the egg weight. The lack of difference in egg weight in the present study could have been due to similarities between the two strains that possibly originated from a common ancestor. The shell thickness values (0.4-0.5mm) obtained from the strain of layers in this present study was better than values recorded earlier (0.35-0.36mm) by Hagan and Eichie (2019) from similar strains of layers on the same farm. Differences in shell thickness could be due to reduction in feed intake, amount of calcium in the diet, egg weight and the egg collection date among breeds, according to Joubrane *et al*. (2019).

The internal egg characteristics according to Hagan and Eichie (2019) are the major determinants of its quality and Lohmann brown layers produced eggs with superior albumen weight and height compared to those of the Lohmann white, culminating in a better Haugh unit for the Lohmann brown layers.

Even though yolk colour is affected mainly by diet, there was a difference ($p<0.05$) in yolk colour between the two strains. However, the yolk colour observed in the present study was below the minimum value of 9.0 accepted by the International Markets (Jones *et al*., 2002). There is therefore need to add green vegetables in the diets of intensively kept layers at the Teaching and Research Farm of the School of Agriculture, University of Cape Coast in Ghana, in order to improve the colour of the yolk.

*Effect of the housing system on egg quality*

Egg weight, according to Vlčková *et al*. (2019) is one the fundamental indicators of egg quality. In the present study, eggs from hens in the deep litter system were heavier than those in the battery cage system and this was consistent with the findings of Zita *et al*. (2018). However, Englmaierová *et al*. (2014) and Kraus and Zita (2019) found heavier eggs under the battery cage housing system. The different results, according to Vlčková *et al*. (2019) might be due to variable conditions in the experiments, such as environment, strain or feeding. The interactive effects of age and housing system did not influence egg weight in this study, an observation which disagrees with the findings of Vlčková *et al*. (2019), Yilmaz *et al*. (2017). Egg weight from birds under a free-range system, according to Samiullah *et al*. (2014), increased at the start of the laying period then remained constant while eggs from conventional cages steadily increased with age. The variation in egg weight as explained by Lewko and Gornowicz (2011) was as a result of physio-chemical changes known as egg ageing. They explained that water and gases begin to move both inside the egg and between the internal and external environment of the egg. In smaller eggs water loss is swift especially when the surface area in relation to volume is more which links to higher egg weight loss. Grashorn (2016) observed that faster quality changes in albumen and yolk occur with higher egg weight loss. Water loss from the eggs according to Caner and Yüceer, (2015) begins the sequence of changes in its quality and the changes include thinning of the albumen, increased pH, weakening and stretching of the vitelline membrane, increased dry matter content in the albumen and increased water content of the yolk.

Ledvinka *et al*. (2012), Kraus and Zita (2019) and Ogunshola *et al*. (2018) reported higher internal egg quality from birds under deep litter housing systems, an observation which the present study corroborated. Benton and Brake (2000) and Roberts (2004) in explaining the effects of housing system on internal egg quality concluded that eggs from birds under deep litter were exposed to higher concentrations of ammonia, which might negatively affect albumen quality and the Haugh unit. It could be inferred that there was reduced exposure of the eggs laid by birds under deep

litter to ammonia in the present study.

*Effects of age of layer on egg quality*

Results from the present study showed no significant influence of age on egg weight, an observation which disagrees with earlier observation by Kraus and Zita (2019). Results obtained from the current study also showed that the Haugh unit significantly declined with increasing age of the layers, confirming the observations of Jones *et al.* (2018), Padhi and Haunshi, (2015) and Samiullah *et al.* (2017). According to Toussant and Latshaw (1991) the decline in albumen quality, which is a determinant of the Haugh unit, with advancing layer age is as a result of decreasing protein content in albumen. Wang *et al.*, 2019 however attributed it to albumen fraction ovomucin because of high correlation between ovomucin content and the Haugh unit. Kraus and Zita (2019) and Samiullah *et al.* (2015) indicated that age and housing system significantly influenced eggshell thickness and also validated the interaction between age and housing system which differed from the findings in this study.

Yolk weight, according to Kraus *et al*, (2019) often increased with age in eggs from both housing systems which confirms the findings in this study. Sokołowicz *et al.* (2018) showed that housing system affected the yolk weight and Dikmen *et al.* (2017) claimed that interaction between age and housing systems significantly influenced yolk weight which disagreed with the results in this study.

*Interaction effects of age, strain and housing system on egg quality*

There were no significant interaction effects of the strain of layers, age of layers and housing system on egg weight (Table 4). There was also no significant interaction effect between age of layer and the housing system on egg weight, an observation which disagrees with earlier observations by Vlčková *et al.* (2018) who found a significant interaction effect between genotype and housing system on egg weight.

## Conclusion

The findings from this study indicated that egg weight an important quality indicator, is not affected by the strain and age of layer used at the Teaching and Research Farm of the School of Agriculture, University of Cape Coast in Ghana. However, the housing system under which the birds are kept can influence the size of the egg, with birds on the deep litter system laying bigger eggs than their counterparts on the battery cage system. There is therefore need to promote the use of the deep litter system among farmers if bigger egg sizes are preferred. The freshness of the eggs which is determined by the Haugh unit was also found to be influenced by the strain and age of layers and the housing system employed. It is therefore recommended that in order to improve on egg freshness, Lohmann white layers be reared under the deep litter system and that they should not be kept too long into lay.

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# INNOVATIONS TO HARNESS THE POTENTIAL OF AFRICAN ANIMAL AGRICULTURE IN A GLOBALIZING WORLD – EMPOWERING PROFESSIONAL NATIONAL ANIMAL PRODUCTION SOCIETIES

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## Abstract

The All Africa Society for Animal Production (AASAP) is an association of individuals, groups and institutions which have interest in the art, science and practice of animal sciences relevant for animal agriculture. These include animal nutrition and feeding, genetics and breeding, health, welfare, and other aspects of husbandry. An online survey was carried out to put together suggestions on how best National Animal Production Societies (NAPS) can be strengthened and contribute to sustaining the AASAP. Key findings indicated the need to improve on the visibility of the AASAP and the need for AASAP to establish a Secretariat and develop a framework to co-ordinate the activities of NAPS. The large imbalance in the gender of respondents (16% female to 84% male) emphasized the need to encourage more females to go into animal production. NAPS need to raise their publicity drives as many people were not even aware of their existence and the procedures for enrolment. In terms of membership, over two thirds of the respondents (67%) were from research institutions or the universities indicating the need for NAPS to reach out to people in industry and the private sector. About 66% of the respondents indicated that the societies were seen as relevant by their countries. In some countries, NAPS had some legal backing but close to 26% of the respondents were not aware of the existence of the AASAP. Registering and coordinating national associations and supporting weak ones to get established (28%), regional livestock trade shows/ conferences to enhance visibility of AASAP (20%), lobbying for government support and making inputs into the Research and Development (R&D) agenda (15%), online conferences/discussion (13%), supporting local associations to undertake training programmes and various entrepreneurship programmes (12%), production of a journal and a quarterly newsletter (12%) were some of the potential activities AASAP can engage in between the quadrennial conferences.

**Keywords:** livestock, conferences, associations, opportunities, membership, networking

# INNOVATIONS POUR EXPLOITER LE POTENTIEL DE L'ÉLEVAGE AFRICAIN À L'ÈRE DE LA MONDIALISATION - HABILITER LES SOCIÉTÉS PROFESSIONNELLES NATIONALES DE PRODUCTION ANIMALE

## Résumé

La société africaine de production animale (AASAP : All Africa Society for Animal Production) est une association de personnes, de groupes et d'institutions qui s'intéressent à l'art, à la science et à la pratique des sciences animales pertinentes pour l'élevage. Elle s'intéresse notamment à la nutrition et l'alimentation animales, la génétique et l'élevage, la santé, le bien-être et d'autres aspects de l'élevage. Une enquête en ligne a été réalisée dans l'objectif d'élaborer des suggestions sur le meilleur moyen de renforcer les Sociétés nationales de production animales (NSPA : National Animal Production Societies) pour qu'elles puissent contribuer au maintien de l'AASAP. Les principaux résultats ont fait ressortir la nécessité d'améliorer la visibilité de l'AASAP et la nécessité pour cette dernière de créer un secrétariat et d'élaborer un cadre

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de coordination des activités des NSPA. Le grand déséquilibre concernant le genre des répondants (16 % de femmes contre 84 % d'hommes) a souligné la nécessité d'encourager davantage de femmes à se lancer dans la production animale. Les NSPA ont besoin de renforcer leurs campagnes de publicité, car on a constaté que beaucoup de personnes n'étaient même pas au courant de l'existence de ces sociétés ni de procédures d'inscription. En termes d'adhésion, plus des deux tiers des répondants (67 %) provenaient d'instituts de recherche ou d'universités, une indication de la nécessité pour les NAPS de sensibiliser les acteurs du domaine et du secteur privé. Environ 66 % des répondants ont indiqué que les sociétés étaient considérées comme pertinentes par leur pays. Dans certains pays, les NSPA avaient un certain soutien juridique, mais près de 26 % des répondants n'étaient pas au courant de l'existence de l'AASAP. Quelques-unes des activités éventuelles dans lesquelles l'AASAP peut s'engager entre les conférences quadriennales comprennent : l'enregistrement et la coordination des associations nationales et l'assistance aux sociétés faibles (28%) pour ce qui est de leur établissement, les expositions/conférences régionales sur l'élevage pour améliorer la visibilité de l'AASAP (20%), la recherche de l'appui du gouvernement et la contribution au programme de recherche et développement (15 %), l'organisation de conférences / discussions virtuelles (15 %), l'appui aux associations locales pour l'exécution de programmes de formation et divers programmes d'entreprenariat (12 %) ; la production d'un journal et d'un bulletin trimestriel (12 %).

**Mots-clés :** élevage, conférences, associations, possibilités, adhésion, collaboration en réseau

## Introduction

The potential of animal agriculture in Africa has not received the attention it deserves. Policies and associated institutions promoting livestock development have been weak or absent in most African countries. FAO and AU-IBAR reports indicate that livestock-derived food items alone contribute, on average, 30 percent to agricultural GDP in Africa (FAO, 2015; AU-IBAR, 2019). This estimate does not include non-food livestock products such as draught power, manure, and transportation, which enhance the productivity of crop production, nor does it consider intangible livestock contributions to rural communities through risk mitigation and wealth accumulation (savings). There are persistent food shortages arising from the rapidly increasing human population, amidst inability of the continent to significantly increase productivity. This is compounded by a host of other trends: globalization, agricultural policy and associated impacts particularly on small producers with limited abilities to compete in input and output markets, urbanization and the aging farming community, climate change and its complex relationships with crop and animal agriculture and low investments in agriculture. It is projected that demand for livestock products in sub-Saharan Africa will increase several folds by 2050 (AU-IBAR, 2019). The trend of increased demand is currently not matched by increase in productivity within Africa. Yet, this growing demand for livestock products presents an opportunity for the continent: in the form of contribution towards economic growth of African nations, as well as to the resilience and productivity of producers' livelihoods, and to the food security of the continent.

The effects of climate change as well as food and nutritional security deficits in many parts of Africa have invigorated research and extension in African animal agriculture in the last decade. The realization of the full benefits from these efforts will require greater cooperation and linkages among farmers, researchers, governments, and the private sector. Professional societies are a key driver to these linkages and are best placed to lead bio-innovation, extension, and to initiate and drive the requisite policy formulations that are key to accelerated development in the sector. Among the key stakeholders in animal agriculture are the professional animal scientists and industry players. Unfortunately, in most countries there is poor organisation of national animal production societies (NAPS) which are supposed to provide platforms for stakeholders in the animal industry to among others influence policies to bring about the

desired changes.

The main objective of the All African Society of Animal Production (AASAP) is to facilitate the use of technical, policy and institutional innovations to address current and emerging challenges of African animal agriculture through engagement of communities of practitioners in Africa and beyond. At the same time NAPS made up of professional Animal Scientists exist in many African countries. These societies among others play advocacy roles to influence the animal production agenda to ensure a sustainable livestock sector. They also organise annual general meetings where they share information and discuss the latest innovations in animal agriculture. They are normally funded by annual dues paid by their members or donations and are managed by executives who work voluntarily to ensure that the societies remain relevant to their countries. Additionally, the societies also provide a unique environment for grooming the next generation of future animal scientists and entrepreneurs as well as providing opportunities for networking and information sharing. Unfortunately, in some of the countries, NAPS are not active and membership dues are not paid whilst in some countries they do not exist at all. This is a disturbing situation which needs to be addressed as we seek innovative ways to harness the potential of African animal agriculture. Furthermore, no formal links exist between NAPS and AASAP which, since its formation, has lacked a permanent Secretariat. In this study the views of stakeholders in Africa were collated to identify options for strengthening the NAPS and enhancing synergy between their activities and those of AASAP. This represents the first attempt to document the strengths and challenges of professional animal production societies in Sub-Saharan Africa. A dscussion of the results of the survey during the 7th All African Conference on Animal Agriculture, held in Accra, Ghana in July 2019, should facilitate the formulation of frameworks for the regulation and networking of the national associations and the AASAP.

## Methodology

An online survey was conducted to determine the functional efficiencies of national animal production societies (NAPS) across Africa, their preparedness to drive change in animal agriculture and to identify key opportunities to strengthen and position the NAPS to drive animal agriculture innovation, extension, and policy. The survey was conducted within April and May 2019 to gather information on the NAPS, solicit views for their effective functioning and how the AASAP could be strengthened by the NAPS. The data gathered were analysed and the frequencies and various descriptive statistics computed using the SPSS software (Version 26; SPSS, 2019).

## Results and Discussion

A total of 74 respondents made up of 12 females and 62 males submitted responses between April and May 2019. These were from Benin (1), Cameroon (1), DR Congo (2), Ethiopia (4), Ghana (7), India (1), Kenya (21), Liberia (2), Malawi (3), Nigeria (7), Rwanda (1), Sierra Leone(1), South Africa (7), Sudan (2), Tanzania (1), Uganda(1), United Kingdom(3), Zambia(1), Zimbabwe(1). All the African countries represented indicated they had NAPS in their countries. However, one respondent each from Cameroon, Ghana, South Africa, Zimbabwe and 2 respondents from Kenya were not aware of the existence of such associations in their respective countries. In terms of knowledge of AASAP 26% of the respondents were ignorant. Table 1 gives the breakdown of those interviewed by professional position and sex of the respondents.

The majority of respondents (79%) were members of the associations in their various countries (Figure 1).

There was very little or no effort to encourage student and youth participation as the majority of the members (over 85%) were master's or Doctorate degree holders (Table 2). It is suggested that special efforts be made to encourage the countries to form student and youth wings of the NAPS.

The contribution of livestock to agricultural GDP in the respondent countries was assessed to gauge the importance of livestock in the countries' agriculture. The majority of respondents (45%) indicated this to be between 10-30%, followed by 0-10% (28% of respondents), 30-60% (24% of respondents) with 3% of the respondents estimating it to be between 60 and 100%. Figure 2 shows the break down by the sex of the respondents. Interestingly the views of male and female respondents were very different.

In order to determine how active members of NAPS were in the various countries, the respondents were asked to estimate what percentage of members they perceived as active in their respective NAPS. Thirty eight percent (38%) reported that between 50 and 75% of members were active, 27% indicated this as 25-50%, 19% reported >75% were active while 16% indicated unfortunately, that <25% of the members were active. These results indicated that on the average, there are some committed members working hard

**Table 1:** Sex of respondent by professional position

| Position | Membership | | Total | |
|---|---|---|---|---|
| | Female | Male | Number | % |
| Animal Production Worker/Manager | 0 | 10 | 10 | 13 |
| Student | 1 | 4 | 5 | 7 |
| Extension Worker | 1 | 8 | 9 | 12 |
| Lecturer/Professor | 1 | 21 | 22 | 30 |
| Researcher | 9 | 19 | 28 | 38 |
| Total (%) | 12(16%) | 62(84%) | 74 | 100 |

**Table 2:** Membership of NAPS by professional position

| Qualification | Membership | | Total | |
|---|---|---|---|---|
| | Yes | No | Number | % |
| Diploma/BSc/BA | 5 | 3 | 8 | 11 |
| MSc/MSC/MBA | 25 | 9 | 34 | 47 |
| PhD | 27 | 3 | 30 | 42 |
| Total (%) | 57(79%) | 15(21%) | 72 | 100 |

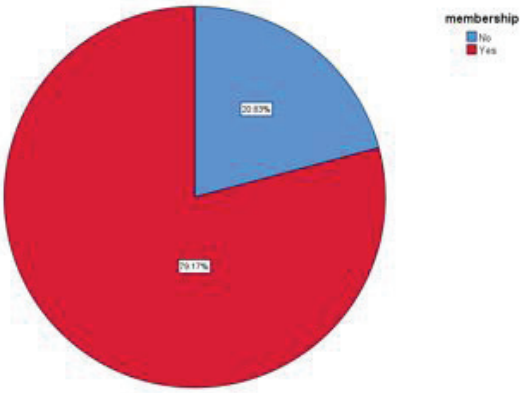


**Figure 1:** Pie chart showing proportion of respondents who were members of NAPS in sampled African countries

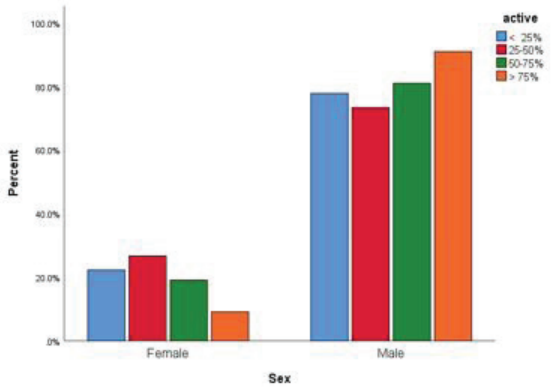


**Figure 2:** Bar Graph showing contribution of livestock to agricultural GDP from the perspective of the respondents

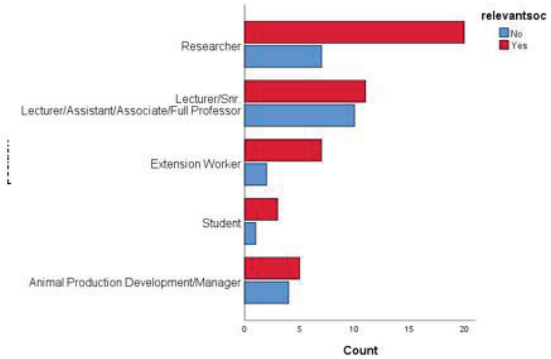


**Figure 3:** Bar Graph showing the respondents' perceptions on relevance of NAPS

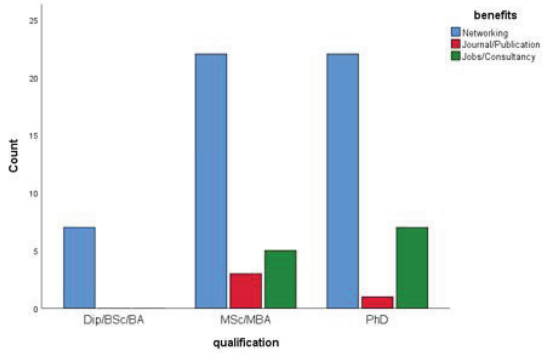


**Figure 4:** Bar Graph showing benefits of being a member of NAPS

to support NAPS and any support in terms of leadership, co-ordination and direction from the AASAP would help to enhance the output of such groups. In addition, about 65% of the respondents indicated that their associations were already actively engaging governments and other stakeholders in advocacy to influence policy formulation. Unfortunately working with actual policy makers remained weak (79% of the respondents) or non-existent (4% of the respondents) with only 18% indicating that the working relationship was very good. Establishing good and innovative strategies to enable national animal production societies to work actively with policy makers in the various countries will go a long way to enhance animal agriculture on the continent. To achieve this objective, it is important for national governments and civil society organisations to recognize the relevance of the national societies. This question elicited affirmative responses from about two thirds of the respondents (66%). However, as shown in Figure 3, several professionals believed that a lot still needs to be done to enhance the relevance of the national societies in their respective communities and countries.

Irrespective of qualifications, networking seems to be an important benefit which motivates people to join NAPS and therefore in countries where communication between the executives and the members happens only during conferences, the associations were weak. Not surprisingly job and consultancy opportunities were considered a big motivation particularly for those with higher degrees. It is therefore important for the NAPS to have a platform from which to communicate such important opportunities to their members in a timely manner.

Eighty-eight percent (88%) of the respondents indicated that their associations had the requisite legal backing. This should not deceive us into thinking that all is well with all the countries as the majority of the respondents were from countries (Kenya, South Africa and Nigeria) where the associations were well established. Interaction with participants at the 7th All African Conference on Animal Agriculture (AACAA), Accra 2019 indicated that many of the associations are yet to get legal backing from their respective governments. This is understandable judging from the fact that a good number of the respondents in the current study came from countries with well-established NAPS.

## Entry requirements

On what it takes for one to become a member of NAPS, 80% of the respondents indicated that Graduates of Animal Science/ Production who apply and pay the necessary fees are registered. In the remaining instances (12%) people in industry with the requisite experience and recommended students may be registered. A few NAPS (8%) also had special induction ceremonies for new members whilst some also recognised members who had made substantial contributions by conferring

fellowship honours on them.

*How can NAPS support the AASAP?*

Participants shared their views on how the NAPS can support the AASAP as follows:

- Share information on the societies' activities/advertisements/publications (39%);
- Organise programmes in line with activities of AASAP/AASAP should coordinate all NAPS/all NAPS to come under the umbrella of AASAP so they can help implement the plan of work (27%);
- Encourage their members to be active in AASAP programmes and activities/ publicise activities of AASAP (15%);
- Support livestock production in the various countries and work with all stakeholders to achieve the same (11%);
- Pay annual subscriptions/dues to AASAP (8%);

*What other activities can AASAP engage in beyond the quadrennial conferences?*

- Registering and coordinating NAPS and supporting weak ones to get established (28%),
- Organising regional livestock trade shows/ conferences to enhance visibility of AASAP (20%),
- Lobbying for government support and making inputs into the R&D agenda (15%), online conferences/discussion (13%),
- Supporting NAPS to undertake training programmes and various entrepreneurship programmes (12%),
- production of a journal and a quarterly newsletter (12%).

*How can AASAP help the NAPS?*

- Encourage all livestock personnel to be active in local associations/ Encourage student and private sector participation in activities/Sensitize, support and link them up to perform (23%);
- Provide/source funding opportunities/ Create a database of active researchers/ Support research collaboration/Training opportunities/Research mentoring for younger scientists (19%);
- Help solve animal production challenges in Africa/ link societies to animal development and marketing opportunities (17%);
- Help increase visibility and relevance of NAPS to national governments (15%);
- Enhance networking among NAPS and with the AASAP/Establish a permanent secretariat and play a co-ordinating role/ Regular meetings/dialogue/3-4 times a year (13%)
- Ensure that there is an Act of Parliament/ LI on the establishment of all national societies/ Help get a legal framework to regulate animal production profession/ licence all technicians etc. (11%);
- Reduce registration fees of AASAP Conferences (2%);

*Recommendations to ensure sustainable NAPS and AASAP*

- All countries should be encouraged to form NAPS/Strengthen NAPS with more local support/Establish a database of available donors so that national societies can seek support from time to time (19%);
- More awareness and publicity/ Encourage more advocacy, research and collaboration on African Animal Agriculture (14%);
- Provide opportunities for more interaction with well-established regional bodies/ association such as the European Association of Animal Production (EAAP) to help learn from them/ Link up with NGOs with interest in Animal Production/ learn from current best practices (13%).
- Encourage networking between and within NAPS/Form a committee from selected NAPS to steer the affairs of the AASAP for an agreed period/ Set realistic targets and seek support to achieve them (13%).
- Undertake Newsletters and publications (10%);
- Support more students to attend AASAP conferences (10%);
- Share the outcome of this survey/Discuss these issues further during the 7th AACAA as most members may not respond to the survey (6%);

- Seek collaborative labs where young scientists can carry out research (6%);
- Abolish subscription fees to encourage more participation in NAPS and AASAP programmes (6%);
- Make inputs into national policy formulation and implementation on animal production issues (3%).

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# HIGH QUALITY CASSAVA PEEL® MASH SUPPLEMENTED WITH DIRECT FED MICROBIAL AS AN ALTERNATE SOURCE OF ENERGY: EFFECT ON GROWTH AND ECONOMICS OF WEANED PIGS

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## Abstract

The use of Direct fed microbial (DFM) in livestock production has the potential of enhancing feed utilization and promoting growth. Experiments were conducted to investigate the effect of Direct fed microbial (DFM) on High quality cassava peel® (HQCP) utilization. The HQCP was used to replace maize up to 15% of total maize (40%) in the diet of weaned pigs. The experimental animals were assigned to five dietary treatments (T1-T5) in a 2 × 2 factorial design including a control diet with eighteen weaned pigs per treatment. Pigs on T1 were given a 40% maize-based diet without HQCP, while T2 had 7.5% HQCP (to replace 18.75% of the total maize) and T3 had 15% HQCP (to replace 37.5%) of the total maize. The pigs on T4 were given the same diet as those on T2 with the addition of multi-strain DFM and pigs on T5 were given the same diet as those on T3 with the addition of multi-strain DFM. The results indicated that addition of DFM had no significant (P>0.05) effect on the body weight gain, final weight, average daily feed intake and feed conversion ratio. Additionally, there was no significant (P>0.05) effect of DFM on the performance of the pigs, as the treatments (T4 and T5) with DFM inclusion did not give improved performance over the treatments (T2 and T3) without the DFM. There was neither significant (P>0.05) effect of HQCP level nor DFM inclusion on all the performance parameters. These results indicated that weaned pigs can utilize up to 7.5% HQCP without DFM and 15% HQCP with DFM, without any deleterious effect on their performance.

**Key Words:** Multi-strain, processed cassava peel, weaned pig, feeding, performance, costs.

# MÉLANGE DE PEAUX DE MANIOC DE HAUTE QUALITÉ COMPLÉTÉ PAR DES PROBIOTIQUES COMME SOURCE ALTERNATIVE D'ÉNERGIE : EFFET SUR LA CROISSANCE ET L'ÉCONOMIE DES PORCELETS SEVRÉS

## Résumé

L'utilisation de probiotiques dans la production animale a le potentiel d'améliorer l'utilisation d'aliments pour animaux et de promouvoir leur croissance. Des expériences ont été menées dans l'objectif d'étudier l'effet des probiotiques sur l'utilisation de mélange de peaux de manioc de haute qualité (PMHQ). Le PMHQ a été utilisé pour remplacer le maïs jusqu'à 15 % du maïs total (40 %) dans le régime alimentaire des porcelets sevrés. Les animaux utilisés dans l'expérience ont été affectés à cinq traitements diététiques (T1à T5) dans un schéma factoriel 2 × 2 comprenant un régime témoin, avec dix-huit porcelets sevrés par traitement. Les porcelets sous T1 ont reçu une alimentation à base de maïs à 40 % sans PMHQ, alors que le groupe sous T2 a reçu 7,5 % de PMHQ (pour remplacer 18,75 % du maïs total) et T3 15 % de PMHQ (pour remplacer 37,5 % du maïs total). Les porcelets sous T4 ont reçu le même régime alimentaire que ceux soumis au T2 avec ajout de probiotiques multi-souches, et les porcelets sous T5 ont reçu le même régime que ceux sous T3 avec ajout de probiotiques multi-souches. Les résultats indiquent que

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l'ajout de probiotiques n'a pas eu d'effet significatif (P > 0,05) sur le gain de poids corporel, le poids final, la consommation journalière moyenne et l'indice de consommation. En outre, on n'a pas relevé d'effet significatif (P > 0,05) des probiotiques sur la performance des porcelets, puisque les traitements (T4 et T5) complétés par les probiotiques n'ont pas donné une meilleure performance par rapport aux traitements (T 2 et T 3) sans probiotiques. Il n'y a pas eu non plus d'effet significatif (P > 0,05) du niveau de PMHQ ni de l'inclusion de probiotiques sur tous les paramètres de performance. Ces résultats indiquent que les porcelets sevrés peuvent utiliser jusqu'à 7,5% de PMHQ sans probiotiques et 15% de PMHQ avec probiotiques, sans effet nocif sur leur rendement.

**Mots-clés :** multi-souches, peaux de manioc transformés, porcelet sevré, alimentation, performance, coûts.

## Introduction

Maize is well known as the major source of energy in the diets of monogastric animals in Nigeria. Competition between animals and humans for maize consumption, its industrial uses, the seasonality effect on production and insurgency in the northern part of Nigeria where most of the maize in the country is produced, has led to its scarcity and consequently resulted in its high cost (Umar *et al*., 2019). There is therefore a need for suitable alternative sources of energy that can totally or partly replace maize in monogastric animal diets in order to reduce the costs of production. As a result of increasing prices of maize, the use of agricultural by-products in livestock diets has become more imperative (Makinde and Sonaiya, 2011, Oguadimma *et al*., 2018).

A good alternative source of energy for animal feeding that can replace maize is cassava peel (Akinfala and Tewe, 2001). The cassava species Manioc, [Manihot esculenta Crantz or Manihot utillisima Pohl] is believed to have originated from Brazil and was introduced into West Africa by the Portuguese (Odunfa, 1985, Silvestre, 1989). More than 95 percent of cassava processing requires peeling to get rid of two outer coverings: a thin brown outer covering and a thicker leathery parenchymatous inner covering which generates up to 14 million metric tonnes of waste annually, since these peels could make up to 10% of the wet weight of the roots (Okike *et al*., 2015). The wastes generated at present pose a disposal problem in spite of its potential use as animal feed and this could be even more problematic in the future with the increased industrial production of cassava products if proactive measures are not taken to convert it to useful products. If properly processed, the generated peel can be used as an energy source in monogastric feeds (Iyayi and Dorothy, 2001, Obadina *et al*., 2006, Okike *et al*., 2015). Recent advances in feed technology have led to the development of a new feedstuff from cassava peels known as high quality cassava peel® (HQCP) (Okike *et al*., 2015) which has brought about an improvement in the quality of the peel.

In a diet that contains 30% maize, HQCP has been reported to replace up to 75% of it for growing pigs while it can only replace up to 15% of it in the diet of weaner pigs (Adesehinwa, *et al*., 2016). The inability of weaner pigs to effectively utilize HQCP may be due to their age, since utilization of fiber by pigs has been reported to increase with advancement in age (Noblet *et al*., 1994). The use of feed additives such as direct fed microbial (DFM) has been reported to aid digestion and may enhance the utilisation of HQCP by weaned pigs.

Direct-fed microbials are live microorganisms, which when administered in adequate amounts confer health benefits on the host. Supplementing pig feed with DFM has been proven to give more positive and consistent effects in weaned piglets than in grower or finisher pigs (Jensen, 1998). This is because the microbiota in the gut is unstable during the first week post-weaning and it takes 2-3 weeks post-weaning for the gut microbes to fully develop their fermentative capacity and to reach a high level of stability (Vanbelle, 2001, Giang *et al*. 2011). Proliferation of the microorganisms in the gastrointestinal tract has been reported to stimulate metabolic

activities and improve the digestion of fibre from various sources (Kerr *et al*., 2013, Fatufe *et al*., 2016). This study was therefore conducted to investigate the performance of weaned pigs fed HQCP mash supplemented with DFM as an alternative source of energy.

## Materials and Methods

Ninety crossbred (Large White × Landrace) weaned pigs sourced from the Institute of Agricultural Research and Training, Ibadan, Nigeria with an average initial weight of 11.10±0.4 kg (± SE) were used for the study. The study was carried out at AK Research farm, Ibadan with latitude 7°25´N and longitude 3°50´E and lasted for eight weeks. The experimental animals were assigned to five dietary treatments (T) in a 2 × 2 factorial design with a control and eighteen weaned pigs per treatment. The factors were two (2) levels of HQCP (i.e. 7.5 and 15 kg), two (2) levels of multi-strain DFM inclusions and one (1) control without HQCP and DFM inclusion. Each treatment had three replicates with six pigs per replicate. The experimental diets were formulated using the nutrient requirement recommendations for weaned pigs (National Research Council, 1998). The diets were formulated based on maize, groundnut cake and palm kernel cake. High Quality Cassava peel partially replaced maize in the basal diet in treatments 2 to 5.

Pigs on T1 were given a 40% maize-based diet without HQCP and multi-strain DFM, T2 had 18.75% of the total maize replaced by HQCP and T3 had 37.5% of the total maize replaced by HQCP. The pigs on T4 were given the same diet as in T2 with the addition of multi-strain DFM and pigs on T5 were given the same diet with those on T3 with the addition of multi-strain DFM (Table 1). The multi-strain DFM used in the study contained 99.9% water, 1×108 CFU/g Lactobacillus sp, 4 × 1012 CFU/g Bacillus sp and 11× 105 CFU/g Saccharomyces cerevisiae and was supplemented at 1.6 ml/kg diet. The DFM was mixed with the feed during milling. The animals were allowed ad libitum access to feed and water in the concrete-floored pens where they were grouped based on sex throughout the 56-day duration of the trial.

The pigs were weighed at the onset of the trial and weekly thereafter. The growth performance parameters recorded including body weight (BW) and daily feed intake (DFI). Daily weight gains (DWG), average daily feed intake (ADFI) and feed conversion ratio (FCR) were calculated. Feed intake was recorded daily. The daily feed intake of the pigs was recorded as the calculated amount of feed offered daily less the remaining quantity in the feeder the next morning and expressed on a dry matter basis. The chemical composition and gross energy value of the test ingredients and the experimental diets were carried out

**Table1:** Ingredient Composition of the Experimental Diets

| Ingredients (%) | T1 | T2 | T3 | T4 | T5 |
|---|---|---|---|---|---|
| Maize | 40 | 32.5 | 25 | 32.5 | 25 |
| HQCP | 0 | 7.5 | 15 | 7.5 | 15 |
| Wheat bran | 10.5 | 9.5 | 8.5 | 9.5 | 8.5 |
| Palm oil | 1.5 | 2.5 | 3.5 | 2.5 | 3.5 |
| Groundnut cake meal | 20.00 | 20.00 | 20.00 | 20.00 | 20.00 |
| Fish meal | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 |
| Palm kernel cake | 20.00 | 20.00 | 20.00 | 20.00 | 20.00 |
| Limestone | 1.30 | 1.30 | 1.30 | 1.30 | 1.30 |
| Bone meal | 2.50 | 2.50 | 2.50 | 2.50 | 2.50 |
| Salt | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |

| Ingredients (%) | T1 | T2 | T3 | T4 | T5 |
|---|---|---|---|---|---|
| DL-Methionine | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |
| L-Lysine | 0.40 | 0.40 | 0.40 | 0.40 | 0.40 |
| Vitamin-Mineral Premix | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Total | 100 | 100 | 100 | 100 | 100 |
| Calculated analysis | | | | | |
| Crude fibre (%) | 5.06 | 5.48 | 5.90 | 5.48 | 5.90 |
| Crude protein (%) | 20.55 | 20.20 | 19.86 | 20.20 | 19.86 |
| Metabolizable energy (kcal/kg) | 2754.85 | 2782.47 | 2810.10 | 2782.47 | 2810.10 |
| Calcium | 1.67 | 1.68 | 1.69 | 1.68 | 1.69 |
| Phosphorus | 0.80 | 0.79 | 0.79 | 0.79 | 0.79 |
| Lysine | 1.09 | 1.07 | 1.04 | 1.07 | 1.04 |
| Methionine | 0.38 | 0.36 | 0.35 | 0.36 | 0.35 |

** HQCP - High quality cassava peel.*

as described by the Association of Official Analytical Chemists (AOAC, 1990). Energy was determined with a bomb calorimeter. The fibre fractions which included the neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), hemicellulose and cellulose were assessed as described by Van Soest *et al*. (1991) The data obtained were subjected to analysis of variance (ANOVA) appropriate for a factorial design, using the general linear model procedures of the Statistical Analysis System (SAS) software 9.1 for Windows (SAS, 2004). Statistical significance was assessed at P <0.05 (95% confidence) and significant means were separated using Duncan's Multiple range Test.

## Results and Discussion

Tables 2 and 3 show the chemical composition and the fibre fraction of the test ingredients and experimental diets, respectively. The analysed chemical composition values of the test ingredients were in agreement with the nutrient composition of feed ingredients reported by NIAS (2016). Both the calculated chemical composition values (Table 1) and the analysed chemical composition values (Table 2) of the diets were similar.

Table 4 shows the performance of weaner pigs fed the experimental diets. No significant (P>0.05) differences were observed in all the performance parameters across the treatments. The final weights ranged from 26.97 to 24.42 kg with pigs fed 7.5% HQCP without DFM (T2) and 15% HQCP without DFM (T3) having the highest and lowest values respectively. The daily weight gain did not follow a similar trend as the final weight. Pigs fed with 7.5% HQCP without DFM (T2) and 7.5% HQCP with DFM (T4) had the highest (285.87 g) and lowest (236.57 g) daily weight gain values respectively. The values for average daily feed intake and feed conversion ratio ranged from 672.24 to 769.50 g and 2.60 to 3.10 respectively. The least average daily feed intake (672.24 g) was recorded in animals that were fed on a diet with 7.5% HQCP with additive (T4) while animals that fed on a diet with 7.5% HQCP without additive (T2) recorded the highest average daily feed intakes (769.50 g). The pigs that had the highest final weights (T2) also recorded the highest average daily feed intake with the best feed conversion ratio.

Multiple approaches have been used by the feed industry to improve the utilisation of fibre in feedstuffs. Exogenous carbohydrases are likely the most used in practice but the successful implementation

**Table 2:** Chemical composition of the test ingredients and experimental diets

| Parameters (%) | T1 | T2 | T3 | T4 | T5 | Maize | HQCP |
|---|---|---|---|---|---|---|---|
| Dry matter | 90.67 | 90.76 | 90.86 | 90.81 | 90.90 | 90.65 | 90.94 |
| Crude Protein | 17.53 | 17.74 | 18.64 | 17.83 | 18.84 | 11.21 | 6.63 |
| Crude Fibre | 5.84 | 6.14 | 6.29 | 6.08 | 6.24 | 1.27 | 8.7 |
| Crude fat | 3.6 | 3.64 | 3.70 | 3.68 | 3.74 | 4.12 | 2.47 |
| Ash | 6.32 | 6.41 | 6.51 | 6.45 | 6.60 | 3.76 | 3.28 |
| NFE | 57.40 | 56.85 | 55.74 | 56.78 | 55.50 | 70.3 | 70 |
| Gross energy (kcal/kg) | 4009.5 | 4041.5 | 4024.5 | 4037.0 | 4033.0 | 3784 | 2985 |

*NFE - Nitrogen Free Extract*

**Table 3:** Fibre fraction of the test ingredients and experimental diets

| Parameters (%) | T1 | T2 | T3 | T4 | T5 | Maize | HQCP |
|---|---|---|---|---|---|---|---|
| NDF | 45.14 | 47.61 | 48.67 | 47.54 | 48.40 | 9.59 | 42.07 |
| ADF | 20.06 | 23.58 | 25.15 | 23.51 | 25.10 | 6.88 | 20.71 |
| ADL | 6.60 | 6.67 | 6.90 | 6.64 | 6.78 | 0.19 | 6.77 |
| Hemicellulose | 25.08 | 24.04 | 23.52 | 24.03 | 23.30 | 2.71 | 21.37 |
| Cellulose | 13.46 | 16.91 | 18.25 | 16.86 | 18.32 | 6.69 | 13.94 |

*NDF- Neutral Detergent Fibre, ADF- Acid Detergent Fibre, ADL- Acid Detergent Lignin, % - percent*

of this practice is highly dependent on the ability to characterise and understand the fibrous composition of different feedstuffs and diets and the inherent properties of enzymes (Kim *et al*., 2003; Olukosi *et al*., 2007). The application of feed processing methods and the use of exogenous feed additives such as DFM and enzymes in an effort to improve nutrient digestibility of plant-based feed ingredients for swine has been studied for decades (Whitney and Shurson, 2004; Adesehinwa *et al*., 2016). Limited research has been reported on the impact of DFM on nutrient digestibility or pig performance when fed fiber-based diets. In this study, there were no significant effects of DFM and HQCP levels on the growth parameters tested for weaned pigs. Taking together the diversity and concentration of the chemical characteristics that exist among plant-based feed ingredients such as HQCP, as well as the interactions among constituents within HQCP, DFM and diets, it was previously suggested that improvements in nutrient digestibility and pig performance from adding exogenous feed additives to diets fed to weaned pig depends on a better understanding of some characteristics of the fiber source and other characteristics in order for nutrient digestibility or voluntary feed intake to be alleviated.( Brian and Gerald, 2013).

The amounts of fibre in feedstuffs vary considerably within and between plant species, Moreover, the type of fibre may also vary, thus affecting how fibre can be utilised by the animal. There is also some variation in the fibre concentrations in agricultural by-products because the amount of fibre present in such feedstuffs depends, in part, on the processing conditions of the raw materials and the ability to convert starch into fuel, among other factors. Dietary supplementation of DFM modulated the gastrointestinal bacterial populations by stimulating the proliferation and metabolic activity of beneficial microbes such as Bifidiobacteria and Lactobacilli, and this also increased the fibre digestion from various fibre sources such as soybean hulls and corn dried distiller grain with solubles (Kerr *et al*., 2013). The non-significant effect of the DFM on HQCP fiber in the present study may have been due to the source of the fiber. This observation was similar to that of Fatufe *et al*. (2016) in which DFM improved utilization of palm kernel cake but had no positive effect on rice bran

in growing pig diets. It is also well known that the utilization of crude fibre by non- ruminants varies considerably depending on the degree of lignification (Trowell *et al*., 1976; Berrocoso *et al*., 2015), level of inclusion and extent of processing (McNab, 1975; O'Doherty *et al*., 2002; Agyekum and Nyacho, 2017).

Direct fed microbials are administered to livestock to improve performance and health status of the animals by controlling pathogens through the modification of the microflora (Prescott *et al*., 2005), increased nutrient digestibility (Giang *et al*., 2011) and enhanced immune regulation and response (Liu *et al*., 2018). There was a non-significant (P>0.05) effect of DFM on the final weight, daily weight gain and average daily feed intake. Direct fed microbial however increased the final weight and daily weight gain of pigs fed 15% HQCP by 8.2 and 10% respectively when compared to final weight and daily weight gain of pigs fed 15% HQCP without direct fed microbial. In contrast, direct fed microbial reduced the final weight and daily weight gain of pigs fed 7.5% HQCP by 8.4 and 7.0 % respectively when compared with that of pigs fed 7.5% HQCP without DFM. The underlying principle for this observation remains unclear. This result shows that the DFM has an effect (not significant) on the levels of HQCP used in this study. This could be as a result of the ability of the DFM especially the Bacillus sp to degrade non-starch polysaccharides to reducing sugar thereby enhancing the digestibility of the feed for improved performance (Jarworski *et al*., 2017).

Table 5 shows the effects of HQCP levels, DFM inclusion and their interaction on the growth performance of weaned pigs. There were no significant (P>0.05) effects of HQCP levels, DFM inclusion and their combination on the final weight, body weight gain, daily weight gain, average daily feed intake and feed conversion ratios of the pigs across the treatments. It was observed that replacing maize with more than 7.5% HQCP in the diets reduced the final weight, daily weight gain and

**Table 4:** Performance of Weaned Pigs Fed the Experimental Diets

| Parameters | T1 | T2 | T3 | T4 | T5 | SEM | P-value |
|---|---|---|---|---|---|---|---|
| Initial weight (Kg) | 10.97 | 10.96 | 10.88 | 11.46 | 11.53 | 0.50 | 0.10 |
| Final weight (Kg) | 25.95 | 26.97 | 24.42 | 24.71 | 26.42 | 0.85 | 0.87 |
| BWG (Kg) | 14.98 | 16.01 | 13.54 | 13.25 | 14.89 | 0.46 | 0.33 |
| DWG (g) | 267.49 | 285.87 | 241.78 | 236.57 | 265.87 | 8.23 | 0.33 |
| ADFI (g) | 716.51 | 769.50 | 691.80 | 672.24 | 753.29 | 26.35 | 0.77 |
| FCR | 2.80 | 2.60 | 2.91 | 3.10 | 2.98 | 0.10 | 0.65 |

*DWG - Daily weight gain, ADFI - Average daily feed intake, FCR – feed conversion ratio, SEM - Standard error of mean*

**Table 5:** Single and Combined Effect of High-Quality Cassava Peel Levels and Direct Fed Microbial on Performance of the Pigs Fed Experimental Diets

| Parameters | T2 | T3 | T4 | T5 | SEM | P(ANOVA) | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | DFM | HQCP | DFM* HQCP |
| Initial weight (Kg) | 10.960 | 10.877 | 11.458 | 11.528 | 0.601 | 0.6420 | 0.9957 | 0.9507 |
| Final weight (Kg) | 26.968 | 24.417 | 24.706 | 26.417 | 1.4226 | 0.9502 | 0.8417 | 0.3134 |
| DWG (g) | 285.87 | 241.78 | 236.57 | 265.87 | 14.0536 | 0.5183 | 0.7045 | 0.0631 |
| ADFI (g) | 769.50 | 691.80 | 672.24 | 753.29 | 41.8569 | 0.7843 | 0.9796 | 0.2269 |
| FCR | 2.5996 | 2.9145 | 3.1014 | 2.9819 | 0.2054 | 0.2516 | 0.6927 | 0.38068 |

*BWG - Body weight gain, DWG - Daily weight gain, TFI - Total feed intake, ADFI - Average daily feed intake, FCR – feed conversion ratio, SEM - Standard error of mean, DFM - Effect of direct fed microbial, HQCP - Effect of high quality cassava peel level, DFM*HQCP – Interaction between level of high quality cassava peel and direct fed microbial.*

**Table 6:** Economic Analyses of Feeding High Quality Cassava Peel and Direct Fed Microbial in the Diet of Weaned Pigs

| Parameters | T1 | T2 | T3 | T4 | T5 | SEM |
|---|---|---|---|---|---|---|
| Cost of feed (N/Kg) | 137.37[b] | 134.56[d] | 131.75[e] | 137.76[a] | 134.95[c] | 0.2348 |
| Total cost of feeding (N) | 5511.9 | 5798.3 | 5104 | 5186 | 5692.7 | 199.351 |
| Cost of feeding per day (N) | 98.43 | 103.54 | 91.14 | 92.61 | 101.65 | 3.5598 |
| Feed cost/Kg weight gain (N/Kg) | 385.20 | 349.79 | 383.98 | 427.25 | 402.40 | 14.2594 |

*SEM - Standard error of mean* *US$ 1 =* N*350.00*

resulted in poor feed conversion ratios (T2 vs T3). Replacing 15% of maize with HQCP caused depression in final weight and daily weight gain of the animals, but this was ameliorated with the inclusion of DFM. However, addition of DFM to diets containing 7.5% HQCP had no positive effect and the reason for this trend was not known. The use of DFM as an enhancer for nutrient utilization in the diets containing 15% HQCP resulted in improved performance. This corroborated the report of Cai *et al*. (2015), who reported improvement in the growth response of weaned pigs and attributed it to inclusion of DFM in the diet of the pigs which invariably enhanced the absorption of nutrients. There was no mortality recorded during the feeding trial.

The economics of production of weaned pigs fed HQCP diets supplemented with multi-strain direct fed microbial were shown in Table 6. Cost in Nigerian Naira (N) of feed per kilogram, total cost of feeding (N), average cost of feed per day (N) and cost of feed per kilogram weight gain (N/kg) were calculated by using the market cost of the ingredients at the time of the study. The highest cost of feed (137.76 N) was recorded for treatment 4 due to additional cost of DFM, but an increment in the inclusion level of the HQCP reduced the price of feed cost in T5. The cost of feeding per unit weight gain was comparable across the groups, mainly due to the savings on account of the lower cost of HQCP compared to maize grains. In summary, there were no significant (P>0.05) differences in the total cost of feeding (N), average cost of feed per day (N) and the cost of feed per kilogram weight gain (N/kg). The inclusion of additives in the diet of livestock may increase the cost per unit of the diet but this effect should be compensated for in the response of the animals to the diet in terms of health, growth and other response variables. The use of direct fed microbial in the diet containing 7.5 and 15% HQCP increased the feed cost per kilogram as well as the cost per kilogram weight gain of the weaned pigs by 22% and 4.8% respectively when compared with the same diet without DFM supplementation. This contrasts with the report of Dersjant-Li *et al*. (2014) and Wealleans *et al*. (2018) in which inclusion of DFM in the diets of broiler chickens was observed to be economically beneficial. Based on the results of the present study, it is suggested that the development of processing techniques or exogenous feed additives that degrade fibre, and thereby improve energy digestibility or voluntary feed intake, will be both metabolically and economically beneficial to pork production.

## Conclusions

The addition of direct fed microbial to weaned pigs diet containing 15% HQCP improved the growth performance of the crossbred weaned pigs. The pigs were also able to better utilize diet containing 7.5% HQCP than 15% HQCP without direct fed microbial. Therefore, weaned pigs can utilize 7.5% HQCP without DFM and 15% HQCP if supplemented with DFM.

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# THE USE OF FIBRE-DEGRADING ENZYMES IN CORN COB-BASED DIETS FOR LABORATORY RATS: EFFECTS ON GROWTH, ORGAN PARAMETERS AND BLOOD PROFILE

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## Abstract

The need to explore alternative feed resources for use in monogastric diets is important due to growing competition between man and livestock for scarce resources. The aim of this trial was to determine the effects of the inclusion of corn cobs (CC), with or without exogenous enzymes in diets for laboratory rats on growth, organ parameters and blood profiles. Thirty-five Sprague Dawley© albino rats were randomly assigned to seven dietary treatments in a trial that lasted 28 days. The control diet (T1), had 0% CC and 6% wheat bran. T2, T4 and T6 had CC replacing 25, 50 and 75% of the wheat bran concentration of the control diet respectively, without enzyme supplementation. T3, T5 and T7 had the same levels of CC inclusion as T2, T4 and T6 respectively but with enzyme supplementation. The enzyme cocktail contained phytase, xylanase and β-glucanase. The experiment was set up as a completely randomized design (CRD), and all data collected were analysed using SAS 9.4 edition. On day 29 all the rats were euthanized and organ and blood parameters studied. The average daily feed intake (ADFI) of rats on T1 (22.91g), was higher ($P<0.05$) than those of rats on the CC treatments (17.32 – 19.47 g). No differences were observed in the average daily gain (ADG) and the feed conversion efficiency (FCE). The empty gastrointestinal tract (GIT) weight of rats on T6 (17.41g) and T7 (21.40g) were heavier ($P<0.05$) than those of rats on the other dietary treatments. No significant differences ($P>0.05$) were observed in the blood profiles between the treatments. It was concluded that corn cobs can be included up to a level of 4.5% in the diets of albino rats without any adverse effects on growth, organ and blood parameters, even without exogenous enzyme supplementation.

**Key words:** Albino rats, Corn cob, Enzymes, Gastro intestinal tract, Non-starch polysaccharides

# L'UTILISATION D'ENZYMES DÉGRADANT LES FIBRES DANS LES RÉGIMES À BASE D'ÉPIS DE MAÏS POUR RATS DE LABORATOIRE : EFFETS SUR LA CROISSANCE, LES PARAMÈTRES D'ORGANES ET LES PROFILS SANGUINS

## Résumé

La nécessité d'explorer les ressources alimentaires alternatives destinées aux régimes monogastriques est importante en raison de la compétition croissante entre l'homme et le bétail pour des ressources devenues rares. Le but de cet essai était de déterminer les effets de l'inclusion d'épis de maïs (CC : corn cobs) - avec ou sans enzymes exogènes dans les régimes alimentaires des rats de laboratoire - sur la croissance, les paramètres d'organes et les profils sanguins. Trente-cinq rats Sprague Dawley albinos ont été répartis de manière aléatoire à sept traitements diététiques dans un essai d'une durée de 28 jours. Le régime témoin (T1) avait 0% de CC et 6% de son de blé. Dans les régimes T2, T4 et T6, les CC ont remplacé respectivement 25, 50 et 75 % de la concentration de son de blé, sans supplémentation enzymatique. Les T3, T5 et T7 présentaient les mêmes niveaux d'inclusion de CC que T2, T4 et T6, mais avec des suppléments enzymatiques. Le cocktail enzymatique contenait la phytase, la xylanase et la β-glucanase. L'expérience a été mise en place en tant que schéma entièrement aléatoire, et toutes les données recueillies ont été analysées

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à l'aide de SAS édition 9.4. Au jour 29, tous les rats ont été euthanasiés et les paramètres des organes et du sang ont été étudiés. La consommation moyenne quotidienne des rats sous T1 (22,91 g) était supérieure (P < 0,05) à celle des rats sous régimes de CC (17,32 - 19,47 g). Aucune différence n'a été observée dans le gain quotidien moyen et l'indice de consommation. Le poids du tractus gastro-gastro-intestinal vide des rats sous T6 (17,41 g) et T7 (21,40 g) était plus lourd (P < 0,05) que celui des rats soumis aux autres traitements diététiques. Aucune différence significative (P > 0,05) n'a été observée dans les profils sanguins pour les différents traitements. Il a été conclu que les épis de maïs peuvent être inclus jusqu'à 4,5% dans les régimes alimentaires des rats albinos sans effets nocifs sur la croissance, les paramètres d'organes et les profils sanguins, même sans supplémentation exogène enzymatique.

**Mots-clés :** rats albinos, épis de maïs, enzymes, tractus gastro-intestinal, polysaccharides non amylacés

## Introduction

Competition between man and domestic animals, including pigs, for conventional feed ingredients such as cereals and grains (Tyner and Taheripour, 2007) has resulted in high prices of these ingredients worldwide, especially in developing countries such as Ghana. This translates to high feed costs, and invariably high costs of production (Yenesew *et al*., 2015). High cost of production is a major reason for the relatively higher prices of locally produced pork and broiler meat compared to the imported ones. There is the need therefore for livestock and poultry farmers to rely more on agro-industrial by-products (AIBPs) which could serve as potential feed ingredients for pigs (Aniola *et al*., 2009). Such by-products are not used by humans as food and can therefore potentially result in lower feed costs if found to be suitable. Corn cob, a by-product of corn after it is shelled, is such an AIBP which could be incorporated into feed for monogastric animals. According to Božović *et al*. (2004) there is a substantial amount (approximately 180-200 kg) of corn cobs that can be obtained per ton of grains. This quantity of cobs is significant and is readily available in Ghana.

A high amount of fibre in the diets of pigs is not desirable, since monogastric animals require energy dense diets. However, when the right type and amount is included in the diet, fibre is known to improve gut health, increase satiety and improve the overall well-being of pigs (de Lange *et al*., 2010) with no negative effects on growth performance, blood chemical and haematological indices (Adesehinwa *et al*., 2008). Corn cobs are cheap and high in fibre and may, in addition to reducing feed costs, also enhance the positive effects of dietary fibre in monogastric diets (Jansen, 2012). However, high non-starch polysaccharide (NSP) levels and other anti-nutritional factors (ANFs) such as phytic acid and protease inhibitors may influence the performance of monogastric animals. These ANFs are undesirable and are known to reduce nutrient digestion, absorption and utilization (Gemede and Ratta, 2014). This is due to the fact that pigs do not have the endogenous enzymes needed to effectively digest nutrients bound to ANFs (Schedle *et al*., 2012). Exogenous enzymes, when added to pig diets can counteract the negative effects of ANFs present in most feedstuffs. For example, fibre-degrading enzymes nullify the effect of non-starch polysaccharides, reduce the nutrient encapsulating effect of cell walls and reduce intestinal viscosity (O'Neill *et al*., 2014). Addition of fibre degrading enzymes to a corn-soybean-wheat-based diet improved the average daily gain and feed conversion efficiency of growing pigs (Ao *et al*., 2010).

Because of physiological similarities to humans (Siddiqui *et al*., 2016) and other animal species such as poultry and pigs, laboratory rats have been used in research as models of these animals. Laboratory rats have therefore been used extensively in experiments in nutrition, toxicology and physiology (Guvva *et al*., 2017). For example, Dongowski *et al*., (2002) used male Wistar rats as an animal model to study the effect of dietary fibre in barley-based products on intestinal tract morphogenesis, whereas Nortey *et al*., (2015) used Sprague Dawley rats to study the effect of cassava root meal on their performance, organ characteristics and

blood parameters.

In the current study, laboratory rats were used as a model for pigs. It was hypothesized that the partial replacement of wheat bran with corn cobs, supplemented with exogenous enzymes, would not negatively affect their growth performance, internal organs and blood parameters. The objectives of this study were therefore to investigate the effects of exogenous enzymes supplementation in corn cob-based diets on the: 1) growth; 2) internal organs, and 3) blood parameters of laboratory rats.

## Materials and Methods

*Experimental site and duration of study*

The experiment was conducted at the Noguchi Memorial Institute for Medical Research (NMIMR), at the University of Ghana, Accra. The institute is the leading biomedical research facility in Ghana and serves as a centre for conducting medical research of importance to Ghana and other countries. The growth trial lasted for 4 weeks, from mid-October to mid-November, 2018. This was followed by a one-day organ and blood profile analyses.

*Experimental diets*

Corn cobs were obtained from the Livestock and Poultry Research Centre (LIPREC) of the College of Basic and Applied Sciences (CBAS), University of Ghana. After shelling and sun-drying to a moisture content of about 10%, the corn cobs were further milled using a hammer mill fitted with an 8-mm sieve before being incorporated in the various experimental diets. There were seven experimental diets. The control diet, (T1) was a regular diet formulated to meet the recommended nutrient levels of laboratory rats based on their age and weight and contained 0% corn cobs and 6% wheat bran. Treatment 2 (T2), T4 and T6 had 25%, 50% and 75% of wheat bran replaced with corn cobs respectively without enzyme supplementation. Treatment 3 (T3), T5 and T7 followed the same inclusion rate of corn cobs as in T2, T4 and T6 respectively, but with enzyme supplementation. The trade name of the enzyme used was ZIGMA®. It was an enzyme cocktail containing endo-1,4-β-xylanase, endo-1,4-β-glucanase and phytase enzyme with activities of 336,000 TXU, 150,000 TGU and 350,000 FTU respectively. It was added at the manufacturers' recommended rate of 1 kg/ton of complete feed. All diets were pelleted. The composition and calculated nutrient content of the control diet is shown in Table 1.

*Management of experimental animals*

The protocols used in this trial conformed to the principles recommended by the Institute of Animal Care and Use Committee (IACUC) of NMIMR. Thirty-five 8 to 10-week old male albino rats (Sprague Dawley© strain) were obtained from the Animal Experimentation unit of NMIMR and used for the experiment. The rats were housed in cages in groups of five and allowed to acclimatize to their new environment for 7 days before the start of the trial. On day eight, thirty-five male rats with an initial body weight of 200 g (± 5 g) were selected for the trial. There were seven dietary treatments (T1-T7) and five replicates per treatment. Each replicate was made up of five individually tagged, group-housed rats, which were randomly assigned in a completely randomized design (CRD). The rats were housed in open-sided stainless-steel cages of dimensions (38 cm x 25 cm x 19 cm: length, width and height respectively) which allowed the rats a floor space of 950 cm2. The rats were provided with floor bedding in the form of wood shavings which was autoclaved and dried before being used. Each cage had a metallic feeding trough and a nipple drinker. Feed and water were provided ad libitum. The room temperature was maintained between 20-23°C and the rats were kept under a 24-hour period of light throughout the experiment.

*Growth trial*

The growth trial lasted 28 days. The individually tagged rats were weighed at the start of the experiment and weekly on days 7, 14, 21 and 28. The rats (replicates) within a treatment were collectively fed daily with a

**Table 1:** Dietary composition of control diet

| Ingredient | Amount (kg/100kg) |
|---|---|
| Maize | 53.04 |
| Soybean meal | 31.3 |
| Wheat bran | 6 |
| Soybean oil | 6 |
| Corn cobs | 0 |
| Dicalcium phosphate | 1.1 |
| Oyster shell | 1.25 |
| Salt | 0.4 |
| Lysine | 0.25 |
| Methionine | 0.26 |
| Vitamin Premix[1] | 0.4 |
| Total | 100 |
| **Calculated Nutrient Content** | |
| Crude Protein (CP) | 20.33 |
| ME, MJ/kg | 12.5 |
| Total Lysine | 1.2 |
| Digestible Lysine | 1.06 |
| Total Methionine | 0.48 |
| Digestible Methionine | 0.42 |
| Total Phosphorus | 0.62 |
| Available Phosphorus[2] | 0.43 |
| Calcium | 0.8 |

*[1]Provided the following per kilogram of diet vitamin A 8,250 IU, vitamin D3 825 IU, vitamin E 40 IU, niacin 35 mg, D-pantothenic acid 15 mg, riboflavin 5 mg, menadione 4 mg, folic acid 2 mg, thiamine 1 mg, D-biotin 0.2 mg, and vitamin B12 0.025 mg*
*[2]Calculated from analysed phytate contents in corn, wheat bran and soybean meal (Sauvant et al., 2004)*

weighed amount of fresh feed and left-over feed was collected and weighed every morning to determine the feed intake. At the end of every week, the ADFI, ADG and corrected FCE were calculated.

*Organ parameters*

On day 29, all thirty-five albino rats were weighed and euthanized by cervical dislocation for organ analysis. Organs of interest were the full GIT, empty GIT, heart, liver, spleen, right and left kidneys and lungs and these were excised and weighed. Samples of the liver and heart of rats on T1, T2, T4 and T6 were sectioned, stained and photographed. These treatments were chosen because they represented the control diet, and the diets with corn cobs but without enzymes.

*Haematology and serum chemistry analyses*

Two ml of blood was drawn from each rat by cardiac puncture and one ml transferred into vacutainer tubes with Ethylene Diamine Tetra Acetic Acid (EDTA) as an anticoagulant. The rest of the blood was transferred into blood serum separator vacuum tubes for the haematology and serum chemistry analyses respectively. The samples were placed on ice and immediately transported to the Small Animal Teaching Hospital (SATH), School of Veterinary Medicine at the University of Ghana and assayed the same day. Parameters evaluated for blood

haematology were; white blood cells (WBC), red blood cells (RBC), haemoglobin (HGB), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and platelet count (PLT). Those for serum chemistry included; triglycerides, cholesterol, high density lipoprotein, calcium and urea.

*Chemical analysis*

The pelleted diets were crushed into a mash form using a mortar and pestle. The samples were further ground using a Retsch mill (Model ZM200, Retsch GmbH, Retsch-Allee 1-5, Haan Germany) over a 0.5 mm sieve. Proximate analysis was carried out on the experimental diets according to the Association of Official Analytical Chemist (AOAC, 1984) procedures, to determine the dry matter, crude protein, ether extract (fat), crude fibre and ash contents. Neutral detergent fibre and acid detergent fibre contents were determined according to the procedures described by Georing and Van Soest (1970) (Table 2).

*Statistical analysis*

All data collected was analysed as a Completely Randomized Design using the Generalized Linear Model (GLM) procedure of SAS 9.4 edition. Means with significant differences at a 5% probability level were separated using the Student Newman-Kuels' (SNK) Test.

## Results

The analysed chemical composition of the experimental diets is shown in Table 2.

*Growth performance*

Rats on the control diet ate the most feed (P<0.05: 22.91 g/day) compared to rats on T2 (19.47 g/day) and T7 (18.69 g/day). Those on T3 (17.18 g), T4 (17.44 g), T5 (17.69 g) and T6 (17.32 g) had similar ADFI. No significant differences (P>0.05) were observed in the ADG and FCE among the various treatments (Table 3).

**Table 2:** Analysed chemical composition of experimental diets

| Nutrient (%) | Treatments | | | | | | |
|---|---|---|---|---|---|---|---|
| | T1 Control diet | T2 25%CC -Enz | T3 25%CC +Enz | T4 50%CC -Enz | T5 50%CC +Enz | T6 75%CC -Enz | T7 75% +Enz |
| Dry matter | 87.61 | 87.12 | 87.84 | 87.53 | 87.94 | 87.82 | 87.41 |
| Crude protein | 20.61 | 20.57 | 20.74 | 20.74 | 20.58 | 20.62 | 20.53 |
| Ether extract | 13.58 | 12.26 | 13.00 | 11.61 | 13.55 | 13.55 | 13.11 |
| Ash | 7.33 | 8.65 | 4.94 | 6.04 | 6.01 | 5.88 | 5.94 |
| Crude fibre | 2.79 | 2.46 | 3.39 | 3.55 | 3.36 | 3.45 | 3.85 |
| NDF | 6.66 | 6.15 | 9.21 | 6.83 | 8.15 | 7.57 | 8.35 |
| ADF | 4.46 | 4.3 | 4.95 | 4.17 | 5.08 | 4.61 | 5.49 |

*CC: Corn cob, -Enz: no enzymes, +Enz: plus enzymes, NDF: Neutral detergent fibre, ADF: Acid detergent fibre*

**Table 3:** Effect of corn cob on growth performance

| Parameters | Treatment | | | | | | | SEM | P-Value |
|---|---|---|---|---|---|---|---|---|---|
| | T1 Control Diet | T2 25%CC - Enz | T3 25%CC + Enz | T4 50%CC - Enz | T5 50%CC + Enz | T6 75%CC - Enz | T7 75%CC + Enz | | |
| ADFI1 (g) | 22.91[a] | 19.47[b] | 17.18[c] | 17.44[c] | 17.69[c] | 17.32[c] | 18.69[d] | 0.16 | 0.0001 |
| ADG2 (g) | 2.46 | 1.81 | 1.93 | 2.32 | 2.64 | 2.13 | 3.13 | 0.38 | 0.22 |
| FCE3 | 0.11 | 0.09 | 0.11 | 0.13 | 0.15 | 0.12 | 0.17 | 0.02 | 0.15 |

*CC: Corn cob, -Enz: no enzymes, +Enz: plus enzymes, 1Average daily feed intake, 2Average daily gain, 3Feed conversion efficiency, abc: Means with different superscripts in a row are significantly different*

**Table 4:** Effect of corn cob on internal organ weights

| Parameters | Treatment | | | | | | | SEM | P-Value |
|---|---|---|---|---|---|---|---|---|---|
| | T1 Control Diet | T2 25%CC - Enz | T3 25%CC + Enz | T4 50%CC - Enz | T5 50%CC + Enz | T6 75%CC - Enz | T7 75%CC + Enz | | |
| Full GIT[1] | 25.24 | 23.70 | 23.95 | 24.68 | 26.15 | 25.49 | 27.71 | 1.78 | 0.73 |
| Empty GIT | 12.65[a] | 13.36[a] | 12.87[a] | 14.51[a] | 12.76[a] | 17.41[b] | 21.40[b] | 1.64 | 0.005 |
| Left kidney | 0.91 | 0.82 | 0.89 | 0.91 | 1.04 | 0.87 | 0.87 | 0.06 | 0.24 |
| Right kidney | 1.07 | 0.90 | 0.94 | 0.93 | 1.01 | 0.95 | 0.90 | 0.07 | 0.63 |
| Heart | 1.13 | 0.95 | 0.96 | 0.90 | 1.00 | 1.07 | 0.87 | 0.06 | 0.09 |
| Lungs | 1.98 | 1.67 | 1.71 | 1.53 | 1.63 | 1.92 | 1.85 | 0.17 | 0.47 |
| Spleen | 0.66 | 0.67 | 0.65 | 0.59 | 0.63 | 0.68 | 0.58 | 0.04 | 0.39 |
| Liver | 10.67 | 9.14 | 10.57 | 10.58 | 10.61 | 10.26 | 11.14 | 0.58 | 0.36 |

*CC: Corn cob, -Enz: no enzymes, +Enz: plus enzymes, [1]Gastrointestinal tract, [ab]: Means with different superscripts in a row are significantly different*

**Table 5:** Effect of corn cob on blood haematology

| Parameters | Treatment | | | | | | | SEM | P-Value |
|---|---|---|---|---|---|---|---|---|---|
| | T1 Control Diet | T2 25%CC - Enz | T3 25%CC + Enz | T4 50%CC - Enz | T5 50%CC + Enz | T6 75%CC - Enz | T7 75%CC + Enz | | |
| WBC(109/L) | 7.59 | 7.22 | 8.54 | 5.86 | 6.23 | 10.15 | 5.91 | 1.91 | 0.66 |
| LYM (%) | 51.56 | 59.57 | 58.10 | 52.18 | 48.12 | 47.24 | 60.44 | 5.01 | 0.34 |
| MON (%) | 6.83 | 5.93 | 7.27 | 9.21 | 8.22 | 6.39 | 5.91 | 1.41 | 0.60 |
| NEU (%) | 33.36 | 29.86 | 29.21 | 32.50 | 37.65 | 40.09 | 27.53 | 4.20 | 0.34 |
| EOS (%) | 7.80 | 4.23 | 5.17 | 5.75 | 5.70 | 6.03 | 5.90 | 1.52 | 0.80 |
| BASO (%) | 0.45 | 0.41 | 0.25 | 0.37 | 0.29 | 0.24 | 0.21 | 0.11 | 0.60 |
| LYM (#) | 1.85 | 1.84 | 2.13 | 1.55 | 1.25 | 1.90 | 1.59 | 0.42 | 0.82 |
| MON (#) | 0.26 | 0.17 | 0.26 | 0.29 | 0.23 | 0.34 | 0.16 | 0.08 | 0.76 |
| NEU (#) | 1.10 | 0.87 | 1.04 | 0.97 | 0.92 | 2.48 | 0.71 | 0.53 | 0.30 |
| EOS (#) | 0.26 | 0.14 | 0.19 | 0.15 | 0.17 | 0.47 | 0.16 | 0.13 | 0.58 |
| BASO (#) | 0.02 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.004 | 0.003 | 0.45 |
| RBC(1012/L) | 9.91 | 9.49 | 10.00 | 9.49 | 9.28 | 9.31 | 8.79 | 0.41 | 0.46 |
| HGB (g/DL) | 15.66 | 14.96 | 15.88 | 14.94 | 14.92 | 14.64 | 13.96 | 0.75 | 0.63 |
| HCT (%) | 47.60 | 45.80 | 48.30 | 45.38 | 45.28 | 44.52 | 42.44 | 2.23 | 0.61 |
| MCV(fL) | 47.98 | 48.36 | 48.32 | 47.94 | 48.84 | 47.84 | 48.16 | 0.64 | 0.94 |
| MCH (pg) | 15.78 | 15.76 | 15.88 | 15.76 | 16.08 | 15.72 | 15.84 | 0.22 | 0.92 |
| MCHC (g/dL) | 33.00 | 32.80 | 33.00 | 33.00 | 33.00 | 33.00 | 33.00 | 0.08 | 0.44 |
| PLT (109/L) | 1073.80 | 1238.40 | 1124.20 | 1216.60 | 1150.20 | 1113.60 | 906.00 | 148.77 | 0.77 |
| MPV (fL) | 5.44 | 5.36 | 5.44 | 5.74 | 5.54 | 5.08 | 5.84 | 0.27 | 0.55 |

*CC: Corn cob, -Enz: no enzymes, +Enz: plus enzymes, WBC: White Blood Cells, LYM: Lymphocytes, MON: Monocytes, NEU: Neutrophils, EOS: Eosinophil, BASO: Basophils, RBC: Red Blood Cells, HGB: Haemoglobin, HCT: Haematocrit, MCV: Mean Corpuscular volume, MCH: Mean Corpuscular Haemoglobin, MCHC: Mean Corpuscular Haemoglobin concentration, PLT: Platelet, MPV: Mean Platelet Volume*

**Table 6:** Effect of corn cob on blood chemistry

| Parameters | Treatment | | | | | | | SEM | P-Value |
|---|---|---|---|---|---|---|---|---|---|
| | T1 Control Diet | T2 25%CC - Enz | T3 25%CC + Enz | T4 50%CC - Enz | T5 50%CC + Enz | T6 75%CC - Enz | T7 75%CC + Enz | | |
| Triglyceride (mmol/L) | 0.59 | 0.47 | 0.50 | 0.53 | 0.47 | 0.55 | 0.53 | 0.08 | 0.93 |
| Cholesterol (mmol/L) | 1.84 | 1.80 | 1.69 | 1.89 | 1.85 | 1.90 | 2.14 | 0.13 | 0.41 |
| Calcium (mmol/L) | 3.02 | 2.85 | 2.94 | 3.01 | 3.01 | 2.98 | 3.08 | 0.07 | 0.47 |
| HDL (mmol/L) | 0.73 | 0.64 | 0.66 | 0.77 | 0.69 | 0.73 | 0.84 | 0.08 | 0.61 |
| Creatinine (umol/L) | 59.75 | 53.59 | 52.08 | 56.72 | 57.99 | 55.78 | 57.27 | 3.96 | 0.85 |
| Urea (mmol/L) | 2.96 | 3.80 | 3.24 | 4.02 | 2.92 | 3.62 | 3.96 | 0.50 | 0.53 |

*CC: Corn cob, -Enz: no enzymes, +Enz: plus enzymes, HDL: High density lipoprotein*

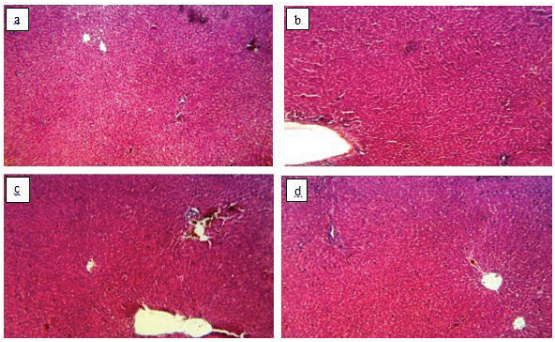


**Figure 1:** Sectioned and stained liver of rats on T1 (a), T2 (b), T4 (c) and T6 (d) (magnification x40)

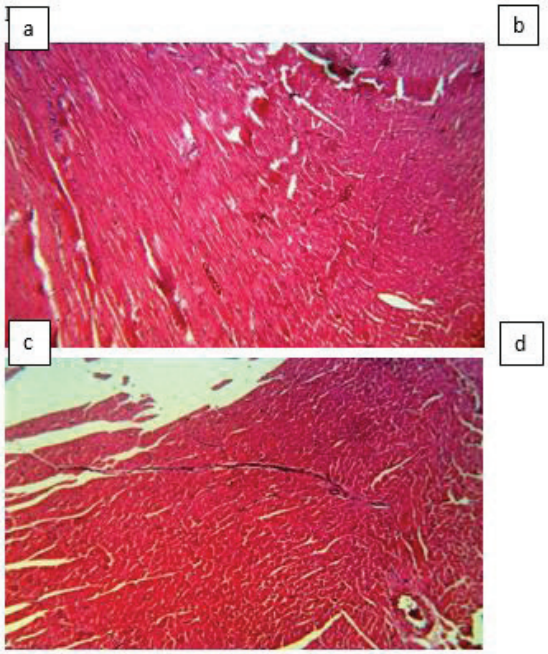


**Figure 2:** Sectioned and stained heart of rats on T1 (a), T2 (b), T4 (c) and T6 (d) (magnification x40)

*Internal organ weight*

The empty GIT weights of rats on T6 (17.41 g) and T7 (21.40 g) were similar, but higher (P<0.05) than that of rats on the other treatments which were all similar in weight. The other organ weights were not significantly different (P>0.05) from each other (Table 4).

Stained slides of the liver and heart of rats on T1, T2, T4 and T6 are shown in Figures 1 and 2 respectively.

*Blood haematological and biochemical parameters*

There were no dietary effects (P>0.05)

on any of the blood haematological and biochemical parameters as shown in Tables 5 and 6 respectively. However, rats on T2 to T7 (corn cob-based diets) had lower triglyceride levels than rats on T1 (control diet).

## Discussion

Rats on the control diet had the highest ADFI followed by rats on T2 (25% corn cob without enzymes). The observed differences in ADFI are similar to the findings of Ndubuisi *et al*. (2008) who found significant differences in the ADFI of pigs fed with various levels of corn cobs. Certain types of fibre namely arabinoxylans, galactans, galactomannans, arabinogalactans, mannans, arabinans and β-glucans are known to decrease the rate of passage of digesta through the GIT (Theander *et al*., 1989). Fibres of this type can cause the intestinal contents to be viscous (Ikegami *et al*., 1990; Campbell and Bedford, 1992; Choct and Annison, 1992). When digesta passage rate slows down, it can lead to a prolonged period of satiety, which invariably leads to a decrease in feed intake (Wenk, 2001; Jimenez-Moreno *et al*., 2010). The types of fibre in corn cobs include cellulose, hemicellulose and xylan (Ebringerová and Heinze, 2000; Vázquez *et al*., 2006; Metzler and Mosenthin, 2008; Kumar *et al*., 2010), which are known to reduce digesta transit time in the GIT (Montagne *et al*., 2003). In this trial, the rate of passage was not determined, but it can be speculated that the reason for the high ADFI of rats on T1 compared to the other treatments may have been due to higher levels of fibre in the other diets resulting in a slower digesta transit time.

Enzymes are biological catalysts that speed up the rate of biological reactions. Fibre degrading enzymes (like cellulases, β-glucanases and xylanases) have been shown to remove the deleterious effects of fibre (cellulose, β-glucan and xylan). A desirable effect of fibre degrading enzymes is a reduction in intestinal viscosity, leading to an increase in gastric emptying and thus ADFI (Jia *et al*., 2009; O'Neill *et al*., 2014). This phenomenon may explain the higher ADFI of rats on T7 (which contained enzymes) compared to T6 (which had no enzymes). Again, for enzyme effects to be felt the most, there needs to be an appropriate substrate (Wilson, 2014). The phenomenon above was not observed in the other treatments because perhaps the level of fibre (from corn cobs) was not high enough for the full effects of the enzymes to be felt.

With the exception of the empty GIT weight, no differences (P>0.05) were observed in the weights of the other organs, an indication that corn cobs had no detrimental effects on the organs of the rats. A similar trend was reported by Nortey *et al*. (2015) where feeding laboratory rats with increasing levels of whole cassava root meal, including the peels (and hence fibre) resulted in no significant differences (P>0.05) in the organ weights. The empty GIT weights of rats on treatments 6 (17.41 g) and 7 (21.40 g) were similar (P<0.05) but higher than those on diets T1 to T5. Dietary fibre is needed in a pig's diet to ensure proper development of the GIT. Depending on the type, dietary fibre may increase or decrease retention time of digesta in the GIT. When fibre tends to increase retention time in the upper GIT, there is an increase in both the weight and length of the GIT (Jimenez-Moreno *et al*., 2011; Mateos *et al*., 2012; Jha and Berrocoso, 2016). This is an adaptation and that better equips the animal to handle the extended transient time of digesta or the higher levels of fibre in the diet (Gerritsen *et al*., 2012; Kheravii *et al*., 2018). This adaptation is a result of well-developed musculature and size of the GIT in order to withstand the bulky nature of dietary fibre, as was seen in T6 and T7.

An abnormal deviation from the normal range of blood parameters indicates a disease condition or an infection (WebMD Medical Reference, 2010). The absence of significant differences in the blood haematological and biochemical parameters (Tables 5 and 6) showed that, the diets did not have any negative health and physiological effects on the rats. The WBC counts fell within the normal range of Sprague-Dawley© rats (5.00 to 14.45x109/L) (Said and Abiola, 2014). Lymphocytes, monocytes, neutrophils, basophils and eosinophils are

haematological indicators which are used to detect the presence of allergies, infections, stress and diseases. All of these fell within the normal ranges, a clear indication that the diets presented no stress to the rats. A deviation from normal counts of RBC, MCH, MCHC and MCV, indicate an anaemic condition or some form of oxygen-related stress. However, all of these indicators fell within the normal ranges for Sprague-Dawley© rats (Said and Abiola, 2014), indicating an absence of any abnormal physiological state in the rats. Work by Adesehinwa *et al*. (2008), also showed no significant effects of diet on the haematological parameters when growing pigs were fed with a cassava peel-based diet (hence hemicellulose). According to Theuwissen and Mensink (2007) and Maki *et al*. (2009), an increase in dietary fibre results in a decrease in serum cholesterol. This trend was not observed in this study (Table 6). It could be speculated that the levels of corn cobs were not high enough to elicit such an effect. High density lipoprotein, also known as "good cholesterol" transports low density lipoprotein (bad cholesterol) and triglycerides from the blood to the liver to be discarded from the body (Berry, 2017). Creatinine and urea levels are used as indicators for detecting kidney and liver malfunctions. Normal levels of these indicators and the normal sizes of the kidneys and livers observed in all the experimental rats showed no dietary effects on these organs. The triglyceride levels across all the treatments were lower than the reference range (Ihedioha *et al*., 2013). Fibre can bind to dietary lipid constituents thereby reducing triglyceride levels by voiding them through the faeces (Wilfart *et al*., 2007; Sarikhan *et al*., 2009). Ben Slama *et al*. (2011) also reported that, fibre inclusion in diets reduces absorption of fat in the small intestine, resulting in lower triglyceride levels in the blood of humans. These could be reasons for the results observed in this study.

## Conclusion

This study has shown that corn cobs can partially replace up to 75% of wheat bran in the diets of Sprague Dawley© albino rats even without enzyme supplementation. Such inclusion levels will not cause any negative effects on their performance, organ integrity and blood profiles.

## Recommendations

A similar trial using other monogastric species like chickens can be done and cost analyses performed to determine the economics of using corn cobs in diets for broilers or layers. The inclusion levels of corn cobs may also be elevated to determine the point at which it becomes detrimental, even in the presence of exogenous enzymes.

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# SHEEP RED BLOOD CELL (SRBC) ANTIGEN AS AN INDICATOR TRAIT FOR DISEASE RESISTANCE IN INDIGENOUS GUINEA FOWLS (NUMIDA MELEAGRIS)

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## Abstract

Studies have proved that birds, especially chickens, selected for high antibody response to sheep red blood cells (SRBC) showed higher resistance to some infectious diseases. Very little research has been done on Guinea fowl antibody response to antigens. The objective of this study was to determine the effect of variety, sex, days of post injection, Sheep Red Blood Cell (SRBC) concentration and route of inoculation on antibody response to SRBC antigen in indigenous Guinea fowls reared under an intensive system. Three hundred and twenty (320), 12 - week old keets, comprising 40 males and 40 females from each of four indigenous Guinea fowl varieties (Pearl, Lavender, White and Black) were randomly selected and assigned to a 4 X 2 factorial experiment (4 = varieties and 2 = SRBC concentrations). Half of each variety as well as sex, were intravenously injected with 0.1ml (100µl) of 1.0% SRBC antigen and the other half given 0.1ml 0.25% intramuscularly. Blood samples were collected at 5, 7, and 9 days post-immunization. Total antibody titers were measured by agglutination assays. Antibody titers after inoculation were significantly ($p<0.05$) higher in the Pearl variety compared to the Lavender, White and Black varieties. Females and intravenous injection elicited significantly ($p<0.05$) better antibody titers than the males and intramuscular injection respectively. The antibody response to SRBC antigen was not significantly ($p>0.05$) influenced by post- injection days and SRBC antigen concentrations. These results imply that differences in T cell subpopulations, sex hormones, ellipsoid-associated cells and peritoneal cavity might have brought about variations in antibody response to the SRBC antigen in this study. The antibody response to SRBC antigen was better in females than in males. Intravenous injection was more effective in presenting SRBC antigen to immunocompetent cells than intramuscular injection. The Pearl variety has high potential for immune competence.

**Keywords:** Antibody response, variety, sex, inoculation, agglutination assays

# L'ANTIGÈNE ÉRYTHROCITAIRE DE MOUTONS COMME CARACTÉRISTIQUE DE RÉFÉRENCE POUR LA RÉSISTANCE AUX MALADIES CHEZ LA PINTADE INDIGÈNE (NUMIDA MELEAGRIS)

## Résumé

Des études ont démontré que les oiseaux, en particulier les poulets, sélectionnés pour une réponse d'anticorps élevée aux globules rouges de moutons (SRBC : Sheep Red Blood Cells), ont fait preuve d'une plus grande résistance à certaines maladies infectieuses. Très peu de recherches ont été effectuées sur la réponse anticorps de la pintade aux antigènes. L'objectif de cette étude était de déterminer l'effet de la

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variété, du sexe, des jours post-injection, de la teneur en globules rouges de moutons (SRBC) et de la voie d'inoculation sur la réponse d'anticorps à l'antigène SRBC chez les pintades indigènes élevés en système intensif. Trois cent vingt (320) pintadeaux de 12 semaines, dont 40 mâles et 40 femelles de chacune des quatre variétés indigènes de pintade (Pearl, Lavender, White et Black) ont été choisis de manière aléatoire et affectés à une expérience factorielle de 4 X 2 (4 = variétés et 2 = concentrations de SRBC). La moitié pour chaque variété et sexe a reçu par injection intraveineuse 0,1 ml (100 µ l) de 1,0% de l'antigène SRBC et l'autre moitié 0,1 ml de 0,25% par voie intramusculaire. Des échantillons de sang ont été prélevés à 5, 7 et 9 jours après l'immunisation. Les titres d'anticorps totaux ont été mesurés par des essais d'agglutination. Les tigres d'anticorps après l'inoculation étaient significativement ($p < 0,05$) plus élevés dans la variété Pearl par rapport aux variétés Lavander, White et Black. Les femelles et l'injection intraveineuse ont montré de meilleurs ($p < 0,05$) titres d'anticorps respectivement par rapport aux mâles et à l'injection intramusculaire. La réponse d'anticorps à l'antigène SRBC n'était pas significativement ($p > 0,05$) influencée par les jours post-injection et les concentrations d'antigènes SRBC. Ces résultats impliquent que les différences au niveau des sous-populations de cellules T, des hormones sexuelles, des cellules associées à l'ellipsoïde et la cavité péritonéale peuvent avoir entraîné des variations dans la réponse immunitaire à l'antigène SRBC dans cette étude. La réponse immunitaire à l'antigène SRBC était meilleure chez les femelles que chez les mâles. L'injection intraveineuse a été plus efficace pour présenter l'antigène SRBC aux cellules immunocompétentes par rapport à l'injection intramusculaire. La variété Pearl possède un potentiel élevé de compétence immunitaire.

**Mots-clés :** réponse d'anticorps, variété, sexe, inoculation, essais d'agglutination

## Introduction

Guinea fowl production plays momentous roles in elevating the rural economy in Ghana. Most of the people in Northern Ghana (Upper East, Upper West and Northern Region) derive their immediate cash needs from Guinea fowl rearing. The ability of most of the traditional people to purchase farm inputs for crop production and food to sustain their families depends on the immediate cash needs (Annor *et al*., 2013). The Guinea fowls, found in many traditional homes, not only serve as source of income to the people but also provide meat and eggs (Avornyo *et al*., 2013). The meat is a delicacy and a source of quality protein because it contains less cholesterol and fats (Dei *et al*., 2014). In addition, the bird is used culturally for different purposes such as in funeral celebrations, sacrifices, courtship, and as a token for settling disputes. Thus, Guinea fowl production is lucrative because there is high demand for both the meat and eggs.

Guinea fowls have been proved to be resistant to most poultry diseases (Kusina *et al*., 2012). However, most researchers have reported high mortality rates in these birds during brooding (Jacob and Pescatore, 2013). Farmers sometimes recorded over 90% deaths of Guinea fowl keets (GNA, 2013). The high keet mortality in the Guinea fowl industry in Ghana may be attributed to lack of technical knowhow in terms of nutrition, genetic improvement of the immune system and medication (Moreki and Radikara, 2013).

One of the traits that should be considered in setting breeding goals during animal improvement programs is resistance to diseases. This should go along with improvement in docility, growth rate, conformation and reproduction (Annor at al., 2013). Production costs in general will reduce when disease resistance in animals is improved as there will be a reduction in the costs of vaccination and other disease prevention measures, mortality control and reduced loss of performance during disease outbreaks. Besides the economic benefits from improving disease resistance, the possibility of reducing medication would be attractive from the standpoints of public health, ethics, product quality and animal welfare (Prajwalita *et al*., 2018).

Good immune systems provide vertebrates with an important mechanism to fight pathogens and to reduce the incidence of diseases. Antibodies are important in protecting against pathogens because of their role in facilitating the lysis of microorganisms,

neutralizing toxins, and agglutinating microorganisms to improve phagocytosis (Oral *et al*., 2002). Many kinds of antigens (pathogenic and non-pathogenic) have been used to monitor immune responsiveness in poultry (Li *et al*., 2000). Non-pathogenic antigens include Sheep Red Blood Cells (SRBC), Mollusk Haemocyanin, Chicken Egg White Lysosome and Bacterial Lipopolysaccharides (Prajwalita *et al*., 2018).

SRBC is the most commonly used antigen to study immune-competence in poultry (Vanderzipp, 1983) Previous studies have proved that birds, especially chickens, selected for high antibody response to sheep/goat red blood cells (SRBC) showed higher resistance to some infectious diseases, for example, Marek's and Newcastle (Arun De *et al*., 2013). It was therefore hypothesized that Guinea fowls selected for high antibody response to sheep red blood cells would show higher resistance to some infectious poultry diseases. The objective of this immunological study therefore was to determine whether the four varieties of indigenous Guinea fowls, reared under an intensive system would differ in their responses to SRBC antigen based on their sex, days post injection, SRBC concentration and route of inoculation.

## Materials and Methods

The study was conducted at the Poultry Section of the Animal farm of the Department of Animal Science Education, University of Education, Winneba, Mampong-Ashanti campus, Ghana, from 2016 to 2017. Mampong-Ashanti lies in the transitional zone between the Guinea savanna zone of the north and the tropical rain forest of the south of Ghana along the Kumasi-Ejura road. Mampong lies on latitude 07° 03' N and longitude 01° 24'W on an altitude of 289.7m above sea level. The rainfall pattern is bimodal, with the major rainfall season occurring from April to July with 1000mm of rainfall while the minor season occurs from August to November with 350mm of rainfall. The average daily temperature is between 25°C and 30°C (MSD, 2015).

*Experimental Design*

Four indigenous Guinea fowl varieties (Pearl, Lavender, White and Black) were used in this experiment. The Pearl Guinea fowl has a round-shouldered, clad in sheer dark feathers with delicate white polka-dots, the Lavender variety is similar to the Pearl but with white plumage that is light gray or dotted with white, the White Guinea fowl has pure white feathers and the Black Guinea fowl has black feathers. Three hundred and twenty (320) 12 - week old keets, comprising 80 (40 males and 40 females) each of the four indigenous Guinea fowl varieties (Pearl, Lavender, White and Black) were randomly selected and assigned to 4 X 2 factorial experimental design (4= strains and 2= SRBC concentrations). Each of the four varieties was replicated four times with twenty (20) keets per replicate.

*Management of experimental birds*

The birds were fed similar diets containing 22% of protein and 2950 kcal/kg metabolizable energy. Feed and water were given ad libitum. All the birds were intermingled, and not vaccinated before the experiment (Li *et al*., 2000). Half of each variety as well as sex were intravenously injected with 0.1ml (100µl) of a 1.0% suspension of SRBC antigen diluted in Phosphate Buffered Saline (Pinard *et al*., 1992) through the medial wing vein and the other half was given 0.1ml of a 0.25% suspension of SRBC antigen intramuscularly through the breast muscles. Blood samples were collected from inoculated keets at 5, 7, and 9 days post-immunization. The blood samples were centrifuged at 3000rpm for 5 minutes after which the plasma was extracted into Eppendorf tubes using pipette and stored at -20oC. Total antibody titers were measured by agglutination assays (Parmentier *et al*., 2002). Wells of microtitre plates each were filled with 50µl of Phosphate Buffered Saline (PBS). Fifty microlitres (50µl) of the thawed test plasma was added and serially diluted using the two (2) fold serial dilutions. The last volume of the diluents in the multi-channel pipette was discarded. Fifty microlitres (50µl) of two percent (2%) SRBC suspension was

added to all the wells starting from the lowest concentrated wells. A controls experiment was set (plasma of untreated birds was used). The microtitre plates were shaken and incubated for 45 minutes at 37oC. Microtitre wells that showed partial or complete Haemaglutination Inhibition (HAI) were taken as the end point and expressed as Log 2x units/50µl, where x =the highest plasma dilution with complete or partial HAI. Total antibody titers to SRBC were determined by routine agglutination procedures (Van der Zijpp and Leenstra, 1980).

*Data analysis*

The data collected was analyzed using the General Linear Model (GLM) procedure of Statistical Analysis System (SAS for Windows, version 7). Significance was considered at P<0.05. The means were separated by using the probability of difference (PDIFF) procedure of SAS (SAS, 2008). The linear model below was considered.

$$Y_{ijklmn} = \mu + G_i + C_j + D_k + E_l + F_m + e_{ijklmn}$$

Where

$Y_{ijklmn}$ = performance of the ith variety group of jth concerntration

µ = overall mean common to all the observations

$G_i$ = effect due to ith strain (I = 1, 2, 3, 4)

$C_j$ = effect due to jth sex (1, 2)

$D_k$ = effect due to kth days (5, 7, 9)

$E_l$ = effect due to lth concentration (1, 2)

$F_m$ = effect due to mth

$e_{ijklmn}$ = the random error term

## Results

Two way interactions among fixed factors showed no significant differences and these were therefore ignored. The control experiment showed no Haemaglutination Inhibition (Plate 1) and was not considered in the discussion.

The variety and sex effects on antibody titers of the local Guinea fowls are shown in Table 1. There were significant (p<0.05) influences of varieties and sex with respect to antibody titers in the birds. Antibody titers were significantly (p<0.05) higher in the pearl variety compared to their counterparts. Similar (p>0.05) antibody titers were recorded among the Lavender, White and the Black varieties. The female Guinea fowls had higher antibody titers than the males.

Table 2 shows the effects of days and SRBC concentration on antibody titers in local

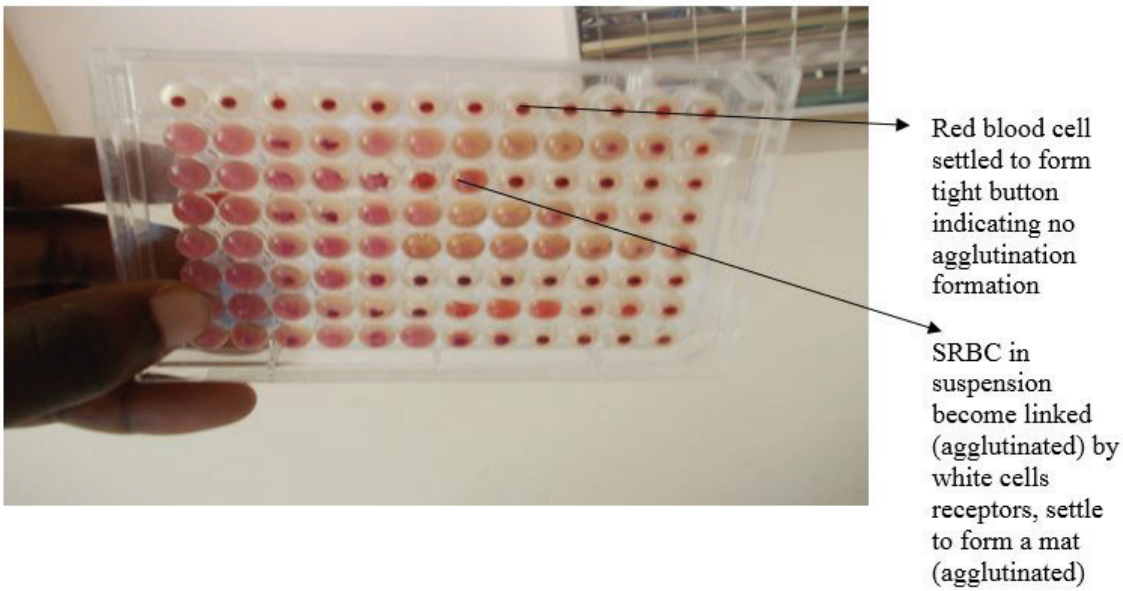



**Plate 1:** SRBC Hemaglutination (HA) test in indigenous Guinea fowls.

**Table 1:** Effect of Variety and Sex on Antibody Titres of Four Guinea Fowl Varieties Inoculated with SRBC Antigen

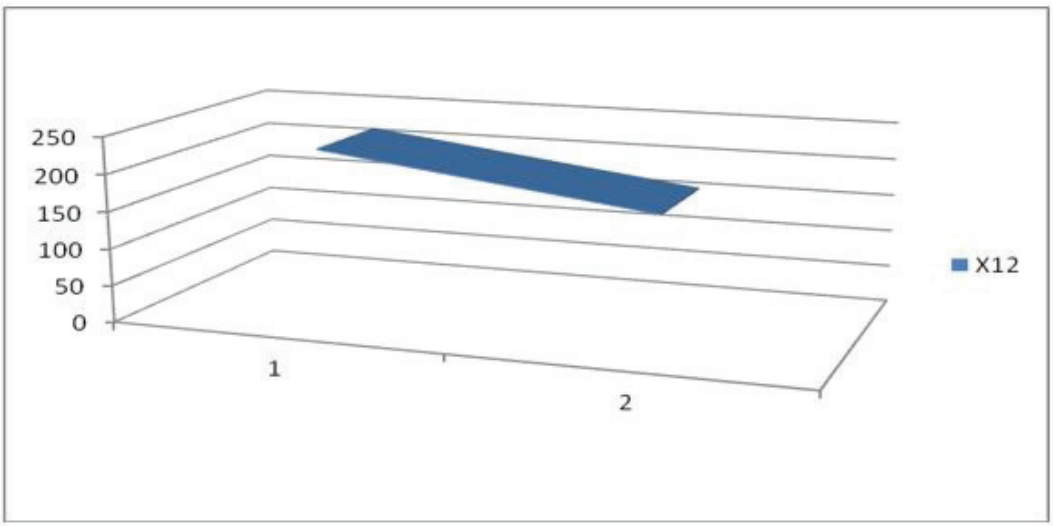
| Factor | Mean Antibody Titre ABR |
|---|---|
| **Variety** | |
| Pearl | $8.16^{a} \pm 0.181$ |
| Lavender | $5.93^{b} \pm 0.181$ |
| White | $6.31^{b} \pm 0.181$ |
| Black | $5.96^{b} \pm 0.181$ |
| P-value | 0.003 |
| **Sex** | |
| Female | $7.16^{a} \pm 0.220$ |
| Male | $6.02^{b} \pm 0.226$ |
| P-value | 0.005 |

*a–b Means within a column for variety and sex with different superscripts differ at $P \leq 0.05$.*

**Table 2:** Effect of Days and SRBC Concentration on Antibody Titers in indigenous Guinea Fowls

| Factor | Level | Antibody Titre ABR |
|---|---|---|
| Day | | |
| | 5 | $6.12 \pm 0.296$ |
| | 7 | $6.67 \pm 0.159$ |
| | 9 | $6.98 \pm 0.290$ |
| P-values | | 0.24 |
| Concentration | | |
| | 1 | $6.67 \pm 0.129$ |
| | 2 | $6.51 \pm 0.128$ |
| P-values | | 0.37 |

*1= 1.0% concentration of SRBC and 2= 0.25% concentration of SRBC*




*Route 1 and 2 (1= Intravenous, produced 200 antibodies; 2= Intramuscular, produced 150 antibodies)*

**Figure 1:** Effect of Route of SRBC Antigen Administration on Antibody Titres in indigenous Guinea Fowls

Guinea fowls. Both days and concentration had no statistical ($p<0.05$) effects on antibody titers in the local Guinea fowls studied in this experiment.

Figure 1 shows the effect of route of administration of SRBC antigen on the varieties of local Guinea fowls. Route of administration significantly ($p<0.05$) influenced antibody titers in the local Guinea fowls. Intravenous injection was more effective than the intramuscular route-in antibody titers production of the birds.

## Discussion

The relatively higher antibody titers (8.16, 5.93, 6.31 and 5.96 for Pearl, Lavender, White and Black respectively) observed in this study than those obtained in previous reports (3.79, 2.17 and 1.17) for normally feathered, naked neck and frizzle chickens respectively (Baclmans *et al*., 2005) and (6.8, 5.3 and 2.8) in White leghorn lines by (Boa-Amponsem *et al*., 2001) may explain the findings of Houndonougbo *et al*. (2017) and Kusina *et al*. (2012) who stated that the Guinea fowl species is resistant to most poultry diseases. Evaluation of T lymphocyte subpopulations may provide some clues in understanding the variation of the antibody responses among the varieties. This is because the SRBC antigen is classified as a thymus-dependent (TD) antigen that obviously needs the help of T lymphocytes to produce antibodies (Mosier and Subbarao, 1982). It seems that growth selection might have resulted in correlated changes of T cell subpopulations, therefore affecting antibody production (Li *et al*., 2000). The better response in antibody titers of the Pearl variety than the other varieties in the present study could be attributed to the stronger genetic disease resistant potential (higher levels of T lymphocytes subpopulations) the birds in this variety possessed which explains the reason why they are common in all the areas Guinea fowls are reared in the country (Issaka and Nartey, 2016)

The significant influence of variety with respect to antibody titers in the present study compares well with the findings of Arun De *et al*. (2013) who reported significant differences among the pure breeds of Black Nicobari WhiteNicobari and Vanaraja fowls in an investigation to study the antibody response to goat erythrocytes. Similar results were reported by Baclmans *et al*. (2005).

The differences in antibody responses between the sexes may be ascribed to the influence of sex hormones. According to the report of Krzych *et al*. (1981) and Eiginger and Garrett (1972), estrogen and androgen have distinct influence on the immune systems of organisms. This difference might also be due to male and female antibody titers being genetically different traits, due to the genes being located on the sex chromosome (Saxena *et al*., 2012). The significant sex effect observed on the antibody responses to SRBC antigen in this study compares well with the result of Li *et al*. (2000) who reported that there were significant sex differences in total titers at 4, 7, and 14 days in turkey lines they studied. The outstanding female response to SRBC antigen in antibody production in the present experiment conforms to the statement that females usually exhibit a higher capacity for antibody formation after immunization (Paavonen *et al*., 1981). Ose-Amponsah *et al*. (2013) reported comparable results in Ghanaian local, Sasso T-44 and broiler chickens. Contrary to this finding, sex wise HAI results showed that males had higher mean (7.925 ± 0.1463) than females (7.57± 0.161) for HA (Saxena *et al*., 2012)

Non- significant ($p>0.05$) differences in antibody titres on post injection days (5, 7 and 9) in the current experiment might have been due to the shorter post injection duration of less than 2 weeks. Production of induced antibodies is slow – often not peaking at 2-4 weeks after exposure to a pathogen or an antigen. Prajwalita *et al*. (2017) also reported non- significant ($p>0.05$) differences in antibody titres at day 10 after inoculation between Kadaknath and IBL-80 breeds of chickens.

The non- significant ($p>0.05$) influence of different concentrations of SRBC on antibody response recorded in this study could have been due to non-variation in maternally transferred

antibodies. Maternally transferred antibodies are an important source of phenotypic variation in a range of offspring traits as well as influencing response to infections across generations (Coakley *et al*., 2014). This finding is incongruous with the observation of Osei-Amponsah *et al*. (2013) who reported that increasing the concentration of SRBC antigens resulted in increased antibody titres in all the ecotypes except the savannah local chickens. The better antibody response to SRBC following intravenous injection compared to intramuscular injection reported previously and in the current study may be attributed to differences in the functions of the ellipsoid-associated cells and peritoneal cavity cells. Van der Zijpp *et al*. (1986) postulated that the ellipsoid-associated cells were more effective in presenting SRBC antigen to immunocompetent cells than the antigen-presenting cells of the tissues or the peritoneal cavity. The significant effects of the route of SRBC inoculation on the primary antibody response of local Guinea fowls in the current investigation was in agreement with the findings of Boa Amponsem *et al*.(2001) who reported that within the HA line antibody titers were consistently higher in intravenously injected chickens than in those injected intramuscularly.

## Conclusion

The pearl variety has high potential for immune competence. The antibody response to SRBC antigen was better in the female than in the male Guinea fowls. Intravenous injection was more effective in presenting SRBC antigen to immunocompetent cells than the intramuscular injection. Different post injection days and SRBC concentrations did not influence the antibody response. This study concluded that SRBC antigen can potentially be used as an indicator trait for the survival traits in Guinea fowls. Future studies should look at the effects of variation in T cell subpopulations and secondary inoculation on antibody response in these birds.

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# ANTIBIOTIC RESISTANCE OF FASTIDIOUS AND NON-FASTIDIOUS MICROORGANISMS ISOLATED FROM SHEEP AND GOATS VISITING THE TAMALE VETERINARY CLINIC

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## Abstract

The treatment of animals at most veterinary facilities in Ghana relies on history and physical observation without laboratory confirmation. This study employed tentative diagnosis of sheep and goats visiting the Tamale Veterinary Clinic for treatment with history of diarrhoea, anorexia, and peste des petits ruminants (PPR). Faecal and lachrymal secretion samples were swabbed, stored under 4oC and transported to the laboratory for analyses. Microbial analysis for fastidious and non-fastidious microorganisms were done according to the USA-FDA Bacteriological Analytical Manual. Antibiotic susceptibility tests were performed using the disc diffusion method. The fastidious microorganisms isolated from sheep were highly resistant to oxacillin (100%), tetracycline (100%), ceftriaxone (90%) and erythromycin (70%). Susceptibility to suphamethoxazole/trimethoprim and chloramphenicol were 60% and 50%, respectively. Intermediate resistances as high as 40% were observed for ampicillin and ciprofloxacin. Non-fastidious microorganisms of sheep origin also exhibited a high resistance to oxacillin (100%), erythromycin (90%), tetracycline (90%) and suphamethoxazole/trimethoprim (70%), but susceptible to ciprofloxacin (70%), ceftriaxone (60%) and gentamicin (60%). In the goats, a higher proportion of the fastidious microorganisms of faecal origin were resistant to oxacillin (100%), tetracycline (100%), gentamicin (80%), erythromycin (80%) and chloramphenicol (60%) while a greater level of susceptibility was observed for suphamethoxazole/trimethoprim (80%). A higher proportion of non-fastidious microorganisms isolated from goat faeces were also resistant to chloramphenicol (100%), oxacillin (100%), tetracycline (100%) and suphamethoxazole/trimethoprim (80%). Susceptibility was 100% for chloramphenicol and gentamicin. Microorganisms isolated from the lachrymal secretions of goats and sheep were highly resistant to oxacillin (100%), erythromycin (100%), tetracycline (100%) and ciprofloxacin (75%), but susceptible to gentamicin (75%). Based on these results, no single antibiotic could effectively treat all the microorganisms that were examined in this study. Ciprofloxacin, ceftriaxone, chloramphenicol, gentamicin and suphamethoxazole/trimethoprim could be effective depending on the source of isolation.

**Keywords:** Antibiotics, diagnosis, small ruminants, veterinary facilities

# RÉSISTANCE AUX ANTIBIOTIQUES CHEZ DES MICRO-ORGANISMES FASTIDIEUX ET NON FASTIDIEUX ISOLÉS PARMI LES OVINS ET CAPRINS VISITANT LA CLINIQUE VÉTÉRINAIRE DE TAMALE

## Résumé

Le traitement des animaux dans la plupart des établissements vétérinaires du Ghana repose sur les antécédents et l'observation physique sans confirmation par un laboratoire. Cette étude a permis d'établir un diagnostic provisoire des ovins et des caprins qui visitent la clinique vétérinaire de Tamale pour traitement, ayant des antécédents de diarrhée, d'anorexie et de peste des petits ruminants (PPR). Des échantillons de sécrétion fécale et lachrymale ont été prélevés en utilisant des écouvillons, et conservés à 4oC et transportés au laboratoire pour des analyses. L'analyse microbienne des microorganismes fastidieux et non fastidieux a été effectuée selon le manuel d'analyse bactériologique américain de la FDA. Des tests

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de sensibilité aux antibiotiques ont été effectués à l'aide de la méthode de diffusion sur disque. Les micro-organismes fastidieux isolés des moutons sont très résistants à l'oxacilline (100%), à la tétracycline (100%), à la ceftriaxone (90%) et à l'érythromycine (70%). La sensibilité à la suphamethoxazole /triméthoprime et au chloramphénicol était respectivement de 60 % et de 50 %. Des résistances intermédiaires aussi élevées que 40% ont été observées pour l'ampicilline et la ciprofloxacine. Les microorganismes non fastidieux d'origine ovine présentaient également une forte résistance à l'oxacilline (100%), à l'érythromycine (90%), à la tétracycline (90%) et à la suphamethoxazole /triméthoprime (70%), mais étaient sensibles à la ciprofloxacine (70%), à la ceftriaxone (60%)) et à la gentamicine (60%). En ce qui concerne les chèvres, une proportion plus élevée de micro-organismes fastidieux d'origine fécale étaient résistante à l'oxacilline (100%), à la tétracycline (100%), à la gentamicine (80%), à l'érythromycine (80%) et au chloramphénicol (60%), tandis qu'un niveau de sensibilité plus élevée a été observé pour la suphamethoxxéthxine azole/triméthoprime (80 %). Une proportion plus élevée de microorganismes non-fastidieux isolés des excréments de chèvres étaient également résistants au chloramphénicol (100%), à l'oxacilline (100%), à la tétracycline (100%) et à la suphamethoxazole/triméthoprime (80%). La sensibilité était de 100 % pour le chloramphénicol et la gentamicine. Les micro-organismes isolés des sécrétions lachrymales de chèvres et de moutons sont très résistants à l'oxacilline (100%), à l'érythromycine (100%), à la tétracycline (100%) et à la ciprofloxacine (75%), mais sensibles à la gentamicine (75%). Sur la base de ces résultats, aucun antibiotique ne pourrait traiter efficacement tous les microorganismes examinés dans cette étude. La ciprofloxacine, la ceftriaxone, le chloramphénicol, la gentamicine et la suphamethoxazole/triméthoprime pourraient être efficaces selon la source d'isolement.

**Mots-clés :** antibiotiques, diagnostic, petits ruminants, établissements vétérinaires

## Introduction

Ruminants suffer from a number of conditions and diseases including diarrhoea, anorexia and peste des petits ruminants. Diarrhoea (scours) is an increased frequency, fluidity, or volume of fecal excretion caused by microorganisms (Navaneethan *et al*., 2008). Diarrhoea often lasts for a few days and can result in dehydration due to fluid loss (World Health Organization, 2017). This can progress to decreased urination, loss of skin colour, a fast heart rate, and a decrease in responsiveness as it becomes more severe (World Health Organization, 2017). Animals suffering from scours have severe colitis characterized by abdominal pain, pasty faces, severe enteritis which may culminate into death due to severe dehydration (Henderson, 2011; Gould and Grooms, 2014).

Anorexia is a condition where animals refuse to feed or reduce feed intake drastically. This can cause reduction in weight, loss of meat and reduced profit to the farmer. It is caused by infectious and non-infectious agents. Peste des petits ruminants (PPR) is a highly contagious animal disease mostly affecting sheep and goats (Munir, 2014). It is a viral disease that can infect up to 90% of an animal herd with a mortality rate of 30-70% (Food and Agriculture Organization, 2020). It is characterized by oral necrosis, mucopurulent nasal and ocular discharges, cough, pneumonia, diarrhoea and death in some cases (Taylor, 2015).

Antibiotics are used to manage diarrhoea conditions caused by microorganisms. Similarly, antibiotics can be used to control secondary infections as a result of peste des petits ruminants. Treating ruminants suffering from diarrhoea and peste des petits ruminants could help boost their appetite and reduce anorexia. With the increase in population and demand for increased production of small ruminants, the use of antibiotics has become crucial in order to prevent and to treat diseases to promote growth (Ezenduka *et al*., 2014). Also, livestock farmers are conscious of adapting and using improved management practices to increase production. Despite improvements in management practices and disease prevention and treatment strategies, diarrhoea is still the most common condition affecting small ruminants (Faerber, 2004).

The indiscriminate usage of antibiotics in animals for the treatment of various microbial infections has led to the emergence

of antibiotic resistant strains of bacteria (Summers, 2002; Adzitey, 2015). Antibiotic resistance is a global problem, but demand for antibiotics continues to rise. Animal health delivery services is a challenge in communities where veterinary facilities and personnel are inadequate or lacking. In Tamale Veterinary Clinic, animals with infections are given broad spectrum antibiotics. However, a specific antibiotic for controlling or managing animals with diarrhoea, anorexia, peste des petits ruminants and other infections has not been identified Therefore, this work was carried out to determine the antibiotics that could be used to achieve this. The specific objective was to determine the antibiotic resistance of fastidious and non-fastidious microorganisms of fecal and lachrymal secretion origin from sheep and goats visiting the Tamale Veterinary Clinic with diarrhoea, anorexia, and peste des petits ruminants.

## Materials and methods

*Study area*

The study was conducted at Tamale Metropolis Veterinary Clinic in the Northern Region of Ghana and microbiological/antibiotic analyses were carried out at the Spanish Laboratory of the University for Development Studies, Nyankpala Campus, Ghana.

*Sampling*

The samples examined were faecal (sheep n=20; goat n= 10) and lachrymal secretions (sheep n=2, goat n= 2) from small ruminants presented to the Tamale Metropolitan Veterinary Clinic with a history of diarrhoea, anorexia and peste des petits ruminants.

*Antibiotic resistance test*

A slightly modified procedure of the disk diffusion method of Bauer *et al.* (1996) was used to determine the antibiotic resistance of fastidious and non-fastidious bacteria from faecal and lachrymal secretions of goats and sheep against the following 9 antibiotics: 30µg tetracycline (Te), 30µg chloramphenicol (C), 5µg ciprofloxacin (Cip), 10µg gentamicin (Cn), 30µg ceftriaxone (Cro), 22µg Sulphamethoxazole (Sxt), 10µg ampicillin (Amp), 15µg erythromycin (E), and 1µg oxacillin (Ox).

For the antibiotic resistance tests for fastidious microorganisms, the swabs were dipped in 10ml sterilized Trypticase Soy Broth and spread plated on Müller Hinton Agar. Four or five antibiotic discs were placed on the Müller Hinton Agar at a distance to prevent overlapping of the inhibition zones. The Müller Hinton Agar plates were incubated at 37°C for 24 h. Plate 1 shows trypticase soya broths with swabs of faecal samples while plate 2 shows Müller Hinton Agar with antibiotic disks.

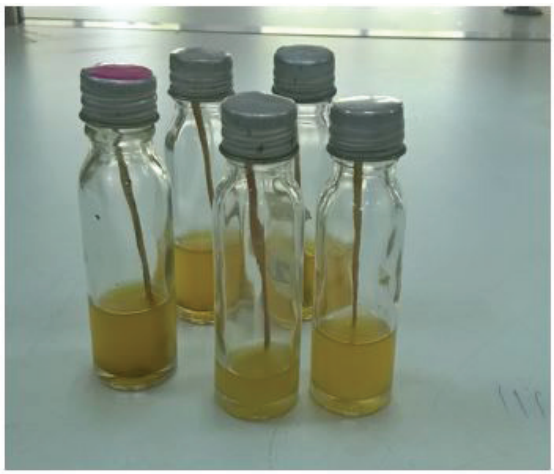


**Plate 1:** Trypticase Soy Broths with swabs of faecal samples

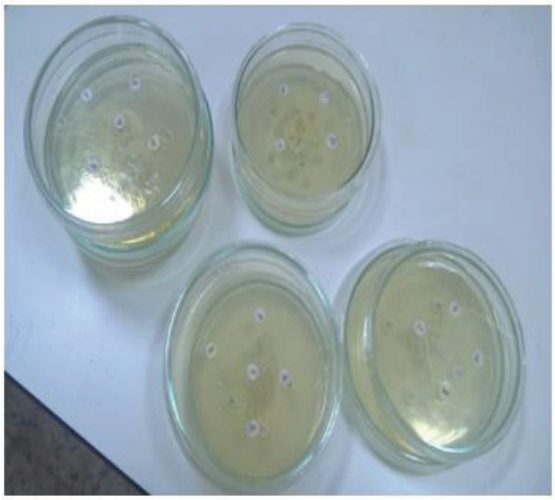


**Plate 2:** Müller Hinton Agar with antibiotic disks

For the antibiotic resistance tests for the non-fastidious microorganisms, the swabs were dipped in sterile 10ml Trypticase Soy Broth and incubated at 37°C for 18 h. After this, the medium was adjusted to 0.5 McFarland turbidity using sterile Trypticase Soy Broth and spread plated on Müller Hinton Agar. The Müller Hinton Agar plates were incubated at 37°C for 24 h.

After incubation of the Müller Hinton Agar plates (for both fastidious and non-fastidious microorganisms), the inhibition zones were measured and the results interpreted as susceptible, intermediate resistant or resistant according to the Clinical Laboratory Standard Institute (2008). The multiple antibiotic resistance (MAR) index was calculated and interpreted according to Krumperman (1983) using the formula: a/b, where 'a' represents the number of antibiotics to which a particular isolate was resistant and 'b' the total number of antibiotics tested. All media and antibiotic discs used were purchased from Oxoid Limited, Basingstoke, UK.

## Results

*Resistance of non-fastidious microorganisms isolated from faeces of sheep*

The antibiotic resistance of the non-fastidious microorganisms isolated from the faeces of sheep is shown in Table 1. In general, 61.1% of the non-fastidious microorganisms showed resistance. Furthermore, a high proportion of the non-fastidious microorganisms were resistant to oxacillin 10 (100%), erythromycin 9 (90%), tetracycline 9 (90%) and sulphamethoxazole 7 (70%). Also, the non-fastidious microorganisms exhibited a low level of intermediate resistance to some of the antibiotics examined. High susceptibility greater than 50% occurred for ciprofloxacin 7 (70%), ceftriaxone 6 (60%) and gentamicin 6 (60%).

*Resistance of fastidious microorganisms isolated from the faeces of sheep*

In general, 61.1% of the fastidious microorganisms showed resistance (Table 2). A high proportion of the fastidious microorganisms were resistant to oxacillin 10 (100%), erythromycin 7 (70%), ceftriaxone 9 (90%) and tetracycline 10 (100%). Intermediate resistance was 2 (20%) and 1 (10%) of the microorganisms for erythromycin and gentamicin, respectively. A moderate susceptibility was shown by sulphamethoxazole 6 (60%) and chloramphenicol 5 (50%).

*Resistance of non-fastidious microorganism isolated from the faeces of goats*

Table 3 shows the antibiotic resistance of non-fastidious microorganisms isolated from the faeces of goats. The overall resistant, intermediate resistant, and susceptible were 60% (27/45), 4.44% (2/45) and 35.6% (16/45), respectively. A higher proportion of the non-fastidious microorganisms were resistant to chloramphenicol 5 (100%), oxacillin 5 (100%) and tetracycline 5 (100%).

*Resistance of fastidious microorganisms isolated from the faeces of goats*

Overall, 55.6% (25/45) of the fastidious microorganism isolated from the faeces of the goats in this study were resistant, 20% (9/45) were of intermediate resistance and 24.4% (11/45) were susceptible. A higher proportion of the fastidious microorganisms were resistant to oxacillin 5 (100%), tetracycline 5 (100%), gentamicin 4 (80%), erythromycin 4 (80%) and chloramphenicol 3 (60%) (Figure 1). A greater level of susceptibility of the isolates to sulphamethoxazole 4 (80%) was observed. The rests (chloramphenicol, ciprofloxacin and ceftriaxone) were below 50%. The fastidious microorganisms isolated from the faeces of the goats showed intermediate resistances to ciprofloxacin 3 (60%) and ampicillin 3 (60%).

*Resistance of fastidious and non-fastidious microorganisms isolated from the lachrymal secretions of sheep and goats*

The overall resistance, intermediate resistance and susceptibility of fastidious and non-fastidious microorganisms isolated from the lachrymal secretions of sheep and goats were 66.7%, 25%, and 8.3%, respectively.

**Table 1:** Antibiotic resistance of non-fastidious microorganisms isolated from the faeces of sheep

| Antimicrobial | Non-fastidious microorganism | | | |
|---|---|---|---|---|
| | *n/10 | R% | I% | S% |
| Ampicillin (Amp) 3µg | 6 | 60 | 0 | 40 |
| Chloramphenicol (C) 30µg | 6 | 60 | 0 | 40 |
| Ciprofloxacin (Cip) 5µg | 2 | 20 | 10 | 70 |
| Ceftriaxone (Cro) 30µg | 3 | 30 | 10 | 60 |
| Gentamicin (Cn)10µg | 3 | 30 | 10 | 60 |
| Erythromycin (E) 15µg | 9 | 90 | 10 | 0 |
| Oxacillin (Ox) 1µg | 10 | 100 | 0 | 0 |
| Sulphamethoxazole (Sxt) 22µg | 7 | 70 | 0 | 30 |
| Tetracycline (Te) 30µg | 9 | 90 | 0 | 10 |

**n-number of resistant isolates, R-resistant, I-intermediate resistant, S-susceptible*

**Table 2:** Antibiotic resistance of fastidious microorganisms isolated from the faeces of sheep

| Antimicrobial | Fastidious microorganism | | | |
|---|---|---|---|---|
| | *n/10 | R% | I% | S% |
| Ampicillin (Amp) 30µg | 4 | 40 | 40 | 20 |
| Chloramphenicol (C) 30µg | 4 | 40 | 10 | 50 |
| Ciprofloxacin (Cip) 5µg | 3 | 30 | 40 | 30 |
| Ceftriaxone (Cro) 30µg | 9 | 90 | 0 | 10 |
| Gentamicin (Cn) 10µg | 5 | 50 | 10 | 40 |
| Erythromycin (E) 15µg | 7 | 70 | 20 | 10 |
| Oxacillin (Ox) 1µg | 10 | 100 | 0 | 0 |
| Sulphamethoxazole (Sxt) 22µg | 4 | 40 | 0 | 60 |
| Tetracycline (Te) 30µg | 10 | 100 | 0 | 0 |

**n-number of resistant isolates, R-resistant, I-intermediate resistant, S-susceptible*

**Table 3:** Antibiotic resistance of fastidious microorganisms isolated from the faeces of goats

| Antimicrobial | Fastidious microorganism | | | |
|---|---|---|---|---|
| | *n/5 | R% | I% | S% |
| Ampicillin (Amp) 30µg | 2 | 40 | 60 | 0 |
| Chloramphenicol (C) 30µg | 3 | 60 | 0 | 40 |
| Ciprofloxacin (Cip) 5µg | 1 | 20 | 60 | 20 |
| Ceftriaxone (Cro) 30µg | 1 | 60 | 20 | 20 |
| Gentamicin (Cn) 10µg | 4 | 80 | 20 | 0 |
| Erythromycin (E) 15µg | 4 | 80 | 20 | 0 |
| Oxacillin (Ox) 1µg | 5 | 100 | 0 | 0 |
| Sulphamethoxazole (Sxt) 22µg | 4 | 20 | 0 | 80 |
| Tetracycline (Te) 30µg | 5 | 100 | 0 | 0 |

**n-number of resistant isolates, R-resistant, I-intermediate resistant, S-susceptible*

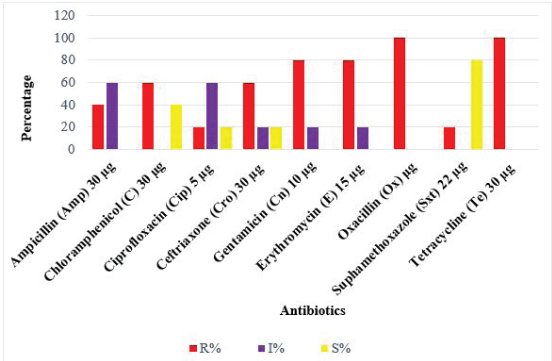
**Table 4:** Antimicrobial resistance profile and MAR index of fastidious and non-fastidious microorganisms

| Serial No. | Source | Isolates | Antibiotic resistant profile | No. of antibiotics |
|---|---|---|---|---|
| 1 | faecal | S1 | EOx | 2 |
| 2 | faecal | S2 | TeCCnSxtAmpEOx | 7 |
| 3 | faecal | S3 | TeCroSxtAmpOx | 5 |
| 4 | faecal | S4 | TeCCipCnCroSxtAmpEOx | 9 |
| 5 | faecal | S5 | TeCSxtAmpEOx | 6 |
| 6 | faecal | S6 | TeCSxtEOx | 5 |
| 7 | faecal | S7 | TeCCipCroSxtAmpEOx | 8 |
| 8 | faecal | S8 | TeCCipCroSxtAmpEOx | 8 |
| 9 | faecal | S9 | TeEOx | 3 |
| 10 | faecal | S10 | TeCSxtAmpOx | 5 |
| 11 | faecal | S1a | TeCnCroAmpOx | 5 |
| 12 | faecal | S2a | TeCCipCnCroSxtAmpEOx | 9 |
| 13 | faecal | S3a | TeCCipCroEOx | 6 |
| 14 | faecal | S4a | TeCCipCnCroOx | 6 |
| 15 | faecal | S5a | TeCroSxtOx | 4 |
| 16 | faecal | S6a | TeCroSxtAmpEOx | 6 |
| 17 | faecal | S7a | TeCroEOx | 4 |
| 18 | faecal | S8a | TeEOx | 3 |
| 19 | faecal | S9a | TeCnCroEOx | 5 |
| 20 | faecal | S10a | TeCCipCnCroSxtAmpEOx | 9 |
| 21 | faecal | G1 | TeCSxtAmpEOx | 6 |
| 22 | faecal | G2 | TeCSxtAmpEOx | 6 |
| 23 | faecal | G3 | TeCSxtAmpEOx | 6 |
| 24 | faecal | G4 | TeCCipox | 4 |
| 25 | faecal | G5 | TeCCipSxtOx | 5 |
| 26 | faecal | G1a | TeCSxtAmpEOx | 6 |
| 27 | faecal | G2a | TeCCroSxtOx | 5 |
| 28 | faecal | G3a | TeCSxtAmpEOx | 6 |
| 29 | faecal | G4a | TeCipEOx | 4 |
| 30 | faecal | G5a | TeSxtEOx | 4 |
| 31 | lachrymal secretions | SE1 | TeCCipSxtAmpEOx | 7 |
| 32 | lachrymal secretions | SE1a | TeCipCroEOx | 5 |
| 33 | lachrymal secretions | GE1 | TeCCipCnCroSxtAmpEOx | 9 |
| 34 | lachrymal secretions | GE1a | TeEOx | 3 |

The microorganisms were highly resistant to oxacillin 4 (100%), erythromycin 4 (100%), tetracycline 4 (100%) and ciprofloxacin 3 (75%), with moderate resistance to chloramphenicol 2 (50%) and ampicillin 2 (50%). The microorganisms exhibited average intermediate resistances to ampicillin 2 (50%), chloramphenicol 2 (50%) and ciprofloxacin 1 (25%). However, they were susceptible to gentamicin 3 (75%).

*The antimicrobial resistance profiles*

The antimicrobial resistance profile and multiple antibiotic resistance (MAR) index of microorganisms (fastidious and non-fastidious) are shown in Table 4. Four of the microorganisms (S4, S2a, S10a, and GE1) were resistant to all the antibiotics. Two isolates each were also resistant to 8 (S7 and S8) and 7 (S2 and SE1) antibiotics.




*R-resistant, I-intermediate resistant, S-susceptible*

**Figure 1:** Resistance of fastidious microorganisms isolated from the faeces of goats

## Discussion

Antibiotics play an important role in the reduction of infections associated with microorganisms in small ruminants. However the use of antibiotics for treatment and therapeutic purposes has been the major cause of the emergence and spread of drug-resistant microorganisms (Summers, 2002; Adzitey *et al*., 2012). The development and spread of antibiotic resistant microorganisms have become of great concern to the public (Gilbert and MacBain, 2003).

The non-fastidious microorganisms (Table 1) exhibited resistances of ≥60% to ampicillin, chloramphenicol, erythromycin, sulphamethoxazole, tetracycline and oxacillin. Also, the fastidious microorganisms from the faecal samples showed resistance to most of the antibiotics with a resistance of 100% to oxacillin and tetracycline, followed by ceftriaxone and erythromycin (Table 2). The antibiotic resistance results observed for the non-fastidious and fastidious microorganisms suggested that, these antibiotics cannot be the best choice to be used in treating sheep with diarrhoea or to manage secondary infections associated with peste des petits ruminants in sheep in this study. Several studies have shown that, there is variation of resistance levels in microorganisms (Van der Meer and Gyssens, 2001; Gilbert and MacBain, 2003; Byarugaba *et al*., 2011; Adzitey *et al*, 2015; Raja and Adzitey, 2017; Adzitey *et al*., 2018). According to Van der Meer and Gyssens (2001), the development of resistance to antibiotics by microorganisms could be due to the fact that the antibiotics have been inappropriately used. In the United States, there have been historical changes in antimicrobial drug resistance in microorganisms isolated from food animals of which sheep are part and a significant upward trend in resistance was observed for ampicillin, sulphonamide and tetracycline (Tadesse *et al*., 2012). Intermediate resistances between 10 to 40% were observed for the non-fastidious and fastidious microorganisms of sheep faecal origin. Intermediate resistant microorganisms have the tendency of becoming resistant and are difficult to treat when they are involved in an infection (Adzitey *et al*., 2018). Susceptibility of ≥60% was recorded for gentamicin, ceftriaxone and ciprofloxacin for non-fastidious microorganisms and sulphamethoxazole for fastidious microorganisms from sheep. This suggests that, these antibiotics can be used to manage diarrhoea and secondary infections resulting from peste des petits ruminants in sheep in the Tamale Metropolis. Susceptibility occurring in these antibiotics could be linked to the fact that, they are not commonly used and/or have not been abused in their usage.

According to Byarugaba *et al.* (2011), in Tanzania, antibiotics that are not commonly used in the animal industry have less resistance.

The non-fastidious microorganisms isolated from faeces of goats in this study demonstrated resistance of ≥60% to tetracycline, oxacillin, chloramphenicol, sulphamethoxazole, erythromycin and ampicillin. Similarly, the fastidious microorganisms of goat origin exhibited resistance of ≥60% to tetracycline, oxacillin, erythromycin, gentamicin, ceftriaxone, and chloramphenicol. These antibiotics are therefore not recommended for the treatment of goats experiencing diarrhea and secondary infections resulting from peste des petits ruminants in the Tamale Metropolis. A study by Katakweba *et al.* (2012) found macrolides and tetracycline to be commonly used and stored in livestock keeper's stores due to their low cost and ready availability thus contributing to their resistance by microorganisms. Adjiri-Awere and Van Lunen (2005) indicated that, the therapeutic use of antibiotics has made microorganisms to undergo genetic change, antibiotic selection, and spread of antibiotic resistance. Ciprofloxacin, ceftriaxone and gentamicin were the antibiotics to which the non-fastidious microorganisms of goat origin were susceptible (≥60%). The fastidious microorganisms of goat origin were susceptible (80%) to suphamethoxazole. These results implied that these antibiotics could be used for the treatment of diarrhoea in goats caused by microorganisms. They can also be used to manage secondary infections in goats suffering from peste des petits ruminants. Similarly, Adzitey *et al.* (2019) reported high susceptibility of non-fastidious bacteria of pig origin to gentamicin (100%). They also found that fastidious bacteria of pig origin were susceptible to gentamicin (70%) and suphamethoxazole/trimethoprim (70%).

The non-fastidious and fastidious microorganisms from the lachrymal secretions of sheep and goats were mostly resistant (67%), as compared to intermediate resistance (25%) and susceptibility (8%) observed in this study. Adzitey *et al.* (2019) found that fastidious bacteria isolated from pigs were mostly resistant (53.75%) to antibiotics, but the non-fastidious bacteria were mostly susceptible (60%).

The non-fastidious and fastidious microorganisms of sheep and goat origin were resistant to 2 to 9 different antibiotics (MAR index of 0.22 to 1). The resistance of microorganisms to two or more antibiotics has been reported by other researchers (Alhaji *et al.*, 2007; Lim *et al.*, 2009; Adzitey *et al.*, 2012; Adzitey *et al.*, 2015). An isolate is considered to have multidrug resistance if it is resistant to three (3) or more different classes of antibiotics (Foley and Lynne, 2008; Lim *et al.*, 2009). In this study 97.06% (33/34) exhibited multidrug resistance. The emergence of multidrug resistance decreases therapeutic options in cases of infections and is an increasingly important issue world-wide. Multidrug resistant microorganisms are seen to be more dangerous as compared to non-multidrug resistant microorganisms (Foley and Lynne, 2008). Multidrug resistance among the microorganisms observed in this study may present challenges to the management of diarrhoea and secondary infections associated with peste des petits ruminants.

## Conclusions

The fastidious and non-fastidious microorganisms from faecal and lachrymal secretions of sheep showed different antibiotic susceptibility and resistance patterns. Higher resistances occurred for oxacillin, tetracycline, erythromycin, ampicillin, sulphamethoxazole, chloramphenicol and ceftriaxone, while higher susceptibility occurred for gentamycin and ciprofloxacin. Therefore, gentamycin and ciprofloxacin are recommended for the treatment of diarrhoea and secondary infections in sheep. In goats, higher susceptibility occurred for gentamycin and ceftriaxone, however, higher resistance occurred for tetracycline, chloramphenicol, sulfamethoxazole, erythromycin, oxacillin and ampicillin. This implies that gentamycin and ceftriaxone will be good for the treatment of diarrhoea and secondary infections in goats. The

findings of this study provided an insight into the right antibiotics for use in the treatment of diarrhea and the management of secondary infections in small ruminants to improve their health in the Tamale Metropolis in Ghana.

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## Public brief

Indiscriminate usage of antibiotics in animals for the treatment of various microbial infections has led to emergence of resistant strains. In Tamale Veterinary Clinic, animals with infections are given broad spectrum antibiotics. The fastidious and non-fastidious microorganisms isolated from faecal and lachrymal secretions of sheep and goats that visited the clinic in this study showed different susceptibility and resistance patterns. No single antibiotic can destroy all the microorganisms that was observed in this study, however, ciprofloxacin, ceftriaxone, chloramphenicol, gentamicin and suphamethoxazole/ trimethoprim could be effective for treating/ managing ruminants suffering from diarrhoea and pet des petite ruminant (PPR) depending on the source of isolation. Findings of the study have provided an insight into the right antibiotics that can be used for treating diarrhea and managing secondary infections in small ruminant to improve their health.

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# EFFECTS OF SEASON, GRADED DIETARY PROTEIN AND ARTIFICIAL INSEMINATION ON SEMEN CHARACTERISTICS AND REPRODUCTIVE PERFORMANCE OF INDIGENOUS GUINEA FOWL (NUMIDAMELEAGRIS)

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## Abstract

The study was undertaken to assess the effects of graded dietary protein, season and artificial insemination on semen characteristics and reproductive performance of indigenous Guinea fowl in the middle belt of Ghana. A total of twenty-four (24) male Pearl Guinea fowls aged 12 weeks old were selected at random from a flock. Four groups of two (2) Guinea cocks were fed diets of 16% CP, 18% CP, 20% CP and 22% CP. Each group was replicated three times and reared under three seasons (Dry: December-March, Major Rains: April-July and Minor Rains: August-November) in a 3x4 factorial experiment. Semen was collected using the dorso-ventral massage method from birds randomly selected from each of the replicates every two weeks. Ejaculates were subjected to physical evaluation to ascertain their quality and quantity and the semen was pooled for the artificial insemination on Guinea hens. Data were analyzed using the General Linear Model procedure of SAS. Season had a significant effect ($p<0.05$) on semen volume, pH and sperm concentration. Varied dietary protein had a significant effect ($p<0.05$) on semen volume but not on pH, motility and sperm concentration. Sperm morphological abnormalities were affected ($p<0.05$) by both season and dietary protein. Fertility and hatchability were both higher ($p<0.05$) in artificially inseminated hens than natural mating. The results of this study indicated that graded dietary protein, seasons of semen collection and artificial insemination significantly influenced the semen quality and fertility and the hatchability of eggs.

**Keywords:** Guinea fowl, dietary protein, season, semen characteristics, artificial insemination

# EFFETS DE LA SAISON, DES PROTÉINES ALIMENTAIRES CLASSÉES ET DE L'INSÉMINATION ARTIFICIELLE SUR LES CARACTÉRISTIQUES DE SPERME ET LA PERFORMANCE REPRODUCTRICE DE PINTADES INDIGÈNES (NUMIDAMELEAGRIS)

## Résumé

L'étude a été réalisée afin d'évaluer les effets de protéines alimentaires classées, de la saison et de l'insémination artificielle sur les caractéristiques de sperme et la performance reproductrice des pintades indigènes dans la ceinture centrale du Ghana. Au total, vingt-quatre (24) pintadeaux mâles de race Pearl âgés de 12 semaines ont été choisies de manière aléatoire dans un troupeau. Quatre groupes de deux (2) coqs (pintades) ont été soumis à des régimes alimentaires de 16 % CP, 18 % CP, 20 % CP et 22 % CP. Chaque groupe a été répété trois fois et élevé sous trois saisons (Sèche : décembre-mars ; Fortes pluies : avril-juillet ; et Faibles pluies: août-novembre) dans une expérience factorielle 3 x 4. Le sperme a été recueilli à l'aide de la méthode de massage dorso-ventral chez les oiseaux choisis de manière aléatoire dans chacune des répétitions toutes les deux semaines. Les éjaculats ont été soumis à une évaluation physique pour vérifier leur qualité et quantité, et le sperme a été regroupé pour insémination artificielle sur les poules (femelles). Les données ont été analysées au moyen de la procédure de Modèle linéaire général SAS. La saison a eu un effet significatif ($p < 0,05$) sur le volume du sperme, le pH et la concentration du sperme. Des protéines

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alimentaires variées ont eu un effet significatif (p < 0,05) sur le volume du sperme mais non sur le pH, la motilité et la concentration du sperme. Les anomalies morphologiques des spermatozoïdes ont été affectées (p < 0,05) par la saison et les protéines alimentaires. La fertilité et l'éclosion étaient toutes les deux plus élevées (p < 0,05) chez les poules inséminées artificiellement par rapport aux accouplements naturels. Les résultats de cette étude indiquent que les protéines alimentaires classées, les saisons de collecte de sperme et l'insémination artificielle ont influencé de façon significative la qualité du sperme et la fertilité et la capacité d'éclosion des œufs.

**Mots-clés :** pintade, protéines alimentaires, saison, caractéristiques du sperme, insémination artificielle

## Introduction

The Guinea fowl is indigenous to Africa and the name Guinea fowl is derived from the Guinean coast of Africa where the birds are believed to have originated. Guinea fowl has great potential for producing animal protein for the ever-growing rural population in Africa. FAO (2014) reported that the three Northern Regions of Ghana alone produce not less than 2.0 million Guinea fowls annually and contribute7.10% to the national poultry population. The annual per capita consumption of Guinea fowl meat for Ghana in 1995 was 9.5kg which increased to 10.6 kg by 2005 with the total production reaching 2,574,996 in 2010 (FAO, 2013). Available data indicate that Guinea fowl eggs have low fertility and hatchability (Annor *et al*., 2013) attributed to poor management practices including feeding and housing, the monogamous nature of the male, seasonality of breeding, and other environmental factors such as temperature, rainfall, humidity and photoperiod (Konlan *et al*., 2011). The seasonality in reproduction has been recognized as one of the major constraints to commercial Guinea fowl production (Oke *et al*., 2003) as relative humidity, low rainfall and high temperature result in a reduction of semen production and quality. Inadequate dietary protein results in lower testosterone levels, inhibition of spermatogenesis, production of immature testes, and delayed puberty compared to cocks fed with adequate protein diet. Similarly, the negative effect of seasonal variation on semen quality of domestic fowl has been documented (McDaniel *et al*., 1996). Assisted Reproduction Technologies (ART's) such as Artificial Insemination (AI) contribute to increase poultry production, as they allow a wider use of genetically superior cockerels with a high reproductive performance (Vishwanath and Shannon, 1997). The fertility of Guinea fowl eggs ranges from 49 to 58% in naturally mated stock, while using artificial insemination results in egg fertility ranging from 70 to 88% (Galor, 1983; Ayorinde *et al*., 1989). The low fertility in naturally mated stock is also associated with the monogamous sexual behavior of the Guinea fowl in addition to the fertility constraints with the male (Annor*et al*.,2013). Currently, there is scanty information on the effects of protein nutrition and season on the semen characteristics of Indigenous Guinea fowl. The present study aimed at determining the effect of dietary protein, season and artificial insemination on semen characteristics and reproductive performance of Guinea fowl in the middle belt of Ghana.

## Materials and Methods

*Location and Duration of Study*

The study was conducted at the Poultry Unit of the Department of Animal Science Education, University of Education, Winneba, Asante Mampong campus between December 2016 and December 2017. Asante Mampong is located 60 km North-East of Kumasi on the Kumasi - Ejura road and lies between latitude 07 040" degrees North and longitude 01 24" degrees West with an altitude of 457.1 m above sea level. The climatic condition is the wet semi-equatorial type, with a bi-modal rainfall of 1224mm per annum and a temperature range of 22.3°C-30.6°C. The main seasons are April to July (Major Rain Season), August to November (Minor Rainy Season) and December to March (Dry Season).

*Animals, Treatment and Experimental design*

A total of fifty-six (56) Pearl Guinea fowls made up of twenty-four (24) male and thirty-two (32) females aged 12 weeks old were randomly selected from a flock at the Animal Science Research Farm of the University of Education Winneba, Mampong Campus for the study. Two (2) Guinea cocks per replicate were each subjected to 16% CP, 18% CP, 20% CP and 22% CP treatment groups. The 16 % CP level serve as the control treatment. Each group was replicated three times and reared under three seasons (the Dry season: December-March, Major Rains: April-July and Minor Rains: August-November) in a 3x4 factorial experiment. The thirty-two (32) females were randomly divided into two (2) groups (Group I: Natural mating/ control and Group II: Artificial Insemination) and each were fed on a 16 % CP diet. Groups I and II were each divided into four sub-groups of 4 birds each. The Group I sub-groups each received one male, selected at random for mating. The four (4) sub-groups in Group II were each artificially inseminated using semen pooled from the Guinea cocks of the four (4) dietary treatments. Each Guinea hen was inseminated with 0.03ml of raw semen pooled from the cocks. Insemination was performed immediately after collection every five days after 12.00 pm. Eggs were collected each day and were stored at room temperature for seven days for incubation. Candling was done on the 18th day to determine fertility rates and records on hatchability and the rates of hatch were taken.

*Management of experimental birds*

The birds were weighed at 12 weeks of age and were housed individually in three-tier wooden cages measuring 90x40x60 cm. The birds were fed with 100g per bird of treatment diets and water was served daily ad libitum under the intensive management system.

*Data collection*

Semen was collected starting at 7:00 AM using the dorso-ventral massage method described by Burrows & Quinn (1937) from birds randomly selected from each of the replicates. Semen analysis was done immediately after collection at the Science Laboratory of the College of Agriculture of the University of Education, Winneba, Mampong. Semen volumes were measured by using calibrated micro pipette and sperm concentrations were examined using a Neubauer haemocytometer. The percent live and abnormal spermatozoa were counted after preparing smears and staining them with eosin and nigrosin according to the methods described by Lake and Stewart (1978). The sperm motility was assessed by examination of a drop of semen (5µl) under the microscope at 10X magnification as described by Hutt (2003) using an Olympus BX43-Standard Laboratory Microscope. The semen pH was measured by using a pH meter. The sperm morphology was determined as the measurement of the shape of sperm cells and was reported as the percentage of normal sperm cells and abnormal sperm cells. Maximal leucocyte and erythrocyte concentrations were determined as the number of white blood cells and red blood cells per milliliter of semen sample ($1\times 10^6$cells/ml).

*Data analysis*

Data collected were analyzed using the General Linear Model (GLM) procedure of Statistical Analysis System (SAS for Windows, version 7). The means were separated by using the probability of difference (PDIFF) procedure of SAS at 5% probability level.

**Table 1:** Weather Records during the Study Period for the Mampong-Ashanti Municipality

| Variables | Dry season (December-March) | Major Rainy Season (April-July) | Minor Rainy Season (August-November) |
|---|---|---|---|
| Temperature (°C) | 32.0 | 30.25 | 27.0 |
| Rainfall (mm) | 27.28 | 130.0 | 125.05 |
| Humidity (%) | 65.5 | 81.25 | 84.5 |
| Cloud cover (%) | 37.50 | 60.00 | 65.25 |
| Sun Hours (hr.) | 103.10 | 89.30 | 62.45 |

*Source: World weather online (2017)*

**Table 2:** Ingredients and Proximate Composition (%) of experimental diets

| Variables | Diet 1 (16% CP) | Diet 2 (18% CP) | Diet 3 (20% CP) | Diet 4 (22% CP) |
|---|---|---|---|---|
| **Ingredients** | | | | |
| Maize | 61 | 60 | 58 | 55.5 |
| Fish meal (Russia) | 2.5 | 5 | 6 | 9.5 |
| Fish meal (Tuna) | 7 | 8 | 10 | 11.0 |
| Soya bean | 7 | 8.5 | 10 | 10.0 |
| Wheat bran | 18.5 | 14.5 | 12.5 | 10.0 |
| Oyster shell | 2.5 | 2.5 | 2 | 2.0 |
| Dicalcium phosphate | 0.5 | 0.5 | 0.5 | 0.5 |
| Vitamin Premix | 0.5 | 0.5 | 0.5 | 0.5 |
| Salt | 0.5 | 0.5 | 0.5 | 0.5 |
| Total | 100kg | 100kg | 100kg | 100kg |
| **Proximate Composition %** | | | | |
| Moisture content | 10.5 | 10.5 | 10.0 | 11.0 |
| Protein content | 15.76 | 17.78 | 19.75 | 21.49 |
| Ether extract | 4.0 | 5.5 | 4.0 | 5.0 |
| Ash content | 11.0 | 8.5 | 10.0 | 9.5 |
| Crude fibre | 5.42 | 4.91 | 4.37 | 4.28 |
| ME, kcal/kg | 2,750 | 2,750 | 2,750 | 2,750 |

## Results

The dietary crude protein concentration showed no significant ($p<0.05$) effect on semen pH, motility, sperm concentration and clumps (Table 3). The dietary protein concentration influenced the volume of semen produced and the difference was statistically significant ($p<0.05$). The highest semen volume was recorded in 16% and 20% crude protein levels whilst the lowest volume was recorded in the 18% crude protein diet. Season had significant ($p<0.05$) effect on semen volume, pH and sperm concentration, but not sperm motility and clumps ($p>0.05$). The cocks produced the highest semen volume of 0.03ml during the minor rainy season, followed by the major rainy season. The lowest semen volume of 0.01ml was recorded by the cocks during the dry season. Season significantly ($p<0.05$) increased semen pH in the dry season. The highest pH of 7.40 was observed in cocks reared during the dry season. The values for the major rainy season and those of the minor rainy season

were similar. The spermatozoa concentration was highest ($p<0.05$) in the minor rainy season. This was followed by the hot dry season while the major rainy season recorded the lowest. The dietary protein concentration and season interaction was not significant ($p>0.05$) in all the physical semen characteristics.

*Effect of graded dietary protein concentrations and season on semen morphological characteristics*

The dietary protein concentration had a significant ($p<0.05$) effect on sperm maturation (Table 4). No significant ($p>0.05$) effects were discovered in sperm abnormality, head and tail malformation with increasing dietary protein concentrations. Abnormal spermatozoa percentages were noticed to be highest ($p<0.05$) in the dry season and this was followed by the minor rainy season. The percentage abnormal spermatozoa tended to decrease ($p<0.05$) in the major rainy season. A significant ($p<0.05$) positive relation was observed between the number of immature sperm cells and season of collection. Significantly ($p<0.05$) higher immature sperm cells were identified in the dry season follow by the minor rainy season. The cocks produced significantly ($p<0.05$) higher mature sperm cells in the major rainy season but the season of ejaculation did not significantly ($p>0.05$) affect the morphology (big-head and double-tail) of sperm cells. There were significant ($p<0.05$) interaction effects between dietary protein concentration and season of rearing on sperms maturation.

*Effect of graded dietary protein concentrations and season on semen round cell differential characteristics*

Dietary protein concentration had a significant ($p<0.05$) effect on epithelial cells concentration in semen (Table 5). No significant ($p>0.05$) effect was observed in the red blood cells and white blood cells as a result of increasing the dietary crude protein concentration. The epithelial cells' concentrations were highest in birds fed 20% diet and reduced epithelial cells concentration was observed in birds fed 18% and 22% diets. The season resulted in statistically significant ($p<0.05$) effects on the red blood cells concentration in semen and this was highest in the dry season and lower in the major and minor rainy seasons. There was no significant effect ($p>0.05$) observed in epithelial cells and white blood cells across the seasons. Season × dietary protein concentration was not significant ($p>0.05$) in the round cell differential characteristics.

*Effect of artificial insemination on reproductive performance*

Artificial insemination (GII) generally increased ($p<0.05$) fertility. Naturally mated hens (GI) produced eggs with a fertility rate of 61.88% whilst hens which were artificially inseminated produced eggs with a higher fertility rate of 81.33%. The hatchability of total eggs set was higher ($p<0.01$) in artificially inseminated hens than in hens that were naturally mated. Artificial insemination produced higher ($p<0.05$) hatchability of fertile eggs than the control group. The hatchability of fertile eggs was 79.08% in artificially inseminated hens whilst natural mating resulted in 54.79%.

## Discussion

The highest semen volume (0.03ml) obtained with 16% and 20% crude protein levels was similar to the 0.03 ml obtained in Exotic Golden Sovereign Guinea fowl but lower than 0.05ml (Mohan *et al*., 2016). Zhang *et al*. (1999) also showed that the spermatozoa volume was unaffected by different crude protein levels in the diet before sexual maturity and this was attributed to no change in testicular weight. However, Hocking (1990) reported that when naturally mated broiler breeder males were fed a high (16.1%) or low (11.3%) protein diet, a suboptimum spermatozoa volume was observed. It has been demonstrated that there is no significant effect of diet on testosterone concentration which is the hormone responsible for semen production (Elmaz*et al*., 2007). However, there is evidence that the reproductive axis does not seem to be intricately linked with dietary intakes of amino

**Table 3:** Effect of graded dietary protein concentration and season on semen physical and chemical characteristics

| Variables | Volume (ml) | pH | Motility (%) | Sperm concentration X10⁶/ml | Clumps (%) |
|---|---|---|---|---|---|
| **Dietary protein** | | | | | |
| 16% | 0.03[a] | 7.32 | 73.00 | 62.67 | 2.55 |
| 18% | 0.01[c] | 7.30 | 71.94 | 54.90 | 1.89 |
| 20% | 0.03[a] | 7.27 | 76.11 | 63.57 | 2.33 |
| 22% | 0.02[b] | 7.22 | 77.22 | 66.04 | 2.11 |
| SEM | 0.01 | 0.05 | 4.97 | 8.01 | 0.34 |
| P-value | 0.02 | 0.59 | 0.85 | 0.78 | 0.56 |
| **Season** | | | | | |
| Dry Season | 0.01[c] | 7.40[a] | 72.50 | 53.66[b] | 2.25 |
| Major Rainy Season | 0.02[b] | 7.20[b] | 78.75 | 44.05[c] | 2.08 |
| Minor Rainy Season | 0.03[a] | 7.22[b] | 72.45 | 87.67[a] | 2.33 |
| SEM | 0.01 | 0.05 | 4.31 | 6.94 | 0.30 |
| P-value | 0.01 | 0.05 | 0.50 | 0.01 | 0.83 |
| Protein x season | Ns | Ns | Ns | Ns | Ns |

**Table 4:** Effect of graded dietary protein concentrations and season on semen morphological characteristics

| Variables | Abnormal (%) | BHC (%) | DTC (%) | Immature (%) |
|---|---|---|---|---|
| **Dietary protein** | | | | |
| 16% | 15.00 | 4.44 | 4.56 | 6.11 c |
| 18% | 20.55 | 5.11 | 6.77 | 8.66 b |
| 20% | 22.22 | 5.77 | 5.33 | 11.22 a |
| 22% | 16.56 | 4.11 | 4.11 | 8.44 b |
| SEM | 2.41 | 0.80 | 0.98 | 1.18 |
| P-value | 0.15 | 0.40 | 0.25 | 0.04 |
| **Season** | | | | |
| Dry Season | 21.50[a] | 4.58 | 6.00 | 11.00[a] |
| Major Rainy Season | 14.67[c] | 4.58 | 4.08 | 6.16[c] |
| Minor Rainy Season | 19.58[b] | 5.4 | 5.50 | 8.67[b] |
| SEM | 2.09 | 0.69 | 0.85 | 1.02 |
| P-value | 0.05 | 0.62 | 0.27 | 0.01 |
| Protein x season | Ns | Ns | Ns | S |

*Means bearing different superscripts in the same column are significantly different (P<0.05)*
*AC= Abnormal cells; BGC= Big head cells; DTC= Double tail cells*

**Table 5:** Effects of different dietary protein concentrations and season on semen cell differential characteristics

| Variables | EC (%) | RBC (%) | WBC (%) |
|---|---|---|---|
| **Dietary protein level** | | | |
| 16% CP | 1.66[b] | 1.11 | 3.77 |
| 18% CP | 1.33[c] | 2.17 | 4.00 |
| 20%CP | 2.00[a] | 1.56 | 8.83 |
| 22% CP | 1.44[c] | 1.22 | 5.20 |
| SEM | 0.14 | 0.32 | 0.25 |
| P-value | 0.01 | 0.11 | 0.42 |
| **Season** | | | |
| Dry Season | 1.75 | 2.13[a] | 3.37 |
| Major Rainy Season | 1.58 | 1.25[b] | 4.70 |
| Minor Rainy Season | 1.50 | 1.17b | 8.27 |
| SEM | 0.12 | 0.27 | 2.04 |
| P-value | 0.34 | 0.05 | 0.23 |
| Protein x season | Ns | Ns | Ns |

*Means bearing different superscripts in the same column are significantly different (P<0.05)*
*EC= Epithelial cells; RBC= Red blood cells; WBC= White blood cells*

**Table 6:** Effect of artificial insemination on reproductive performance

| Variables | Fertility (%) | HOES (%) | HOFE (%) |
|---|---|---|---|
| Natural Mating(GI, Control) | 61.88[b] | 34.33[b] | 54.79[b] |
| Artificial Insemination(GII) | 81.33[a] | 64.67[a] | 79.08[a] |
| SEM | 1.64 | 4.43 | 5.27 |
| P-value | 0.01 | 0.01 | 0.05 |

*Means bearing different superscripts in the same column are significantly different (P<0.05)*
*HOES= Hatchability on eggs set; HOFE= Hatchability on fertile eggs*

acids (Kheradmand *et al*., 2006) and may not influence semen volume. The study of Zhou *et al*. (2015) indicated that a semen pH of 7.2 maximized the mean quality of spermatozoa whereas a pH of 6.2 reduced semen quality significantly. The average semen pH of7.2 recorded in this study falls within the range reported by Ting *et al*. (2015). However, the percentage sperm motility increased in alkaline pH in turkey and in quails (Holm *et al*., 1988). This study suggests that a dietary protein of 16% was sufficient to support the optimum pH for successful sperm motility and fertilization. Increasing the dietary protein did not show any significant increase in sperm concentration.The results of this study are supported by Zhang (1999) who similarly reported no increase in spermatozoa concentration in male broiler breeders fed varied protein diets.

Shanmugam *et al*. (2016) concluded that reduced dietary crude protein level had no effect on semen morphological characteristics and suggested that lower crude protein diets containing required amino acid such as lysine, methionine and threonine are sufficient for quality semen morphological output. It may be deduced that the dietary protein level can be reduced without affecting the morphology of sperm cells of the breeder males (Zhang *et al*., 1999).

This study showed that increased dietary protein levels increased the maturity

of spermatids to spermatozoa. The highest maturity obtained in birds fed 20% diet in this experiment may mean that proteins in the form of amino acids are syntheses of epididymal epithelium and lack of protein may be detrimental to its availability and may affect the maturation and viability of sperm (Cheah and Yang, 2011).

The mean WBC number was 5.45% and was lower than 10%-20% which has been identified as a major cause of infertility in males and it has been reported that WBC in semen has the potential to damage sperm function and ovum penetration and has been associated with decreased sperm numbers, reduced sperm velocity and impaired sperm fertility (Close *et al*., 1990). Johanisson *et al*. (2000) reported that quality semen may have only leucocytes and epithelial cells present when evaluated. The mean RBC of 1.51 was higher than the normal reference values of none reported by Johanisson *et al*. (2000).

The lowest volume of semen during the dry season in this study was supported by an earlier study by McDaniel *et al*. (1996) which reported that an ambient temperature above 31°C depressed the semen volume, sperm motility, viability and fertilization potential of roosters. Even the environmental temperature at ejaculation has an important effect on exogenous physiological factors influencing avian sperm motility (Ashizawa and Sano, 1990). The optimum temperature stimulates testicular growth and promotes increased semen volume whereas a high temperature suppresses the reproductive capacity as a result of decreased seminiferous epithelial cell differentiation which is manifested in semen volume (McDaniel *et al*., 1996).

The values of semen pH in this study were similar to the range of pH for poultry semen (Uchechukwu *et al*., 2015). The pH of semen observed in this study pointed to moderately alkaline. The values were similar to those reported earlier of 7.45±0.01 for Erbro cocks and 7.5±0.1 for Denizli cocks (Tuncer *et al*., 2006). However, a significant effect was observed by Sonseeda *et al*. (2013) who reported higher sperm motility during winter and lower motility in the hot dry season in Thai indigenous chickens. This study contradicts an earlier report that mass motility reduced during summer (Elagib *et al*., 2012) in white leghorn cocks in conditions in Sudan. There was an increase in sperm concentration as reported earlier by Sonseeda *et al*. (2013) which showed significant effects of season on Thai indigenous chicken across seasons. Studies in the Sahel region on birds showed a significant reduction in sperm concentration during the summer season ((Elagib *et al*., 2012).

The season contributed significantly to proportions of sperm morphological characteristics. The results indicated that abnormal and immature sperm cells increased in the hot dry season and decreased during the major and the minor rainy seasons. This is supported by an earlier study which reported lower value of normal spermatozoa during the summer than in other seasons in White Leghorn cocks (Elagib *et al*., 2012). The low performance in the hot dry season may be ascribable to higher ambient temperature during the period. Higher temperatures affect the testes by reducing the scrotal circumference and may cause degeneration in seminiferous tubules (Kumi-Diaka *et al*., 1981) which will result in production of inferior semen. In the hot season the feed intake is reduced thereby causing inhibition in the release of GnRH, FSH and LH (Khodaei-Motlagh *et al*., 2017). It was observed that the season of semen collection significantly affected the red blood cells in the semen but not the epithelial cells. There was a significantly higher amount of red blood cells in semen collected in the dry season as compared to those observed in other seasons. However, there were no significant differences in the season of semen collection on the amount of epithelial cells and white blood cells in the semen.

Artificial insemination had a positive relationship with the fertility of eggs set. Values for the percentage fertility of eggs for Guinea fowl hens that were artificially inseminated in this study were higher than earlier reports by Fani *et al*. (2004) and Khairun *et al*. (2016) of 80% and Odukwe and Onunkwu (2016) who also

obtained 55.64% in pearl Guinea fowls raised intensively. The fertility values for the naturally mated hen were similar to values of49% to 58%for Guinea fowls obtained by Ayorinde *et al*., 1989. Comparatively, the percentage fertility of eggs in artificially inseminated birds was found to be higher than birds which were subjected to natural mating. The reason for the variation in the two systems of mating may be attributable to the monogamous behaviour of Guinea cocks and the possibility that some of the hens may not be mated (Saina, 2005). The volume of 0.03ml applied in each inseminate may be higher than the average ejaculate of 0.02ml in pearl Guinea fowls (Keethy *et al*., 2017) and that might increase the chances of fertility of artificially inseminated birds.

Hens which were artificially inseminated had their eggs having significantly higher percent hatchability than those which were mated naturally. The results agreed with earlier report by Gumuka *et al*. (2005) who also reported a higher percentage hatchability of eggs from artificially inseminated hens. However, this result disagrees with the results of Habibullah *et al*. (2015) who reported no significant difference in percentage hatchability of eggs from naturally mated and artificially inseminated Hubbard classic broiler parent stock. Hatchability rates following artificial insemination were significantly lower than those obtained with natural mating in broiler breeder pullets (Hughes, 1978). Sayyazadeh *et al*. (2005) showed that when hens were artificially inseminated, they produced eggs with significantly thinner shells and this decreased the average weight of eggs hence, resulting in the reduced percentage hatchability. The report indicated that artificial insemination increased the bi-yolk egg production percentage and non-standard eggs production which are all factors that cause poor hatchability in Guinea fowls.

## Conclusion

It may be concluded that season, dietary protein and artificial insemination may be used to improve the reproductive performance of Guinea fowl in colonies.

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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
June 2020

**Aims and scope**

The Bulletin of Animal Health and Production in Africa (BAHPA) of the African Union Interafrican B*ureau* 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 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.

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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.
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- 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.
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**Types of contribution**

Full papers providing accounts of original work: Research containing significant new findings. The material presented should be original and not have been published elsewhere, except in a preliminary form. Papers will be reviewed by three referees familiar with the subject matter of the paper.

*Short Communications:* are intended to provide quick publication of highly relevant and interesting information. Manuscripts will be peer reviewed by two reviewers and the Editor.

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*Key notes and special calls:* The editor will, from 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.

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

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Full papers of original research

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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.
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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.
10. State the conclusions, and any implications that may be drawn from the study.

*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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3. Use 1 inch margins on top, bottom, left and right margins,
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**Examples of References**

- *Journal Articles:* Ouyang D, Bartholic J, Selegean J, 2005. Assessing sediment loading from agricultural croplands in the Great Lakes basin. Journal of American Science, 1(2): 14-21.
- *Books:* Durbin R, Eddy SR, Krogh A, Mitchison G, 1999. Biological Sequence Analysis: Probabilistic Models of Proteins and Nucleic Acids. London, Cambridge University Press.

- *Chapter in a Book:* Leach J, 1993. Impacts of the Zebra Mussel (Dreissena polymorpha) on water quality and fish spawning reefs of Western Lake Erie. In Zebra Mussels: Biology, Impacts and Control, Eds., Nalepa T, Schloesser D, Ann Arbor, MI: Lewis Publishers, pp: 381-397.
- *Reports:* Makarewicz JC, Lewis T, Bertram P, 1995. Epilimnetic phytoplankton and zooplankton biomass and species composition in Lake Michigan, 1983-1992. US EPA Great Lakes National Program, Chicago, IL. EPA 905-R-95-009.
- *Conference Proceedings:* Stock A, 2004. Signal Transduction in Bacteria. In the Proceedings of the 2004 Markey Scholars Conference, pp: 80-89.
- *Thesis:* Strunk JL, 1991. The extraction of mercury from sediment and the geochemical partitioning of mercury in sediments from Lake Superior, Unpublished PhD thesis, Michigan State University, East Lansing, MI.
- *Web links:* Cerón-Muñoz M F, Tonhati H, Costa C N, Rojas-Sarmiento D and Solarte Portilla C 2004 Variance heterogeneity for milk yield in Brazilian and Colombian Holstein herds. Livestock Research for Rural Development. Volume 16, Article #20 Visited June 1, 2005, from http://www.lrrd.org/lrrd16/4/cero16020.htm

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