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CONTENTS

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7

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

Mathias Afayoa[1]*, David Kalenzi Atuhaire[1,2], Sylvester Ochwo[1], Julius Boniface Okuni[1], Majid Kisekka[1], William Olaho-Mukani[3], Lonzy Ojok[1]*

[1]College of Veterinary Medicine, Animal Rsources and Biosecurity, Makerere University, P.O.BOX 7062 Kampala, Uganda

[2]National Agricultural Research Organization, National Livestock Resources Research Institute, P.O.BOX 96, Tororo, Uganda

[3]African Union-Interafrican Bureau of Animal Resources, P.O.Box 30786, Nairobi, Kenya

Abstract

African swine fever (ASF) is a highly contagious often fatal viral disease of pigs caused by asfivirus. The disease causes marked leucopaenia, depletion of lymphocytes in the lymphoid tissues, changes in biochemical parameters, haemorrhages and necrosis in multiple organs of the infected pigs. We studied the pathogenic effect of three different Ugandan ASF virus (ASFV) isolates on twelve infected and six uninfected pigs. Each pig in the infected group was inoculated per os with 2 mls of ASFV culture solution containing 1X 108 H.A.D.U/ml of the viral culture solution while the control group were given 2 mls of uninfected porcine alveolar macrophages culture per-os. Clinical parameters were monitored daily and blood samples collected for leucocytes count and biochemical tests.

In the present study, the incubation period of the disease ranged from 7 - 15 days and in average the clinical disease lasted for 5 days. On the eighth day post infection, all test pigs had significant leucopaenia (p = 0.000) and number of lymphocytes reduced significantly (p =0.001,). Band neutrophils progressively increased in number as the disease progressed, however when the changes in mean band neutrophils in the three groups were compared it was not statistically significant (p= 0.52). There were no significant variations in the mean basophils and eosinophil counts in all experimental groups during study period (p = 0.30 and p = 0.32 respectively). Nevertheless, mean monocytes counts significantly increased in infected pigs (p = 0.01), while in uninfected group there was no significant variation in the mean monocytes counts. The majority of the pigs, 83.3% (n = 10) in the test groups had elevated levels of gamma-glutamyl transferase (GGT). The Level of Alanine Amino Transferase (ALT) at 8 days post infection was elevated in all infected pigs in the three groups. In 66.7% (n = 8) infected pigs, Albumin (ALB) levels were elevated in the serum samples above the normal range of 18 – 33 g/l. The levels of other biochemicals in the serum samples such as Creatine kinase (CK), Creatinine (CREA), and Alkaline Phosphatase (ALKP) remained within the normal range (50- 3531 µ/L, 44 -186µmol/L, 92 - 294 µ/L, respectively).

We concluded that ASF causes significant deviation in leucocytes counts, increased levels of GGT, ALT and ALB and clinical parameters in pigs infected with Ugandan isolates of ASF virus.

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

Corresponding authors email: afayoa@vetmed.mak.ac.ug and lonzy@vetmed.mak.ac.ug

L'APPLICATION de la protéine anti virale VP73 IGY de la peste porcine africaine extraite DU JAUNE D'ŒUF DE POULET, DANS UN TEST D'AGGLUTINATION à la CARTE pour le DIAGNOSTIC SEROLOGIQUE DE LA PESTE PORCINE AFRICAINE

Résumé

La peste porcine africaine (PPA) est une maladie endémique hémorragique virale pathogène très infectieuse des porcs domestiques dans de nombreux pays africains. Il n'y a pas de vaccin ou de traitement pour la PPA, de ce fait, un diagnostic rapide est nécessaire pour l'établissement d'autres mesures de contrôle. Le but de la présente étude est d'évaluer l'utilisation de la protéine virale VP73 IGY de la peste porcine africaine extraite du jaune d'œuf de poulet dans le test d'agglutination à la carte (TAC) pour le diagnostic de la peste porcine africaine. La protéine virale vp73 de la PPA purifiée à partir d'un isolat ougandais du virus de la peste porcine africaine a été utilisée pour produire des anticorps chez les poules pondeuses. L'immunoglobuline IgY du jaune d'œuf de poulet a été extrait et purifié avec NaCL 8,8% par la technique de précipitation saline et couplée de manière passive à l'aldéhyde / Sulfate perles de latex de polystyrène dans un milieu acide 2- (N-morpholino) éthanesulfonique (MES) (pH 6,6) et utilisé pour détecter les antigènes viraux de la peste porcine africaine dans des échantillons de sérum. La sensibilité diagnostique, la spécificité, les valeurs prédictives positives et négatives du test mis au point ont été calculées pour évaluer les performances du test. Le test a révélé une sensibilité et une spécificité respectivement de 77,3% et 86,9%, tandis que les valeurs prédictives positives et négatives étaient de 74,7% et 88,4% dans le même ordre. En conclusion, le test d'agglutination à la carte de la PPA à base de l'immunoglobuline IgY du poulet pourrait être utilisé conjointement avec d'autres tests pour le diagnostic sur le terrain de la PPA, ce qui pourrait contribuer à la lutte contre la maladie dans les zones où le virus de la peste porcine africaine est endémique. Cependant, la sensibilité du test mis au point devrait être améliorée et validée en outre dans des conditions de laboratoire et de terrain différents en utilisant un plus grand échantillon des sérums positifs et négatifs de la PPA référencée.

Mots clés : la peste porcine africaine, le jaune d'œuf de poule IgY, PPA-VP73, le test d'agglutination a la carte

Introduction

African swine fever (ASF) is a highly infectious, trans-boundary haemorrhagic disease of pigs caused by a large icosahedral DNA virus of the genus Asfarvirus (Dixon *et al.,* 2005). The disease most commonly appears in domestic pigs as haemorrhagic fever and virulent isolates of ASF virus kill domestic pigs within 7-10 days, post infection. Mortalities in domestic pigs are usually close to 100% (85-100%) and pigs of all ages are affected. However, sub-acute and chronic forms of the disease do exist (Penrith and Nyakahuma, 2000). Pigs that survive the disease are resistant to the same virus isolates but they do not produce classical neutralizing antibody (El-Hicheri, 1998).

At present there is neither vaccine nor effective therapy for ASF. Therefore the control and eradication of the disease is based on rapid and accurate laboratory diagnosis followed by institution of strict sanitary measures (Reis *et al.,* 2007; Costard et al. 2009). Currently in Uganda, there is lack of readily available rapid and reliable diagnostic test for early detection of ASF. Hence in event of an outbreak of the disease, samples are sent to the national diagnostic centre or foreign laboratories for confirmatory diagnosis. It usually takes long for results from these laboratories to reach farmers and for the institution of timely control measures.

Several diagnostic tests have been developed to detect ASF in infected pigs. Some of the common assays include: the long established haemadsoption test (Malmquist, 1960), polymerase chain reaction (Agüero *et al.,* 2003), ELISA (Perez-Filgueira et al. 2006), immunofluorescence test (Heuschele and Hess, 1973; Arias *et al.,* 2002), immunohistochemistry and in situ hybridization (Oura *et al.,* 1998). Each of these diagnostic techniques has its strengths

and flaws. In recent years, the continued presence of ASF virus of low virulence has resulted in an increased prevalence of sub-acute, chronic and in apparent infections. These circumstances require reliable serological tests for diagnosis (Pastor *et al.,* 1992). For any diagnostic test used for disease control and eradication programme to be useful in the field, it must be simple, affordable, fast and the results easily interpreted by the local animal health workers. Card agglutination test (CAT) is one of such simple immunoassays that can be performed at room temperature or in the field without requirement for special equipment. It works on the principle of antigen antibody binding leading to immune complex formation. The antigen in the serum binds to the specific antibodies coupled onto surfaces of latex beads. Due to multi-valence of immunoglobulins and multiple epitopes on an antigen, several antibodies could bind to a single antigen as each antibody binds specifically to distinct epitopes on the antigen. This results into complex formation seen macroscopically as fine granules. The intensity of agglutination is directly proportional to the concentration of the target antigen in the serum. However, the result of CAT should be read and interpreted immediately to minimize false positive reaction associated with evaporation hence concentration of the CAT reagents (Behera, 2014)

Antibodies used in immune assays for diagnosis of mammalian diseases are commonly raised in mammals such as rabbits, rats, mice, guinea pigs, goats or sheep. Unfortunately, mammals are phylogenetically closely related. Recent studies suggest that animals that are phylogenetically distant from mammals are better sources of antibody production for immunodiagnostic assays intended to be used on mammalian samples (Pokorova *et al.,* 2000). Chickens are some of the animals that are phylogenetically distant from mammals that have been used to raise antibodies against antigens of mammalian pathogens for immune-diagnosis and immunotherapy, for example in the prevention of colibacillosis in piglets (Asemota *et al.,* 2013) and streptococci infection in rats (Losonczy and Batke, 1997).

Immunization of hens intramuscularly in the pectoral muscles followed by 2-3 booster doses often result into high antibody titres in eggs, ranging from 1 : 100000 to 1 : 1000000. The level of immune response depends on the immunogenicity of the antigen used (Pauly *et al.,* 2011).

Avian species concentrate antibodies in the egg yolk to provide passive immunity to the chicks in their early stages of growth (Hodek *et al.,* 2013). Immunoglobulin Y (IgY) is the dominant antibody type produced by chicken and accumulates in the egg yolk. Other important immunoglobulin classes in chicken include IgM and IgA. In addition, IgD and IgE exist in chicken eggs though in negligible levels (Chen *et al.,* 1982). Similar to mammalian IgG, avian IgY consists of two light and two heavy chains. Avian IgY light chain is 18.66KD and heavy chain is 65.105KDa compared to 25 KDa and 55 - 77 KDa of light and heavy chains of mammalian IgG respectively (da Silva and Tambourgi, 2010).

There are several distinct advantages for using chickens over mammals to produce polyclonal antibodies: Firstly, higher titres against highly-conserved mammalian gene products. Since chickens are at least 100 million years removed from mammals, they tend to recognize any mammalian gene product as foreign, and mount vigorous immune responses Secondly, double immunostaining is easier to perform in histochemical manipulations and chicken IgYs can be used together with mouse and rabbit antibodies, without the danger of cross-reactivity. Secondary antibodies against chicken IgYs don't cross react with mammalian IgG's. Thirdly, the procedure of producing chicken IgY is animal-friendly because antibodies are purified from eggs, not serum, which is simply a more humane way to produce antibodies (Asemota, *et al.,* 2013). Furthermore, the process is cheaper than the custom mammalian antibodies as antibody preparations are >90% IgY, and shelf-life of 5 years or more at 4 °C. In contrast, mammalian serum has only a limited shelf-life at 4 °C (measured in weeks-to-months), moreover some biological activity is lost during freezing (Asemota *et al.,* 2013). Nearly unlimited quantity of antibodies could

be obtained from eggs with little cost required for purification. The cost of having mammalian antibodies purified to comparable purities (i.e., protein A-purified) is usually very high. In addition, there is no "Fc domain" within the stem portion of a chicken IgY. This provides several advantages over mammalian IgG's as it reduces the likelihood of having false positives in diagnostic applications, since it is the mammalian Fc domain that binds "rheumatoid factors" or other naturally occurring anti-Fc antibodies. Chicken IgY does not activate mammalian complement systems, allowing its use in in vivo applications; and does not bind to mammalian Fc receptors, avoiding non-specific binding to cells expressing such receptors (e.g., macrophages and dendritic cells) (Hodek *et al.,* 2013). Finally, Chicken IgY contains a larger glycosylation index, allowing more labelling with enzymes like horseradish peroxidase and other antibody tags like latex beads, which produces a higher signal contact.

Various egg yolk IgY extraction and purification methods have been described and compared in the recent studies (Meenatchisundaram and Michael, 2010). Egg yolk protein livetins can be removed from yolk suspension by several methods such as ammonium sulphate precipitation plus centrifugation method, alcohol precipitation or by use of ion exchange chromatography (Hatta *et al.,* 1990; Asemota *et al.,* 2013). The choice of IgY purification technique depends on the availability of appropriate laboratory facilities, reagents and cost implications including the impact of the purification method on the environment (Pauly, *et al.,* 2011). Avian immunoglobulin purification generally is done in two phases. In the first phase, water soluble fractions are separated from lipophilic components, followed by the second phase in which IgY is isolated from other hydrophilic compounds.

Material and Methods

Isolation of ASF virus

African swine fever virus was isolated from an infected pig and cultured in swine alveolar macrophages following established protocol (Carrascosa *et al.,* 2011). The virus was purified from the culture supernatant using PEG 6000 technique and gel column chromatograph using Sephadex G-200. Proteins in the solution were separated in 12 % acrylamide gel as previously described (Garfin, 2003) and presence of the viral protein of interest vp73 demonstrated by western blot.

Separation of ASF virus induced proteins by SDS - PAGE

Purified African swine fever virus samples were sonicated and solubilized in buffer containing 20mM Tris- HCl, 5mM EDTA-Na, and 1x protease inhibitor, PH 7.4 at 4^0C for 30 minutes, and the proteins separated by SDS- PAGE (Janson 2012). The viral proteins were separated using 12% separating gel at constant voltage of 200V for 70 hours. The bands were stained with Coomassie brilliant blue (R 250) over night. The gel was then de-stained in two changes of de-staining solution I composed of 40% methanol and 10% acetic acid in distilled water. Further de-staining was done in de-staining solution II composed of 5% methanol and 7% acetic acid in distilled water. The concentration of the separated viral envelop proteins, vp30, Vp54 and Vp73 were estimated using densitometer (Smith 1996). The antigen of interest (Vp73) was then titrated against conjugated antibody directed against it to achieve optimal concentration using checkerboard titrations. Bands of Vp73 were cut and pooled in separate Eppendorf tubes, crushed and sonicated to fine particles and used to immunize chicken.

Production and purification of chicken anti ASF virus polyclonal antibodies

Two six month old unvaccinated healthy local hens were selected for polyclonal antibody production and immunized intramuscularly with purified viral protein vp73. Each hen received intramuscularly 500 µl of the antigen in Freund's complete adjuvant at pectoral muscles on both muscles. Three booster doses in Freund's incomplete adjuvants were administered at interval of 14 days. Eggs were collected daily from the immunized birds and yolk immunoglobulin harvested.

Egg yolk IgY was extracted based on established procedure (Hodek *et al.,* 2013). This optimized method of chicken egg IgY purification is known to produce electrophoretically homogenous preparations. The method is easy, affordable and utilizes most available reagents such as tap water or mineral water and sodium chloride, compared to other methods that require expensive reagents such as chloroform, polyethylene glycol or ammonium sulphate (Hodek *et al.,* 2013). Briefly, the procedure involved washing of the surfaces of eggs in warm water and the shell carefully broken and albumen discarded. Traces of albumen and chalazae on the yolk membrane were removed by rolling the yolk on sterile absorbent paper and the yolk pooled. The yolk membrane was ruptured, its content diluted in sterile water, mixed by stirring and the pH of the mixture adjusted to 5.0 and frozen at − 20 °C overnight. The frozen yolk material was thawed at room temperature and filtered through Whatman filter paper No.1 (WhatmanTM-1 UK. 125 mm pore size). To the filtrate was added 1.5M NaCl to make 8.8% of the total volume of the suspension. The pH was adjusted to 4.0 and the mixture left to precipitate at room temperature for two hours and yolk immunoglobulin pelleted by centrifugation at 4000 x g for 20 minutes. The sediment of IgY pellet was then dissolved in 0.01M PBS, dialysed in same buffer and concentrated by osmosis using PEG 6000. The level of purity of the extracted IgY was assessed by SDS – PAGE.

Estimation of the amount of the purified IgY in solution

Presence of antibodies in the egg yolk extract was determined and its concentration estimated by Bradford assay (Bradford, 1976; Stoscheck, 1990).

Coupling of Chicken anti ASF virus IgY to latex beads

Fifteen (15) mls, 4% w/v of 0.5μm diameter aldehyde/Sulfate polystyrene latex microspheres, (Sigma Aldrich, USA product), used in the current study was kindly donated by Prof. Naoaki Misawa, University of Miyazaki, Japan. The beads suspension was cleaned and re-suspended in distilled water. Chicken egg yolk anti ASF vp73 IgY were coupled to the latex particles passively based on procedure described by (Yap 1994) and Sigma Aldrich USA, product information sheet mumber LB-1 to LB -6. Two millimetres of pre-cleaned latex beads were suspended in 50mM 2-(N-morpholino) ethanesulfonic acid (MES) buffer, PH 6.6 (same for the isoelectric point of chicken IgY, to give optimal binding) to form 1% bead suspension. The bead suspension was dispersed using an ultrasonic disintegrator model, Soniprep 150 Plus, MSE (UK) for short periods. The latex beads surface saturation capacity was estimated following an established procedure (Maron *et al.,* 1954; Roose and De Doncker, 2004). Thereafter, purified immune chicken IgY solution was added at a concentration three times in excess of the total latex surface area capacity for binding IgY (Yap, 1994). The suspension was vortexed for a minute and the mixture was incubated at room temperature over night while stirring gently. The unbound proteins were removed by repeated washing of the beads suspension using the MES buffer containing 0.1% glycine. Presence of residual protein in the supernatant was tested using Bradford assay. Centrifugation and re-suspension of the coupled beads in MES buffer was repeated until the free protein was no longer detected in the supernatant (Hechemy and Michaelson 1984). Sodium azide was added (0.05%) and the CAT reagent stored at 4 °C until used.

Card agglutination test

To test for the presence of ASF antigen in a sample using card agglutination test, 50 μl of the sample was placed on latex card and equal amount of latex reagent added and mixed using applicator stick. The mixture was spread to a circular area of about 2.0 cm diameter. The card was rocked gently and the mixture observed for agglutination with unaided eye. The intensity of agglutination observed was scored as ± (borderline or equivocal), + (+1), positive result with the least intensity of reaction, ++ (+2), positive result with moderate agglutination, +++ (+3), positive result with

high agglutination and ++++ (+4) extensive agglutination that represented a positive CAT result with highest level of agglutination characterized by extensive clumping of latex particles.

Data quality control and evaluation of assay performance

As quality control and to improve the reliability of the results, positive and negative control samples were included in each card agglutination test in every run of the assay. The performance characteristics of the CAT developed was evaluated based on established procedures (Altman and Bland, 1994; Jacobson, 1998; Fears and Pope, 2001): True positive (TP), True negatives (TN), False positive (FP) and false negative (FN) test outcomes including assay sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were used in assessing the assay performance. In evaluating CAT results, the visual score of agglutination ≥1+ within 2 – 5 minutes was considered positive reaction for ASF antigen while those that score ≤1+ was considered negative.

Ethical Statement

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

Results

Determination of the purity of chicken IgY by SDS - PAGE

Chicken egg yolk IgY was purified using 8.8% sodium chloride precipitation method from ten-fold diluted yolk. The purity

of prepared yolk protein (IgY) was evaluated by using SDS – PAGE under reducing condition. The light and heavy chains of IgY were clearly separated as shown in Figure 1.

Card agglutination test result evaluation

Equal amounts of test sample and CAT reagent were mixed and rocked gently on card and the reaction observed for agglutination with unaided eye. The intensity of agglutination observed was scored as ++++ (+4) represented a positive CAT result with highest level of agglutination characterized by extensive clumping of latex particles, + (+1) was the positive result with the least intensity of reaction while ± represented doubtful (equivocal) reaction. The CAT test result was red within 2 – 5 minutes after rocking the mixture. CAT positive result was seen as fine granular agglutination visible with unaided eyes while there was no agglutination in negative result. The CAT result showed that majority 77.3% (n = 68 at 95% CI) of samples that were positive with PCR were also positive while 86.9% of the negative samples similarly tested negative by card agglutination test. The visible positive, negative and doubtful CAT results were as shown in Figure 2.

Evaluation of the developed CAT

The performance of the CAT developed was evaluated by computing the assay sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV). In evaluating CAT results, the visual score of agglutination ≥1+ was considered positive reaction for ASF antigen and that ≤1+ was considered negative. The diagnostic sensitivity (DSe) and specificity (DSp) were 77.3% (95% CI) and 86.9% (95% CI), respectively. The positive predictive value (PPV) was 74.7% while negative predictive value (NPV) was 88.4% as showed in Table 1.

Discussion

African swine fever is viral haemorrhagic fever of pigs that is often confused with other swine haemorrhagic diseases due to the similarities observed at clinical presentations and necropsy lesions.

Figure 1: SDS – PAGE of chicken IgY. Heavy and light chains of IgY were distinctly separated as indicated by arrows; 10μl of IgY solution (0.5μg/μl) was loaded per well and SDS – PAGE run under reducing conditions.
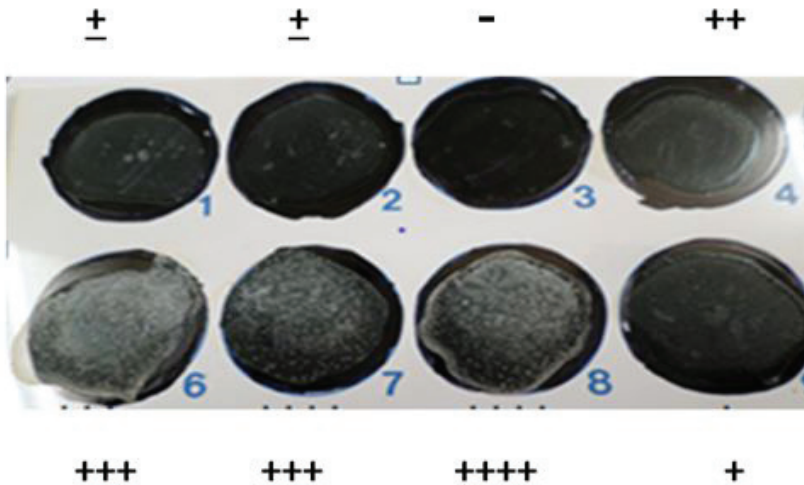
Figure 2: Card agglutination test scoring: Samples 1 & 2 were unclear result, sample 3 was CAT negative, 4 was moderate positive, 6, 7 & 8 strongly positive while sample 9 shows weak positive CAT reaction.

Table 1: A 2 x 2 table for evaluating the sensitivity, specificity, positive and negative predictive values of developed card agglutination test (CAT) using pig serum of known sero- status and PCR as the Gold test.

CAT	No. of PCR positive samples	No. of PCR negative samples	Predictive Values (%)
CAT Positive	68	23	PPV = 68/91 = 74.7%
CAT Negative	20	153	NPV = 153/173 = 88.4%
Total	**88**	**176**	

Some of those pig diseases that should be differentiated from ASF by using appropriate laboratory techniques include: classical swine fever, septicaemic salmonellosis, Trypanosoma simie infection. Several diagnostic methods have been established and used in detection of ASF, these include the traditional haemadsoption test (Malmquist and Hay, 1960), polymerase chain reaction (Agüero *et al.,* 2003; King *et al.,* 2003), ELISA (Lubisi *et al.,* 2009), immunoblot, immunofluorescence (Pastor *et al.,* 1992; Arias, *et al.,* 2002), immunohistochemistry and in situ hybridisation (Oura, et al. 1998). Antibody detecting ELISA has been used extensively in

ASF control and eradication campaign (Pastor *et al.,* 1990; Lubisi *et al.,* 2009). However, the disadvantage of antibody ELISA is that the accuracy of ELISA results depends on the origin and the quality of the sample used (Pokorova *et al.,* 2000). Despite of the existence of several diagnostic techniques for ASF, confirmatory diagnosis of the disease has been a big challenge in developing countries where ASF burden is the biggest. This is probably because most of the available diagnostic techniques are expensive and cumbersome.

Recent studies have identified several cell surface immunogenic proteins induced by ASF virus in infected macrophages with diagnostic potential (Cubillos *et al.,* 2013). Identification of highly antigenic ASF viral protein in infected cells is important in development of diagnostic immune-assays (Cubillos *et al.,* 2013). Out of over 100 ASF virus induced proteins in infected macrophages, about 50 of them are known to be antigenic (Esteves *et al.,* 1986). Amongst the ASF viral capsid proteins, vp73 is highly immunogenic and has been used in several studies to detect antibodies against ASF virus using immunoassays. Other immunogenic capsid proteins include vp54, vp30, and vp17 (Kollnberger *et al.,* 2002; Gallardo *et al.,* 2006). We separated these structural proteins using SDS – PAGE and used vp73, a known immune-dominant protein to raise antibodies in chicken. The applicability of ASF viral structural protein vp73 and vp54 in immunodiagnostics has been documented (Carrascosa *et al.,* 1985; Gallardo *et al.,* 2006). Previous studies and observations suggested that antibodies induced against vp54 recognize linear epitopes of the target antigens (Cubillos et al. 2013). Further investigation by Gallardo, et al. (2006), suggested that sero-detection of vp54 and vp30 in poorly preserved samples was not effective. This was associated with loss of stability of the antibodies against these proteins in poorly preserved samples.

A number of studies have successfully used chicken immunoglobulins in immunodiagnostics and to prevent bacterial infection in animals for instance, colibacillosis in piglets and streptococci infection in rats (Asemota *et al.,* 2013). In average each laying hen produces 20 eggs per month equivalent of about 2000mg of IgY per hen per month. The equivalent amount of IgY can be obtained from 600mls of total blood of chicken, because in average IgY concentration in blood of adult chicken is maintained within the range of 5-7mg/ml of blood (Shimizu et al. 1994). Therefore, the use of chicken eggs as source of immunoglobulin production in the current study was based on the benefits stated above among which include; the less expensive and non-invasive method of antibody production and. the revelation that immunoassay performances tend to be better when antibodies used are raised in animals that are phylogenetically distant from the one in which antibodies are used as diagnostic tool (Bollen and Hau, 1996; da Silva and Tambourgi, 2010).

Chicken Anti-ASF vp73 antibodies (IgY) in this study were produced in laying hens by intramuscular (intra-pectoral muscles) administration of the antigen (vp73) in three boosts. Antibody concentration of 53mg/egg was attained in ten days after the third boost. Route of administration of antigens into animals tend to influence immune response, similarly, immunization of chicken through intramuscular route is reported to have produced higher antibody titres with high specificity by 28th day post infection compared to response to subcutaneous administration of similar antigens in chicken (Woolley and Landon, 1995). (Horton *et al.,* 1985) further reported that chicken immunized through intramuscular route continuously produced antibodies for over 200 days post inoculation and the antibodies produced were highly specific to the antigen used.

Earlier studies showed that chicken were tolerant to administration of commonly used adjuvants such as Freund's adjuvant, Hunters and Specol Adjuvants when used with similar or related antigens (Polson *et al.,* 1980). For timely intervention so as to minimize effects of ASF, diagnostic results needs to be prompt if not instant. This is especially true for acute overwhelming ASF which sporadically occur in different areas of Eastern Africa. Unfortunately, majority of local governments in Uganda that offer veterinary services directly to livestock farmers do not have capacity

neither facilities for confirmatory diagnosis of livestock diseases including ASF. This often results into reliance on tentative diagnosis to make immediate decisions. Confirmatory diagnosis of ASF in Uganda could only be done at the single national diagnostic laboratory or at research institutions located far away from majority of farmers. In the countryside where the majority of pigs are kept, decisions in regards to suspected cases of ASF are often taken based on tentative diagnosis by looking at clinical symptoms, clinical history and necropsy findings. Worst still, there are no established livestock hospitals in Uganda and the few animal clinics in the country do not have the capacity to perform confirmatory diagnosis. This unfavourable situation often results into unnecessary disposal of herds of pigs based on false clinical and or pathologic diagnosis.

An ideal diagnostic test should be simple, efficacious, affordable and applicable at laboratory and field levels. Common pen side diagnostic tests include; immune-strips, latex agglutination tests, dip stick and lateral flow, all of which are portable and convenient for field diagnosis (Behera *et al.,* 2014). Application of chicken egg yolk IgY in diagnosis of ASF using latex agglutination technique in the current study would serve as a simple rapid, sensitive and reliable pen side diagnostic assay. The added benefit of card agglutination test over other immunoassays is that, CAT could be performed and results interpreted by least skilled animal health workers because of its simplicity and the results are read instantly by unaided eyes. It does not require expensive reagents and special facilities and the test is performed individually, therefore CAT is convenient when few samples are to be tested. Furthermore CAT generates minimal amount of biomedical west (Behera *et al.,* 2014) and is portable, affordable and therefore provides a better option to the diagnosis of ASF in developing countries, especially in the resource constraint Sub-Saharan Africa. In addition, coated latex particles are known to be stable for a long period at 4 °C (Behera *et al.,* 2014). The long shelf life of coated latex beads in addition to the rapid and easy–to-conduct CAT procedure could make latex agglutination assay a desirable

diagnostic technique for ASF. However, antigen detecting CAT has some short comings which include; the need for samples that should be collected, handled and preserved well so that the conformational antigen structures are preserved (though good sample handling and preservation is a requirement for most diagnostic assays). Another weakness of CAT could be the high antigen detection limit; hence the assay might not effectively detect low levels of ASF antigens in circulation that occur in chronic or subclinical infection. This could reduce positive predictive values, unlike PCR with better detection limits due to DNA amplification process involved in PCR. The moderate sensitivity of the CAT in the current study could be attributed to the high detection limit of the assay. Therefore doubtful CAT results should be confirmed with highly sensitive and specific diagnostic assays such as PCR, Viral isolation and immune-peroxidase monolayer assay.

Several techniques for protein coupling to latex beads have been described (Sigma Aldrich® USA product information, Life technologies® USA protein coupling protocols 2015). Covalent coupling of the proteins to latex beads is one of viable methods when passive adsorption of the proteins to the latex bead surfaces is not convenient. Carboxylate modified beads could be used and protein could be coupled to polystyrene beads using water soluble carbodiimide coupling agent (1- ethy-3-(3- dimethyl aminopropyl carbodiimide, EDAC) (Quash *et al.,* 1978). Coupling could be achieved by reacting the beads with diaminopentane (spacer) and protein adsorbed to surfaces of latex beads by glutaraldehyde which is a cross linking agent. (http://www.introgen.com/site/us/en/home/products, Sept. 28th 2011, Latex beads protein coupling). In this study, IgY was suspended in 2(N- morpholino) ethane sulfonic acid (MES) buffer and successively coupled on to latex beads passively at pH of 6.6 to match with the iso-electric point of chicken IgY. The MES buffer was chosen for its mid pka range, maximum solubility, minimum salt effect and stability (Sigma Aldrich® USA product information, 2015). Passive coupling is cheaper procedure of adsorbing antibodies to

latex beads as the procedure is simple, flexible and does not require spacer molecules, unlike covalent coupling. However, covalent coupled beads tend to form more stable antibody – latex beads complexes compared to passively adsorbed ones (Mahat *et al.,* 2014).

The developed CAT test in the present study was evaluated based on its diagnostic sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV). The diagnostic sensitivity of the assay was moderate (77.3% at 95% CI). This also indicated that there was 77.3% (95% CI) agreement between CAT and PCR in detecting ASF antigen and DNA, respectively, in ASF virus positive sera. The specificity (86.9% at 95% CI) of the developed assay was higher than the sensitivity (77.3% at 95% CI). The positive predictive value (PPV) and negative predictive value (NPV) was 74.7% and 88.4%, respectively. The most important consideration in assay validation is the capacity of known positive and negative test results to accurately predict the infection status of individuals in population (Wright et al. 1993; Jacobson, 1998). The capacity of a test to predict infection status is influenced to large extent by the prevalence of the disease in a given population. Therefore, positive and negative predictive values should be interpreted based on the disease prevalence in the targeted population (Wright, *et al.,* 1993; Jacobson, 1998).

Recommendations

The diagnostic specificity (86.9%) of chicken egg-yolk IgY-based CAT for detecting ASFV antigens was high while the sensitivity was moderate (77.3%). The sensitivity of the test could be improved by using high affinity monoclonal or polyclonal antibodies and the test validated using more positive and negative reference pig serum from different geographic regions. CAT therefore could be a good pen-side immune-assay that could be used for diagnosis of ASF and contribute towards control of ASF in Uganda. There is also need to compare the performance of CAT in detection of acute, sub-acute, chronic cases and carrier states of ASF infection in pigs.

These aspects should form the basis for future studies to improve the field diagnosis of ASF. This is the first assay where chicken IgY has been for a CAT for the diagnosis of ASF. The use of chicken IgY antibodies in this technique is a commendable approach to diagnosis as it has a high animal welfare compliance.

Conflict of interest

There was no conflict of interest identified.

Ethical statement

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

Acknowledgement

Public Policy Brief

The Latex Agglutination Card Test reported in the present study is the first quick pen-side antigen detection test for African swine fever infection, which utilises chicken egg York IgY and could aid veterinarians and animal health workers to make quick decisions as regards the control of the disease. The procedure of producing chicken IgY is animal-friendly because antibodies are purified from eggs, not serum, which is simply a more humane way to produce antibodies. The test is simple to perform and does not require sophisticated equipment. When fully tuned and validated, this test could be incorporated into routine African Swine Fever Control Programmes.

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INCIDENCE OF UDDER ABNORMALITIES IN WEST AFRICAN DWARF AND KALAHARI RED GOATS: INFLUENCE OF TEAT NUMBER ON MILK PRODUCTION

Bemji M N, Tukur H A and Umejesi S I

Department of Animal Breeding and Genetics, Federal University of Agriculture, P.M.B 2240, Abeokuta, Nigeria

Abstract

A total of 646 goats comprising 580 West African Dwarf (WAD) sampled across Abeokuta South, Abeokuta North and Odeda Local Government Areas of Ogun State and 66 Kalahari Red (KR) goats from the Institute of Food Security, Environmental Resources and Agricultural Research (IFSERAR), Federal University of Agriculture, Abeokuta (FUNAAB), Nigeria were utilized for this study. The udder was appraised for shape, attachment, symmetry, teat number, teat shape and teat placement. Notable for udder abnormalities in the two breeds were asymmetrical udder, pendulous udder and supernumerary teats in varying frequencies. Frequencies of udder abnormalities in KR goats were asymmetrical udder (10.6%), pendulous udder (1.5%) and supernumerary teats with variations from three teats (15.2%), to four teats (57.6%) and five teats (9.1%). Percentage individuals with symmetrical udder and normal two teats were 87.4% and 18.2% respectively. Udder shapes were bowl (9.1%) and cylindrical (86.4%). Udder shapes could not be determined (undifferentiated) in 4.5% of the KR does. Teat shapes were cylindrical (39.4%) and funnel (60.6%) while teat placements were oblique (65.2%) and vertical (34.8%). In WAD goats, 6.4% and 0.3% had asymmetrical and pendulous udders correspondingly while majority (93.3%) of does had symmetrical udder. Percentage individuals with two teats, three teats, four teats and five teats were 72.8%, 16.2%, 9.5% and 1.6% respectively. Udder shapes were bowl (56.7%), cylindrical (34.0%) and round (0.3%) while 9.0% were in the indifferent stage. Teat shapes were cylindrical (64.0%) and funnel (36.0%) while teat placements were oblique (71.7%) and vertical (28.3%). The study revealed a preponderance of four teats (58.82%) in KR goat compared with the normal two-teat condition prevalent in WAD goat. A high proportion of the supernumerary teats in KR were functional while rudimentary teats in WAD were non-functional. Daily milk offtake and yield were not significantly (P>0.05) influenced by teat number in both breeds. Variations in teat number may be useful in setting breed standard for both populations. A study on inheritance of supernumerary teats recommended.

Key words: Goat, udder characteristics, milk offtake, milk yield

INCIDENCE DES ANOMALIES DE LA MAMELLE CHEZ LA CHÈVRE NAINE AFRIQUE DE L'OUEST ET LE KALAHARI ROUGE : INFLUENCE DU NOMBRE DE TÉTINE SUR LA PRODUCTION LAITIÈRE

Résumé

Un total de 646 chèvres comprenant 580 naine d'Afrique de l'Ouest (WAD) échantillonné Abeokuta du Sud, du Nord de Abeokuta et oltra Local gouvernement zones de l'état d'Ogun et 66 chèvres Kalahari rouge (KR) de l'Institut de la sécurité alimentaire, ressources environnementales et Nigéria, Abeokuta (FUNAAB), Université fédérale de l'Agriculture, Agricultural Research (IFSERAR) ont été utilisés pour cette étude. Le pis a été évalué pour la forme, attachement, symétrie, nombre de tétine, forme de tétine et placement de la tétine. Remarquables pour les anomalies de la mamelle chez les deux races étaient asymétriques pis, mamelle pendante et mamelles surnuméraires à des fréquences variables. Fréquences des anomalies de la mamelle chez les caprins KR étaient pis asymétrique (10,6 %), des mamelles pendantes (1,5 %) et des tétines surnuméraires avec variations de trois tétines (15,2 %), à quatre trayons (57,6 %) et cinq mamelles (9,1 %). Pourcentage individus avec pis symétrique et normales deux tétines étaient respectivement de 87,4 % et 18,2 %. Formes de mamelle étaient bol (9,1 %) et cylindrique (86,4 %). Formes de la mamelle ne peuvent être déterminées (indifférenciées) à 4,5 % de la KR fait. Formes de tétine étaient cylindrique

(39,4 %) et l'entonnoir (60,6 %) tandis que des placements tétine étaient obliques (65,2 %) et vertical (34,8 %). Chez les caprins WAD, 6,4 % et 0,3 % avaient asymétriques et pendantes pis conséquence tandis que la majorité (93,3 %) n'avait pis symétrique. Pourcentage de personnes avec deux tétines, trois tétines, quatre tétines et des cinq tétines étaient de 72,8 %, 16,2 %, 9,5 % et 1,6 % respectivement. Mamelle formes étaient bol (56,7 %), cylindrique (34,0 %) et rond (0,3 %) tandis que 9,0 % étaient au stade indifférent. Formes de tétine étaient cylindrique (64,0 %) et l'entonnoir (36,0 %) tandis que des placements tétine étaient obliques (71,7 %) et vertical (28,3 %). L'étude a révélé une prépondérance des quatre trayons (58,82 %) chez les caprins KR par rapport à l'état normal de deux-tétine répandue chez les caprins WAD. Un high proportion des supernumerary tétines dans KR nere functional tandis que les tétines rudimentaires wad étaient non fonctionnel. Dunejely mj'ailk offtunke nd yj'aield nere pas signej'aificantly (P > 0,05) néoclassicismeenced by teat numéro dans les deux breeds. Une étude sur l'héritage des trayons surnuméraires recommandé.

Mots clés : Chèvre, u les caractéristiques de dder, prélèvements de lait, production laitière

Introduction

The Kalahari Red (KR) goat is native to South Africa and imported to Nigeria in 2011 to explore the possibility of improving meat and milk production traits of the local populations. It was developed mainly for meat production (Kotze *et al.,* 2004; Simela and Merkel 2008). Its shares attributes such as hardiness, prolificacy and good mothering abilities with the WAD goat. While the WAD goats have coat colours varying from black, brown, white and mixtures of the listed colours, KR goats are uniformly red, resembling the Nigerian Red Sokoto goat. Studies by Amao et al. (2003) and Bemji and Popoola (2011) focusing on udder traits (udder shapes, teat shapes and teat placement) and abnormalities in WAD goats reported bowl, round, cylindrical and funnel shaped udders. For teat shapes, funnel, bottle and cylindrically shaped teats were reported in WAD and RS goats (Amao *et al.,* 2003; James *et al.,* 2009).

The presence of abnormalities including supernumerary teats, asymmetrical udder and pendulous udder has also been reported with varying incidences. Incidence of supernumerary teats in WAD goats was estimated at 44% (Ozoje, 2002), 24% (Amao *et al.,* 2003) and 7.3% (Bemji and Popoola, 2011). Similar information is not available for the Kalahari Red population. Information on udder traits is important as udder and teat characteristics have been shown to be good determinants of milk yield and ease of milking in dairy animals (Rogers and Spencer, 1991; Peris *et al.,* 1996; De la Fuente *et al.,* 1999; Makovicky *et al.,* 2014). Even though several studies have reported the effects of genetic and non-genetic factors on milk production traits

in goats (Ehoche and Buvanendran, 1983; Bemji *et al.,* 2007), there is paucity of information on effect of supernumerary teats on milk yield. This study was therefore designed to evaluate and compare the incidence of udder abnormalities in KR and WAD goats and also to evaluate the effect of teat number on milk offtake and yield, since most studies have focused more on evaluating genetic and other non-genetic factors such as parity, stage of lactation, sex and number of kids suckled.

Materials and methods

Animals and management

This study involved a total of 646 goats comprising 580 West African Dwarf (WAD) sampled across Abeokuta South, Abeokuta North and Odeda Local Government Areas of Ogun State and 66 Kalahari Red (KR) goats raised at the Institute of Food Security, Environmental Resources and Agricultural Research (IFSERAR) of the Federal University of Agriculture, Abeokuta (FUNAAB) located within the same region in Nigeria. The region is 76 m above sea level and falls within latitudes 7°5.5'N-7°8'N and longitudes 3°11.2'-3°2.5'E. The climate is humid within the Derived Savannah vegetation zone of South Western Nigeria. It receives a mean precipitation of 1,037 mm with a mean annual temperature of 34.7°C.

The first study on appraisal of udder traits involved all the animals sampled. The WADs sampled from different villages were raised on free range consisting of grasses and browse plants and supplemented with kitchen wastes, cassava peels or corn chaff by the

farming families who also provided minimal housing to serve as shelter. For the second study which involved evaluation of effect of teat number on milk offtake and yield, 38 does consisting of 17 KR and 21 WAD were semi-intensively managed at IFSERAR farm. They were housed in semi-open pens, allowed to graze on mixed pastures comprising of Panicum maximum, Chloris gayana, Stylosanthes spps and supplemented with concentrate feed containing 15% crude protein at 200-700g/head/day depending on breed requirement and physiological status: whether dry, pregnant or lactating. The provision of water was on *ad libitum* basis. The animals were released daily to graze at about 10.00 am and brought back to their pens around 16 00 hours, except on rainy days when they were kept in their pens. There was routine treatment against ecto- and endo-parasites and vaccination against Peste des Petits Ruminants. Animals were ear-tagged for ease of identification and age of does initially determined by dentition method (Saini *et al.,* 1992) following acquisition. Natural mating of does was carried out and non-return of does to oestrus was a confirmation that the does were pregnant.

Data collection
Appraisal of udder traits

For the first study, all goat udders were visually appraised and classified based on shape, attachment, symmetry, teat number, teat shape and teat placement following pictorial descriptions of Amao et al. (2003) and James et al. (2009). Based on udder shapes, the frequencies of goats with bowl, cylindrical and round udders were recorded for both populations. Udder symmetry was also determined as either symmetrical with equal halves or asymmetrical with unequal halves. Well attached and firm udders were considered normal as opposed to loosely attached udders which were classified as pendulous. For teat characteristics, teat number ranged from 2-5 and were either cylindrical or funnel in shape, vertically placed or oblique (tilted).

Estimation of milk offtake and yield

At IFSERAR farm, 38 does from the two populations (17 KR and 21 WAD) that kidded were additionally investigated for supernumerary teat functionality (being able to allow passage of milk) and its influence on daily milk offtake (DMO) and daily milk yield (DMO). Hand-milking of does commenced after first week postpartum to allow the kids freely suckle colostrum which is essential for their survival. Data were collected once every week in the morning over 12 weeks duration per animal. DMO was the measure of milk obtained after hand-milking the doe dry immediately after separating dam from kid(s) at about 8.00 am. This initial milking was carried out to empty the udder of residual milk after kid(s) have suckled the dam. Both halves of the udder were milked until no more milk was produced by stripping. The quantity of milk obtained was recorded to the nearest ml using a 100ml calibrated measuring cylinder. The balance of milk left in the udder of suckled does per day was a measure of DMO (Bemji *et al.,* 2007). For DMY estimation, the does were hand-milked again after washing the udder and drying with towel following 3 hours of separation from their kids. Both halves of the udder were rapidly milked until no more milk was obtained by stripping. Total milk yield for 24-hour period was calculated in proportion to the time interval between the initial and final milking by multiplying the yield for 3 hours' interval with a factor of 8 according to the procedure adopted by Bemji et al. (2007). This approach of determining daily milk yield from part yield was based on the finding that rate of accumulation of milk in the mammary glands of small ruminants is constant throughout the day (Linzell, 1966). The technique of measuring daily milk yield at weekly intervals, instead of on daily basis was justified based on the finding that milk production is not likely to vary significantly within a span of eight days (Cardellino and Benson, 2002). The essence of this technique was also to shorten the duration of separation of kids from their dams in order to minimize any negative impact on survival.

Statistical analysis

The data obtained on udder and teat characteristics were analysed with the descriptive statistics of Statistical Package and

Solution Software (SPSS) version 16.0. Number of individuals in each sub-class including those with abnormal udder was expressed as a percentage of the total number of animals sampled. The effects of teat number DMO and DMY were investigated by analysis of variance of data using the GLM Procedure of SAS program release 8.0 (SAS, 1999). Genetic and other non-genetic factors affecting milk production were also included in the model: $Y_{ijklmnop} = \mu + D_i + A_j + W_k + S_l + L_m + X_n + T_o + \varepsilon_{ijklmnop}$, where, $Y_{ijklmnop}$ = trait of interest; μ = overall mean; D_i = effect of ith breed of dam (I = WAD or KR); A_j = effect of jth age of dam (j = ≤2; 3 or ≥4 years); W_k = effect of kth week of lactation (k = 1, 2, … 12th week); S_l = effect of lth month of kidding (I = April/May or Dec/Jan); L_m = effect of mth litter size (m = single birth or twins); X_n = effect of nth sex of kid (n = male, female or mixed); T = effect of oth teat number (o = 2 or 3-4 or 5); $\varepsilon_{ijklmnop}$ = random error assumed to be normally and independently distributed with mean 0 and homogeneous variance equal to $\sigma 2$. Preliminary analysis indicated that there were no significant interactions among factors.

Results

Udder and teat characteristics

Table I shows a summary of percentages of individuals in different categories based on udder shape, teat shape and teat placement for KR and WAD goats. Cylindrical shaped udder (Figure 1a) predominated in KR goats (86.4%) as against 34.4% in WAD while the latter had higher percentage (56.7%) of bowl shaped udder compared with 9.1% recorded for KR goat. Udder shapes could not be determined in 4.5% KR does and 9% of WAD does as they were in the indifferent stage of udder development, most of them being in their first parity. Funnel shaped (Figure 1a) and cylindrical shaped teats were present in both breeds with indication that funnel shaped teat predominated (60.6%) in KR goat while cylindrical shaped teat predominated (64%) in WAD does. Similar patterns of teat placement either vertical or oblique (tilted) were observed in both breeds (Figure 1a). Does with oblique teat placement were most frequent (65.2% and 71.7% for KR and WAD, respectively) while corresponding estimates for vertical teat placement were 34.8% and 28.3%.

Incidence of udder abnormalities

The incidence of udder abnormalities (asymmetrical/pendulous udders and supernumerary teats) are shown in Table 1. Incidence of asymmetrical udder (Figure 1c) was 10.6% and 6.4% in KR and WAD goats respectively. Majority of individuals in both breeds had symmetrical udder while those with parity greater than 4 had pendulous udder (Figure 1b). Teat number in both breeds ranged from 2-5 (Figures 2 and 3). The incidence of normal two-teat condition was low (18.2%) in KR goats while high (72.8%) in WAD goats. Majority of KR goats (57.6%) had four-teats followed by two teats (18.2%), three teats (15.2%) and five teats (9.1%). The four teats observed in some KR individuals appeared similar in shape and placement (Figure 1d).

Effect of supernumerary teats on milk offtake and yield

The additional (rudimentary) teats beyond the normal two-teats in WAD goats were non-functional while a high proportion of supernumerary teats were functional in KR does. Four functional teats (Figure 1d) were most frequent (58.82%) while one out of the seventeen KR does had five functional teats. The summary of mean values from least squares analysis of variance (Table 2) revealed that KR breed produced about twice (P<0.05) the estimate of DMO and DMY produced by the WAD breed. However, both DMO and DMY were insignificantly (P>0.05) influenced by teat number, even though both traits increased with increasing teat number.

Discussion

There were variations in udder shapes identified, similar to those earlier reported by Steele (1996); Amao et al. (2003) for WAD and Red Sokoto goats; Bhuiyan et al. (2004) for dairy cows and James et al. (2009) for WAD goats and sheep. Globular shaped udder included in

the report of Steele (1996) and funnel shaped udder in the report of Amao et al. (2003) and James et al. (2009) were however not detected in the current study. The domination of bowl shaped udder in WAD goats is a characteristic of the breed as corroborated by the reports of Amao et al. (2003) and James et al. (2009), compared to the higher incidence of cylindrical-shaped udder recorded for Kalahari Red. In comparison with dairy cow, low incidence of bowl-shaped udder was reported by Bhuiyan *et al.* (2004) with further indication that dairy cows with bowl-shaped udder yielded the maximum test milk yield which needs to

be investigated with goat breeds. Teat shapes in both KR and WAD goats were similar to those reported by Amao et al. (2003); Tilki et al. (2005) and James et al. (2009) with the exception of additional shapes (bottle and balloon) included in the preceding reports. The predominance of funnel shaped teat in KR goat corroborated a report on Saanen goat (Horak, 1971) while preponderance of cylindrical shaped teat in WAD goats supported previous reports by Amao et al. (2003) and James et al. (2009). A review by Tilki et al. (2005) on dairy cattle showed that cows with funnel shaped teats produced 10.9-15.4% more milk than

Table 1 Incidence of udder traits in Kalahari Red and West African Dwarf goats

Udder trait	Sub-Class	Incidence, %	
		Kalahari Red	**West African Dwarf**
Udder shape	Bowl	9.1	56.7
	Cylindrical	86.4	34.4
	Undifferentiated	4.5	9.0
	Round	0.0	0.3
Teat shape	Cylindrical	39.4	64.0
	Funnel	60.6	36.0
Teat placement	Oblique (tilted)	65.2	71.7
	Vertical	34.8	28.3
Udder symmetry	Symmetrical	87.4	93.3
	Asymmetrical	10.6	6.4
	Pendulous	1.5	0.3
Teat number	2	18.2	72.8
	3	15.2	16.2
	4	57.6	9.5
	5	9.1	1.6

Figure 1 a: Symmetrical and cylindrical shaped udder with funnel-shaped teat and oblique teat placement in Kalahari Red goat; b = pendulous udder; c = asymmetrical udder; d = bowl shaped udder with 4 functional teats

Figure 2. Teat types in West African Dwarf goats; A = normal two teats; B = primary teat with a spur; C and D = primary teats with ancillary teats located anteriorly
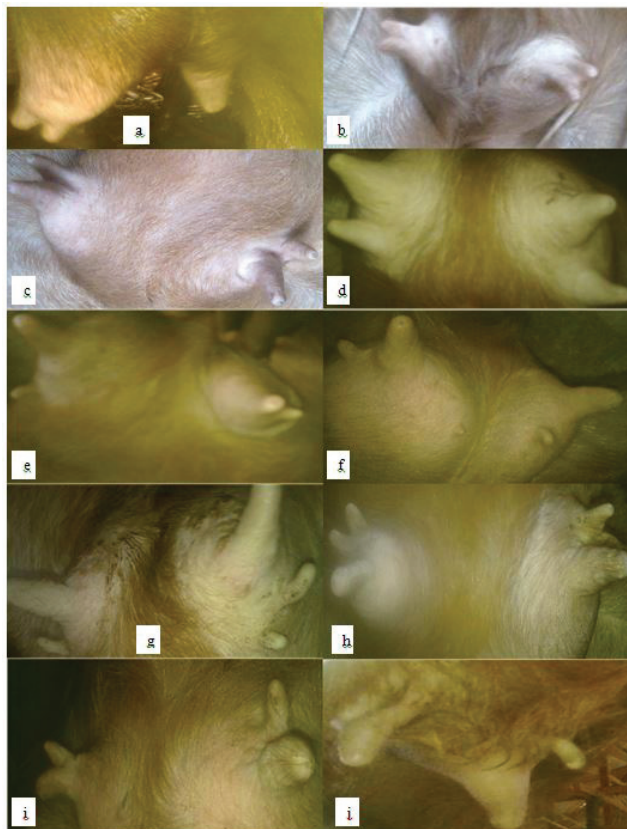
Figure 3: Teat types in Kalahari Red goats; a and b = fish teats (all functional); c, d and i = four teats (all functional); e = primary teat with non-functional ancillary teat on right half of udder and fish teats on left half of udder; f and g = two primary functional teats and three rudimentary non-functional teats; h = five teats all functional; j = two functional teats and a rudimentary teat

Table 2: Effect of breed, week of lactation, litter size, age of dam, sex of kid and teat number on daily milk offtake and daily milk yield of West African Dwarf and Kalahari Red goats

Source of variation	n	DMO(ml)	DMY(ml)
Overall	489	18.39 ± 0.90	482.62 ± 15.97
Breed			
West African Dwarf	305	13.64 ± 0.65[b]	354.18 ± 11.24[b]
Kalahari Red	184	26.27 ± 2.01[a]	695.52 ± 32.58[a]
Week of lactation			
1	36	34.22 ± 5.92[a]	702.89 ± 92.78[a]
2	36	20.25 ± 4.06[b]	595.78 ± 71.20[ab]
3	36	15.14 ± 3.89[b]	514.22 ± 65.02[bc]
4	41	19.73 ± 3.39[b]	600.00 ± 57.12[ab]
5	44	18.16 ± 2.49[b]	513.82 ± 54.53[bc]
6	45	16.24 ± 1.77[b]	457.42 ± 51.19[c]
7	44	14.07 ± 2.14[b]	406.91 ± 38.61[c]
8	44	17.55 ± 2.41[b]	448.73 ± 43.02[c]
9	44	17.52 ± 2.73[b]	433.27 ± 41.10[c]
10	41	15.98 ± 2.55[b]	376.59 ± 39.16[c]
11	39	15.69 ± 2.23[b]	393.64 ± 45.23[c]
12	39	18.46 ± 2.57[b]	395.90 ± 42.63[c]
Litter size			
1	256	22.00 ± 1.53[a]	559.97 ± 26.88[a]
2	233	14.43 ± 0.79[b]	397.63 ± 13.63[b]
Age of doe			
≤2	100	15.35 ± 1.73[b]	407.36 ± 20.90[b]
3	168	14.83 ± 1.12[b]	420.62 ± 21.04[b]
≥4	221	22.48 ± 1.58[a]	568.80 ± 29.19[a]
Sex of kid			
Male	221	20.90 ± 1.61[a]	563.62 ± 30.65[a]
Female	186	15.22 ± 1.08[b]	382.15 ± 16.93[c]
Mixed	82	18.84 ± 1.87[ab]	492.20 ± 26.46[b]
Teat number			
2	264	14.53 ± 0.85	366.76 ± 13.30
3-4	170	20.58± 1.81	574.49 ± 32.11
5	55	30.15 ± 3.55	754.76 ± 57.68

[abc]Means in the same column with different superscripts for each sub-class differ significantly
(p<0.01); DMO=Daily milk offtake; DMY=Daily milk yield; n=Number of observations

cows with cylindrical shaped teats at similar lactation stage and age. The latter authors further reported from their investigation that cows with cylindrical shaped teat showed higher incidence of mastitis, while the effect of teat shape on milk yield was insignificant, corroborating an earlier report by Ozbeyaz et al. (1998). The predominance of oblique teat placement in both breeds was similar with the findings of De la Fuente et al. (1999) on sheep. Teat placement could serve as useful indicators for milk yield. Vertical teats comply with most

milking machines (Milkus, 1978) and hence preferable for dairy animals.

The observation that majority of individuals in both breeds had symmetrical udder is considered normal for both breeds; those with parity greater than 4 had pendulous udder is expected with increasing parities in view of the physiological changes that accompany lactation and drying up of the udder following successive parturitions. The low incidence of pendulous udder in WAD goat agreed with the observation of Chineme and Addo (1984); Kawu et al. (1992); Egwu et al. (1994) and Amao et al. (2003). Goats with pendulous udders are of little economic importance to dairy farmers because they are more susceptible to injuries (while grazing) and they are more difficult to milk (Mavrogenis *et al.,* 1988). Alawa and Oji (2008) observed that pendulous udder developed as a result of untreated mastitis. The frequency of asymmetrical udder recorded in WAD goats is higher than 0.4 % earlier reported by Amao et al. (2003) for the same breed while the estimate for KR does was similar to earlier report by the same authors for Red Sokoto goats. This disparity in the estimate for WAD goats could be attributed to increase in the frequency over time or differences in sample size.

The South African Boer Goat Breeders' Association (2013) reported variation in teat number of Boer goat ranging from two to eight, their acceptability depending on whether the teat is functionally effective (doe must be able to suckle their kids effectively). It is interesting from the current finding that the four teats observed in KR does appeared similar in shape and placement and were functional. Generally, most supernumerary teats were functional, with the exception of few that were very rudimentary in development (Figure 3f). There are indications from a report by Campbel (2003) that South Africa had two lines of KR goats of which one line was developed from red-head Boar goats while the other was developed from unimproved indigenous goats. This is likely to explain the high frequency of supernumerary teats in KR goats despite the small data size considered. The predominance of two teats in WAD is well supported by previous reports (Odubote, 1994; Ozoje, 2002; Amao *et al.,* 2003;

Bemji and Popoola, 2011). The high frequency of functional supernumerary teats in the KR breed could be considered an advantage since two or more kids could be suckled at a time in cases with higher litter size, compared to the WAD. Even though the presence of non-functional supernumerary teats could pose danger of malnutrition to neonates which get emotionally attached to such teats as reviewed by Bemji and Popoola (2011), it is not likely that the populations under consideration will be affected given that the frequency of individuals with supernumerary teats was low in the WAD population, while most supernumerary teats were functional in KR population.

Extra number of teats, whether functional or non-functional may not affect quantity of milk produced based on the current study in both breeds. It has been observed in cattle that about 50% of all cows have extra teats, referred to as supernumerary teats, some of which open into a normal gland, but many do not (http://ansci.illinois.edu/static/ansc438/Mamstructure/anatomy_4.html), a pseudo-teat has no streak canal, and therefore, no connection to the internal structures of the gland. Kukovics et al. (2006) similarly observed in sheep that udder traits based on types had little or no effect on milk yield but affected milkability, while udder traits based on size had strong effects on milk yield. The udder of goats consists of two halves each with a single mammary gland drained through a single teat (Džidic, 2004). Teats have no physiological roles in milk production but are connected to the gland cistern of the udder and through their orifices, serve to drain the udder of milk during suckling or milking. Based on observations during the current experiment, the presence of supernumerary teats caused difficulties during hand milking. The problems encountered in milking does and other species with supernumerary teats is also applicable even where machine milking is practiced; To avoid this situation, some commercial dairy farmers take the option of removing the additional teats surgically in the early stages of the animal's life (Brka *et al.,* 2002; Akpa *et al.,* 2010).

Conclusion

Comparatively, majority of KR goats had cylindrically shaped udders while more WAD goats having bowl shaped udders. With respect to teat shape, more KR had funnel shape while more WAD had cylindrical shaped teat. Both populations however had more individuals with oblique teat placement. Majority of individuals in both breeds had symmetrical udder which is considered normal while less that 2% of the all the breeds, mainly those with parity greater that 4 had pendulous udders. A striking feature about teat number is the preponderance of the normal 2-teat condition in WAD does while the 4-teat condition is most common in KR does. DMO and DMY were not significantly affected by teat number while breed effect was significant with indication that KR does produced about twice the amount of milk estimated for WAD does. Even though variation in teat number may be used as a parameter for milk yield, the result from this study and other similar studies could be used to set breed standard for KR and WAD populations. Further investigation should focus on inheritance of supernumerary teats in both breeds.

Acknowledgement

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GROWTH PERFORMANCE AND IMMUNITY STATUS OF STARTER BROILER BIRDS SUPPLEMENTED WITH NEEM (AZADIRACHTA INDICA) AND GARLIC (ALLIUM SATIVUM)

Muhammad S B[1], Sobayo R A[1], Adegbenjo A A, Sogunle O M[2], Oso O M[1] and Adeyemi O A[2]

[1]Department of Animal Nutrition

[2]Department of Animal Production and Health, Federal University of Agriculture, Abeokuta

Abstract

This study was conducted to investigate the effects of feeding diets containing Neem Leaf Meal (NLM), Garlic Meal (GM) and their combinations (NLM + GM) on growth performance and serum parameters of starter broiler birds. A total of 180 day-old Cobb broiler chickens were divided into twelve groups of fifteen chicks with three replicate of five chicks each. The diet contained NLM, GM and NLM + GM at four levels of inclusion (0ppm, 500ppm, 1000ppm and 1500ppm). The experiment was arranged in a 3 × 4 factorial layout in a completely randomized design. Additives and levels of inclusion had significant ($P<0.05$) influence on growth performance parameters. Final live weight (FLW) (725.69g/bird), weight gain (WG) (718.72 g/bird), daily weight gain (DWG) (25.67 g/bird/day) and feed intake (61.02 g/bird) of birds were increased ($p<0.05$) by NLM + GM compared to GM which was decreased. Weight gain (716.19g/bird) and daily weight gain (25.57g/bird) of birds were influenced at 1500ppm levels of inclusion than that of 1000ppm levels of inclusion. Highest ($P<0.05$) Feed intake of birds was recorded at 1000ppm (60.92 g/bird/day) but similar to 1500ppm (60.89g/bird/day) levels of inclusion compared to that of 0ppm inclusion levels which was lowered. Feed conversion ratio (FCR) was superior at 0ppm and 1500ppm levels of inclusion compared to 1000ppm inclusion levels. Improved ($P<0.05$) FLW (806.00 g/bird), WG (759.60 g/bird) and DWG (27.12 g/bird/day) were recorded at 1000ppm inclusion levels of NLM + GM compared to GM at 1000ppm levels of inclusion. Feed intake (67.32 g/bird/day) and mortality (20.00) of birds at 1500ppm inclusion of NLM + GM were elevated compared to 500ppm inclusion of NLM + GM which was depressed. Inclusion at different levels of added additives significantly ($P<0.05$) influenced FCR but was superior at 500ppm inclusion levels of NLM + GM than that of GM at 500ppm levels of inclusion. Additives and levels of inclusion had no significant ($P>0.05$) influence on serum parameters. Highest albumin content (3.30 g/dl) was recorded at 0ppm levels of inclusion of added additives but lowest at 1500ppm inclusion levels of NLM + GM. Decreased ($P<0.05$) amount of cholesterol (81.00mg/dl) and high density lipo-protein were achieved at 1500ppm inclusion levels of GM but was increased at 0ppm of added additives.

Additives had no significant ($P>0.05$) influence on serum parameters except MCHC which was influenced by NLM +GM. Inclusion levels at 1500ppm increased PCV, Hb and RBC but was decreased at 0ppm inclusion levels.

It can be concluded that feeding of NLM + GM at 1000ppm improved growth performance and additives at different levels affected the serum biochemical of the starter broiler chickens.

Keywords: Neem, garlic, starter broilers, growth performance, serum.

LA PERFORMANCE DE CROISSANCE ET LE PROFIL IMMUNITAIRE DES POULETS DE CHAIR DÉMARRAGE SUPPLÉMENTÉS AU GAROUSIER (AZADIRACHTA INDICA) ET L'AIL (ALLIUM SATIVUM)

Résumé

Cette étude a été réalisée pour étudier les effets des régimes alimentaires contenant la farine de feuille de garousier (FFG), la farine d'ail (FA) et leurs combinaisons (FFG + FA) sur la croissance et les paramètres sanguins des poulets de chair en démarrage. Les poulets de chair Cobb de 180 jours ont été divisés en douze groupes de quinze poussins avec trois répétitions de cinq poussins chacun. Le régime

Corresponding author: babatunde.muhammad9@gmail.com

alimentaire contenait la FFG, la FA et la FFG + FA à quatre niveaux d'inclusion (0mg/kg, 500mg/kg, 1000mg/kg et 1500mg/kg). L'expérience était effectuée suivant un dispositif factoriel de 3 × 4 complètement aléatoire. Les additifs et les niveaux d'inclusion ont eu une influence significative (P <0,05) sur les paramètres de croissance. Le poids vif final (PVF) (725,69 g / poulet), le gain de poids (GP) (718,72 g / poulet), le gain de poids quotidien (GPQ) (25,67 g / poulet / jour) et la consommation alimentaire journalière (61,02 g / poulet) des poulets FFG + FA ont augmenté (p <0,05) par rapport aux poulets FA. La consommation alimentaire des poulets la plus élevée (P <0,05) a été enregistré à 1000mg/kg (60,92 g / poulet / jour), mais similaire aux niveaux d'inclusion de 1500mg/kg (60.89g / poulet / jour) par rapport à celui des niveaux d'inclusion de 0mg/kg. Le taux de conversion des aliments (TCA) était supérieur aux niveaux d'inclusion de 0mg/kg et 1500mg/kg par rapport aux niveaux d'inclusion de 1000mg/kg. La consommation alimentaire (67,32 g / poulet / jour) et la mortalité (20.00) des poulets à l'inclusion 1500mg/kg de la FFG + FA étaient élevés par rapport à l'inclusion 500mg/kg de la FFG + FA. L'inclusion à des niveaux différents des additifs a de manière significative (P <0,05) influencé le TAC, qui était supérieure au niveau d'inclusion 1500mg/kg de la FFG + FA comparé à ceux de la FA à 500mg/kg des niveaux d'inclusion. Les additifs et les niveaux d'inclusion n'ont aucune influence significative (P> 0,05) sur les paramètres sanguins. La plus haute teneur en albumine (3,30 g / dl) a été enregistrée à 0mg/kg des niveaux d''inclusion des additifs mais plus bas à 1500mg/kg des niveaux d'inclusion de la FFG + FA. La quantité de cholestérol (P <0,05) diminuée (81.00mg / dl) et la haute densité de lipoprotéines ont atteints des niveaux d'inclusion de 1500mg/kg de la FA, mais augmenté à 0mg/kg d'additifs. Les additifs n'ont pas d'influence (P> 0,05) sur les paramètres sanguins sauf le MCHC qui était influencé par la FFG + FA. Les niveaux d'inclusion à 1500mg/kg ont augmenté les PCV, Hb et RBC comparé au niveau 0mg/kg. On peut donc conclure que l'alimentation des FFG + FA à1000mg/kg améliore la performance de croissance et les additifs à des niveaux différents affectent le profil sanguin des poulets de chair en démarrage.

Mots-clés : le garousier, l'ail, poulets de chair en démarrage, croissance, profil sanguin.

Introduction

Feed additives are products used in animal nutrition for purposes of improving the quality of feed, animal's performance and health. The use of antibiotics in diets arose from the discovery in the late 1940's, in the United States that including the fermentation products of Streptomyces aureofaciens (a strain of bacteria) in the diets of simple-stomached animals such as pigs and poultry resulted in growth responses (Frost, 1991). The use of antibiotic-based growth promoters is presently facing serious criticism and has raised global concern as some reports revealed their ill effects among which are development of microbial resistance to the products and their potential harmful effects on human health (Rahmatnejad et al. 2009). The practice of complementary and alternative medicine is now on the increase in developing countries in response to World Health Organization directives culminating in several pre-clinical and clinical studies that have provided the scientific basis for the efficacy of many plants used in folk medicine to treat infections (Iwalokun

et al., 2004). Medicinal plants are cheap and renewable sources of pharmacological active substances and are known to produce certain chemicals that are naturally toxic to bacteria (Basil et al., 1999). Availability, easy usage and non-side effects of herbs make it possible for the treatment of diseases since long time ago. Some of the medical effects of herbs are related to their secondary metabolites such as phenols, necessary oils and saponins, etc. (Fritz et al., 1993).

They also exert certain immunological consequences in birds (kong et al., 2006). Research on the use of herbal mixtures in bird's diets has produced inconsistent results (Fritz et al., 1993).Improvement in nutrients digestibility of broiler diets using medicinal plants mixtures was reported by(Abaza 2001, Al-Harthi, 2002 and ElHusseiny et al. 2002).Findings by Lewis et al. (2003) showed that addition of plant extracts to broilers' diet has some effects on performance and microbial activity of intestinal tract. This was also supported by Peric et al., (2008) where they reported significant positive effects on performance. Whereas Cross et al., (2007) and Ocak et al., (2008) established

no influence on gain, feed intake and feed conversion. Garlic (Allium satium) is very rich in aromatic oils, which enhance digestion and positively influence respiratory system being inhaled into air sacs and lungs of birds. Also, it was found that garlic has strong anti-oxidative effects (Gardzielewska *et al.,* 2003).

Neem (Azadiracta indica) belongs to the family Meliaceae family and a fast growing evergreen tree as a potential to provide medicinal and nutritive value to broiler (Schmutterer, 1990). Various parts of the tree have been reported to contain certain chemicals like azadiractine, nimbin, nimbindin, quercetin among others (Makeri 2007).

Hence, the current study focuses on examining the effect of neem and garlic leaf meal on growth parameters and serum biochemical of starter broiler chickens.

Materials and Methods

This experiment was carried out at the Directorate of University Farms, Federal University of Agriculture, Abeokuta, Ogun State. The area lies on latitude 7010'N and longitude 30 2'E. It is 76m above sea level and located in the tropical rain forest vegetation zone with an average temperature of 34.70C and relative humidity of 82% (Google Earth, 2012). Allium sativum (garlic) powder was prepared by cutting garlic bulbs into small pieces, followed by sun-drying for 14 days (≤ 90% DM) and pulverised using laboratory mill (1mm sieve) while Azadirachta indica leaves were removed from the stalk and air dried under a shed (29±20C) until they are crispy to touch, while still retaining their greenish colouration, milled using a laboratory mill (1mm).

Management of experimental birds and diets

Twelve experimental diets were formulated with the inclusion of neem, garlic and their combination for finishing broiler.

Diet 1 - 4 contained inclusion of NLM at 0 mg/kg, 500 mg/kg, 1000 mg/kg, and 1500 mg/kg, Diet 5 - 8 contained inclusion of GM at 0 mg/kg, 500 mg/kg, 1000 mg/kg, and 1500 mg/kg, Diet 9 - 12 contained inclusion of NLM + GM each at 0 mg/kg, 250 mg/kg, 500mg/kg and 750mg/kg.

One hundred and eighty (180) unsexed day old Cobb broilers were used for the experiment. They were divided into twelve treatment groups of fifteen birds. Each treatment group was replicated thrice with five birds per replicate in a 3×4 factorial experimental design. Brooding of birds was done for three weeks using charcoal and bulbs as source of heat. The birds were fed *ad libitum* and managed intensively throughout the duration of the experiment.

Data collection

Records of weight gain (g)/bird: (final weight - initial weight), feed intake (g)/bird: (feed supplied – left over/ number of birds), mortality: (number of dead birds/total number of birds × 100) and feed conversion ratio: (total feed intake/total body weight gain) were obtained.

At 4th weeks of the study, 2.5 ml of blood was collected into labelled sterile sample bottles without anticoagulant and used to determine the serum biochemical indices following standard clinical procedures (Olorede *et al.,* 1996). Cholesterol content of blood was determined using analytical method as described by Coles (1986).

Statistical analysis

Data collected were subjected to 3×4 factorial arrangement in a completely randomized design. Significant (p<0.05) differences among treatment means were determined using Duncan Multiple Range Test (Duncan 1955) as contained in Statistical Analysis Software (SAS 2000) package.

Results

Table 2 shows the effect of additives and levels of inclusion on growth performance of starter broiler chickens. There was significant (P<0.05) effect on final live weight, weight gain, daily weight gain and feed intake of birds. Improved Final live weight (764.31g/bird), weight gain (718.72g/bird), daily weight gain (25.67g/bird) and feed intake (61.02g/bird) were recorded in birds fed NLM + GM diets

Table1: Gross Composition (%) of experimental Diets (Starter)

Dietary treatments	NLM (mg/kg)				GM(mg/kg)				NLM + GM (mg/kg)			
Ingredients	0	500	1000	1500	0	500	1000	1500	0	500	1000	1500
Maize	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00
Wheat offal	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00
SBM	22.00	22.00	22.00	22.00	22.00	22.00	22.00	22.00	22.00	22.00	22.00	22.00
PKC	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
GNC	10.30	10.30	10.30	10.30	10.30	10.30	10.30	10.30	10.30	10.30	10.30	10.30
FM (72%)	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Bone meal	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Oyster shell	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Lysine	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Methionine	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
GM	-	-	-	-	-	+	++	+++	-	-	-	-
NLM	-	+	++	+++	-	-	-	-	-	-	-	-
NLM +GM	-	-	-	-	-	-	-	-	-	+	++	+++
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated Chemical Composition (%)												
ME(kcal/kg)	2835.13	2835.13	2835.13	2835.13	2835.13	2835.13	2835.13	2835.13	2835.13	2835.13	2835.13	2835.13
Crude Protein	22.40	22.40	22.40	22.40	22.40	22.40	22.40	22.40	22.40	22.40	22.40	22.40
Crude Fibre	3.98	3.98	3.98	3.98	3.98	3.98	3.98	3.98	3.98	3.98	3.98	3.98
Fat	4.29	4.29	4.29	4.29	4.29	4.29	4.29	4.29	4.29	4.29	4.29	4.29
Calcium	1.66	1.66	1.66	1.66	1.66	1.66	1.66	1.66	1.66	1.66	1.66	1.66
Phosphorus	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82

Table 2: Main effects of NLM, GM, NLM + GM and Levels of inclusion on growth performance of starter broiler chickens (0-4weeks)

Parameters	Additives				Levels of inclusion (mg/kg)				
	NLM	GM	NLM + GM	SEM	0	500	1000	1500	SEM
IW(g/bird)	47.00	46.50	45.58	0.764	45.89	46.22	47.11	46.22	0.882
FLW(g/bird)	725.69ab	687.92b	764.31a	18.949	740.74	707.22	693.52	762.41	21.881
WG(g/bird)	678.69ab	641.42b	718.72a	18.779	694.85ab	661.00ab	646.41b	716.19a	21.684
DWG(g/bird/day)	24.24ab	22.91b	25.67a	0.671	24.82ab	23.61ab	23.09b	25.57a	0.774
FI(g/bird/day)	59.13ab	58.08b	61.02a	0.668	57.18b	58.66ab	60.92a	60.89a	0.772
MOR	8.33	6.67	11.67	2.72	6.67	6.67	11.11	11.11	3.143
FCR	2.47	2.57	2.38	0.07	2.30b	2.51ab	2.67a	2.39b	0.079

ab means on the same row having different superscripts were significantly different (P<0.05)
IW=Initial Weight, FLW=Final Live-weight, WG=Weight Gain, DWG=Daily Weight Gain, FI=Feed Intake, MOR=Mortality, FCR=Feed Conversion Ration
GM= Garlic Meal NLM= Neem Leaf Meal

Table 3: Interaction effects of NLM, GM, NLM + GM and Levels of inclusion on Growth performance of starter broiler chickens (0-4 weeks)

Additives	NLM				GM				NLM + GM				SEM
Inclusion Levels (mg/kg)	0	500	1000	1500	0	500	1000	1500	0	500	1000	1500	SEM
IW(g/bird)	46.67	47.33	48.00	46.00	46.00	46.00	47.33	46.67	45.00	45.33	46.00	46.00	1.528
FLW(g/bird)	752.22ab	710.00abc	662.00bc	778.00ab	730.00abc	675.00bc	613.00c	733.00abc	740.00abc	737.00abc	806.00ab	775.00ab	37.899
WG(g/bird)	705.60ab	662.60abc	613.60bc	732.80ab	684.00abc	629.00bc	566.00c	686.70abc	695.00ab	691.30ab	759.60ab	729.00ab	37.558
DWG (g/bird/day)	25.20ab	23.70abc	21.91bc	26.71ab	24.43abc	22.46bc	20.21c	24.52abc	24.82ab	24.70ab	27.12a	26.03ab	1.341
FI(g/bird/day)	58.17cde	57.29cde	61.50bc	59.61cd	56.17de	64.14ab	56.25de	55.8de	57.18cde	54.54e	65.04ab	67.32a	1.336
MOR	6.67ab	6.67ab	6.67ab	13.33ab	6.67ab	13.33ab	6.67ab	0.00b	6.67ab	0.00b	20.00a	20.00a	5.443
FCR	2.31c	2.44bc	2.81b	2.29c	2.29c	2.89a	2.79ab	2.31c	2.30c	2.21c	2.40bc	2.59abc	0.136

abcde means on the same row having different superscript were significantly different (P<0.05)
IW=Initial Weight, FLW=Final Live-weight, WG=Weight Gain, DWG=Daily Weight Gain, FI=Feed Intake, MOR=Mortality, FCR=Feed Conversion Ration
GM= Garlic Meal   NLM= Neem Leaf Meal

Table 4: Main effect of NLM, GM, NLM + GM and Levels of inclusion on Serum parameters of broiler chickens at starter phase (0-4 weeks)

Parameters	Additives				Levels of inclusion (mg/kg)				
	NLM	GM	NLM + GM	SEM	0	500	1000	1500	SEM
Total protein (g/dl)	5.14	5.21	5.09	0.237	5.15	5.13	5.33	4.97	0.274
Albumin (g/dl)	2.96	3.06	3.05	0.190	2.93	2.98	3.30	2.88	0.220
Globulin (g/dl)	2.18	2.15	2.04	0.120	2.22	2.15	2.03	2.08	0.139
Glucose (mg/dl)	120.63	114.75	121.00	3.599	118.67	122.67	115.33	118.50	4.156
AST (u/l)	51.50	60.75	60.13	4.829	49.50	62.33	62.83	55.17	5.576
ALT (u/l)	27.00	26.50	25.00	1.729	27.00	23.17	28.17	26.33	1.997
Urea (mg/dl)	1.91	2.10	2.36	0.194	2.25	2.10	2.00	2.15	0.224
Cholesterol (mg/dl)	101.38	106.75	100.50	3.407	105.00	101.33	101.10	104.17	3.934
Triglyceride (mg/dl)	110.13	107.00	108.00	1.924	104.00	110.67	109.83	109.00	2.222
HDL (mg/dl)	61.50	68.63	62.13	3.776	66.50	61.17	62.00	66.67	4.360
LDL (mg/dl)	17.85	16.73	16.78	0.971	17.70	18.03	17.03	15.70	1.121
VLDL (mg/dl)	22.03	21.40	21.60	0.385	20.80	22.13	21.97	21.80	0.444

GM= Garlic Meal   NLM= Neem Leaf Meal AST: Aspartate transaminase ALT: Alanine transaminase HDL: High density lipo-protein LDL: Low density lipo-protein VLDL: Very low density lipo-protein

Table 5: Interaction effect of NLM, GM, NLM + GM and Levels of inclusion on Serum parameters of broiler chickens at starter phase (0-4 weeks)

Inclusion Levels (mg/kg)	NLM				GM				NLM + GM				
	0	500	1000	1500	0	500	1000	1500	0	500	1000	1500	SEM
Total protein (g/dl)	5.20	4.65	4.40	3.85	5.20	4.30	4.80	3.95	5.20	4.35	4.60	3.85	0.485
Albumin (g/dl)	3.30a	2.70ab	2.35ab	2.10ab	3.30a	2.30ab	2.95ab	2.45ab	3.30a	2.80ab	2.70ab	2.05b	0.354
Globulin (g/dl)	1.90	1.95	2.05	1.75	1.90	2.00	1.85	1.50	1.90	1.90	1.90	1.80	0.284
Glucose (mg/dl)	119.00	128.50	112.50	112.50	119.00	109.00	111.00	112.00	119.00	128.00	105.00	123.00	8.142
AST (u/l)	70.00	70.50	62.00	53.50	70.00	71.50	56.50	55.50	70.00	75.00	56.50	51.00	7.220
ALT (u/l)	25.50	33.50	28.00	32.00	25.50	24.00	31.00	25.50	25.50	26.50	29.00	26.00	3.247
Urea (mg/dl)	2.40	1.95	2.35	1.60	2.40	1.45	2.10	2.50	2.40	1.90	1.95	2.25	0.494
Cholesterol (mg/dl)	101.50a	92.00ab	98.00a	96.00ab	101.50a	92.50ab	98.00a	81.00b	101.50a	105.00a	98.00a	100.50a	4.495

Inclusion Levels (mg/kg)	NLM				GM				NLM + GM				SEM
	0	500	1000	1500	0	500	1000	1500	0	500	1000	1500	
Triglyceride (mg/dl)	107.50	109.50	110.00	110.00	102.00	107.50	108.00	111.50	113.50	107.50	107.50	106.50	5.590
HDL (mg/dl)	61.65[a]	57.90[ab]	58.55[ab]	60.75[a]	61.65a	51.50[ab]	56.00[ab]	41.85[b]	61.65[a]	66.85[a]	57.40[ab]	60.30[a]	5.273
LDL (mg/dl)	18.35	17.70	17.45	14.85	18.35	19.40	19.70	16.95	18.35	16.65	19.10	19.00	1.775
VLDL (mg/dl)	21.50	21.90	22.00	20.40	21.50	21.60	22.30	22.70	21.50	21.50	21.50	21.20	1.118

[ab] means on the same row having different superscript were significantly different (P<0.05)
GM= Garlic Meal   NLM= Neem Leaf Meal   GM= Garlic Meal   NLM= Neem Leaf Meal   AST:Aspartate transaminase ALT:Alanine transaminase HDL: High density lipo-protein LDL: Low density lipo-protein VLDL:Very low density lipo-protein

compared to GM diets which had the least values (687.92g/bird, 641.42g/bird, 22.91g/bird, 58.08g/bird) respectively. Levels of inclusion revealed significant (P<0.05) effect on weight gain, daily weight gain, feed intake and feed conversion ratio. WG (716.19g/bird) and DWG (25.57g/bird) were influenced at 1500ppm levels of inclusion compared to 1000ppm levels of inclusion (646.41g/bird, 23.09g/bird). Highest FI was recorded at 1000ppm levels of inclusion (60.89g/bird/day) but similar to that of 1500ppm (60.89g/bird/day) compared to 0ppm levels of inclusion (57.18g/bird/day). Feed conversion ratio (2.30) of birds on 0ppm levels of inclusion and1500ppm levels of inclusion (2.39) were similar and superior than that of1000ppm inclusion levels (2.67)

Data on interaction of additives and levels of inclusion on growth performance of starter broiler chickens are presented in Table 3. Final live weight, weight gain, daily weight gain, Feed intake, mortality and feed conversion ratio were significantly (P<0.05) affected. FLW (806.00g/bird) of birds fed 1000ppm inclusion of GM + NLM was elevated compared to 1000ppm inclusion of GM (613.00g/bird) which was depressed. WG (759.60g/bird) and DWG (27.12g/bird) followed the same trend with 1000ppm inclusion of NLM+ GM better than that of 1000ppm inclusion of GM (566.00g/bird, 20.21g/bird). Birds on 500ppm inclusion of NLM + GM recorded lower FI (54.54g/bird/day) and mortality (0.00) compared to that of NLM + GM at 1500ppm inclusion levels. FCR was influenced by additives at various inclusion levels but superior in birds fed 500ppm inclusion of NLM + GM (2.21).

Table 4 shows the effects of additives and levels of inclusion on serum parameters of starter broiler chickens revealed no significant (P>0.05) effect on serum parameters.

Data on interaction effects of additives and levels of inclusion on serum parameters of starter broiler chickens are presented in Table 5. Albumin, cholesterol and HDL were significantly (P<0.05) affected. Albumin (3.30g/dl) of birds was influenced at 0ppm inclusion levels compared to NLM + GM at 1500ppm levels of inclusion(2.05g/dl).Decreased cholesterol (81.00mg/dl)and HDL (41.85mg/

Table 5: Main effects of Additives and Levels of inclusion on Haematological parameters of broiler chickens at starter phase (0-4 weeks)

Parameters	Additives				Levels of inclusion (mg/kg)				
	NLM	GM	NLM + GM	SEM	0	500	1000	1500	SEM
PCV (%)	29.38	31.75	30.63	1.696	28.00[b]	30.83[ab]	28.00[b]	35.50[a]	1.958
Hb (g/dl)	7.75	7.74	8.45	0.441	7.25[b]	8.32[ab]	7.32[b]	9.03[a]	0.509
RBC (×10^{12}/l)	1.98	2.11	2.04	0.119	1.75[b]	2.08[ab]	1.85[b]	2.48[a]	0.137
WBC (×10^{9}/l)	11.20	10.90	11.74	0.345	10.80	11.87	10.77	11.68	0.398
HET (%)	27.75	29.25	29.38	1.434	30.00	29.67	27.33	28.17	1.656
LYM (%)	70.88	69.63	69.50	1.339	58.50	69.83	72.00	69.67	1.546
EOS (%)	0.38	0.25	0.38	0.177	0.50	0.167	0.00	0.67	0.204
BAS (%)	0.25	0.63	0.63	0.270	1.00	0.167	0.17	0.67	0.312
MONO (%)	1.00	0.50	0.38	0.331	1.00	0.167	0.50	0.83	0.382
MCV (fl)	151.00	150.20	155.54	6.492	159.80	150.20	157.10	141.89	7.497
MCH (Pg)	39.76	36.99	43.21	2.724	41.30	40.45	41.53	36.67	3.145
MCHC (g/dl)	26.26[ab]	24.66[b]	27.59[a]	0.900	25.85	26.83	26.02	25.98	1.039

[ab] means on the same row having different superscript were significantly different (P<0.05)
GM= Garlic Meal   NLM= Neem Leaf Meal, PCV: Packed cell volume Hb: haemoglobin, RBC: Red blood cell WBC,: White blood cell, HET: Heterophil, LYM: Lymphocyte, EOS: Eosinophil, BAS: Basophil, MONO: Monocyte, MCV: Mean corpuscular volume MCH: Mean corpuscular haemoglobin MCHC: Mean corpuscular haemoglobin concentration.

Table 6: Interaction effect of NLM, GM, NLM + GM and Levels of inclusion on Haematological parameters of broiler chickens at starter phase (0-4 weeks)

I Levels (mg/kg)	NLM				GM				NLM + GM				SEM
	0	500	1000	1500	0	500	1000	1500	0	500	1000	1500	
PCV (%)	25.00	22.50	27.00	22.50	25.00	24.00	24.00	22.50	25.00	23.50	23.50	25.00	2.650
Hb (g/dl)	6.40	5.85	7.35	6.10	6.40	6.70	6.40	7.60	6.40	5.95	8.00	7.15	0.730
RBC(×1012/l)	1.85	1.65	2.20	1.80	1.85	1.55	1.90	1.95	1.85	1.60	1.80	1.80	0.225
WBC(×109/l)	10.05	10.10	11.05	10.95	10.05	10.70	10.60	11.10	10.05	11.55	9.40	11.60	0.676
HET (%)	30.50[abc]	33.50[ab]	36.50[a]	23.00[c]	30.50[abc]	28.50[abc]	25.00[bc]	28.50[abc]	30.50[abc]	25.00[bc]	24.50[bc]	33.50[ab]	3.003
LYM (%)	68.50[ab]	65.50[ab]	61.50[b]	75.00[a]	68.50[ab]	70.00[ab]	74.00[a]	69.50[ab]	68.50[ab]	72.50[a]	74.50[a]	65.50[ab]	2.905
EOS (%)	0.00[b]	0.50[ab]	0.50[ab]	0.50[ab]	0.00[b]	0.50[ab]	0.00[b]	0.50[ab]	0.00[b]	1.50[a]	0.00[b]	0.50[ab]	0.382
BAS (%)	0.50	0.00	0.00	1.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.00	0.433
MONO (%)	0.50	0.50	1.50	0.00	0.50	0.50	0.50	1.00	0.50	0.50	0.50	0.50	0.540
MCV (fl)	134.65[abc]	137.25[abc]	125.85[bc]	125.25[bc]	134.65[abc]	154.60[a]	125.85[abc]	120.00[c]	134.65[abc]	147.20[bc]	130.00[abc]	139.30[abc]	7.431
MCH (Pg)	84.80	36.05	34.00	34.10	84.80	46.80	33.45	39.80	84.80	37.00	44.65	39.65	24.371
MCHC(g/dl)	25.80[b]	26.20[b]	27.10[b]	27.15[b]	25.80[b]	28.15[ab]	26.50[b]	33.65[a]	25.80[b]	25.15[b]	34.15[a]	28.55[ab]	1.887

[abc] means on the same row having different superscript were significantly different (P<0.05)   I Levels: Inclusion levels
GM= Garlic Meal NLM= Neem Leaf Meal GM= Garlic Meal   NLM= Neem Leaf Meal PCV: Packed cell volume Hb: haemoglobin RBC: Red blood cell WBC: White blood cell HET: Heterophil LYM: Lymphocyte EOS: Eosinophil BAS: Basophil MONO: Monocyte MCV: Mean corpuscular volume MCH: Mean corpuscular haemoglobin MCHC: Mean corpuscular haemoglobin concentration.

dl) were recorded at 1500ppm inclusion of GM compared to NLM + GM at 500ppm levels of inclusion which was increased.

Haematological parameters of broiler chickens fed experimental diet at starter phase (0-4 weeks)

Haematological parameters of broiler chickens fed NLM, GM, NLM + GM and levels of inclusion are presented in Table 6. There was significant ($P<0.05$) effect on MCHC of birds fed NLM, GM, NLM + GM. MCHC of birds at NLM + GM recorded the highest value (27.59g/dl) compared to GM which had the least value (24.66g/dl).

Levels of inclusion revealed significant ($P<0.05$) effect on PCV, Hb and RBC. PCV (35.50%), Hb (9.03g/dl), RBC (2.48×10^{12}/l) of birds on 1500ppm levels of inclusion recorded the best values compared to 1000ppm levels of inclusion which had the least values (28.00%, 7.25g/dl, 1.75×10^{12}/l) respectively.

Interaction effects on haematological parameters of broiler chickens fed NLM, GM, NLM + GM and levels of inclusion are presented in table 7. Interaction showed significant ($P<0.05$) effect on Heterophil, Lymphocytes, Eosinophil, Mean Corpuscular Volume (MCV) and Mean Corpuscular Haemoglobin Concentration (MCHC). Heterophil of birds at 1000ppm inclusion of NLM had the highest (36.50%) and lowest value (23.00%) at 1500ppm inclusion of NLM. Also, Lymphocytes of birds at 1500ppm inclusion of NLM had the highest (75.00%) and lowest value (61.50%) at 1000ppm inclusion. Eosinophil of birds at 500ppm inclusion of NLM + GM recorded the highest value (1.50%) compared to 0ppm inclusion of the additives which had the least value (0.00%). MCV of birds at 500ppm inclusion of GM had the highest (154.60fl) and lowest value (120.00fl) at 1500ppm levels of inclusion. MCHC (34.15g/dl) of birds at 1000ppm inclusion of NLM + GM recorded the best compared to 0ppm inclusion of all the additives which had the least values (25.80g/dl), respectively.

Discussion

The improvement achieved in starter broiler chickens fed NLM + GM was in line with the findings of Ademola et al. (2009) who reported increase in final body weight and weight gain of broilers fed a mixture of garlic and ginger. These observations were also reported by Shi et al. (1999) and Javandel et al. (2008). This result was against the findings of Hernandez et al.(2004) who reported that herbs, plant extracts, essential oil and/or the main components of the essential oil do not affect body weight gain, feed intake or feed efficiency in broilers.

Interaction effect revealed significant effect of NLM + GM inclusion at 1000ppm on final live weight, weight gain and daily weight gain. The increase in weight could be due to improved intestinal secretion of mucus in broilers, an effect that was assumed to impair adhesion of pathogens and thus contribute to stabilizing the microbial eubiosis in the gut of the animals (Jamroz *et al.,* 2006). The decreased in feed intake by NLM + GM at 500ppm levels of inclusion was against the results of Fadlalla *et al.,*(2010) and Onibi *et al.,* (2009); who found no difference between control group and broilers fed with garlic in both body weight gain and feed intake. Improved feed conversion ratio achieved by NLM + GM at 500ppm was in consonance with findings of Endens et al. (2003) who reported that probiotics improved digestion, absorption and availability of nutrition accompanying with a positive effect on intestine activity and increasing digestive enzymes.

The non- significant differences observed in additives at starter broiler chickens on serum parameters could be that the immune system of the birds was adequate. This agreed with result of Awosanya et al. (1999) that blood protein depends on the quality and quantity of dietary protein.

Observed values at interaction levels of inclusion of the added additives in albumin were a bit higher than the range of 1.25g/dl to 2.20g/dl observed by Akinmutimi and Onen (2008).

The significant effect of GM inclusion at 1500ppm on cholesterol goes with evidence in the literature that garlic has cholesterol-lowering effect in humans and animals due to the presence of sulphur-containing bioactive compounds in its homogenates (Chowdhury

et al., 2002). The increase in HDL achieved with NLM + GM at inclusion levels of 500ppm could be as a result of improved harmonious gut environment suitable for the release and assimilation of digestive nutrients necessary for growths (Elangovan *et al.,* 2000).

The non-significant differences in haematological indices that occurred at starter broiler chickens in this experiment could infer that the health status were within safety limits for broiler. This was in line with the results of (Ologhobo *et al.,* 2013) that Moringer oleifera leaf meal had no significant influence on haematological parameters of broiler chickens.

The normal PCV, Hb and other haematological values portray the nutritional status of the broiler chicken and thus indicating adequate nourishment of the birds Church et al. (1984). The haematological parameters of birds at different levels of inclusion of starter broiler chickens revealed 1500ppm levels of inclusion increased PCV, Hb and RBC. The obtained PCV values were within the reference range of 24.9 - 45.2% for healthy birds reported by Mitruka and Rawnsley (1977). The Hb values were close to the range of 7.40g/dl - 13.10g/dl for healthy birds (Mitruka and Rawnsley, 1977) suggesting anaemia. The RBC values were less than the range 3.7 - 7.5x106µl reported by Hewitt et al. (1989).

Interaction effect shows that haematological indices were not significant except for Heterophil, Lymphocytes, Eosinophil, MCV and MCHC. However the values were in harmony with the normal range for healthy broiler chickens as reported by Awaad and Zouelfeker (2001) and Campbell et al. (2003). This also portrays the nutritional status of the broiler chicken and thus indicating adequate nourishment of the birds (Church *et al.,* 1984).

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EFFECT OF YOGHURT WASTE ON GUT MORPHOLOGY AND GROWTH PERFORMANCE OF PIGLETS WEANED AT 7 WEEKS.

Nortey T N[1], Danquah H P[1], Naazie A[2], Tudeka A[2] and Kpogo A L[1*].
[1]Department of Animal Science, School of Agriculture College of Basic and Applied Sciences, University of Ghana, P. O. Box LG 226 Legon, Accra.
[2]Livestock and Poultry Research Centre, University of Ghana, P. O. Box LG 38, Legon
[§]Present address: Maridav Ghana Ltd, PMB KA 144, Airport, Accra-Ghana.

Summary

The experiment was carried out to determine the effect of yoghurt waste on intestinal morphology and growth performance of pigs weaned at 7 weeks of age. A total of 20 weaned pigs (15.6 ± 2kg, initial body weight {BW}) were randomly assigned in groups of four, to 5 experimental treatments in a randomized block design. Pigs were fed one of five diets for 28d. Treatment 1 (T1) was a standard weaner diet containing 28% soybean meal (SBM) and 2369 Kcal/kg net energy (NE), 19.25% crude protein (CP), 0.98% standardized ileal digestible (SID) lysine (Lys), 0.91% calcium (Ca) and 0.6% phosphorus (P). Treatment 2 (T2) to T5 contained 2.5, 5, 7.5 and 10% yoghurt waste (89% dry matter {DM}) at the expense of SBM resulting in a total dietary lactose content of 0, 0.6, 1.2, 1.8 and 2.4 % for T1 to T5 respectively. Pigs on T5 recorded the highest (P<0.05) average daily feed intake (ADFI) and the best average daily weight gain (ADG: 1.42kg/d and 0.85kg/d respectively). Intestinal microbial populations were not affected by treatment. However at the terminal ileum, villi heights of pigs on T5 were greater than all other treatments. Formulating weaner diets with 10% yoghurt waste can improve ADG and feed conversion efficiency (FCE) and also improve villi heights in the terminal ileum.

Keywords: Dietary yoghurt waste; Weaned pigs; Growth performance; Gut morphology.

L'EFFET DES DÉCHETS DE YAOURT SUR LA MORPHOLOGIE DE L'INTESTIN ET LA PERFORMANCE DE LA CROISSANCE DES PORCELETS SEVRÉS À 7 SEMAINES.

Résumé

L'expérience a été effectuée pour déterminer l'effet des déchets de yaourt sur la morphologie intestinale et la croissance des porcs sevrés à l'âge de 7 semaines. 20 porcelets sevrés (15,6 ± 2 kg, poids corporel initial {PC}) ont été répartis au hasard en groupes de quatre, à 5 traitements expérimentaux dans une conception de blocs aléatoires. Les porcs nourris avec un des cinq régimes pour 28 jours. Le Traitement 1 (T1) était un régime standard de l'animal sevré contenant 28% de tourteau de soja (TDS) et 2369 Kcal / kg d'énergie nette (EN), 19,25% de protéines brutes (PB), 0,98% d'iléal digestible normalisée (IDN) la lysine (Lys), 0,91% de calcium (Ca) et 0,6% de phosphore (P). Le Traitement 2 (T2) au T5 contenait 2,5, 5, 7,5 et 10% de déchets de yaourt (89% de matière sèche {MS}) en substitution de TDS résultant et avait une teneur en lactose alimentaire totale de 0, 0,6, 1,2, 1,8 et 2,4% pour le T1 au T5 respectivement. Les porcs sur T5 ont enregistré la plus forte (P <0,05) consommation alimentaire moyenne quotidienne (CAMQI) et le meilleur gain de poids moyen quotidien (GMQ) de 1,42 kg / j et 0,85 kg / j respectivement. Les populations microbiennes intestinales n'étaient pas affectées par le traitement. Cependant, à l'iléon terminal, les hauteurs de villosités de porcs sur le T5 étaient supérieures à tous les autres traitements. Formuler les régimes de sevrage avec 10% de déchets de yaourt peut améliorer le GMQ et l'efficacité de la conversion alimentaire (ECA) et aussi améliorer les hauteurs de villosités dans l'iléon terminal.

Mots-clés: les déchets de yaourt diététique; les porcs sevrés; Les performances de croissance; la morphologie intestinale.

*Corresponding author email: tnortey@ug.edu.gh

Introduction

Feed costs make up 60 to 75% of overall pig production and is a major contributor to the cost of raising pigs (Gillespie and Flanders, 2010). Thus for maximum return on investment in any swine operation, there is the need for efficient management of feeding and feed resources. Efficient feeding entails providing the right quantity and quality of feed at any particular stage of physiological growth. Weaning and the transition to solid diet are stressful to the piglet and according to Milan and Kreing (2011) the ideal time for piglets to be introduced to solid feed successfully without any noticeable change in growth or health is around four weeks of age. This is possible if highly digestible and complex diets which take into consideration the under-developed GIT of the piglet, are fed. However for most rural farmers in Africa, this can be a challenge because the majority of feed ingredients are based on agro-industrial by-products. These by-products are high in fibre and are not easily digestible by the young piglet. Such ingredients when fed to young pigs present major challenges by imposing severe post-weaning-growth-checks and by pre-disposing the piglet to bouts of severe diarrhoea. At this time, major changes occur in the digestive physiology and immune status of the young animal and as a result, the digestive system must adapt with respect to pH regulation, enzyme secretions, motility and absorption (Makkink, 1993). The subsequent development of the pig, its feed efficiency and effective growth is entirely dependent on how successful the period immediately after weaning is handled.

Diets which include ingredients like milk and dairy products, fishmeal, soy proteins, spray-dried-plasma, and an excellent balance of essential amino acids are preferred immediately post-weaning. However high quality protein feed sources are very expensive and their inclusion in early weaner diets can result in high cost of production. Most of these ingredients are not available in some developing countries like Ghana, and where present tend to be very expensive and unaffordable by most small to medium scale farmers. For this reason most pig farmers tend to wean at 6 - 8 weeks of age. By this age, it is hoped that the piglets' GIT'S would have developed well enough to handle diets based on available and less digestible nutrients. This practice is however counterproductive, since by weaning late the period from farrowing to oestrus is extended. This results in less farrowings per sow per year and an eroding away of farmers' profits. The case for making farmers wean their piglets earlier than 7 weeks will be made stronger if cheap alternative high quality protein sources which are highly digestible for use in weaner diets are identified and proved to be effective.

One such product which shows potential is yoghurt waste. It is a by-product of yoghurt manufacture and is produced by a multi-national milk processing plant based in Accra. This multi-national company also has branches in most West African countries including Nigeria, Liberia and Sierra Leone, to name a few. In addition to yoghurt, this dairy processing plant produces ice cream, chocolate milk and citrus fruit drinks. At least 1.5 tonnes of dairy waste is produced a day, of which approximately 1 tonne is yoghurt waste. Yoghurt waste consists of dairy assembly line "spills", specific product line "streams" which have slight amounts of other product "streams" mixing with it, and products which may not meet the strict quality control standards. Thus yoghurt waste, poses no health risk when fed to weaner pigs.

In addition to lactose, which has been shown to have beneficial effects when fed to weaned pigs, yoghurt also contains live bacterial cultures such as Lactobacillus acidophilus, Lactobacillus bulgaricus, Sterptococcus thermophilus, and Bifidobacterium animalis all of which ferment milk sugars. Fermentation of lactose by these bacteria produces lactic acid, which acts on milk protein thus giving yoghurt its flavour and characteristic taste (Bashiti, 2010). The presence in the digestive tract of the bacteria species mentioned earlier ensures that the Gastro-Intestinal-Tract (GIT) stays healthy and is able to perform the primary function of nutrient absorption in an efficient manner. A highly stable immune status has also been attributed to the GIT being colonized with

Lactobacillus spp. In this regard, the nutritional benefits of yoghurt are believed to outweigh the benefits of milk.

The hypothesis of the present study is that feeding a diet with added yoghurt waste to 4-week weaned pigs will improve performance, increase the lactobacilli populations in the GIT, and increase intestinal villi height. The objectives of this study were to determine the effect of a diet based on yoghurt waste on: 1) ADFI, ADG and FCE, 2) intestinal microbial populations, and 3) villi height.

Materials and Methods

The trial was carried out at the Livestock and Poultry Research Centre (LIPREC), School of Agriculture, College of Basic and Applied Sciences (CBAS) of the University of Ghana (UG), Legon Accra. The Centre is part of the Coastal Savannah and is located on latitude 05o 40`N and longitude 00o 16`W on the Accra Plains. Annual rainfall at the Centre is 785 mm with a range of 128-1,709 mm distributed bimodally. The long rainy season usually occurs between March and July with a peak in June, and the short rainy season occurs between August and November with a peak in October. Mean monthly temperatures range from 24.8oC in August to 28.3oC in February with a mean of 26.9oC. Relative humidity ai 1500 h ranges between 58% and 83.7% and is slightly lower at 0900 h. The area is gently rolling with low elevation and covered by natural grassland of medium tussock growth with scattered fire resistant trees and shrubs.

Storage of yoghurt waste

Fresh yoghurt waste (80% moisture) was obtained from a milk processing factory in Accra, transported in 100 l plastic-lined cardboard drums to LIPREC and stored in a cold room at 50 Celsius prior to incorporation into the experimental diets. The yoghurt waste represented waste from the previous day's production and was relatively fresh.

Experimental diets

Five experimental diets were used in this trial. Diet 1, representing Treatment 1 (T1)

was a standard weaner diet containing 28% SBM and 2369 Kcal/kg NE, 19.25% CP, 0.98% SID Lys, 0.91% Ca and 0.6% P. T2 contained 2.5% yoghurt waste on a DM basis and was included at the expense of SBM (Table 2). T3, T4 and T5 contained 5, 7.5 and 10% yoghurt waste respectively and were included in the diets at the expense of SBM.

Experimental pigs and design

The animal protocol used followed principles recommended by the Institutional Animal Care and Use Committee of the Noguchi Memorial Institute for Medical Research, University of Ghana. The study does not contain clinical studies or patient data. Twenty piglets (15.6 ± 2kg, initial BW) were weaned from three sows on the same day and randomly assigned to one of five diets. Piglets were individually tagged and assigned to the five diets in a Completely Randomized Design. The experimental period was 28 days. Each group was housed in an open-sided pen 2m by 4m (L x W) with concrete floors and with open sides and which provided enough space for movement. To allow the pigs to adapt to the new solid diets, pigs in each treatment were fed the respective experimental diet for a period of 7 days prior to the start of the experiment. A known amount of feed was provided ad-lib to the piglets every morning. Prior to feeding, any leftover feed from the previous day was collected, dried and weighed to determine ADFI. To T1, T2, T3 and T4, water was added in order to obtain a wet mash similar in consistency to T5. On d7, 14, 21 and 28 all pigs were weighed, and together with ADFI measurements, the FCE were calculated.

Microbial analysis

On d 29 all the pigs were slaughtered and the GIT's carefully removed. The terminal ileum and caecum were identified and approximately 30 ml contents from these sections obtained, put into glass containers, capped and immediately stored on ice, prior to microbial analysis. The samples were immediately transported to the lab for microbial analysis. 100µl of the intestinal content was diluted serially in 900µl of sterile phosphate buffer solution (v/v) into a 1.5mL

tube. The samples were diluted serially into six tubes. The various diluents were then vortexed and 100µL of the diluted sample transferred aseptically and plated on Count Medium (PCA: oxoid, Hampshire, England) and DeRogosa, Sharpe Medium (MRS; Hampshire, Germany) aerobically at 350C using the pour plate method (Murray *et al.,* 1995). Enumerations of the bacteria were performed on the two media respectively for 72hr. The number of colony forming units (CFU) are expressed as CFU per 100 µl.

Villi height determination

Within 10 minutes of slaughter, 6-8 cm segments of the jejunum and terminal ileum were cut, rinsed in distilled water, and immediately placed in 10% formalin fixative solution for further morphometric analysis. The samples were then prepared according to the method of Bejo (1990). To summarize, the intestinal samples were removed from the fixative and sliced using a microtome blade to expose the cross- and length- sections of the intestine surface. These were then transferred into cassette cases and placed under running water for 30 minutes to wash off the fixative. The samples were dehydrated by dipping them into graded concentrations of ethanol (75%, 80%, 95%, and 100%) for 20 minutes at a time and subsequently in chloroform for 30 minutes. Tissue samples were then infiltrated in molten wax at 60oC and kept overnight. Subsequently the embedded tissues were blocked and sectioned at an angle of five degrees and a thickness of about 0.5-0.7µm using a microtome (Model: Bright 5040, Bright Instrument Company Ltd., Huntington, England) . Sections thus obtained were gently spread on water at a temperature of 40oC, fixed onto glass slides and heated until the samples were dry. Tissues were then deparaffinised in xylene hydrated through alcohol, stained with haematoxylin and counter stained with eosin. Tissue sections were finally dehydrated using 95% alcohol and mounted on 1.2 mm double frosted extra-thick micro slides with cover slips.

Statistical analysis

All data gathered were subjected to statistical analysis using the Generalized Linear Model of the Statistical Analysis Systems Institute (SAS, 1999). Initial weight was used as a co-variate to account for any variations in weaner weights. Means with significant differences was separated using SNK.

Results

Table 1 shows the chemical composition of the yoghurt waste. The composition and calculated values of the diets are shown in Table 2

Pigs fed diets 4 and 5 (7.5 and10% yoghurt waste respectively) ate the most feed while those on diets 1, 2 and 3 ate the least (Table 3). However although diet 2 had 2.5% of yoghurt waste added, intake of this diet was lower (P>0.05) than intake of diet 1 (1.0 vs 1.14kg /d respectively). Average daily gain generally followed a similar trend as ADFI, with pigs on diets 4 and 5 gaining more than pigs on diets 1 to 3. There were no differences in FCE among the various treatments.

Intestinal microbial populations and villi height
Total anaerobic and Lactobacilli bacterial populations in both the ileum and caecum were similar (P>0.05) across all the treatments (Table 4). Within the jejunum, villi heights of pigs on the various treatments were not different (P>0.05) from each other. In the terminal ileum however, pigs on diet 5 had an average villi height of 0.530µm and this value was higher (P<0.05) than that of pigs on diets 1 to 4, which ranged in height from 0.341 to 0.351µm (Table 5).

Table 1: Chemical composition of the yoghurt waste used in the trial

Ingredients	Amount
Energy (kcal/kg)	820
Protein (%)	2.9
Fat (%)	2.4
Ca (%)	2
P (%)	1.7
Lactose (%)	24.3

Table 2: Composition of the pig weaner diets

Ingredients (%)	0% YW	2.5% YW	5% YW	7.5% YW	10% YW
Corn	56.1	56.1	56.1	56.1	56.1
Wheat bran	8	8	8	8	8
Soybean meal	30	27.5	25	22.5	20
Waste yoghurt	0	2.5	5	7.5	10
Oyster shell	1.4	1.4	1.4	1.4	1.4
Dicalcium phosphate	1	1	1	1	1
Fat	1.2	1.2	1.2	1.2	1.2
Vitamin/mineral premix[1]	1	1	1	1	1
Salt	0.,5	0.,5	0.,5	0.,5	0.,5
Lysine	0.15	0.15	0.15	0.15	0.15
methionine	0.15	0.15	0.15	0.15	0.15
Chemical Composition (%)					
Crude protein	19.25	18.6	17.9	17.2	16.5
Ca	0.91	0.95	0.98	1.02	1.06
P	0.6	0.62	0.64	0.66	0.69
Lys (SID)[2]	0.98	0.95	0.91	0.88	0.84
Meth (SID)	0.4	0.4	0.4	0.39	0.39
Lactose	0	0.6	1.2	1.8	2.4
NE (kcal/kg)	2369	2374	2379	2384	2389

1 Provided per kilogram of complete diet: vitamin A, 8,250 IU; vitamin D3, 825 IU; vitamin E, 40 IU; niacin, 35mg; D-pantothenic acid, 15mg; riboflavin, 5mg; menadione, 4mg; folic acid, 2mg; thiamine, 1mg; D-biotin, 0.2mg; vitamin B12, 0.0025mg; Zn, 100mg as $ZnSO_4$; Fe, 80mg as $FeSO_4$; Cu, 50mg as $CuSO_4$; Mn, 25 mg as $MnSO_4$; I, 0.5mg as $Ca(IO_3)_2$; and Se, 0.1mg as $Na_2Se)3$.
2SID: Standardized ileal digestibility.
YW: Yoghurt waste

Table 3: Effect of level of yoghurt waste on production parameters

Parameter	0% YW	2.5% YW	5% YW	7.5% YW	10% YW	SEM	P-Value
ADFI (g)	1.14[b]	1.00[c]	1.05[bc]	1.32[a]	1.42[a]	0.05	<0.05
ADG (g)	0.62[b]	0.61[b]	0.63[ab]	0.68[ab]	0.85[a]	0.06	0.04
FCE (g)	0.54	0.60	0.57	0.51	0.51	0.04	0.56

SEM: Standard error of the mean
ab Means with same superscripts within the same row are not significantly different (P > 0.05)
YW: Yoghurt waste

Table 4: Effect of level of yoghurt waste on anaerobic bacteria populations

Parameter	0% YW	2.5% YW	5% YW	7.5% YW	10% YW	SEM	P-Value
Ileal populations (log 10)							
Lactobacilli (cfu)	8.45	7.6	7.43	8.02	8.05	0.40	0.51
Total (cfu)	8.56	8.00	8.45	8.50	8.28	0.34	0.80
Caecal populations (log 10)							
Lactobacilli (cfu)	8.56	8.00	8.45	8.40	8.28	0.35	0.80
Total (cfu)	8.70	8.20	8.57	8.65	8.35	0.29	0.65

SEM: Standard error of the mean
Cfu: Colony forming units
YW: Yoghurt waste

Table 5: Effect of level of yoghurt waste on villus height

Parameter	0% YW	2.5% YW	5% YW	7.5% YW	10% YW	SEM	P-Value
Villi height (μm)							
Jejunum	0.331	0.400	0.369	0.377	0.370	0.027	0.543
Terminal Ileum	0.341[b]	0.346[b]	0.351[b]	0.341[b]	0.530[a]	0.021	<0.001

Discussion

Production parameters studied in this trial included ADFI, ADG and FCE. Intestinal microbial populations and intestinal morphometric parameters were also studied.

The inclusion of milk products in pig weaner diets has been shown to increase ADFI. This in part, is a result of an improvement in palatability due to the enhanced flavour and taste that is associated with milk and milk products (Torrallardona and Roura, 2009). The addition of milk products to weaner diets has also been shown to improve digestibility (Tokach *et al.,* 1988). When transitioning from a diet that is based entirely on milk, to one based on solid ingredients, weaned pigs perform better if they are fed complex diets made up of easily digestible ingredients (Mahan *et al.,* 2004). Feeding such a diet is beneficial to the pig since it places less of a burden on the yet undeveloped GIT. An improvement in digestibility results in a faster disappearance of feed from the GIT. This will invariably lead to quicker return to a state of hunger and subsequently an increase in ADFI (Dong and Pluske, 2007). This phenomena is in line with results of this trial where pigs on T4 and T5 (7.5 and 10% added yoghurt waste respectively) ate the most feed.

One of the biggest determinants of ADG in livestock production is ADFI (Pluske *et al.,* 2003). The more an animal eats the greater it's ADG. As expected in this trial, pigs on T4 and T5 which ate the most feed had the best ADG. Treatments 2 to 5 all added lactose and with the exception of T2, piglets fed on the other three diets had ADG which were higher than, or tended to be higher than the ADG of pigs on T1. The positive results of lactose in pig weaner diets in relation to ADG have been clearly demonstrated by Mahan et al. (2004) who showed that weaners (25kg BW) performed better when fed complex diets containing lactose levels of (between 10 and 15%) from d 21 to 35 post weaning. Efird et al. (1982) also demonstrated that when pigs were weaned at 21d and fed diets based on either lactose or soy protein (24% of the diet), ADG was better when compared to a control group. The lack of difference in FCE was in line with the observed trends in ADG and ADFI.

The results of added lactose in weaner diets have however not always been positive. Molino et al. (2011) did not find any improvements in ADG, ADFI and FCE when piglets were fed diets with 12% added lactose between 21 and 49 days of age.

Yoghurt is a dairy product produced by bacterial fermentation of milk sugar into lactic acid. 'Fermentation of lactose by live cultures of bacteria strains such as Lactobacillus acidophilus, Lactobacillus bulgaricus, Sterptococcus thermophilus, and bifidobacteria produces lactic acid, giving yoghurt its flavour and characteristic taste (Bashiti, 2010). The presence and activity of lactobacilli in the yoghurt have a stimulatory effect on both gut immunity and maturation, enhancing immune protection and reducing gastro-intestinal inflammatory responses (Daly *et al.,* 2013). According to Adolfsson et al. (2004), the benefits of yogurt consumption to gastro-intestinal function are most likely due to effects mediated through the gut microflora, bowel transit, and enhancement of gastro-intestinal innate and adaptive immune responses.

At birth, pigs are free of bacteria, but quickly develop an established microbiota that is acquired from the feed and oral-faecal transmission in their post-birth environment (Heo, 2010). Dominant among these microbiota are Lactobacillus spp., Streptococcus spp. and Helicobacter spp., as they can tolerate the low pH environment (Jensen *et al.,* 2001). These intestinal microbes remain stable until weaning, or when sow milk is no longer available to the piglet at which time the population of Lactobacillus spp dramatically decreases (Franklin *et al.,* 2002; Konstantinov *et al.,* 2006). At this stage a new microbial community is re-established in the piglets' GIT with potentially pathogenic bacteria such as coliforms increasing in numbers. (Yin and Zheng, 2005). Thus feeding complex highly digestible solid diets which incorporate the use of milk-based ingredients, helps to make this transition easier for the pig. It also reduces potentially harmful bacteria from colonizing the GIT too quickly.

In this trial microbial populations from the lower GIT indicated no differences both in the total microbial, as well as the lactobacilli counts amongst pigs on all treatments. It may be speculated that the time of weaning (7 weeks) was such that pigs may have, in addition to sow milk been used to eating part of the solid feed that was being fed to the sow. Thus the transition from sow's milk to solid feed was gradual and did not pose a "shock" to the intestinal microflora dynamics of the piglets

Immediately post-weaning, piglets suffer from villi atrophy and this has a negative effect on nutrient absorption (Soulet, 2012; Campbell *et al.,* 2013). This reduction in nutrient absorption is due to the fact that villi atrophy causes a reduction in the total absorptive surface area of the lower GIT (Loh *et al.,* 2002; Hedemann *et al.,* 2003). In the current trial, villi heights at the terminal ileum of pigs on T5 (10% yoghurt waste) were longer than that of pigs on all other treatments. This increased absorptive surface area, especially at the terminal ileum, could have improved nutrient absorption and may have accounted in part for the improved performance of pigs on T5. This phenomena has been observed in trials by (Pluske and Williams, 1995) who also observed an increase in villi height and crypt depth of pigs fed diets based on milk products.

Conclusions and recommendations

Results from this trial have indicated that feeding weaned pigs with diets containing 10% yoghurt waste improved ADFI, probably due to improved palatability and digestibility. The improved ADG that was observed was likely due a combination of an increase in ADFI and villi height, and not to increased lactobacilli counts in the GIT. In Ghana, this product is thrown away by the company which produces it. This trial has shown some evidence on the benefits of feeding complex milk-based diets on piglet performance. It should be possible in future trials to demonstrate that, farmers can wean their piglets at 4 weeks of age and formulate diets using such cheap industrial by-products, and still maintain piglet health and performance. This will ultimately improve their profits, by causing their sows to return to oestrus quicker and reducing the number of "open" days.

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Impact

The experiment was carried out to find if yoghurt waste, which is a natural consequence of the yoghurt manufacturing industry can be put to good use as a feed ingredient for weaner pigs. It was concluded that adding yoghurt waste at a rate of 10% at the expense of soybean meal in weaner diets results in improved performance in terms of weight gain. In addition overall intestinal health was improved as was manifest by increased villi heights in pigs fed diets with 10% added yoghurt waste.

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EFFECT OF EUPHORBIA HIRTA AND THYMUS VULGARIS POWDERS ON PERFORMANCE AND HAEMATOLOGICAL PARAMETERS OF THE CAMEROON KABIR CHICKEN

Ngantu H Ndzi[1]*, Keambou T Christian[1], Manfo T F Pascal[2] and Kenneth J N Ndamukong[1]

[1]Department of Animal Sciences, Faculty of Agriculture and Veterinary Medicine, University of Buea, P. O. Box 63, Buea, S.W.R. Cameroon

[2]Department of Biochemistry and Molecular Biology, Faculty of Science, University of Buea, P. O. Box 63, Buea, S. W. R. Cameroon

Abstract

The ban placed on the long term use of commercial antibiotics at subtherapeutic levels for diseases control and growth promotion in livestock production necessitated a worldwide search for available, cost effective and efficacious alternatives. Accordingly, the effects of Euphorbia hirta (EH) and Thymus vulgaris (TV) powders were evaluated against Oxykel 80 WP on feed intake, growth performance, carcass characteristics and haematological parameters of the Kabir chicken. For, 144 one-week old Kabir chicks of both sexes divided into 6 groups (n=18), which received a commercial antibiotic (0.5g/L H2O), a basal diet alone (negative control) or supplemented with 0.75% & 1.5% EH and 0.5% & 1.0% TV powders. Feed intake and weight gain were recorded weekly for 9 weeks. Carcass and haematological analysis were evaluated at 45 days post-treatment. Feed intake, weight gain and feed conversion were generally not significantly affected (P>0.05) by the dietary treatments. However, chicken on 0.75% EH had the highest overall feed intake (5324.70g) and weight gain (1451.70g) while those on 1.0% TV and basal diet treatments had the lowest overall feed intake (4060.90g) and weight gain (1150.03g) respectively. The overall feed conversion ratio ranged from 3.19 to 3.99, and was better only during the first 21st days of age. Pre-slaughter and dressed carcass weights of chicken were higher in the 0.75% EH (944.75) and 1.0% TV (588.13), respectively, and both lowest in the negative control. Significant inter-treatment weight differences (P<0.05) occurred in 3 internal organs; liver, proventriculus and pancreas. Not much inter-treatment variations were noticed in the 19 blood parameters studied except for % lymphocytes and% granulocytes, which were higher (P<0.05) when compared to 0.5% TV and 0.75% EH treatments. From these investigations, both TV and EH powders showed varied potentials as growth promoters in local chicken production. The implications of these findings are further discussed.

Key words: Euphorbia hirta, Thymus vulgaris, antibiotic, growth promoter, performance, Kabir chicken

L'EFFET DES FARINES DE L'EUPHORBIA HIRTA ET DE THYMUS VULGARIS SUR LES PERFORMANCES ET LES PARAMÈTRES HÉMATOLOGIQUES DU POULET KABIR DU CAMEROUN

Résumé

L'interdiction sur l'utilisation à long terme d'antibiotiques commerciaux à des niveaux sub-thérapeutiques pour le contrôle des maladies et la promotion de la croissance en production animale nécessitait la recherche dans le monde entier des alternatives efficaces et à coûts effectifs . Par conséquent, les effets des farines de l'Euphorbia hirta (EH) et de Thymus vulgaris (TV) ont été évalués en comparaison à l'Oxykel 80 WP sur la consommation alimentaire, la croissance, les caractéristiques de la carcasse et les paramètres hématologiques du poulet Kabir. En effet, 144 poussins Kabir âgés d'une semaine des deux sexes, répartis en 6 groupes (n = 18), qui ont reçu un antibiotique du commerce (0,5 g / L de H2O), un seul régime de base (témoin négatif) ou additionné de 0,75% et 1,5 % de l'EH et 0,5%, 1,0% des farines de TV. La consommation alimentaire et le gain de poids étaient enregistrés chaque semaine pendant 9 semaines. La carcasse et l'analyse hématologique étaient évaluées à 45 jours après le traitement. La consommation alimentaire, le gain de poids et la conversion alimentaire n'étaient pas dans l'ensemble affectés de façon

Corresponding author email: harrisonngantu@yahoo.com

significative (P> 0,05) par les traitements alimentaires. Cependant, le poulet sur 0,75% d'EH avait la consommation alimentaire totale la plus élevée (5324,70g) et le gain de poids (1451,70g) tandis que ceux sur 1,0% de TV et le régime alimentaire de base des traitements avaient la plus faible consommation totale d'alimentation (4060,90g) et le gain de poids (1150,03g) respectivement. Le taux global de conversion alimentaire variait de 3,19 à 3,99 et était meilleur pendant les premiers 21 jours d'âge. Le poids des poulets avant l'abattage et le poids des carcasses habillées étaient plus élevés respectivement de 0,75% dans l'EH (944,75) et 1,0% de TV (588,13) et les deux plus bas dans le contrôle négatif. Les différences significatives de poids inter-traitement (P <0,05) se sont produites dans 3 organes internes ; le foie, le pro ventricule et le pancréas. On n'a pas vraiment noté de variations inter-traitement dans les 19 paramètres sanguins étudiés à l'exception des% de lymphocytes et des% de granulocytes, qui étaient plus élevés (P <0,05) pour les traitements 0,5% de TV et 0,75% l'EH. De ces enquêtes, les deux farines de TV et d'EH ont montré des potentiels variés comme promoteurs de croissance dans la production de poulet local. Les implications de ces résultats seront discutées plus profondément.

Mots clés : L'euphorbia hirta, le Thymus vulgaris, les antibiotiques, les promoteurs de croissance, la performance, le poulet Kabir.

Introduction

The use of herbs in livestock production (ethno-veterinary medicine) mainly to control diseases dates back thousands of years BC. This became drastically replaced by synthetic drugs known as antibiotics probably due to technological advancements and increasing scale of production, with urbanization and population growth being the drivers of change. The beneficial effects of antibiotics in fighting bacteria related diseases and as growth promoters are well documented (Castanon, 2007; Zulkifli et al., 2012). In poultry production, antibiotics are administered at subtherapeutic doses either in water or feed to control diseases and promote growth. The risks associated with such a practice after long use include toxicity of residual drugs in poultry products, bacterial resistance development, etc (Lu et al., 2006), and have been of great concern for many years. The projected hazardous effects antibiotics can have in consumers of poultry products in the near future led to the systematic withdrawal and finally a complete ban of their usage as growth promoters by the European Union in 2006 (Fertet, 2007).This ban placed on antibiotics has opened a worldwide search for safe and efficacious alternatives. Most of such searches have based on the old practice of using plants, plant products and their extracts not only to combat bacterial and fungal problems but also to promote growth.

Plants contain phytonutrients and phytochemicals known as secondary metabolites (Ogbe and Affiku, 2012) which are applied in nutrition and as pharmacologically active agents (Soetan and Oyewole, 2009). Nutritional Wise, Plants are known to contain high amounts of essential nutrients, vitamins, minerals, fatty acids and fibre (Soetan and Oyewole, 2009; Gafar and Itodo 2011) . Apart from their antimicrobial activities, they act as natural antioxidants (Botsoglou et al., 2002; Muira et al., 2002; Radwan et al., 2008) and provide a stimulate effect on animal digestive systems (Ramakrishna et al., 2003; Ferket, 2007) to increase production of digestive enzymes and improve utilization of digestive products through enhanced liver function (Hernandez et al., 2004; Ertas et al., 2005).

Euphorbia hirta is a small annual herb common to the tropical countries and belongs to the same family as tic, tapioca and the rubber tree (Zulkifli et al., 2012). The plant has been recommended for various therapeutic indications in traditional medicine, such as diseases of the digestive and respiratory systems (Kumar et al., 2010). Invitrostudies of antimicrobial properties of Euphorbia hirta extract demonstrated activity against Salmonella enteriditis, Escherichia coli, *Staphylococcus aureus* and Bacillus subtilis (Somchit et al., 2001).At the dose of 7.5g/Kg feed, Euphorbia hirta showed potentials for use as natural growth promoter in broiler production (Zulkifli et al., 2012). Thymus vulgaris contains many active principles known to have disease

preventing and health promoting properties. These include thymol, one of the important essential oils of Thymus vulgaris, which has been shown to possess antiseptic and anti-fungal characteristics. Leaves of Thymus vulgaris are one of the richest sources of potassium, iron, calcium, manganese, magnesium, and selenium. The herb is also a rich source of many important vitamins such as B-complex vitamins, beta carotene, vitamin-A, vitamin-K, vitamin-E, vitamin-C, and folic acid (USDA-NNDSR u.o.). The addition of 1% Thymus vulgaris to layer diets improved feed conversion and production performance (Radwan *et al.,* 2008).

A number of compounds have been isolated from Euphorbia hirta (Zulkifli *et al.,* 2012) and Thymus vulgaris (Boruga *et al.,* 2014) and chemically characterized, but their potential in local poultry production has not been fully explored. The various therapeutic and nutritional indications of Euphorbia hirta and Thymus vulgaris potentially present an opportunity especially in family/backyard poultry production. To the best of our knowledge, there is lack of information about the potential effect of these 2 plants used as growth promoter on the Cameroon Kabir chicken which have better performances as compared to the common local chicken and is thought to be adapted to most of the local feed resources and local environmental conditions in Cameroon. Hence, the main objective of this study was to evaluate the effects of Euphorbia hirta and Thymus vulgaris powders in comparison with a commercial antibiotic on growth performances, carcass characteristics and haematological parameters of the Cameroon Kabir chicken.

Material and Methods

Study site

This research was conducted at the Green Gold Poultry Research and Production Farm located at Buea, in the South West Region of Cameroon.

Experimental diets

The Kabir chicks were fed on 2 experimental diets formulated to contain graded levels of euphorbia (0.75% and 1.5%) or Thymus vulgaris (0.5% and 1.0%) powders against two control diets (standard diet with a commercial antibiotic and without a plant powder nor commercial antibiotic). The diets used in the experiment were those formulated by the Green Gold feed unit to meet the nutrients requirements of broiler breeds. They varied with the age of the chicks and comprised formulations for pre-starter (30.04% CP and 3000Kcal/Kg), starter (24.05% CP and 3106 Kcal/Kg) and grower (21.92% CP and 3118.06 Kcal/Kg) (Table 1). The various mashes were formulated from ingredients commercially available in Cameroon. Addition of plant powders to basal diet was done daily while the antibiotic (Oxykel 80WP) was added to water following manufacturer's instruction (0.5g/litre of water for 3 continuous days weekly).

Preparation of growth promoter plants

The whole Euphorbia hirta plant (leaves, stem and roots) were harvested from the University of Buea campus, while aerial parts (leaves and stem) of Thymus vulgaris were and purchased from the local market. The plants were dried in an electric oven starting at a temperature of 50°C, which was progressively increased to 70°C within a period of three days. Dried samples were grinded to powder using a hammer mill, transferred to separate and labeled plastic bags, sealed and stored in a cool dry place until use.

Animals and experimental design

The study was carried out on 144 1-week old Cameroon Kabir chicks (local chicken) of both sexes. Chicks were administered antistress (sugared solution and vitamins), distributed according to the treatment groups and identified individually by means of wing tags, and allowed to acclimatize for a seven-day period. It should be noted that the entire poultry house and its premises were thoroughly cleaned with water and disinfected with virunet® solution by means of a knapsack sprayer, prior to placement of the chicks in the pens. The chicks were then followed up as described by (Pelicano *et al.,* 2005). Briefly, the chicks were distributed in pens

measuring 3m long by 2m wide, and standard commercial management applied throughout the experimental period. The floors of the pens were provided with approximately 5cm of wood shaving and equipped with drinkers and feeders. Fluorescent lamps were used for lighting and tungsten filament lamps for heating as necessary. The conventional prophylaxis program was applied. Water and feed were provided ad-libitum. To ensure the maintenance of the bio-safety security of the house, a disinfectant (virunet® solution) was always provided as a dip at the entrance of the house.

For evaluation of Euphorbia hirta and Thymus vulgaris potentials, the chicks (n = 24/group) were allocated into the control and treatment groups with/without different levels of inclusion of the 2 growth promoter plants and reference antibiotics shown in table 1, in a completely randomized design. Animals from each treatment group were balanced based on sex, and their zootechnical performances recorded until 63 days post-treatment.

Evaluation of growth parameters

Feed intake and weight gain by the chicks in each group were monitored on weekly basis and feed conversion efficiency was calculated per treatment group. Weight records were taken using a sensitive electronic balance (3kg x 0.5g). The formulae used in calculating weight gain and feed conversion ratio for each animal were as follows:

Weight gain = Initial weight of the bird (g)–final weight (g) (after a specified feeding period)

Feed conversion ratio=

$$\frac{\text{Amount of feed consumed (g)}}{\text{Weight gain (g)}}$$

Evaluation of carcass parameters

Carcass analysis was done following previously described procedures (Sarica *et al.,* 2005; Ogbe and Affiku 2012). Briefly, carcass weights were determined from six female broiler chicken per treatment at 45 days post-treatment. Selected chicken were those with body weights similar to the group's average.

For, the chicken were fasted for 12 hours (no access to food except water), slaughtered (through cervical dislocation) and eviscerated. Following evisceration and blood collection, each of the internal organs (liver, heart, gizzard, proventriculus, spleen, caeca, kidneys, lungs and pancreas) in each animal were dissected out, weighed and expressed as a percentage of the live body weight. The carcass was thereafter plucked and weighed.

Evaluation of haematologicalparameters

Blood samples collected in heparinized test tubes immediately after slaughtering of the animals, served for haematological analysis, which was done using an automated blood analyser.

Statistical Analysis

Data (feed intake, weight gain, carcass and organ weights and haematological values) were entered into a spread sheet and analyzed with the Statistical Package for Social Sciences software (SPSS, IBM version 21.0). Prior to analysis, appropriate transformations were done to normalize the data following homogeneity of variance test results. Carcass (dressed carcass and organs) and haematological data were square-root transformed while feed intake feed conversion ratio and weight gain were log10 transformed. Analysis of variance (ANOVA) was used to test for significant differences between means at 0.05% confidence level. Where significant, means were compared using the Duncan's New Multiple Range Test (DNMRT).

Results

Growth performance of the chicken (Body weight gain, feed intake and feed efficiency)

Effects of commercial antibiotic and two herbal feed additives in Kabir chicken diets on feed intake body weight gain, and feed efficiency are presented in Table 2.

Feed intake:

Feed intake progressively increased with the duration of the experiment, but was generally not significantly (P>0.05) affected

by the types of additive throughout the follow up period. Though not significant the intermittent and overall feed intake was higher in all the Euphorbia hirta and positive control treatments. Feed intake values (g±SEM/chicken) of over 5324.70±78.00, 5162.60±92.17 and 5018.25±58.88, were recorded during the 63-day feeding period for 0.75% Euphorbia hirta, 1.5% Euphorbia hirta, and commercial antibiotic, respectively. The 0.75% Euphorbia hirta and 1.0% Thymus vulgaris treatments had the highest and lowest feed intake values, respectively.

Weight gain:

With almost similar feed intake values in the different treatment groups, body weight gain was generally not significantly affectedby the dietary treatments during the 63days trial period (P>0.05). However, the higher feed intake values in the Euphorbia hirta and positive control treatments relative to the other treatments, gave them weight gain advantages. Though not significant, chicken receiving 0.75% Euphorbia hirta had the highest weight gain (614.75±73.81) while those receiving 0.5% Thymus vulgaris had the lowest (469.63±27.55) weight gain between days 14 and 35. During the 35 to 63 feeding interval, chicken receiving 1.5% Euphorbia hirta had the highest weight gain (758.30±141.25), while those receiving no additive had the lowest (615.06±90.98) weight gain. Cumulatively, the weight gain for chicken on 0.75% Euphorbia hirta (1451.70±154.79) was the highest while that of chicken on neither feed additive nor antibiotic was the lowest (1150.03±118.90).

Feed conversion:

Feed conversion ratio in all the treatments was generally high. Values of feed conversion ranging from 3.03±0.35 to 4.19±0.68 and 3.48±0.33 to 4.45±0.96 in the 14 to 35 and 35 to 63 day feeding intervals respectively were obtained in this study. Though there were no significant differences (P > 0.05) in feed conversion ratios between dietary treatments irrespective of the feeding interval, feed conversion was better during the first 21 days when the chicks were still very young and received the starter basal diet, and became poor during the last 28 days of the experiment following the change in mash from starter to grower basal diet. Regarding treatments in general, chicken on 0.5 and 1.0% Thymus vulgaris had better feed conversions than the other treatments while 1.5% Euphorbia hirta and negative control had poorer feed conversions.

Relative organs weights and carcass characteristics

The effects of the feed additives on relative weights of internal organs and carcass yields are summarized in Table 3. The pre-slaughter weights at 45 days of age were higher in the 0.75% Euphorbia hirta (944.75±191.65) followed by the 1.0% Thymus vulgaris (863.88±133.25) and the antibiotic (847.56±74.21) treatments. The negative control treatment (602.11±57.25) had the least pre-slaughter and carcass weights. Accordingly, carcass weights were higher in the aforementioned treatments and also lowest in the negative control treatment. Generally, the Thymus vulgaris treatments recorded better carcass percentages than the other treatments. Values of dressing percentage ranged from 59.79±3.56 in the negative control to 69.14±3.78 in the 0.5% Thymus vulgaris treatments. However, these variations remained not statistically significant.

In contrast with carcass weights, significant differences (P<0.05) were obtained in the relative weights of the liver, proventriculus and pancreas. At 45 days of age, relative weights (%) of up to 3.25±0.11 were recorded for the liver, 1.19±0.21 for the proventriculus and 0.45±0.04 for the pancreas. Great variations across treatments occurred in the relative weights of the remaining internal organs studied but were not statistically significant (P > 0.05). Pancreas weight was similar in the 0.5% Thymus vulgaris (0.45±0.04) and negative control (0.44±0.02) treatment. However, with the exception of the spleen, the relative weights of all the internal organs were higher in the negative control treatment compared to the other treatments. Numerical values of the spleen were very similar and higher in the

0.5% (0.18±0.02) and 1.0% (0.18±0.03) Thymus vulgaris treatments and drastically decreased in the other treatments.

Haematological parameters

Haematological parameters in the Kabir chicken following 45 days - treatments with Euphorbia hirta and Thymus vulgaris based basal diets and/or antibiotic are presented in Table 4. a total of 19parameters were studied and significant variations (P<0.05) across treatments occurred only in three of these; including lymphopcite number,lymphopcite percentage, and granulocyte percentage. The 0.5% Thymus vulgaris treatment recorded highest values of lymphocyte number (183.40±1.93) and percentage (73.77±0.46), while the 1.0% Thymus vulgaris treatment recorded the highest value of granulocyte percentage (22.60±3.54). Also, high variations were observed in the studied blood parameters with respect to standard reference ranges, with very few remaining within the range. In this respect, up to 9 parameters (WBC, Lymph#, Mid#, Gran#, Lymph%, Mid%, MCV, MHC and MCHC) were above, 7 (Gran%, HGB, RBC, HCT, RDW-SD, PLT and PCT) were below and 3 (RDW-CV, MPV and PDW) within the standard reference range. Values of 4 blood parameters (WBC, Lymph#, Mid# and PLT) were sufficiently larger, and those of Gran% and PCT were sufficiently lower than the standard reference values. On the other hand, values of Mid%, HGB, RBC, HCT, MCV, MCH, RDW-SD and Lymph% deviated slightly from the reference values.

Discussions

With the huge benefits expected to be obtained from Euphorbia hirta and Thymus vulgaris, it was but logical to evaluate their effects on performance and haemalotogical parameters as potential growth promoters in chicken production. Several studies of this kind adopt the use of either the extract or whole plant powder in feed at different levels/concentrations and taking records to determine their effects on the organism in question. The average feed intake per Kabir chicken obtained

in this is study (Table 2) is very similar to those of broiler chicken (3825-4208.2g) at 42 days of age (Sarica *et al.,* 2005). Many are of the opinion that local chicken rearing is a low input activity since they are adapted to the local environment/feed resources and consumes less food. This study clearly demonstrates that feed intake in local chicken is a function of the manner in which they are reared and accessibility to food. The key activity here therefore is to look for strategies to enable a more efficient utilization of the food being consumed by the local chicken with cheap and readily available materials. Using broiler chicken, several studies carried out using herbs and their extracts have been found not to have any significant effects on feed intake (Engberg *et al.,* 2000; Patil *et al.,* 2000; Van Campenhout *et al.,* 2001; Tucer, 2002; Demir et a., 2005), corroborating our findings. However, it could be seen from the current study that Euphorbia hirta at 0.75% and 1.5% levels of inclusion in the basal diet as well as the commercial antibiotic slightly influenced feed intake. The appetite stimulating abilities of these herbs and antibiotic could have therefore been present but not enough to be visualized significantly.

Variability of weight gain per chicken in the various treatments at different age intervals suggests differential adaptation abilities to feed additives and commercial antibiotic, probably a direct influence of age as well as additive level. Up to date, whether herbs and herbal extracts in maize and wheat based basal diets truly have any significant effects on chicken weight gains remains a debatable issue. In the present study, weight gain was not significantly (P>0.05) influenced by dietary treatments, and this observation was also reported elsewhere (Mottaghitalab 2000; Cross *et al.,* 2003; Demir *et al.,* 2005). Therefore, it would appear that it is the higher feed intake values together with additive or antibiotic effects that gave chicken in the Euphorbia hirta and antibiotic treatments weight gain advantages, and not additive or antibiotic alone. Furthermore, the lack of significant treatment differences could be due to the high nutritive quality of basal diet (Table 1) used in this study. Ogbe and Affiku (2012) attributed the observed

Table 1: Basal diet and treatments allocations to the experimental animals

Composition (g/kg) of basal diets			
Ingredients	**Pre-starter**	**Starter**	**Grower**
Maize	515	610	600
Wheat bran	6	60	120
Soya bean cake	285	165	149
Fish meal	139	110	76
Palm Oil	00	/	/
Oyster shell	02	02	02
Bone meal	02	02	02
Salt	01	01	01
5% broiler concentrate	50	50	50
Total	1000	1000	1000
Estimated composition of essential nutrients			
Metabolizable Energy in Kcal/Kg	3000	3106	3118.06
Crude Protein	30.04	24.05	21.92
Fat	4.61	4.65	4.45
Calcium	2.01	1.70	1.36
Phosphorus	0.98	0.83	0.71
Lysine	1.77	1.35	1.19

Composition (g/kg) of basal diets			
Treatments	**Additive amounts (g/kg)**	**Basal diet amount (g/kg)**	**Total feed composition (g)**
EH 0.75%	7.5	992.5	1000 = 1KG
EH 1.5%	15	985	1000 = 1KG
TV 0.5%	5	995	1000 = 1KG
TV 1.0%	10	990	1000 = 1KG
Positive control	0.5g Oxykel 80 WP/1L H_2O	1000	1000 = 1KG
Negative control	0	1000	1000 = 1KG

EH: *Euphorbia hirta*, TV: *Thymus vulgaris*

weight increases despite the inexistence of a significant difference to the beneficial effects of natural growth promoters and antibiotic. According to Demir et al. (2005), the observed numerical increase in performance following supplementation with herbs may be due to stimulation of pancreatic secretions to increase digestive enzyme activity and the antimicrobial properties of bio-active components found in these herbs.

In contrast to our findings, Guo et al. (2000); Van Campenhout et al. (2001); Jamroz and Kamel (2002); Ertas et al. (2005) reported significant effects of herbs and herbal extracts on broiler weight gains. The use of Thymus vulgaris as additive in broilers diets was reported to have exhibited significant growth promoting effects (Sarica *et al.,* 2005). The plant Euphorbia hirta on the other hand has been shown to have very potent antimicrobial activities (Somchit *et al.,* 2001); Ogbulie *et al.,* 2007; Kumar *et al.,* 2010). The beneficial effects expected to be derived from Euphorbia hirta in local chicken production is therefore their good health status which of course should translate to better growth performances.

Table 2: Effects of antibiotic, EH and TV on gain, feed intake and feed conversion ratio of Kabir chicken.

Variable	Treatments±SEM						Sig.
	0.5%TV	1.0%TV	0.75% EH	1.5% EH	Positive control	Negative control	
Feed intake (g)							
14 to 35 d	1490.05±72.22	1571.60±73.04	2199.90±89.62	2105.90±81.91	1934.85±59.09	1764.80±55.73	NS
35 to 63 d	2698.10±69.48	2489.30±39.06	3124.80±39.40	3056.70±134.17	3083.40±40.51	2706.75±40.19	NS
14 to 63 d	4188.15±81.42	4060.90±68.29	5324.70±78.00	5162.60±92.17	5018.25±58.88	4471.55±52.26	NS
Body weight gain (g)							
14 to 35 d	469.63±27.55	518.58±32.59	614.75±73.81	502.70±107.35	509.50±37.95	473.65±38.21	NS
35 to 63 d	727.75±90.78	714.64±92.56	701.50±123.59	758.30±141.25	701.45±100.31	615.06±90.98	NS
14 to 63 d	1225.20±104.47	1274.00±120.58	1451.70±154.79	1293.20±59.82	1353.10±114.92	1150.03±118.90	NS
Feed efficiency (g:g)							
14 to 35 d	3.17±0.40	3.03±0.35	3.58±0.57	4.19±0.68	3.80±0.10	3.73±0.91	NS
35 to 63 d	3.71±0.43	3.48±0.33	4.45±0.96	4.03±0.65	4.40±0.74	4.40±0.58	NS
14 to 63 d	3.42±0.33	3.19±0.25	3.67±0.60	3.99±0.59	3.71±0.02	3.89±0.90	NS

EH: Euphorbia hirta, TV: Thymus vulgaris, NS: Not significant at P <0.05, SEM: standard error of the mean

Table 3: Effects of antibiotic, EH, and TV on carcass and relative organ weights of Kabir chicken.

Variable	Treatments±SEM						Sig.
	0.5% TV	1.0% TV	0.75% EH	1.5% EH	Positive control	Negative control	
Live body weight (g)	671.10±108.61	863.88±133.25	944.75±191.65	681.38±73.74	847.56±74.21	602.11±57.25	NS
Carcass weight (g)	469.00±84.74	588.13±99.88	569.36±126.77	422.25±52.90	526.44±64.55	367.89±43.68	NS
Dressing percentage	69.14±3.78	67.29±1.77	59.79±3.56	61.55±1.38	61.39±3.97	59.97±2.18	NS
Heart weight (%LWt)	0.62±0.08	0.57±0.17	0.52±0.03	0.54±0.07	0.54±0.04	0.66±0.04	NS
Liver weight (%LWt)	2.71±0.21 [abc]	2.57±0.29 [ab]	2.33±0.26 [a]	3.12±0.06 [bc]	2.53±0.16 [bc]	3.25±0.11 [c]	S
Kidneys weight (%LWt)	0.89±0.13	0.83±0.05	0.63±0.03	0.87±0.04	0.74±0.06	0.99±0.10	NS
Lungs weight (%LWt)	0.60±0.08	0.49±0.02	0.50±0.06	0.50±0.02	0.47±0.03	0.64±0.07	NS
Gizzard weight (%LWt)	2.76±0.06	2.82±0.30	2.45±0.25	2.61±0.10	2.70±0.17	3.02±0.11	NS
Proventriculus weight (%LWt)	0.85±0.08 [ab]	0.67±0.10 [ab]	0.56±0.04 [a]	0.94±0.20 [ab]	0.66±0.06 [ab]	1.19±0.21 [b]	S
Pancreas weight (%LWt)	0.45±0.04 [b]	0.36±0.06 [ab]	0.37±0.04 [ab]	0.38±0.05 [ab]	0.30±0.02 [a]	0.44±0.02 [b]	S
Spleen weight (%LWt)	0.18±0.02	0.18±0.03	0.10±0.02	0.13±0.03	0.14±0.02	0.15±0.02	NS
Caeca weight (%LWt)	1.44±0.22	1.04±0.07	1.22±0.17	1.13±0.11	1.28±0.16	1.47±0.16	NS
Caeca length (%LWt)	2.18±0.37	1.32±0.22	1.73±0.45	2.13±0.33	1.62±0.14	2.50±0.30	NS

EH: Euphorbia hirta, TV: Thymus vulgaris, NS: Not significant at P <0.05, S: significant at P<0.05, SEM: standard error of the mean
[abc]: *means followed by same superscript letter in the same row are not significantly different*

Table 4: Haematological values of Kabir chicken administered a commercial antibiotic, EH, TV and basal diet without additive

Variable	Ref range	Treatments±SEM						Sig.
		0.5% TV	1.0% TV	0.75% EH	1.5% EH	Positive control	Negative control	
WBC•	4.0-11.0 ×10⁹/L	248.57±1.12	209.27±18.37	246.17±2.39	241.63±1.55	238.03±2.17	223.63±1.55	NS
Lymph#•	0.8-8.0 ×10⁹/L	183.40±1.93c	114.83±10.74a	160.10±6.05bc	149.00±13.68b	154.90±8.32b	151.10±5.08b	S
Mid#•	0.1-1.2 ×10⁹/L	40.43±1.13	46.77±3.83	44.10±3.39	48.77±5.47	45.40±2.64	45.20±2.71	NS
Gran#•	2.0-7.0 ×10⁹/L	24.73±1.63	47.67±8.84	41.97±1.97	43.87±9.18	39.10±5.48	48.08±2.90	NS
Lymph%•	20-40%	73.77±0.46b	55.00±3.02a	65.00±1.96ab	61.70±5.91a	64.65±3.41ab	61.24±1.96a	S
Mid%•	3.0-14.0	16.27±0.47	22.40±0.52	17.93±1.41	20.17±2.19	19.03±1.18	18.50±1.09	NS
Gran%••	40.0-75.0%	9.97±0.68a	22.60±3.54c	14.07±2.03ab	18.13±3.75bc	16.32±2.30abc	19.66±1.06bc	S
HGB••	12.0-16.0g/dL	12.17±0.22	10.47±1.07	10.43±1.11	10.30±0.76	10.50±0.29	9.25±1.25	NS
RBC••	3.50-5.50 × 10¹²/L	3.11±0.30	2.3±0.41	2.63±0.23	2.46±0.18	2.61±0.08	2.19±0.32	NS
HCT••	34.5-54.0%	33.87±3.37	21.67±6.05	27.00±3.02	25.73±1.59	27.08±0.72	23.83±3.36	NS
MCV•	80.0-100.0 fL	108.93±1.99	90.03±11.40√	102.67±2.49	106.10±1.61	104.22±2.18	110.23±3.28	NS
MCH•	27.0-34.0 pg	39.60±2.97	46.37±4.57	39.53±1.12	41.77±1.19	40.27±0.73	43.03±1.59	NS
MCHC•	32.0-36.0 g/dL	36.43±2.72	55.00±13.60	38.63±0.58	39.47±0.52	38.73±0.29	39.13±0.67	NS
RDW-CV√	11.0-16.0%	11.10±0.44	11.37±0.73	10.87±0.69	12.07±0.58	11.80±0.47	12.25±0.96	NS
RDW-SD√	35.0-56.0 fL	39.37±2.24	34.37±1.84	34.77±1.11	42.00±2.10	38.95±1.61	50.12±6.84	NS
PLT••	150-400 × 10⁹/L	41.67±12.17	46.33±16.34	34.67±1.86	34.33±1.33	35.50±3.39	29.00±4.30	NS
MPV•	6.5-12.0 fL	9.60±0.32	8.43±0.50	8.77±0.22	8.93±0.17	8.85±0.23	9.06±0.29	NS
PDW•	9.0-17.0	15.10±0.25	14.90±0.31	15.10±0.20	15.03±0.23	15.20±0.13	14.96±0.19	NS
PCT••	0.108-0.282%	0.04±0.013	0.21±0.189√	0.03±0.002	0.03±0.000	0.03±0.003	0.03±0.002	NS

EH: Euphorbia hirta, TV: Thymus vulgaris, NS: Not significant at P <0.05, S: significant at P<0.05, SEM: standard error of the mean

abc: means followed by same superscript letter in the same row are not significantly different

WBC: white blood cell, Lymph#: lymphocyte number, Gran#: granulocyte number, Lymph%: lymphocyte percentage, Gran%: granulocyte percentage, HGB: haemoglobin, RBC: red blood cell, HCT: haematocrit, MCV: Mean corpuscular volume, MCH: mean corpuscular haemoglobin, MCHC: mean corpuscular haemoglobin concentration, RDW-CV Red cell distribution width, RDW-SD: red cell distribution width (actual size), PLT: platelets, MPV: mean platelet volume, PDW: platelets distribution width, PCT: plateletcrit

•Above reference range, • • below reference range, √ within reference range

Dietary differences did not show any significant effects on feed conversion. This corroborates other findings (Engberg *et al.,* 2000; Van Campenhout *et al.,* 2001; Sarica *et al.,* 2005; Demir *et al.,* 2005), who reported that antibiotic supplementation to either maize or wheat-based broiler diets did not significantly influence feed conversion ratio. However, with flavomycin or a xylanase preparation supplementation on a wheat-based broiler chicken diet, Esteve-Garcia et al. (1997) reported a significant improvement on feed conversion ratio. In the present study, the overall feed conversion values ranged from 3.19 to 3.99 and it was expected that groups showing higher weight gains would have better feed conversions. This was however not the case, as treatments with higher weight gains had higher feed intakes and poor feed conversions compared to the Thymus vulgaris treatments with low weight gains and feed intake but better feed conversions. These justifications further suggest that weight gain superiority in the Euphorbia hirta and antibiotic treatments were due to a combination of higher feed intakes additive or antibiotic effects and not only the latter. The poor feed conversion values obtained indicated that intensive rearing of the Kabir chicken with weight gain as targeted output may be counterproductive in terms of feed cost/unit gain. Rather a completely extensive or semi-intensive rearing system could be adopted with supplemental feeding (including proven feed additives) playing an integral role.

The dressing percentage obtained in this study is lower than those reported by other authors. The carcass yields of up to 76.48% [18] and 73.90% (Radwan *et al.,* 2008) were obtained with broilers on maize and wheat-based broiler diets respectively. The discrepancies with our results could however be attributed to several factors, including breed difference. The Kabir chicken like any normal local chicken utilizes a large amount of its energy needs in feather production and grows relatively slower compared to the broiler chicken though consumes basically the same amount of feed under intensive rearing conditions.

With the exception of the liver, proventriculus and the pancreas, the relative weight of internal organs did not significantly vary with treatment, corroborating the other findings (Sarica *et al.,* 2005; Radwan *et al.,* 2008; Ogbe and Affiku 2012). Ali et al. (2007) reported that hens fed Thymus vulgaris or anise had no significant effect on carcass parameters and internal organs. The relative weights of heart, liver, gizzard and spleen obtained in our study were similar to those reported by Radwan et al. (2008) for laying local hens, higher than those reported by Sarica et al. (20050, but sufficiently lower than those reported by Ogbe and Affiku, (2012) for broilers. The later authorsrecorded gizzard, liver, heart and spleen percentages ranging from 8.9-10.64%, 6.35-7.95%, 3.05-3.36% and 1.83-2.02% respectively using feed additives made of extracts from moringa leaves, gum Arabic, wild ganoderma and antibiotic (tetracine). Higher values (except spleen values) in the negative control compared to the other treatments suggest growth suppressing effects of the antibiotic and plant additives on the Kabir chicken's internal organs. The numerical increase of spleen values to almost the same magnitude in the Thymus vulgaris compared to the other treatments provides an indication of the immune-stimulating potentials of the plant. Al-Sultan (2003) reported a higher spleen weight index in broiler chicken fed 1.0% turmeric supplemented compared with the controls. Radwan et al. (2008) observed a similar trend with laying local hens on feed supplemented with 0.5% oregano and 0.5 or 1.0% Curcuma longa. This implies that the absence of statistically significant treatment differences does not necessarily mean a lack of effects on the internal organs. It further shows that different plant additives affect the different organs in specific ways. The weight and length of caeca in the control relative to the other treatments could be resulting from the physiological adaption of the chicken to effectively utilize the feed through bacteria degradation.

The wide variations that occurred in the haematological analysis of the Kabir chicken's blood, is considered a normal phenomenon for avian species (Ogbe *et al.,*

2009). The 0.5% Thymus vulgaris significantly (P<0.05) recorded highest values of lymphocyte number and percentage and the lowest granulocyte percentage compared with the other treatments. Still, the highest granulocyte number occurred in the 1.0% Thymus vulgaris treatment. The use of Thymus vulgaris could therefore, be important in defense. Apart from this, not much could be said regarding Euphorbia hirta since the rest of the deviations observed seemed not treatment related as chicken in the negative control groups deviated in a like manner. Ogbe and Affiku (2012) reported normal ranges of PCV%, HB g/dL, RBC/L and WBC/L with broilers administered polyherbal extracts from moringa leaves, gum Arabic, wild ganoderma and antibiotic (tetracine) in drinking water for 63 days. It is possible that avian haematological parameters may be influenced by the content of their feed as well as their physiological/health status. Defining a reference range for any avian species therefore will only be relative.

Conclusion

The plant powders as feed additives and commercial antibiotic had no statistically significant but rather contributory effects on feed intake weight gain and feed conversions, though all the Thymus vulgaris treatments had better overall feed conversions. It was inferred that treatments with weight gain advantages though not significant were on a one hand as a result of higher feed intake rather than just better feed efficiencies. High relative weight variations in the internal organs across treatments were observed but significant differences occurred regarding only the liver, proventriculus and the pancreas. Blood parameters also showed great deviations from the standard reference range with only few showing significant treatment variations. Summarily, Euphorbia hirta and the commercial antibiotic were noted for their appetite stimulating effects, Thymus vulgaris with better feed conversion, immune stimulating effects and defense enhancing ability. In our own opinion and regarding other reports, the contributory effects of plant additives generally in chicken production depends on three things:

the rearing conditions, feed quality and the additive in question.

Competing interest

There is no conflict of interest among authors.

Author's contributions

NHN, KTC MTFP and KJNN planned the study and NHN and KTC executed it, NHN and KTC carried out data collection and analysis and produced the first draft of the manuscript. MTFP and KJNN edited the manuscript and all the authors read and gave approval for the manuscript.

Acknowledgements

Impact

Long term use of antibiotics at sub-therapeutic levels in livestock production poses a significant risk to livestock product consumers due to the potential development of antibiotic resistant human pathogenic bacteria. This apparent threat resulted into antibiotics ban by the European Union and stimulated a worldwide search for available, cost effective and efficacious alternatives. The various therapeutic and nutritional indications of Euphorbia hirta (EH) and Thymus vulgaris (TV) potentially present an opportunity especially in family/backyard poultry production. For, the Kabir (local) chicken which have better performances as compared to the common local chicken and is thought to be adapted

to most of the local feed resources and local environmental conditions in Cameroon was used as model animal. Our main objective was to evaluate the effects of EH and TV plant powders in comparison with a commercial antibiotic on growth performances, carcass characteristics and haematological parameters of the Cameroon Kabir chicken. The study that lasted 9 weeks employed standard poultry rearing methodologies and experimental procedures. The plant powders were evaluated at two levels of inclusion (0.75% & 1.5% EH and (0.5% &1.0% TV) against a commercial antibiotic (0.5g Oxykel/L H2O) in maize based basal diet which without additive served as a negative control. Despite a general lack of significant inter-treatment differences on feed intake weight gain and feed conversions due to high quality and acceptability of feed used the feed additives showed rather contributory effects. There was no evidence of additive toxicity but had very little or no effect on the internal organs and blood parameters studied. Euphorbia hirta and the commercial antibiotic were noted for their appetite stimulating effects, and Thymus vulgaris with better feed conversion, immune stimulating effects and defense enhancing ability. In line with other studies, we concluded that the contributory effects of plant additives generally in chicken production will depend on three things: the rearing conditions, feed quality and the additive in question.

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FEED VALUE OF ENZYME SUPPLEMENTED CASSAVA LEAF MEAL AND SHRIMP MEAL IN PIGS

Olufemi S. Akinola[1]*, Amos O. Fanimo[1], J. Adeniyi Agunbiade[2], Andreas Susenbeth[3] and Eva Schlecht[4]

[1]University of Agriculture, Department of Animal Production & Health, Abeokuta, Nigeria,
[2]Olabisi Onabanjo University, Department of Animal Production, Nigeria
[3]University of Kiel, Institute of Animal Nutrition and Physiology, Germany
[4]University of Kassel and University of Göttingen, Animal Husbandry in the Tropics and Subtropics, Germany

Abstract

Ten crossbred male pigs of 49.3±3.97 kg body weight were used to evaluate the digestibility, energy value and N (nitrogen) retention of two unconventional protein sources, i.e. cassava leaf meal (CLM) and shrimp meal (SM), with or without the addition of a nonstarch polysaccharide (NSP) enzyme complex (β-glucanase and xylanase). During two trial periods, each lasting 7 days, two pigs each were fed the following five experimental diets: Basal diet (BD), BD+cassava leaf meal with (CLM+E) and without enzyme addition (CLM) and BD+shrimp meal with (SM+E) and without enzyme (SM) supplementation.

Total tract digestibility of Dry matter (DM) was general depressed in pigs fed diets containing the alternate protein sources. Crude protein (CP) and Gross energy (GE) digestibility were depressed in pigs fed cassava leaf meal (CLM) diet. Enzyme supplementation did not improve the digestibility of the energy and other proximate constituents. Faecal N output increased in pigs fed the alternate protein sources while Urine N and N retention were not affected ($P>0.05$) by the use of the alternate protein sources in the diets of pigs. There was reduced ($P<0.05$) conversion to ME in pigs fed diets containing the alternate protein sources. The ratio of DE/GE was lower in pigs fed diets containing CLM as compared to the basal diet. The digestibility energy values obtained for CLM, CLM+E, SM and SM+E were 10.2, 8.8, 10.1 and 10.0 MJ/kg DM respectively. Corresponding metabolizable energy were 9.8, 8.3, 9.0 and 9.3 MJ/kg DM, respectively. It was concluded that SM and CLM can be use individually in feeding growing pigs as partial substitute for the more expensive conventional plant protein feedstuffs, such as soybean; and can replace up to 23% of the diet of growing pigs.

Key words: Cassava leaf meal, shrimp meal, digestibility, N-retention, pigs

LA VALEUR ALIMENTAIRE DE L'ENZYME COMPLETEE DES FARINES DES FEUILLES DE MANIOC ET DE CREVETTES

Résumé

Dix porcs mâles croisés de 49,3 ± 3,97 kg de poids corporel ont été utilisés pour évaluer la digestibilité, la valeur énergétique et la rétention d'Azte de deux sources de protéines non conventionnelles(alternative), à savoir la farine de feuille manioc (FFM) et la farine de crevettes (FC), avec ou sans ajout d'un complexe enzymatique de polysaccharide non amylacé (PNA) ($\square$-glucanase et xylanase). Pendant deux périodes d'essai d'une durée de 7 jours chacune, deux porcs était nourris avec les cinq régimes expérimentaux suivants : l'alimentation basale (AB), AB + la poudre des feuilles manioc avec (FFM + E) et sans addition d'enzyme (FFM) et AB + farine de crevettes avec (FC + E) et sans supplémentation d'enzyme (FC).

La digestibilité totale du système de la matière sèche (MS) était commune chez les porcs nourris avec des aliments contenant les sources de protéines alternative. La digestibilité de la protéine brute (PB) et de l'énergie brute (EB) était réduite chez les porcs nourris à la farine de feuilles de manioc (FFM). La supplémentation enzymatique n'a pas amélioré la digestibilité de l'énergie et d'autres constituants immédiats. Les débits fécaux N ont augmenté chez les porcs nourris des sources alternatives de protéines tandis que l'azote urinaire et la rétention N n'était pas affectée ($P> 0,05$) par l'utilisation des sources

alternatives de protéines dans l'alimentation des porcs. La conversion en énergie métabolisable(EM) était réduite (P<0.05) chez les porcs nourris avec des aliments contenant les sources de protéines alternatives. Le rapport ED / EB était plus faible chez les porcs nourris avec des aliments contenant des FFM par rapport à l'aliment de base. Les valeurs de l'énergie de digestibilité obtenues pour FFM, FFM + E, FC et FC + E étaient respectivement de 10,2, 8,8, 10,1 et 10,0 MJ / kg MS. L'énergie métabolisable correspondant était respectivement de 9,8, 8,3, 9,0 et 9,3 MJ / kg MS. Il a été conclu que la FC et la FFM peuvent être utilisés individuellement dans l'alimentation des porcs en croissance, substitut partiel pour les aliments de protéines végétales classiques plus coûteuses, telles que le soja ; et peut remplacer jusqu'à 23% du régime alimentaire des porcs en croissance.

Mots clés : La farine de feuille de manioc, la farine de crevettes, la digestibilité, la rétention N et les porcs

Introduction

Soybean meal is the preferred source of protein in pig diets due to its content of highly digestible essential amino acids (lysine, threonine, truptophan and isoleucine). Combining it with cereals that is high in methionine (Heuzé, 2012) makes it an ideal protein feedstuff. Soybean meal can feed all classes of pigs and the inclcusion levels gerenerally used are about 30% in growing, finishing and pregnant sows, and slightly lower (20-25%) in piglets (Ewing, 1997). But the price of soybean in the developing economies of the world, like Nigeria, is far beyong the reach of most smallholder pig farmers.

FAO's Meat Price Index, a measure of global meat prices, is currently about 90% higher than ten years earlier, reflecting the impact of higher feed costs, which more than doubled over the decade (OECD-FAO, 2014). Nevertheless, higher meat consumption is brought about by rising incomes and urbanization; these factors are thought to enhance the intake of animal proteins at the expense of foods of vegetal origin in emerging economies (OECD-FAO, 2011). This development thus presents an opportunity for the global 500 million smallholder farmers to increase their livestock production and by this improve their livelihood (IFAD, 2014).

The use of alternative feedingstuffs can be help in ameliorating the effect of high prices of conventional feedstuffs, such as soybean, on the cost of smallholder pig farms. Archimède et al., (2011) view a great potential to produce high yields of livestock protein feeds on farms if tropical species of forage trees and shrubs, and of aquatic plants, are taken into account, even though the digestibility of these feeds might be somewhat lower than of the conventional feedstuffs. Plant foliage and agro-industrial by-products, such as cassava leaf meal and shrimp meal, respectively can used in order to address some of the challenges. Nevertheless, their proximate composition is variable, especially due to their high concentration of dietary fibre, when compared to soybean.

Nigeria is the worlds' largest producer of cassava (Emiko, 2014), from which cassava leaves are obtained, with an average figure of 73.95million tons in 2013 (FOASTAT, 2015). Cassava leaves are rich in protein (Aletor and Fasuyi 1997), minerals, vitamins B1, B2, C, and carotenes (Ravindran and Blair, 1992; Aletor and Adeogun, 1995). Ayodeji (2005) and Blank et al., (2012) reported a favourable balance of both non-essential and essential amino acids, namely lysine, leucine, valine and tryphophan, in cassava leaves, while methionine concentration was low. Despite this, cassava leaves so far remain under-utilized and huge tonnages of cassava leaves are currently discarded as waste after harvesting the roots. Also, Shrimp meal (SM), which is the dried and milled residue of the shrimp industry, consisting of the heads, appendages and exoskeleton of the shrimp (Aktar et al., 2011; Fanimo et al., 1996), has been identified as a potential feed resource for pigs (Rosenfeld et al., 1997). Ferrer et al., (1996) reported that shrimp waste is characterized by relatively high concentrations of protein (34.6% in DM), carbohydrate (chitin, 22.6%) and ash (28.1%). Exploring the use these two alternate protein sources will bring respite to many resource-poor pigs farmers and thereby increase food security.

Some studies have however reported reduced use of cassava leaf meal and shrimp meal by growing pigs, due to their high concentration of non-starch polysaccharides (Ravindran *et al.,* 1987; Oduguwa et al., 2004; Fanimo *et al.,* 2006). The work of Dierick and Decuypere (1994) indicated that the addition of enzymes to feedstuff with high concentrations of non-starch polysaccharides (NSP) caused an improvement in digestibility, by a reduction of their anti-nutritive effects in monogastrics and availability of energy content previously locked in the fibre. There is dearth of information in literature on the use of enzymes to enhance the utilization of both cassava leaf meal and shrimp meal in the diet of growing pigs.

Against the background of improving local resource use by smallholder pig farmers in Nigeria, and in general Africa, this study aimed at determining the nutritive value of cassava leaf meal and shrimp meal. Furthermore, it investigated how enzyme addition to leaf meal and shrimp meal affects nutrient digestibility, nitrogen balance and energy utilization in growing pigs.

Materials and Methods

Site

The study was conducted at the piggery unit of the Teaching and Research Farm of the University of Agriculture, Abeokuta (7°13'32.9"N, 003°25'26.2"E, 76 m a.s.l.), located in the derived savannah vegetation zone of South Western Nigeria. The region's climate is tropical humid, with mean annual precipitation averaging 1037 mm and rainfall occurring from March to October. Daily temperatures average 29.6°C in January (coolest month) and 30.4°C in April (hottest month).

Processing of test feeds

The cassava leaves were collected without petioles from nearby farms (after harvesting of roots) and then sundried for 3 days. The fresh shrimp meal was collected from a seafood processing industry in Lagos, and sun-dried for 4 days. All dried (<10% moisture content) test ingredients were milled to 2 mm particle size and stored in polythene sacks in a moisture-free environment until chemical analysis and subsequent feeding.

Animals and housing

Ten Large White x Duroc crossbred male pigs with an initial body weight (BW) of 49.3 ±3.97 kg were used. The pigs were housed individually in metabolic crates (allowing for separate collection of urine and faeces.) of 1.3 x 0.97m2, for a period of 28 days. The crates were placed on a cemented floor in a roofed shed with dwarf side walls.

Diets and feeding

Shrimp meal (SM) and cassava leaf meal (CLM) were added to the basal diet (BD) at the point of feeding. The latter consisted, on as-fed basis, of 79% maize meal, 18% soya bean meal and 3% minerals plus vitamin premix (Table 1). Pigs were fed the same amount of basal diet (1kg as fed), except for the pigs fed the test materials, which received additional 300 gm of the tested feedstuff, thoroughly mixed and fed in wet mash form (water : feed = 2:1) in two equal meals at 08:00 and 17:00 h. All the other treatments except for the basal group, received the same amount of feed. Water was supplied for *ad libitum* intake. The non-starch polysaccharide (NSP) enzyme complex, Rovabio(R) (Endo-1, 4, β-xylanase: 22,000 Visco. Units/g, β-Endo-1, 3(4) β-glucanase: 2,000 AGL units/g) was added at a concentration of 100 mg per kg DM to one batch of the basal diet, to which the test ingredients were also added at the point of feeding. In this way five test diets, i.e. cassava leaf meal without and with enzyme (CLM, CLM+E) and shrimp meal without and with enzyme (SM, SM+E) were obtained in addition to the basal diet that was also fed alone.

Experimental procedure

In each of the two experimental periods, two pigs each were assigned to the five experimental diets in such a way that no pig received the same experimental diet twice and no sequence of change over from one diet to another was repeated for any pig throughout the two periods (incomplete block crossing over design) which had adaptation

of 7days before and in-between the feeding periods. Thus, there were four pig replicates per treatment over the entire experiment. Pigs were weighed at the beginning and end of each of the 7 days of data collection. Faeces and urine were collected quantitatively twice daily and stored in a freezer (-4°C). To avoid ammonia losses, urine was collected into bottles with H_2SO_4 (20%, v/v) to keep pH below 3. After the collection periods, faeces and urine were thawed and homogenized separately and stored at -4°C until analysis.

The nutrient digestibility for test ingredients CLM, SM, CLM+E, and SM+E was determined using the formula proposed by Adeola (2001) as shown in Eq. 1. The digestible energy (DE), metabolizable energy (ME) and metabolizable energy corrected for zero nitrogen retention (MEn) for the alternative protein feedstuffs were calculated from the determined energy values of the test diets containing these feedstuffs and the basal diet.

$$A = 100 \times \left[\frac{(T \times t) - (B \times b)}{a} \right]$$

(Eq. 1)

Where T = digestibility, in percent, of the component in the total diet (basal diet plus the test feedstuff)
A = Digestibility of a test ingredient.

B = digestibility, in percent, of the component in the basal diet

t = amount of the component in the total diet consumed

a = amount of the component in the test feedstuff added to the basal diet

b = amount of the component in the basal diet consumed

Sample analysis

Following AOAC (1990) protocols, the residual dry matter (DM) of the samples were determined by drying at 105°C for 4 hours. The crude protein (CP) concentration of samples were determined by the Kjeldahl method and the ether extract (EE) by a

Soxhlet apparatus. The crude fibre (CF), neutral detergent fibre (NDF) and acid detergent fibre (ADF) concentration were determined by a modification of the method of Van Soest *et al.,* (1991) using a semi-automated ANKOM200/220 Fiber Analyzer (ANKOM Technology, Macedon, NY, USA). NDF and ADF values are expressed without residual ash. The gross energy (GE) of feeds and faeces were determined using a bomb calorimeter (CAL2K Calorific Value Analyser). The quantity of GE and nutrients consumed in the basal diet and the test diets was related to the corresponding quantity of GE and nutrients voided in faeces and urine. The digestible energy (DE) and metabolizable energy (ME) content of the diets and the nutrient digestibility coefficients were calculated by difference, i.e. DE (Eq. 2) and ME (Eq. 3) were calculated by subtracting gross energy of faeces voided or summation of GE of faeces and urine (in the case of ME) from the corresponding GE of feed ingested during the collection period. Energy loss due to methane production was not considered in this study. For the calculation of metabolizable energy corrected to zero nitrogen retention (MEn; Eq. 4), the value 31.2 MJ/kg (ARC, 1981) was used as the correction factor to zero nitrogen retention.

The formula used for the various energy values are as shown below:

$$DE = \frac{[(F_d \times GE_{fd}) - (F_c \times GE_{fc})]}{F_d}$$ (Eq. 2)

$$ME = \frac{[(F_d \times GE_{fd}) - (F_c \times GE_{fc}) - (U_r \times N_{ur} \times K_n)]}{F_d}$$ (Eq. 3)

$$MEn = \frac{ME - [(F_d \times N_{fd}) - (F_c \times N_{fc}) - (U_r \times N_{ur})] \times K_p}{F_d}$$

(Eq. 4)

where DE is the digestible energy (MJ/kg DM), ME the metabolizable energy (MJ/kg DM), MEn the metabolizable energy corrected for nitrogen retention, Fd the feed intake (kg DM), GE_{fd} the gross energy of feed (MJ/kg DM), F_c the faecal output (kg DM), N_{fd} the nitrogen content in feed (g/kg DM), N_{ur} the nitrogen content in urine (g/kg), GE_{fc} the gross energy of faeces (MJ/kg DM), U_r the urine output (kg), Kn= 40 MJ/kg N in urine (Susenbeth, 1996) and K_p = 31.2 MJ/kg N retained (ARC, 1981), the correction factor.

Statistical Analysis

Statistical analyses were carried out using the mixed model procedure (Proc Mixed) of SAS 9.1 (2003). The following model was used: where Y_{ij} is the observed response, υ the overall mean, D_i the fixed effect of diet i, P_j the random effect of period j animal effects Ak and eij is the residual error. A pair-wise comparison between Least Squares Means was conducted using Tukey–Kramer post hoc test for multiple mean comparisons.

Results

Composition of the alternate protein sources

Table 2 shows the GE concentration and proximate composition of the basal diet and the alternate protein sources. Crude protein content of CLM (284 g/kg DM) was lower than that of SM (406 g/kg DM) while crude fibre concentration was higher in SM compared to CLM. However, the NDF and the ADF concentration were higher in CLM (504 and 423 g/kg DM) than in SM (223 and 182 g/kg DM). In consequence, the concentration of hemicellulose was higher in CLM than in SM. the GE concentration was higher in CLM (15.7 MJ/kg DM) than in SM (14.9 MJ/kg DM).

Nutrient digestibility and nitrogen balance

Table 3 shows the dietary intake and excretion of faeces and urine of pigs fed the various by-products. The quantitative faecal DM excretion of pigs was not affected (P>0.05) by the inclusion of CLM and SM in their diet. Higher (P<0.05) faecal output was however recorded in pigs fed the CLM+E diet as compared to those fed the basal diet, while pigs on other diets had marginally higher faecal output than pigs on BD. Across the two protein

Table 1. Gross Composition (g kg-1 DM) of the basal diet (BD) and the diets containing the test ingredients (shrimp meal (SM) and cassava leaf meal (CLM)), supplemented with enzymes (+E).

Component	BD	SM	SM+E	CLM	CLM+E
Maize	790	790	790	790	790
Soybean	180	180	180	180	180
Vit/Mineral Premix	30	30	30	30	30
SM	-	300	300	-	-
CLM	-			300	300
Total	**1000**	**1300**	**1300**	**1300**	**1300**

Mineral-vitamin premix supplied per kg DM of complete diet: 100 mg Fe as FeSO4; 100 mg Zn as $ZnSO_4$; 20 mg Mn as MnO; 10 mg Cu as $CuSO_4$; 0.30 mg I as CaI; 0.30 mg Se as Na_2SeO_3; 5.506 IU vitamin A; 551 IU vitamin D3; 33 IU vitamin E; 3.6 mg vitamin K; 5.5 mg riboflavin; 25 mg D-pantothenic acid; 33 mg niacin; 27µg vitamin B12; 1.7 mg folic acid; 220 µg biotin; 120 mg choline.

Table 2. Proximate composition (g kg-1 DM) and energy value of the basal diet (BD) and the test ingredients (shrimp meal (SM) and cassava leaf meal (CLM)). Values are means of 3 samples collected during 2 sampling periods.

Component	Basal diet	CLM	SM
Dry matter	905	955	915
Crude protein	169.2	284.0	406.4
Crude fibre	31.3	210.3	258.6
ADFom†	75.0	422.9	182.0
NDFom†	252.0	504.2	223.3
HCom†	220.3	81.3	41.4
Gross Energy (MJ/kg)	16.4	15.7	14.9

CLM: Cassava leaf meal; SM: Shrimp meal; †om, exclusive residual ash; ADF, Acid detergent fibre; NDF, Neutral detergent fibre; HC, Hemicellulose.

Table 3: Daily feed intake, faecal and urine excretion of pigs fed the basal diet (BD) and the diets containing the alternative protein sources. Values are means of 4 pigs collected during 2 sampling periods.

Variable	BD	CLM	CLM+E	SM	SM+E	SEM	P=
Intake (g DM)							
Basal Diet	1358	918	910	909	909	-	-
Protein sources		274	272	271	271	-	-
Excretion							
Faecal Output (g DM)	117[b]	176[ab]	192[a]	161[ab]	163[ab]	9.81	0.033
Urine Output (g)	2240	2500	2370	2510	2390	99.0	0.931

[a,b] *Least square mean values in rows bearing different superscripts are significantly different at the indicated probability level. Where no superscripts are given, treatment means are not significantly different.*
BD, Basal diet; CLM, cassava leaf meal diet; CLM+E, cassava leaf meal diet with enzyme; SM, Shrimp Meal diet; SM+E, Shrimp meal diet with enzyme.

sources, enzyme supplementation did not have a significant effect on faecal DM concentration and quantitative faecal DM excretion. The quantitative urine excretion was not affected by the use of the alternate protein sources.

Table 4 shows the apparent nutrient digestibility of the diet containing the alternative protein sources with and without enzyme supplementation. DM digestibility in pigs that consumed the basal diet was significantly higher (P<0.05) than in pigs consuming diets CLM, CLM+E, SM and SM+E, which were of similar DM digestibility, indicating also that enzyme supplementation did not improve DM digestibility. The digestibility of fibre and it fractions appears to improve (P<0.05) with the inclusion of CLM and SM in the diets of growing pigs. Crude protein and Gross energy digestibility were better (P<0.05) with SM diets compared to CLM diets. In all the use of the multi-enzyme did not yield expected improvement in the digestibility of the proximate constituents of the test ingredients.

Table 5 shows the apparent digestibility of the two alternative protein sources, with or without enzyme supplementation. The digestibility of DM, NDF, CP and GE were not affected with or without enzyme addition to CLM and SM, respectively (P>0.05). Likewise, energy digestibility was not improved with or without the addition of enzyme did not improve the digestibility was marginally reduced with the use of enzyme.

Nitrogen intake (Table 6) was higher

(P<0.01) in pigs fed diets SM and SM+E than in pigs on CLM, CLM+E; nitrogen intake was lowest with the basal diet. Faecal nitrogen output was higher in pigs fed diets CLM and CLM+E than in pigs fed the basal diet, but the values were similar to those of pigs fed diets SM and SM+E. Urine nitrogen excretion was not affected (P>0.05) by the use of the alternate protein sources. As a consequence, total N excretion was similar (P>0.05) for all diets and was not affected by enzyme supplementation. Nitrogen retention therefore was not affected (P>0.05) by the use of alternate protein sources despite the increase in protein intake.

When expressing faecal and urine N excretion as well as N retention as a fraction of N intake, the proportion of faecal N was higher (P<0.01) in pigs on diet CLM+E than on BD, SM and SM+E, while it was similar to CLM. Urine nitrogen and total nitrogen excretion expressed as a fraction of nitrogen intake were not affected (P>0.05) by alternate protein sources and enzyme supplementation. N retention as a fraction of N intake was not affected (P>0.05) by the use of the alternate protein sources and enzyme supplementation.

Table 7 shows the energy value of the basal diet and the diets containing the alternate protein sources with or without enzyme supplementation. The digestible energy (DE) concentration was higher (P<0.05) for the basal diet than for the diets containing the alternate protein sources. Metabolizable energy (ME) concentration of BD was similar to CLM but higher than for CLM+E, SM and

Table 4: Digestibility (%) of the basal diet (BD) and the diets containing the alternative protein sources with or without enzyme supplementation. Values are means of 4 pigs collected during 2 sampling periods.

Variable	Basal Diet	CLM	CLM+E	SM	SM+E	SEM	P=
DMD	91.4[a]	85.2[b]	83.7[b]	86.4[b]	86.1[b]	0.899	0.009
NDFDom†	86.4[a]	81.6[ab]	78.8[b]	86.9[a]	87.2[a]	1.19	0.032
ADFDom†	48.0[b]	70.5[a]	63.1[ab]	76.7[a]	73.1[a]	2.93	0.003
CFD	71.7[ab]	74.2[ab]	68.5[b]	84.7[a]	83.9[a]	2.02	0.010
CPD	90.9[a]	85.3[bc]	83.3[c]	89.6[ab]	89.4[ab]	0.842	0.003
HCDom†	91.9	89.0	89.3	90.7	92.5	0.91	0.634
GED	91.4[a]	85.5[b]	83.7[b]	86.9[ab]	86.3[ab]	0.927	0.009

[a,b] *Least square mean values in rows bearing different superscripts are significantly different at the indicated probability level. Where no superscripts are given, treatment means are not significantly different.*
BD, Basal diet; CLM, cassava leaf meal diet; CLM+E, cassava leaf meal diet with enzyme; SM, Shrimp meal diet; SM+E, Shrimp meal diet with enzyme.
DMD, dry matter digestibility; CPD, crude protein digestibility; NDFD, digestibility neutral detergent fibre; ADFD, digestibility acid detergent fibre; HCD, digestibility of hemicellulose; GED, Gross energy digestibility.
†om, exclusive residual ash

Table 5. Digestibility (%) of the alternative protein sources (as single ingredients) with or without enzyme supplementation. Values are means of 4 pigs collected during 2 sampling periods.

Variable	CLM	CLM+E	SM	SM+E	SEM	P=
DMD	65.7	59.6	69.9	68.8	3.69	0.525
NDFom†	49.6	63.7	73.0	57.3	4.73	0.455
CFD	72.4[ab]	63.9[b]	82.2[a]	88.0[a]	3.10	0.012
CPD	71.2	65.6	80.1	80.1	2.26	0.054
GED	63.2	55.4	62.4	61.8	3.59	0.720

[a,b] *Least square mean values in rows bearing different superscripts are significantly different at the indicated probability level. Where no superscripts are given, treatment means are not significantly different.*
CLM, cassava leaf meal; CLM+E, cassava leaf meal with enzyme; SM, Shrimp Meal; SM+E, Shrimp meal with enzyme; DMD, dry matter digestibility; CPD, crude protein digestibility; NDFD, digestibility neutral detergent fibre; GED, Gross energy digestibility.
†om, exclusive residual ash

Table 6. Nitrogen (N) balance of pigs fed the basal diet (BD) and diets containing the alternative protein sources with or without enzyme supplementation. Values are means of 4 pigs collected during 2 sampling periods.

N balance (g N pig-1 d-1)	BD	CLM	CLM+E	SM	SM+E	SEM	P=
N intake	36.7[c]	37.5[b]	37.2[b]	42.3[a]	42.3[a]	0.583	0.001
Faecal N	3.4[b]	5.5[a]	6.2[a]	4.4[ab]	4.5[ab]	0.298	0.010
Urine N	14.6	12.2	13.5	17.2	14.7	1.030	0.626
Total N excreted	17.9	17.8	19.7	21.5	19.2	1.100	0.834
N retention	18.8	19.7	17.5	20.7	23.1	1.15	0.649
N balance (g g-1 N intake)							
Faecal N	0.092[b]	0.147[ab]	0.167[a]	0.104[b]	0.106[b]	0.008	0.004
Urine N	0.380	0.280	0.412	0.454	0.347	0.020	0.831
Total N excretion	0.488	0.474	0.530	0.512	0.453	0.027	0.926
N Urine / N Faeces	4.57[a]	2.23[b]	2.20[b]	3.92[a]	3.14[ab]	0.309	0.006
N retention	0.512	0.527	0.470	0.489	0.547	0.027	0.926

[a,b] *Least square mean values in rows bearing different superscripts are significantly different at the indicated probability level. Where no superscripts are given, treatment means are not significantly different.*
BD, Basal diet; CLM, cassava leaf meal diet; CLM+E, cassava leaf meal diet with enzyme; SM, Shrimp Meal diet; SM+E, Shrimp meal diet with enzyme.
N, Nitrogen

Table 7: Energy values MJ/kg DM of the basal diet (BD) and diets containing the alternative protein sources with or without enzyme supplementation. Values are means of 4 pigs collected during 2 sampling periods.

Variable	BD	CLM	CLM+E	SM	SM+E	SEM	P=
DE	14.97[a]	13.86[b]	13.56[b]	13.93[b]	13.82[b]	0.161	0.002
ME	14.54[a]	13.45[ab]	13.10[b]	13.35[b]	13.33[b]	0.166	0.009
MEn	14.10[a]	12.93[b]	12.64[b]	12.80[b]	12.71[b]	0.167	0.001
ME/DE	0.971	0.970	0.966	0.958	0.964	0.000	0.493
DE/GE	0.914[a]	0.855[b]	0.837[b]	0.869[ab]	0.863[ab]	0.010	0.011
ME/GE	0.888[a]	0.830[ab]	0.808[b]	0.833[ab]	0.832[ab]	0.010	0.030
MEn/GE	0.861[a]	0.798[b]	0.780[b]	0.799[b]	0.793[b]	0.010	0.005

[a,b]*Least square mean values in rows bearing different superscripts are significantly different at the indicated probability level. Where no superscripts are given, treatment means are not significantly different.*
DE, digestible energy; ME, metabolizable energy; MEn, Metabolizable energy with zero nitrogen retention.
BD, Basal diet; CLM, cassava leaf meal diet; CLM+E, cassava leaf meal diet with enzyme; SM, Shrimp meal diet; SM+E, Shrimp meal diet with enzyme

Table 8. Mean digestible (DE) and metabolizable (ME and MEn) energy concentration (MJ/kg DM) of the alternative proteins sources evaluated with or without enzyme addition. Values are means collected from 4 pigs during 2 sampling periods.

Variable	CLM	CLM+E	SM	SM+E
DE	10.2	8.8	10.1	10.0
ME	9.8	8.3	9.0	9.3
MEn	9.0	7.7	8.0	8.1

DE, digestible energy; ME, metabolizable energy;
MEn, Metabolizable energy with zero nitrogen retention.
CLM, cassava leaf meal; CLM+E, cassava leaf meal diet with enzyme; SM, Shrimp meal diet; SM+E, Shrimp meal diet with enzyme.

SM+E. The metabolizable energy concentration at zero nitrogen retention (MEn) followed a pattern similar to that of DE. All diets had similar ME/DE-ratio. The proportion of ME to GE was higher (P<0.01) in all diets except in CLM+E diet. The efficiency of conversion of GE into MEn was highest (P<0.01) for the basal diet compared to all diets containing the alternate protein sources.

Table 8 shows the mean DE, ME and MEn values of the alternate protein sources calculated from the energy values determined for the test diets containing them. The DE values of the CLM, SM and SM+E are close compared to that of CLM+E.

Discussion

Digestibility of DM, CP, OM and energy

was lower in diets containing CLM and CLM+E compared to those of basal diet. Other studies indicated a similar reduction in digestibility with the use of CLM in the diet of growing pigs (Blank et al. 2012; Agunbiade et al. 2004; Régnier et al. 2013). The lower CP digestibility of CLM and CLM+E as compared to the basal diet is in line with findings of Phuc et al. (1995), and their lower GE digestibility is in accordance with the findings of Noblet and Le Goff (2000), indicating that energy digestibility in growing pigs is reduced by an increase in dietary fibre content. Pigs, although eat voraciously and can consume a considerable quantity of fibre, their hind gut fermentation capacity of fibre is limited compared to ruminants. The enzyme used did not improve fibre digestibility of CLM and SM. Digestibility of CLM+E diet by pigs was comparable to that of pigs fed CLM diets. This is line with the findings of Officer (1995) who used a multi-enzyme complex in a wheat-based

diet for weaner pigs and reported reduced utilization of the enzyme supplemented diet as compared to the unsupplemented one.

In DM digestibility, there was no effect of the enzyme used in pigs fed diet SM+E compared SM diet. The digestibility of other proximate constituents was not affected by the use of SM in our study. Most reports have shown that the major concern when feeding shrimp meal is the high concentration of chitin, a poly-β-($1\rightarrow4$)-N-acetyl-D-glucosamine, that forms part of the protein complex and has been generally considered to be poorly utilized, even in the microcrystalline form (Austin *et al.,* 1981). According to Rosenfeld *et al.,* (1997) the nutritional value of SM depends on its proportion of exoskeletons, and thus the chitin concentration. The result obtained indicated that the enzyme used was not effective in degrading chitin – thereby making the nutrient locked in it unavailable for use by pigs. This reason can be the cause of the non-effectiveness of the enzyme used in enhancing the use of SM by pigs in this study.

The digestibility of proximate constituents and energy of CLM and SM, when considered as single ingredients, were unaffected by enzyme supplementation. The ADF digestibility of SM and SM+E (85.3% and 80.1%) was higher than the 47.1% reported by Fanimo *et al.,* (2006). Normally, the utilization of shrimp meal will improve with prompt processing of these wastes. This involves controlled drying, preferably in a tunnel, at 80oC, since the material still contains large portions of soluble membranes in the head region and other parts. Sun-drying, which takes relatively longer time, may encourage putrefaction, if not carefully handled due to the activities of flies, leading to a significant reduction in the soluble parts and appreciable compositional changes, leaving mainly the chitin portion that is poorly digestible. A tunnel dryer was however employed by Rosenfeld *et al.,* (1997), leading to a good performance in broiler chickens fed SM, comparable to that of soybean meal, which it replaced. In the present study, sun drying of SM was done during the hot dry season and thus within short period of time. But this shortness could not compare

to that achieved by a tunnel dryer employed by Rosenfield, and as a result the impracticability of obtaining the same quality SM product and consequently good animal response under the normal sun-drying conditions in the humid tropics.

There appears to be no difference in the digestibility of the two alternate protein sources tested in this study, as the energy digestibility and most proximate constituents were not affected. Recalculating the amount of CLM and SM added to the diets, as seen in Table 1, while taking the total as 100 percent, showed that each of the alternate protein sources makes up 23% of the diet they contain.

The quantitative excretion of faeces was higher in diets containing the alternative protein sources as compared to the basal diet. This could be related to the effect of the largely insoluble dietary fibre in these feedstuffs, as insoluble dietary fibre (Zhang *et al.,* 2013) and by extension total dietary fibre (Agunbiade and Susenbeth 2006) have been reported to increase faecal bulk. One can safely deduce that the total dietary fibre in the alternative protein sources used in this study consisted of more insoluble than soluble dietary fibre. The former has been reported to have a higher water holding capacity (Serena *et al.,* 2008) and therefore capacity to increases faecal bulk (Zhang, 2013).

The higher fractional excretion of ingested nitrogen in faeces observed with the alternative protein sources is consistent with the results of Ravindran *et al.,* (1987), Agunbiade *et al.,* (2004) and Blank *et al.,* (2012). Increase of bacteria nitrogen in faeces has been shown to occur with an increased proportion of dietary fibre (Bindelle *et al.,* 2009) due to the availability of fermentable fibre in the lower gut, occasioned by the decreased absorption of nutrients (Wilfart *et al.,* 2007) in the upper gut, which could be mainly responsible for the low CP digestibility observed in CLM and CLM+E fed pigs. Absolute faecal nitrogen excretion was lower in the basal diet than in the diets containing the alternative protein sources due to their higher fibre levels (Zhang 2013 and Patráš *et al.,* 2009), and even enzyme supplementation in the diets containing the

alternate protein did not influence faecal nitrogen excretion. Fiber fermentation changed the partitioning of N excretion from urine to faecal N (Hansen *et al.,* 2007; Shriver *et al.,* 2003; Zervas and Zijlstra, 2002). Absolute total nitrogen output and nitrogen retention were however not affected.

When expressed as a fraction of N intake however, faecal N excretion of pigs fed CLM+E and CLM were similar but higher than that of pigs fed SM and SM+E. Protein that escapes digestion in the small intestine is used along with products from fibre digestion by the microflora in the large intestine for the synthesis of microbial protein, resulting in enhanced microbial protein concentration in the faeces (Zervas and Zijlstra 2002; Canh *et al.,* 1997). Notwithstanding, N retention was not affected by the use of the alternative protein sources with or without enzyme supplementation. Phuc *et al.,* (2000) however, reported a significant depression in N retention as protein from soybean was replaced by protein from cassava leaf meal. Unfortunately, the author did not indeed whether the cassava leaves used were with or without petioles. While Fanimo *et al.,* (2006) reported a significant reduction in N retention with SM, the use of SM in the present study compared favourably with the basal diet. It is well understood that the test ingredients provided small amount of amino acids, which could aid the digestion of the diets in which they were added, but the confounding effect of fibre may altogether reduce their optimal use.   However, from the result obtained in this study, it was observed that there was an overestimation of N-retention by N-balance technique.

The observed reduction in DE and ME concentrations of diets CLM, CLM+E, SM and SM+E can be attributed to the increase in fibre intake by pigs on these diets. It is well known that fibrous diets are energy-poor as a result of their low digestibility, as also reported by Agunbidade *et al.,* (2004) and Fanimo *et al.,* (2006).

The DE concentration of CLM obtained in this study was 10.2 MJ/kg DM, while Agunbidade *et al.,* (2004) reported a value of 8.0

MJ/kg DM. This might be so because the CLM used by Agunbiade et al. 2004 included petioles, whereas that used in this study were without. The use of the multicarbohydrase enzyme tends to reduce the energy value of CLM as compared to SM, which appears unaffected and unchanged in its energy values by the enzyme addition.

Masey O'Neill *et al.,* (2014), in their extensive review on the use of multicarbohydrase enzymes for non-ruminants, proved that the use of products containing many mixed enzymes is unwarranted, and can even generate negative effects on animal performance. This might explain some of the responses obtained, especially in the digestibility and energy values of the alternative protein sources when supplemented with the enzyme.

Conclusions

The alternative protein sources, cassava leaf meal and shrimp waste meal, contain high concentrations of antinutritive NSP fractions, which limit their utilization in pig feeding, even though adverse effects on nitrogen retention are not observed. The addition of the multicarbohydrase enzyme to these two feedstuffs lowered their digestibility and did not improve their energy values, but nitrogen retention were not affected in growing pigs. Summarily, our results and some previous studies showed that cassava leaf meal and shrimp meal can replace up to 23% of the diet of growing pigs without any adverse effect on production performance.

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EFFECTS OF DIFFERENT HOUSING SYSTEMS ON GROWTH PERFORMANCE AND CARCASS YIELD OF TWO BREEDS OF TURKEY

*Olajide Mark Sogunle, Muideen Aderemi Ogundele[1], Olufemi Sunday Akinola, Chiemeka Promise Njoku and Abimbola Oludele Oso[2]
Department of Animal Production and Health, Federal University of Agriculture, Abeokuta,   P. M. B. 2240, Abeokuta, Ogun State, Nigeria
[1]Livestock Department, Osun State Ministry of Agriculture, Osogbo, Osun State, Nigeria
[2]Department of Animal Nutrition, Federal University of Agriculture, Abeokuta, P. M. B. 2240, Abeokuta, Ogun State, Nigeria

Summary

In the last few decades, a rapid increase in poultry production is experienced due largely to improvements in the management systems. This study thereby investigated the effects of different housing systems on growth performance and carcass yield of exotic and locally-adapted breeds of turkey. A total of 192 unsexed day-old poults (96 each of British United and locally-adapted Turkeys) were purchased and brooded for three weeks. Each breed was then allocated randomly into two treatments of deep litter and wooden cage with 48 poults each which were further subdivided into four replicates of twelve poults each using a 2 x 2 factorial experimental layout in a Completely Randomized Design. The growth performance was significantly ($p<0.05$) influenced by the breeds with exotic breed having higher final weight (6,305.00 g/b), weight gain (60.67 g/b/d) and feed intake (238.57 g/b/d) compared with the final weight (3,541 g/b), weight gain (34.08 g/b/d) and feed intake (138.55 g/b/d) of the locally-adapted turkey breed. Also, significantly ($p<0.05$) higher final weight and weight gain were recorded for the exotic turkey reared on wooden cage at the starter phase. The results on carcass yield showed a significantly ($p<0.05$) higher plucked weight (5,216.67 g/b) in the exotic breed. A better ($p<0.05$) cost-benefit ratio of 3.29 was obtained in the locally-adapted turkey. The study concluded that growth performance indices were better in the exotic than the locally-adapted turkeys. However, with respect to the cost-benefit ratio, the rearing of the locally-adapted turkey in either of the housing system is recommended.

Keywords: locally-adapted turkey, exotic turkey, growth performance, wooden cage, deep litter

LES EFFETS DES DIFFÉRENTS SYSTÈMES DE LOGEMENTS SUR LES PERFORMANCES DE CROISSANCE ET DE RENDEMENT DE LA CARCASSE DE DEUX RACES DE DINDE

Résumé

Au cours des dernières décennies, une augmentation rapide de la production de la volaille est connue principalement en raison de l'amélioration des systèmes de gestion. Cette étude a ainsi étudié les effets des différents systèmes de logement sur les performances de croissance et le rendement de la carcasse des races exotiques et localement adaptées de dinde. 192 dindonneaux d'un jour non sexes (96 de race British United et 96 localement adaptées) ont été achetées et couvées pendant trois semaines. Chaque race avait ensuite été répartie au hasard en deux traitements litière profonde et cage en bois avec 48 dindonneaux et chacune subdivisée en quatre répétitions de douze dindonneaux, chacune utilisant un dispositif expérimental factoriel de 2 x 2 complètement aléatoire. La croissance était significativement (p <0,05) influencée par les races avec race exotique ayant un poids final plus élevé (6,305.00 g / b), un gain de poids (60,67 g / b / d) et une consommation alimentaire (238,57 g / b / j) par rapport au poids final (3541 g / b), au gain de poids (34,08 g / b / j) et à la consommation alimentaire (138,55 g / b / j) de la race de dinde adaptée localement. En outre, de manière significative, (p <0,05) le gain de poids le plus élevé et le poids final avaient été enregistrés pour la dinde exotique élevée sur la cage en bois à la phase de démarrage. Les résultats sur le rendement de la carcasse ont montré un poids élevé (p <0,05) des plumes (5,216.67 g / b) dans la race exotique. Un meilleur rapport (p <0,05) coût-bénéfice de 3,29 a été obtenu dans la

Corresponding author email: sogunleom@funaab.edu.ng

dinde adaptée localement. L'étude a conclu que les indices de performance de croissance étaient mieux dans l'exotique que les dindes adaptées localement. Toutefois, en ce qui concerne le rapport coût-bénéfice, l'élevage de la dinde localement adaptée soit du système de logement est recommandé.

Mots-clés: la dinde adaptée localement, la dinde exotique, la performance de croissance, la cage en bois, la litière profonde.

Introduction

Indigenous chickens have remained predominant in the developing world (Do, 2005; Bett *et al.,* 2012) despite the introduction of the exotic strain. Local chickens have a slow growth rate thereby attaining a market body weight at a much later age when compared with the exotic meat strain. The slow growth has been shown to result into tastier and tougher meat (Wattanachant et al., 2004; Saowakon *et al.,* 2008); attributes which are concomitant with preferences of many chicken consumers. UBOS (2009) reported an increase in the number of producers and consumers interest in local chicken products whereas Mugga (2007) and Kyarisiima et al. (2004; 2011) reported a big market niche for local chicken products because their meat is perceived to be tastier than that from exotic strain.

In Nigeria, there is no known discriminatory legislation against the production of turkey and consumption of its meat but they are very scarce to find. It was thereby opined that the potentials of locally-adapted turkeys cannot be overlooked considering the huge foreign exchange implication of the imported exotic stocks. However, scarcity of the local strain of turkey could be due partly to the fact that chickens are grown so well that there seems to be no reason to consider any other poultry species, and partly because most exotic turkeys have been so highly bred for intensive production that the resulting birds are inappropriate for extensive production (Peters *et al.,* 2002). These exotic turkeys are characterized by early maturity, high productivity but low tolerance to change in environmental condition; disease, pest and parasites. The problems associated with reliance on exotic breeds for turkey industry in Nigeria include poor performance, importation of disease if birds are not well quarantined and the need to replenish stock by importation which in turn affects the country's foreign exchange earnings.

Alternative poultry production involves a cage-free environment or other access to the outdoors as alternative to conventional poultry housing and cages. It is an important aspect of sustainable agricultural production that enhances farm income while protecting the environment and addressing consumers' concerns. It could be practiced on a larger scale but it is often on a small-scale and sometimes integrated into a diversified farm. In a recent study (Sogunle *et al.,* 2013) on cockerel chickens, it was revealed that free range could be an alternative to confinement housing which was found environmentally sound, economically viable and focused on low-input strategies.

It is noteworthy that housing system is dependent on the market for different categories of birds and the practical experiences with competing model (Tauson, 2005). Therefore, for any housing or production system to be considered good, birds must be free from stress and disease. Birds need to be able to grow, sleep and lay eggs in comfort. Pasture rearing is thereby a cornerstone to this approach. This study thereby seeks to evolve an acceptable recommendation from the comparison of the growth performance and carcass yield of raising exotic and locally-adapted breeds of turkey in different housing systems.

Materials and Methods

Experimental Site

The experiment was carried out at the Turkey Unit of the Directorate of University Farms (DUFARMS), Federal University of Agriculture, Abeokuta, Ogun State, Nigeria. The site is located in the rain forest vegetation zone of Southern - Western on Latitude 70 10' N and Longitude 30 2' E and altitude of 76m

above the sea level (Google Earth 6.0, 2014). The climate is humid with a mean annual rainfall of about 1037 mm and mean temperature and humidity of 34.7⁰C and 83%, respectively.

Experimental birds and management

A total of 192 unsexed day old poults (96 each of locally-adapted and British Unit Turkeys) were purchased from Agrited hatchery Nigeria Limited in South-western Nigeria. The poults were intensively brooded together for three weeks on floor with space requirements of 0.25 square metre per poult. At the end of the third week each breed of poults were on weight equalization basis (balancing for the sexes) and allocated randomly to two treatment groups of deep¬-litter and wooden cage housing systems of 48 poults each. Each treatment group was further divided into four replicates of twelve (12) poults each. Routine and occasional management practices in turkey production were carried out as at when due. Feeders and drinkers were cleaned daily and fresh feed and water were supplied daily. Litter materials (wood shavings) were changed forthnightly and as at when due throughout the experimental period. The birds were fed commercially prepared turkey diets containing 26.6% CP and 12.1 MJ/kg ME at weeks 3 to 8 (starter phase), and 19.15% CP and 12.54 MJ/kg ME at weeks 9 to 16 (grower phase).

Dimensions of the housing systems

Each wooden cage as well as each deep litter pen has a dimension of 1m x9m which is 9m2 that housed 12 turkeys. The height of the wooden cage which was made of wire mesh and wood with galvanized metal at the base was 2.5m from the floor to the roof.

Experimental Design

The experimental layout was a 2x2 factorial arrangement that contained two housing systems (wooden cage and deep litter) and two breeds of turkeys (locally-adapted and exotic).

Data Collection

The following measurements were taken on the growth performance of the poults at starter and grower phase:

Determination of Growth Performance

i.   Weight gain (g)
The initial body weight of each of turkeys was taken while subsequent body weights were recorded on weekly basis.
Weight gain   = Final weight - Initial weight.

ii.   Feed Intake (g)
Feeds were given to each group of poults while left over of feeds were weighted to determine daily feed intake and consequently weekly feed intake. This was calculated using the formula:

(iii)   Feed intake per bird =
$$\frac{\text{Feed supplied   - Left over of feed}}{\text{Number of birds}}$$

(iv)   Feed   conversion   ratio   (FCR) determination
The FCR of each group of poults was determined by calculating the ratio of feed intake to weight gain and thus calculated as:

(v)   Feed conversion ratio (FCR) =
$$\frac{\text{Total Feed intake (g)}}{\text{Total body weight gain (g)}}$$

(v)   Percentage mortality =
$$\frac{\text{Number of dead birds per replicate}}{\text{Initial number of bird per replicate}} \times 100$$

Cost-Benefits ratio

The cost analysis of each breed of turkeys was estimated using the prevailing market prices at the time of the study. Brooding cost and the cost of feed were recorded, and the feed intake of each turkey in the course of the experiment was used to multiply the cost per kg of feed to obtain the cost of feed consumed per turkey. The production cost per kg live weight gain was calculated as an estimate of cost of day old poults, brooding cost, drugs, vaccines and feeds. A dollar was equivalent to N170 at the time of the study

Statistical Analysis

Data collected were subjected to Completely Randomized Design. Significantly (P<0.05) different means were separated using Duncan's Multiple Range Test as contained in SAS (2003) package. Data collected on the cost benefit ratio were subjected to studentized t-test at 5% level of significance while changes in weight of the breeds of turkey in the different housing systems were descriptively expressed using line charts.

$$Y_{ijk} = \mu + A_i + B_j + (AB)_{ij} + E_{ijk}$$

where:

Y_{ijk} = observed value of a dependent variable

μ = Population mean

A_i = Effect of the ith housing systems group (i=1,2)

B_j = Effect of the jth breed (j = 1,2)

$(AB)_{ij}$ = Effect of the interaction between housing systems and breeds

E_{ijk} = Random error associated with each observation.

Results

Growth performance of locally-adapted and exotic turkey breeds on deep litter and wooden cage at the starter phase

The main effects of breed and housing system on the growth performance of turkey at the starter phase (3-8 weeks) are shown in Table 1. The breeds were significantly (p<0.05) different in all the parameters considered including the initial weight and the body temperature which were higher in the exotic turkey from day-old. Although, exotic turkey had a better (p<0.05) feed conversion ratio than locally-adapted turkey but it recorded more mortality (20.33%) than the locally-adapted turkey (11.88%). In the housing system, significantly (p<0.05) higher final weight (g/b) and weight gain (g/b/d) were obtained in turkeys reared on wooden cage.

In the interaction effects between breed and housing system on the growth performance of turkey at starter phase (Table 2), significantly (p<0.05) highest final weight (g/b), weight gain (g/b/d) and feed intake (g/b/d) including mortality (%) were observed in exotic turkey on wooden cage. Locally-adapted turkeys on deep litter recorded lowest values in final weight (g/b), weight gain (g/b/d), and feed intake (g/b/d). The lowest (p<0.05) mortality of 9.10 % was recorded in locally-adapted turkeys on wooden cage.

Growth performance of locally-adapted and exotic turkey breeds on deep litter and wooden cage at the grower phase (9-16 weeks)

In Table 3, the main effects of breed and housing system on the growth performance of turkey at the growing phase are shown. Exotic breed had significantly (p<0.05) higher initial weight (g/b), final weight (g/b), weight gain (g/b/d) and feed intake (g/b/d). On the other hands, significantly (p<0.05) higher initial weight (2026.69 g/b) was recorded in turkeys reared on wooden cage.

In the effects of interaction between breed and housing system on growth performance of turkey at growing phase (Table 4), significant differences were obtained in the initial weight, final weight, weight gain, feed intake and body temperature (0C). The highest (p<0.05) weight gain of 61.45g/b was obtained in exotic breed reared on deep litter while the lowest value of 32.11g/b/d was obtained in locally-adapted turkey reared on wooden cage.

Figure 1 depicts exotic turkey having no marked differences in weight changes in the housing systems for the period of the experiment. This same trend is observed in Figure 2 for locally-adapted turkey in the housing systems at the 16th week. However, from the 6th to the 14th week, locally-adapted turkey reared in wooden cage had relatively higher weight changes than those reared on deep litter. In Figure 3, marked difference could be observed in the weight changes between locally-adapted turkey and exotic turkey reared on deep litter housing system and this same trend is depicted by the two breeds of turkey

reared on wooden cage as shown in Figure 4.

Carcass yield of locally-adapted and exotic turkey breeds on deep litter and wooden cage
        Table 5 shows the main effects of breed and housing system on carcass yield of turkey.

In the breed, higher (p<0.05) significant effects were observed in live weight (5,916.67g), and plucked weight (5216.67g) in the exotic breed than the locally-adapted turkeys but the dressing percentage was not significantly (p>0.05) different. In the cut-up parts, higher

Table 1: Main effects of breed and housing system on the growth performance of turkey at the starter phase (3-8weeks)

Parameter	Breed		Housing System	
	Locally-adapted	**Exotic**	**Deep litter**	**Wooden cage**
Initial Weight (g/b)	208.60±2.39[b]	289.05±14.92[a]	255.12±0.89	242.53±19.47
Final Weight (g/b)	1394.07±62.00[b]	2482.91±61.11[a]	1850.28±244.4[b]	2026.69±251.49[a]
Weight gain (g/b/d)	21.16±1.07[b]	39.17±1.15[a]	28.48±3.99[b]	31.86±4.21[a]
Feed intake (g/b/d)	70.44±2.25[b]	94.17±5.08[a]	78.67±4.69	85.93±7.73
Feed conversion ratio	3.35±0.15[a]	2.40±0.10[b]	2.95±0.30	2.80±0.18
Mortality (%)	11.88±5.54b	20.33±9.11a	13.39±5.08	18.81±9.6
Body temperature (^{0}C)	37.50±00[b]	41.50±0.00[a]	39.50±0.00	39.50±0.89

[a,b] Means in the same row with different superscripts differ significantly (P<0.05)

Table 2: Effects of interaction between breed and housing system on the growth performance of turkey (3-8weeks)

Parameter	Exotic		Locally-adapted	
	Deep litter	**Wooden Cage**	**Deep litter**	**Wooden cage**
Initial Weight (g/b)	303.15±9.78[a]	274.96±28.62[a]	207.10±1.05[b]	210.10±5.02[b]
Final Weight (g/b)	2389.44±78.18[a]	2576.37±61.90[a]	1311.11±45.74[b]	1477.02±101.37[b]
Weight gain (g/b/d)	37.25±1.50[a]	42.01±0.83[a]	19.71±0.79[b]	22.62±1.72[b]
Feed intake (g/b/d)	86.71±5.25[ab]	101.62±6.78[a]	70.64±4.20[b]	70.24±2.72[b]
Feed conversion ratio	2.32±0.07[b]	2.48±0.21[b]	3.58±0.22[a]	3.12±0.13[a]
Mortality (%)	12.13±8.02[b]	28.53±16.84[a]	14.66±7.94[b]	9.10±9.10[c]
Body temperature (0C)	41.50±0.00[a]	41.50±0.00[a]	37.50±0.00[b]	37.50±0.00[b]

[a,b] Means in the same row with different superscripts differ significantly (P<0.05)

Table 3: Main effect of breed and housing system on the growth performance of turkey at the growing phase (9-16weeks)

Parameter	Breed		Housing System	
	Locally-adapted	**Exotic**	**Deep litter**	**Wooden cage**
Wooden cage	1394.07±62.00[b]	2482.91±61.11[a]	1850.28±244.49[b]	2026.69±251.49[a]
Final Weight (g/b)	3541.00±68.28[b]	6305±291.65[a]	4922.17±656.79	4925.00±649.78
Weight gain (g/b/d)	34.08±1.72[b]	60.67±4.14[a]	48.76±6.89	46.00±6.50
Feed intake (g/b/d)	138.55±4.31[b]	238.57±14.27[a]	181.71±24.35	195.40±24.70
Feed conversion ratio	4.14±0.32	3.94±0.10	3.76±0.20	4.32±0.22
Mortality (%)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Body temperature (0C)	40.00±00	43.00±0.00	41.50±0.00	41.50±0.07

[a,b] Means in the same row with different superscripts differ significantly (P<0.05)

Table 4: Effects of interaction between breed and housing system on growth performance (9-16 weeks)

Parameter	Exotic		Locally-adapted	
	Deep litter	**Wooden cage**	**Deep litter**	**Wooden cage**
Initial Weight (g/b)	2389.44±78.18[a]	2576.37±61.90[a]	1311.11±45.74[b]	1477.02±101..37[b]
Final Weight (g/b)	6261.00±589.49[a]	6350.00±275.38[a]	3583.33±130.17[b]	3500.00±68.06[b]
Weight gain (g/b/d)	61.45±8.33[a]	59.90±3.97[a]	36.06±2.66[b]	32.11±1.97[b]
Feed intake (g/b/d)	230.01±24.60[a]	247.12±18.43[a]	133.42±5.28[b]	143.69±6.22[b]
Feed conversion ratio	3.77±0.13	4.12±0.07	3.76±0.0.44	4.52±0.44
Mortality (%)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Body temperature (0C)	43.00±0.00	43.00±0.00	40.00±0.00	40.00±0.00

[a,b] Means in the same row with different superscripts differ significantly (P<0.05)
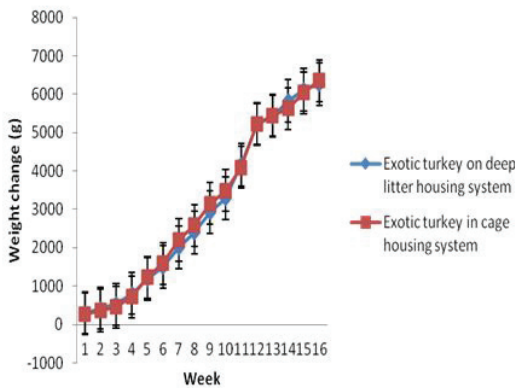
Figure 1: Effects of housing system on weekly weight change of exotic turkey
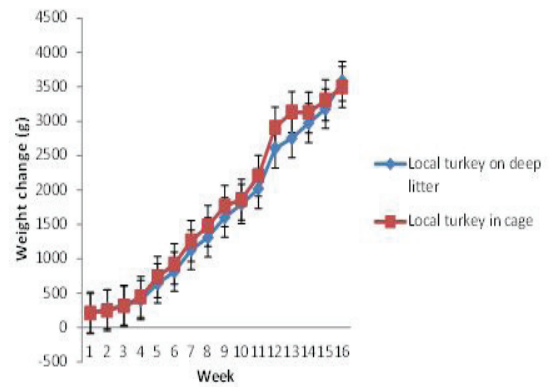
Figure 2: Effects of housing systems on weekly weight change of locally-adapted breed of turkey,
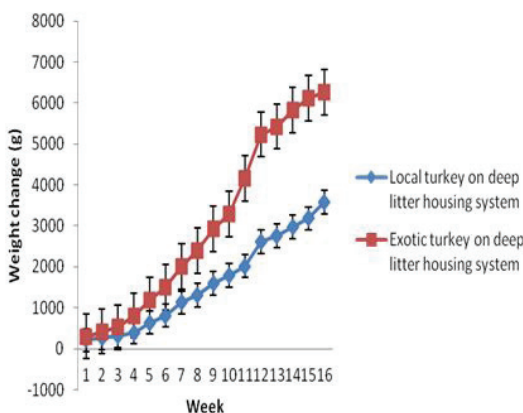
Figure 3: Effects of deep litter housing system on weekly weight change of local and exotic turkey.
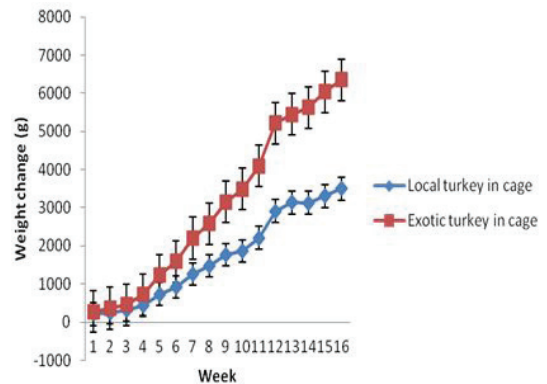
Figure 4: Effects of cage housing system on weekly weight change of local and exotic turkey

significant (p<0.05) differences were obtained in the breast meat (17.90%) and shanks (3.40%) in the exotic breed of turkey than the locally-adapted turkey. However, higher significant (p<0.05) value was obtained in the head (2.78%) of the locally-adapted turkey than the exotic breed (1.72%). Also, in the organs, higher (p<0.05) significant value was obtained in the proventriculus (0.23%) of exotic breed than the locally-adapted turkey. The housing system

did not significantly (P>0.05) influence all the parameters considered.

In the effects of interaction between breed and housing systems on the carcass yield of turkey (Table 6), significant (p<0.05) differences were obtained in live weight, plucked weight, head, neck, drumsticks, thighs, shanks, proventriculus and the small intestine across the treatments. Exotic turkey on wooden cage had the highest (p<0.05) values in live weight, plucked weight, drumsticks and thighs. The dressing percentage, wings, back, breast, liver, heart, gizzard, kidney, lungs, spleen, caecum and large intestine were not significantly (p>0.05) different across the treatments.

Cost-benefit ratio of rearing locally-adapted and exotic turkey breeds

In Table 7, the cost-benefits ratio of rearing locally-adapted and exotic turkeys is shown. Significantly (p<0.05) higher total feed intake/bird (g), cost of feed consumed (N), initial cost, total cost/bird (N), average final weight/bird (kg) and income/bird (N) were obtained in exotic turkey. In addition, a higher but poorer (p<0.05) cost-benefits ratio (3.91) was obtained in exotic turkey than in the locally-adapted turkey.

Table 5: Main effects of breed and housing systems on carcass characteristics of turkey

Parameter	Breeds		Housing Systems	
	Locally-adapted	Exotic	Deep litter	Cage
Live weight (g/b/)	3600±0.00[b]	5916.67±0.00[a]	4750.00±560.80	4766.67±603.13
Plucked weight (g/b)	3200±106.45[b]	5216.67±435.44[a]	4150.00±515.59	4266.67±583.47
Dressing percentage (%)	82.37±1.80	80.68±2.56	80.69±2.48	82.36±1.90
Cut-up parts[1]				
Head	2.78±0.05a	1.72±0.10b	2.23±0.22	2.27±0.27
Neck	9.27±0.62	10.14±0.63	9.11±0.55	10.30±0.46
Wings	9.09±0.51	10.17±0.37	9.41±0.53	9.85±0.46
Drumsticks	9.15±0.15	11.22±0.50	9.61±0.52	10.76±0.69
Thighs	8.43±0.41	9.44±0.63	8.37±0.30	9.50±0.55
Breast	13.91±0.73[b]	17.90±1.52[a]	15.00±1.63	16.81±1.20
Shanks	2.78±0.05[b]	3.40±0.21[a]	4.69±0.30	5.32±0.36
Organs[2]				
Liver	1.16±0.10	1.03±0.85	1.17±0.11	1.02±0.69
Heart	0.35±0.02	0.32±0.02	0.30±0.01	0.37±0.01
Gizzard	2.29±0.19	1.92±0.10	2.16±0.19	2.05±0.01
Kidney	0.36±0.01	0.33±0.02	0.34±0.02	0.35±0.01
Lungs	0.50±0.06	0.44±0.05	0.55±0.05	0.42±0.04
Spleen	0.16±0.03	0.11±0.03	0.13±0.04	0.13±0.03
Ceacum	0.17±0.02	0.17±0.03	0.15±0.02	0.19±0.03
Proventriculus	0.03±0.01[b]	0.23±0.02[a]	0.27±0.01	0.26±0.02
Small intestine	1.98±0.09	1.72±0.11	1.97±0.11	1.73±0.10
Large intestine	0.71±0.03	0.50±0.03	0.66±0.03	0.64±0.04

[a,b] *Means in the same row with different superscripts differ significantly (P<0.05)*
[1,2]: *Percentage of the live weight*

Table 6: Effect of interaction between breed and housing systems on carcass characteristics of turkey

Parameter	Exotic		Locally-adapted	
	Deep litter	Cage	Deep litter	Cage
Live weight (g/b)	5766.67±731.05[a]	6066.67±352.76[a]	3733.33±66.66[b]	3466.67±64.66[b]
Plucked weight (g/b)	4900.00±873.68[ab]	5533.33±290.59[a]	3400.99±57.73[bc]	3000.88±55.47[c]
Dressing percentage	77.47±4.52	83.90±1.40	83.91±0.29	80.82±03.72
Cut-up parts[1]				
Head	1.78±0.20[b]	1.65±0.10[b]	2.68±0.04[a]	2.88±0.05[a]
Neck	4.03±0.13[b]	4.86±0.68[ab]	5.36±0.04[a]	5.77±0.05[a]
Wings	9.85±0.29	10.49±0.70	8.96±1.07	9.21±0.39
Back	10'96±0.70	9.31±0.90	9.64±1,07	8.91±0.81
Drumstick	10.23±0.48[ab]	12.12±0.48[a]	8.91±0.81[b]	9.40±0.57[b]
Thigh	8.53±0.63[ab]	10.35±0.89[a]	8.21±0.16[b]	8.66±0.16[ab]
Breast	16.60±2.90	19.20±1.20	13.40±1.53	14.43±0.27
Shanks	3.57±0.41[a]	3.31±0.20[ab]	2.68±0.04[b]	2.88±0.05[ab]
Organs[2]				
Liver	1.09±0.16	0.97±0.07	1.24±0.18	1.07±0.12
Heart	0.29±0.03	0.36±0.01	0.32±0.02	0.38±0.03
Gizzard	1.97±0.14	1.88±0.18	2.36±0.36	2.21±0.20
Kidney	0.33±0.04	0.34±0.01	0.36±0.03	0.37±0.01
Lungs	0.46±0.08	0.43±0.07	0.63±0.02	0.41±0.08
Spleen	0.13±0.07	0.09±0.03	0.14±0.04	0.17±0.04
Ceacum	0.15±0.04	0.18±0.06	0.14±0.02	0.20±0.04
Proventriculus	0.24±0.03[ab]	0.21±0.03[b]	0.29±0.01[ab]	0.31±0.02[a]
Small intestine	1.80±0.20[ab]	1.63±0.14[b]	2.14±0.03[a]	1.83±0.14[ab]
Large intestine	0.60±0.03	0.58±0.06	0.71±0.04	0.70±0.06

[a,b] *Means in the same row with different superscripts differ significantly (P<0.05)*
[1,2]: *Percentage of the live weight*

Table 7: Cost-Benefits ratio of rearing locally-adapted and exotics breeds of turkey.

Cost parameter	Locally-adapted	Exotic
Total feed intake/bird (kg)	11.32±0.53[b]	21.47±0.02[a]
Cost price/kg feed (N)	98.03±0.00	98.03±0.00
Cost of feed consumed/bird(N)	1109.69±30.06[b]	2104.70±1.98[a]
*Initial cost (N)	950.81±0.00[b]	1700.81±0.00[a]
Total cost/bird (N)	2060.50[b]	3805.51[a]
Average final weight/bird (kg)	3.58±0.13[b]	6.37±0.28[a]
Income/bird (N)	2687.50±97.63[b]	4778.50±213.76[a]
Benefit (N)	626.70±104.51	972.30±215.40
Cost: Benefits	3.29±0.72b	3.91±1.28[a]

Initial Cost = Cost of day old poults + brooding cost + cost of medication/vaccination
1US Dollar ≈ N170 at the time of the experiment

Discussion

From the results of this study, the difference in higher weight gain observed in exotic breed than the locally-adapted breed is certainly breed-specific and in the growth associated with the increase in feed intake. This could also be corroborated by the findings of Saowakon et al. (2008) that locally-adapted birds have a slow growth rate when compared with the exotic meat strain thus attaining a marketable body weight at much later age. The final body weight recorded for locally-adapted breed is in line with the findings of Karki (2005) who reported that at 16th week of age locally-adapted turkey will have highest body weight after which it deteriorates progressively. In addition, the growth performance exhibited by the exotic breed of turkey in this study resulted from early maturity and high productivity. Body weight in the exotic turkey was significantly higher than that of locally-adapted breed in this study and it is supported by the fact that locally-adapted poultry have gone through more of natural selection for survival to the tropical climate rather than artificial selection for productivity (Ibe, 1990). Mortality rate was higher in the exotic turkey on wooden cage housing system which was in contrast with the findings of Garber et al. (2003) that birds reared under deep litter floor systems had higher risk of infections compared to those kept in cage system. However, Namata et al. (2008) reported higher risk of contamination under cage system. The non-significant effects in the growth performance of the breeds in the two housing systems as shown in the interactive effects supported the reports by Fanatico et al. (2005) that showed no differences in the final weight of indoor and outdoor birds. The growth of the exotic breed of turkey was influenced mainly by breed effect and not by housing systems. Exotic and locally-adapted breed of turkeys reared in wooden cage housing systems performed better than those reared on deep litter. This study suggest that the effect of choice of housing systems on feed consumption is of importance as more feed was consumed in wooden cage than deep litter for the exotic breed while consumption was high on deep litter for the locally-adapted breed. The body temperature of the birds in this study fell within the range of 41-42oC for optimal performance of birds. The difference in the temperature of the turkeys in both breeds might have been due to adaptation, it could also be as a result of the thermal stress (high internal/external temperature).

The Exotic breed had higher live weight, plucked weight, proventriculus, shanks and drumstick. The possible explanation for the differences between locally-adapted and exotic breed is the body size. In this study, the cage reared turkey had heavier body weight, lower feed conversion and higher meat yield than those reared on deep litter. This result is in contrast with the report by Sogunle et al. (2008) where broilers reared on deep litter showed better feed conversion ratio and lower mortality compared to those reared in cage. In addition, Santos et al. (2008) also corroborated it in his report that litter reared birds not only have significant heavier gizzard and proventriculus than cage reared birds, but also have improved feed conversion ratio and heavier breast muscle relative to body weight. The exotic breeds had higher value of live weight, thighs and breast meat percentage.

In this study, feed alone accounts for between (60-70%) of the production cost. The rearing of the locally-adapted turkey was found profitable in the long run with a better cost-benefits ratio. The high cost of production of the exotic turkey results narrow profit margin. This could in the long run lead to a collapse of the once prosperous poultry industry with high cost of production occasioned by higher feed intake. Of course, high cost of feed in poultry industry in Nigeria is a major challenge facing the poultry farmer. Hence, frequent and sometimes unwarranted increases in the prices of ingredients have contributed substantially to the difficulties experienced by poultry farmers in Nigeria.

Conclusion

1. There were breed differences in the growth performance indices with better growth performance in the exotic turkey

than the locally-adapted turkey. In addition, exotic turkey on wooden cage performed better than exotic turkey on deep litter and locally-adapted turkey on both housing systems at the starter phase.

2. Exotic turkey yielded more in term of the carcass component than the locally-adapted turkey but recorded similar dressing percentage with the locally-adapted turkey.

3. The locally-adapted turkey recorded better cost-benefits ratio than the exotic turkey.

Recommendations

The followings are recommended based on the result of the study:

1. The two housing systems of deep litter and wooden cage are recommended for turkey production. However, for a better performance and higher meat yield, the exotic turkey could be reared on wooden cage.

2. In terms of the cost-benefits ratio and reduced mortality, the rearing of the locally-adapted turkey in any of the housing systems is recommended.

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EFFECT OF GRADED LEVELS OF CONCENTRATE MIXTURE CONTAINING 4% PALM OIL ON DIGESTION AND NITROGEN RETENTION BY RED SOKOTO GOATS FED BASAL DIET OF *DIGITARIA SMUTSII* HAY

Otaru S M*, Adamu A M, Ehoche O W and Lakpini C A M

National Animal Production Research Institute, Shika, Ahmadu Bello University, P.M.B. 1096, Zaria, Nigeria

Abstract

Sixteen male Red Sokoto goats (RSG) of average weight of 21.50 ± 1.04 kg were used to determine the effects of feeding levels of concentrate mixture containing 4% palm oil on nutrient digestion by Red Sokoto goats fed basal diet of wooly finger grass (*Digitaria smutsii*, Stent) hay. The goats were randomly assigned to four feeding levels of concentrate mixture at 1.0, 1.5, 2.0 and 2.5% of body weight designated as treatments 1%C, 1.5%C, 2%C and 2.5%C, respectively. The animals were individually fed *Digitaria smutsii* hay *ad libitum* and supplemented with concentrate mixture at their assigned feeding level. Voluntary feed and water intakes, total urine and faecal outputs were measured for seven consecutive days after an adjustment period of fourteen days. The results showed that the levels of the concentrate fed significantly (P<0.05) improved the intakes of total DM, OM, CP, EE, and non-structural carbohydrates (NSC) with a linear (P<0.01) trend. The consumption of concentrate did not substitute that of hay until at 2.5%C when hay intake was comparatively lower. Generally, digestibility of nutrients linearly (P<0.05) increased with the level of concentrate supplementation, except that of NSC which had a quadratic trend (P<0.05). Feeding levels of concentrate mixture significantly (P<0.05) increased percent nitrogen retention (range, 13.66 – 41.38) by 83% with a significant (P<0.01) linear response. It is concluded that supplementation of Red Sokoto goats on basal diet of *Digitaria smutsii* hay at 2% of body weight with concentrate mixture containing 4% palm oil enhanced consumption of hay, total dry matter intake and marked increase in digestibility of nutrients and nitrogen retention.

Keywords: Red Sokoto goats, concentrate levels, *Digitaria smutsii*, digestion, nitrogen retention

L'EFFET DES DOSES GRADUELLES DU MÉLANGE CONCENTRÉ CONTENANT 4% D'HUILE DE PALME SUR LA DIGESTION ET LA RÉTENTION D'AZOTE PAR LES CHÈVRES DE SOKOTO NOURRIES AU RÉGIME À BASE DE FOIN *DIGITARIA SMUTSII*

Résumé

Seize chèvres de Sokoto (CS) de poids moyen de 21,50 ± 1,04 kg avaient été utilisées pour déterminer les effets des niveaux d'alimentation de mélange de concentré contenant 4% d'huile de palme sur la digestion des nutriments par les chèvres de Sokoto nourris d'un régime à base de foin laineux (*Digitaria smutsii*, Stent). Les chèvres ont été assignées au hasard à quatre niveaux de mélange de concentré d'alimentation à 1,0, 1,5, 2,0 et 2,5% du poids corporel désigné respectivement comme traitement 1% C, 1,5%C, 2%C et 2,5%C. Les animaux ont été nourris individuellement de foin de Digitaria *ad libitum* et complétées avec le mélange de concentré à leur niveau d'alimentation assigné. Les consommations volontaires d'aliment et de l'eau, l'urine totale et les matières fécales ont été mesurés pendant sept jours consécutifs après une période d'adaptation de quatorze jours. Les résultats ont montré que les niveaux de mélange de concentré ont augmenté de manière significative (P <0,05) la consommation de la matière sèche totale, MO, PB, EE et les hydrates de carbone non structuraux (CNS) avec une tendance linéaire (P <0,01). La consommation de concentré n'a pas substitué celle du foin jusqu'à 2,5% lorsque la consommation de foin était relativement basse. En général, la digestibilité des nutriments a augmenté de manière linéaire (P <0,05) avec le niveau de concentré, à l'exception de celle du CNS qui avait une tendance quadratique (P <0,05). Les niveaux de concentré ont augmenté de manière significative (P <0,05) et linéaire (P <0,01) la rétention d'azote. Il

Corresponding author email: s.m.otaru@napri-ng.org

est conclu que la supplémentation de chèvres de Sokoto sur le régime alimentaire à base de foin *Digitaria smutsii* à 2% du poids corporel avec le mélange de concentré contenant 4% d'huile de palme améliorait la consommation de foin, la consommation totale de matière sèche et une augmentation marquée de la digestibilité des nutriments et la rétention d'azote.

Mots clés : les chèvres de Sokoto, les niveaux de concentré, le *Digitaria smutsii*, la digestion, la rétention d'azote

Introduction

In Nigeria, the productivity of grazing ruminants is low because of the poor quality of available pasture. The native pasture, especially grass which are of C4 type grow and lignify so rapidly with concomitant low soluble carbohydrates and crude protein contents. With these poor nutritive values, they cannot support, in most cases, moderate to high level of production as sole diets. Adamu et al. (1993) had demonstrated that supplementation with energy, nitrogen, phosphorus and common salt could increase the liveweight gain of heifers subsisting on native pasture by a percentage range of 31 – 89%. The magnitude of response reflects the depth of deficiency of nutrients in native pastures. To complement the nutrient of the native pasture, Winter (1993) recommended that as part of supplementation strategies, consideration should be given to the balance of nutrients rather than one nutrient. This, therefore, necessitates the supplementation of grazing ruminants with concentrate mixtures to increase productivity. The level of concentrate to offer is sometimes influenced by the quality of forage fed. Havrevoll et al. (1995) reported that with good quality forage, low to moderate amount of concentrate will suffice for rearing growing dairy goats. The tendency to feed higher levels of concentrate to compensate for low nutrients in poor quality forages can be counterproductive. Llano and Depeters (1985) observed that when concentrate proportion was beyond 50-60 % in the diet, digestibility was impaired in cattle. Results of a similar study by Liu et al. (2005) indicated that grower sheep fed basal diet of corn stalk supplemented with varying levels of concentrate exhibited the greatest ADG when concentrate was fed at 450 g/hd/d. Compared with other levels of supplementation, 350 g/hd/d was however the optimum level with the highest feed efficiency and greatest digestibility of DM, OM, CP, NDF and ADF. The authours emphasized the need to have optimum forage: concentrate ratio in the diets for ruminants for better feed efficiency.

One of the means of increasing the energy density of diets or energy ingestion by farm animals without excessive use of dietary soluble carbohydrates or without high dietary levels of concentrate is the addition of fats to the diet. Several responses of ruminants to dietary inclusion of fats had been reported (Drackley *et al.,* 2003; Appeddu *et al.,* 2004; Sanz Sampelayo *et al.,* 2004). Such reports on indigenous breeds of ruminants in Nigeria are scanty. Otaru *et al.,* (2011) investigated effects of varying levels of palm oil in concentrate mixture on the performance of lactating Red Sokoto goats fed basal diet of *Digitaria smutsii* hay. They found that 4% level of palm oil in the concentrate mixture was the optimum for high voluntary intake and milk production. The authours did not determine the appropriate or optimum level of the concentrate mixture to feed along with *Digitaria smutsii* hay. This study was, therefore, carried out to determine the effect of increasing the feeding level of maize-based concentrate (containing 4% palm oil) on voluntary intake, nutrient digestibility and nitrogen balance in Red Sokoto bucks. We hypothesized that supplementation of Red Sokoto goats fed basal diet of D. smutsii hay with concentrate mixture containing 4 % palm oil will enhance hay intake, digestibility of nutrients and nitrogen retention.

Materials and methods

Location of study

The study was carried out at the Experimental Unit of the Small Ruminant Research Programme, National Animal Production Research Institute (NAPRI), Ahmadu Bello University, Shika – Zaria, Nigeria.

Shika is located within the Northern Guinea Savannah Zone at Latitude 11°11'N and Longitude 7°34'E, and is 640m above the sea level (Macmillan Nigeria, 2006).

Animals and Design of experiment

Sixteen male Red Sokoto goats of average weight of 21.50 ± 1.04 kg were obtained from the Goat Project of the Small Ruminant Research Programme, National Animal Production Research Institute, Shika - Zaria, and used for this trial. The goats were divided into four blocks according to initial body weight and were randomly allocated to four experimental treatments (concentrate levels) within each block. The concentrate mixture (C) feeding levels of 1.0, 1.5, 2.0 and 2.5% of body weight were designated as treatments 1%C, 1.5%C, 2%C and 2.5%C, respectively. There were four animals per treatment group.

Management of animals

The animals were de-wormed one week before the commencement of the study. They were also dipped in acaricide (Steladone® 300EC) solution to control ecto-parasites. On the first day of the study, they were weighed and placed in individual metabolism crates designed for separate collection of total faeces and urine, the latter flowing down a gradient to be collected into 5-litre capacity plastic jericans which protect the urine against draught. The goats were offered wooly finger grass (*Digitaria smutsii*) hay, *ad libitum*, across treatments and were each supplemented with concentrate in accordance with levels commensurate with their respective treatments specified above. Thus, the mean quantity of concentrate offered was 219, 321, 423 and 541 g/hd/d for treatments 1%C, 1.5%C, 2%C and 2.5%C, respectively. The ingredient composition of the concentrate mixture is shown in Table 1. The procedure and duration of feeding the diets each day were as described by Otaru *et al.,* (2011) in an earlier feeding trial with lactating Red Sokoto goats.

After the first 14 d of adjustment period, measurements of feed offered, feed refused, total faeces and urine voided, and amounts of water consumed were taken for a period of seven consecutive days. Feed intake and water consumption were determined by subtracting each day's left over from the quantity offered. For each animal, total urine output was collected into 5-litre capacity plastic jerrycan containing 20 mL of 0.2N HCl to prevent loss of nitrogen (Osuji *et al.,* 1993). Total faecal output from each animal was also collected and weighed fresh. Ten percent of each day's collection was bulked at the end of the trial, and stored in a refrigerator in the case of urine until it was analyzed for nitrogen, while the faeces were dried in the oven for 48 h at 70 °C, milled and stored in polythene bags kept inside tins until required for proximate analyses. Sub samples of feed offered and refused were also taken daily, bulked, milled and stored for chemical analyses.

Chemical analyses

The dried samples of the diets and faeces were ground through 1mm sieve and further dried at 105°C for one hour to determine the dry matter of samples. The nitrogen in the dried samples of feed ingredients, diets, faeces and also urine was determined according to Kjeldahl procedure (AOAC, 1980), while the neutral detergent fibre (NDF) and acid detergent fibre (ADF) contents of the diets, feed ingredients and faecal samples were determined according to the procedure of Goering and Van Soest (1970). The AOAC (1980) procedures were followed to determine the crude fibre (CF), ether extract (EE) and ash contents of the feeds and faecal samples. The samples were ashed by charring in a Muffle furnace at 5000 C for 3 h or until a whitish ash remained.

Non-structural carbohydrate (NSC) concentrations of the diets were estimated by equation: OM - (CP% + NDF% + ether extract %) (Arieli *et al.,* 2005).

Microbial N yield was estimated according to ARC (1984) equation as cited by Kossaibati and Bryant (1994). Microbial N yield = 32 g/kg DOMR, where DOMR is the digestible organic matter in the rumen, and is estimated by multiplying digestible organic matter (DOM) intake by a factor of 0.65 (ARC, 1980).

Statistical analyses

The data on intakes of nutrients, nutrient digestibility coefficients, estimated rumen microbial yield, urine and faecal outputs and nitrogen retention were analyzed using ANOVA of the General Linear Model (GLM) procedures of the Statistical Analysis Systems (SAS, 2000, version 8.1) for a randomized block design with the following statistical model: $Y_{ij} = \mu + b_i + t_j + e_{ij}$, where Y_{ij} is the response of animal i in treatment j, μ is the overall mean, b_i is a fixed effect of the ith block (i= 1, 2, 3, 4), t_j is a fixed effect of the jth treatment (j= 1, 2, 3, 4), e_{ij} is the random error. Orthogonal polynomial contrast was run in accordance with the procedures of SAS (2000) to establish the response relationship between the variables and levels of concentrate offered. After significant F-test, least squares means were separated using the PDIFF OPTION of SAS (SAS, 2000), and differences between least squares means were declared significant at $P<0.05$.

Results

Intake of dry matter and nutrients

The chemical composition of experimental diet is as indicated in Table 2. Table 3 shows the least squares means of daily nutrient intakes of RSG bucks. As evident from Table 3, the levels of the concentrate fed to the goats significantly ($P<0.05$) increased concentrate consumption, total dry matter and nutrient intakes (organic matter, crude protein, ether extract and non-structural carbohydrates). Table 3 also shows that the level of concentrate fed elicited significant ($P<0.01$) linear increase in the intake of total DM, OM, CP, EE and NSC. The hay DM intake was not significantly ($P>0.05$) affected by the treatment so also are the intakes of CF, NDF and ADF. At 2%C, the intake of hay declined but the consumption of concentrate was only greater than that of hay when feeding level was increased to 2.5% of body weight.

Digestibility of nutrients

Table 4 shows the least squares means of total tract nutrient digestibility coefficients, estimated microbial nitrogen yield in the rumen, and nitrogen retention. The digestibility of most of the nutrients increased in goats supplemented at higher levels of concentrate. However, the increase was only significant for crude protein (between 1%C and 2%C or 2.5%C treatments, $P<0.05$). The relationship between the digestibility values of DM, OM, and CP and the level of concentrate supplementation was linear ($P<0.05$). The digestibility of NSC value was significantly ($P<0.05$) improved by levels of concentrate supplementation, but the trend was quadratic ($P<0.05$). Although not statistically significant, the digestibility of DM, OM, EE, NDF and ADF was numerically increased in goats supplemented at higher levels of concentrate mixture (2% and 2.5% of body weight).

Nitrogen intake and retention

There were significant ($P<0.05$) effects of levels of concentrate supplementation on

Table 1: Ingredient composition of concentrate mixture

Ingredient	% level in the mixture
Maize	33.87
Maize offal	18.94
Palm oil	4.00
Cotton seed cake	39.04
Urea	0.15
Bone meal	2.5
Common salt	1.5

Table 2: Chemical composition of experimental diet (%)

Parameter	Experimental Diet	
	Concentrate mixture (C)	*Digitaria smutsii* **Hay**
Dry matter	94.91	96.95
Organic matter	85.91	88.73
Crude Protein	16.30	5.40
Crude fibre	16.24	46.48
Ether extract	14.52	5.10
Neutral detergent fibre	36.37	68.34
Acid detergent fibre	25.00	45.20
Ash	9.00	8.22
Non-structural carbohydrate (calculated)	18.72	9.90
Gross energy (GE, MJ/kg DM)	16.08	17.66

Table 3: Mean daily dry matter and nutrient intake of Red Sokoto bucks fed graded levels of concentrate mixture

Parameter	Concentrate mixture level					Concentrate level effect, P<		
	1%C	1.5%C	2%C	2.5%C	SEM	L	Q	Cu
Dry matter intake, g/d								
Hay	404.94	409.79	398.19	370.94	37.02	NS	NS	NS
Concentrate	207.62[d]	304.42[c]	379.30[b]	511.53[a]	22.29	0.0001	0.0001	0.0001
Total dry matter Intake	612.56[c]	714.21[bc]	777.49[ab]	882.47[a]	44.57	0.002	0.005	0.005
Nutrient intakes, g/d								
Organic matter	558.54[c]	650.60[bc]	707.76[ab]	802.51[a]	40.67	0.002	0.005	0.005
Crude protein	58.13[c]	75.02[b]	87.24[b]	108.43[a]	4.47	0.0001	0.0001	0.0001
Ether extract	53.06[c]	68.13[b]	78.97[b]	97.77[a]	4.05	0.0001	0.0001	0.0001
Crude fibre	229.66	248.55	255.80	265.36	18.42	NS	NS	NS
Neutral detergent fibre	365.00	405.52	426.03	457.50	28.04	0.05	NS	NS
Acid detergent fibre	243.48	271.24	285.55	307.68	18.63	0.03	NS	NS
Non-structural Carbohydrate	82.34[c]	101.93[b]	115.51[b]	138.81[a]	6.00	0.0001	0.0003	0.0003

[a,b,c,d] *Means within the same row bearing different superscript letters differ significantly (P<0.05).*
L=Linear, Q=Quadratic, Cu=Cubic, NS=Not Significant (P>0.05).

the daily nitrogen intake, nitrogen balance and percent nitrogen retention. The three response variables had significant (P<0.01) linear relationship with the levels of concentrate fed which caused a maximum of 45 and 83% improvement in nitrogen intake and percent nitrogen retention, respectively, in the goats when level fed was increased beyond 1.5 % of body weight. The mean daily urine and faecal outputs were not significantly (P<0.05) affected by the levels of concentrate mixture supplementation but tended to be associated with level of water intake.

Discussion

Table 4: Mean nutrient digestibility coefficients and daily nitrogen intake and balance of Red Sokoto bucks fed graded levels of concentrate mixture

Parameter	Concentrate mixture level					Concentrate level effect, P<		
	1%C	1.5%C	2%C	2.5%C	SEM	L	Q	Cu
Digestibility (%)								
Dry matter	47.52	52.20	59.80	60.50	3.39	0.02	NS	NS
Organic matter	50.15	54.70	61.74	62.76	3.14	0.02	NS	NS
Crude protein	34.96[b]	49.76[ab]	60.39[a]	61.10[a]	5.03	0.004	0.01	0.01
Ether Extract	72.11	72.82	78.36	78.10	2.92	NS	NS	NS
Crude fibre	55.05	54.50	58.77	57.66	3.89	NS	NS	NS
Neutral Detergent Fibre	57.22	53.52	64.53	63.99	4.93	NS	NS	NS
Acid Detergent Fibre	48.06	49.68	54.60	54.22	3.94	NS	NS	NS
Non-structural Carbohydrate	15.24[b]	51.34[a]	40.06[ab]	48.53[a]	9.70	NS	0.04	0.04
Estimated rumen microbial Nitrogen yield (g/d)*	5.89[c]	7.42[bc]	9.17[ab]	10.47[a]	0.68	0.001	0.003	0.003
Nitrogen Balance								
Total Urine output (ml/d)	330.53	467.86	224.64	384.79	130.00	NS	NS	NS
Total faecal output (g/d)	324.03	352.50	315.18	360.71	28.40	NS	NS	NS
Water intake (l/d)	1.31	1.40	1.20	1.46	0.18	NS	NS	NS
Total nitrogen intake (g/d)	9.30[c]	12.00[b]	13.96[b]	17.35[a]	0.72	0.0001	0.0001	0.0001
Faecal nitrogen output (g/d)	5.74	5.86	5.45	6.73	0.56	NS	NS	NS
Urine nitrogen output (g/d)	1.77	3.15	2.34	4.10	0.69	NS	0.04	0.04
Total nitrogen output (g/d)	7.51	9.01	7.79	10.82	0.80	0.04	0.02	0.02
Nitrogen Balance (g/d)	1.79[b]	2.99[b]	6.17[a]	6.53[a]	0.63	0.0002	0.004	0.004
Nitrogen retention (%)	13.66[c]	22.67[bc]	41.38[a]	35.20[ba]	5.37	0.01	NS	NS
Non-structural Carbohydrate	82.34[c]	101.93[b]	115.51[b]	138.81[a]	6.00	0.0001	0.0003	0.0003

[a,b,c] *Means within the same row bearing different superscript letters differ significantly (P<0.05).*
* *Calculated as Microbial N yield = 32g/kg DOMR (ARC, 1984).*
*DOMR = DOMI * 0.65 (ARC, 1980)*
DOMR = Digestible organic matter in the rumen
DOMI = Digestible organic matter intake
L=Linear, Q=Quadratic, Cu=Cubic, NS=Not Significant (P>0.05).

The marked increase in consumption of concentrate, total DM and nutrients such as OM. CP, EE, and NSC with increase in level of concentrate offered agrees with similar observation on goats (Mba *et al.,* 1982; Malau-Aduli *et al.,* 2004; Limea et al; 2009; Safari et al., 2009). Since the gross energy content of the concentrate was 16.08 MJ/kg DM and crude protein was 16.7%, goats offered higher levels of concentrate consumed greater energy and protein which translated into greater intakes of DM, OM, CP, EE and NSC in linear response to the levels of dietary concentrate. Goetsch et al. (2001) did not observe variation in DM intake when dairy goats were offered varying concentrate levels during late lactation and dry period. The DMI response in the present study did not support the authours observation. Breed and sex effects may be responsible for the difference in DMI response recorded in our study and the one by Goetsch et al. (2001).

The similarity in hay consumption by the goats across treatments is not consistent with earlier report by Safari et al. (2009) where increase in level of concentrate significantly reduced hay consumption by Small East African goats. In the present study, the consumption of hay was still more than that of concentrate until at feeding level of 2.5% of body weight when substitution effect sets in.

The observed improvement in digestibility of crude protein (CPD) confirms the results of earlier studies on sheep (Murphy *et al.,* 1994; Liu *et al.,* 2005) and goat (Lallo, 1996; Arieli *et al.,* 2005) where increase in CP intake, mediated through increase in concentrate level offered or crude protein concentration, increased digestibility of CP. In the present study, the CPD exhibited linear response to level of concentrate supplementation whereas in the study of Liu et al. (2005) the CPD increased to a maximum at concentrate level of 350 g/hd/d beyond which digestibility declined. The increase in CPD may be attributed to the supply of sufficient nitrogen and energy, simultaneously, in correct ratio with increasing concentrate level, thus resulting into greater microbial growth for high degradation of dietary protein. The concentrate used in this study contained

ingredients as shown in Table 1, and it thus had the necessary ingredients or substrates for microbial protein synthesis. Although our results support the findings of Murphy et al. (1994) on lamb, the approach the authours used was different. They achieved linear increase in nitrogen digestion by restrictively feeding diet which otherwise had its concentrate proportion increased so as to achieve iso-caloric energy intake. They attributed the nitrogen response to the decrease in faecal nitrogen excretion consequent upon reduced or depressed nitrogen intake attendant with restricted feeding of the concentrate. The findings of the present study do not support the studies by Mba et al. (1982), Lallo (1996) and Malau-Aduli et al. (2004) who observed that varying concentrate levels or energy densities did not affect digestibility of CP in goats. The composition of the concentrate and nature of basal diet fed may be responsible for the differences between the present study and those of these authours.

Even though we did not determine microbial protein yield in the rumen, calculated estimates (Table 4) suggest a linear relationship between microbial protein yield and dietary concentrate level. This is in agreement with the assertion that while dietary protein is critical to DM and fibre digestibility by rumen microbes, microbial protein production is itself, proportional to the intake of fermentable energy (Robbinson et al., 1985; Sniffen and Robinson, 1987; Clark *et al.,* 1992). A more recent report indicate that increase in starch intake enhanced microbial protein supply as evidenced by increase in allantoin and total purine derivatives in urine of cows (Keady *et al.,* 1999). Ingredient composition of the concentrate mixture (Table 1) shows that maize grain and maize offal constitute about 33.87 and 18.94%, respectively, with both accounting for a total of about 53% of the mixture. These two ingredients were the sources of starch or non-structural carbohydrate in the diet. As more concentrate was consumed, more of these sources were consumed, thus providing more starch to enhance microbial fermentation. The 4% level of palm oil in the diet had been shown in previous study not to have effect on

digestibility of DM, CP, fibre and NSC in goats (Otaru *et al.,* 2013).

The decline in the digestibility of NSC after a peak at 1.5%C is an indication that feeding concentrate mixture at higher levels could depress non-structural carbohydrate digestion. This agrees with similar findings by Colucci et al. (1989) and Galyean et al. (1979) who fed concentrate and 84% cracked corn diet, respectively, at low or high level, and observed greater digestion of starch in animals fed at low level. Feeding processed corn at low level or low intake of processed corn has been shown to result in substantial improvement in the digestion of DM, OM, CP and starch (Murphy *et al.,* 1994). The maize used in the present study constituted 33.87% of the concentrate and was ground before being mixed with other ingredients. The starch from the maize grain and maize offal being consumed at higher levels of supplementation might have facilitated faster passage of starch or NSC through the rumen, thus reducing residence time and digestion in the rumen. The improvement in intestinal digestibility of grain when it was ground (Lykos et al., 1997) would have counterbalanced depression of digestibility in the rumen, making the total tract digestibility values of NSC at 1.5%C, 2%C and 2.5%C, to be comparable despite slight depression at 2%C and 2.5%C feeding levels.

Increased levels of concentrate mixture supplementation also led to linear increase in nitrogen intake across treatments, thus resulting in significant improvement of about 87% at the highest level of concentrate supplementation compared to the lowest level. This is expected because as the level of concentrate mixture fed was increased, more concentrate was consumed (Table 3), and given the fact that the same concentrate supplement of 16.69% crude protein concentration was offered, goats consuming more concentrate mixture would consume more nitrogen. Our observation supports earlier studies where successive increase by 100 units from 150 to 450 g/hd/d of concentrate supplement was observed to significantly elicit linear increase in nitrogen intake by sheep fed basal diet of corn stalk (Liu *et al.,* 2005). The significant treatment

effect on nitrogen intake contrasts the reports of other studies (Hassan and Bryant, 1986; Murphy *et al.,* 1994). While the data by Hassan and Bryant (1986) showed similar values of nitrogen intake, those of Murphy et al. (1994) showed slight decrease in nitrogen intake as the level of concentrate was increased. Murphy et al. (1994) varied the proportion of concentrate in the diet, but fed the diet at varying levels of *ad libitum* intake to achieve equal intakes of nutrients. Such restricted feeding was not done in the present study.

Nitrogen balance and percent nitrogen retention were significantly improved by levels of concentrate supplementation with a linear response. Most studies show that increase in levels of concentrate resulted in increase in energy intake associated with increase in percent nitrogen retention (Gubert, 1979; Murphy *et al.,* 1994; Lallo, 1996; Liu *et al.,* 2005). From available reports, the nature of the improvement of percent nitrogen retention to increase in level of concentrate is, however, dissimilar. Murphy et al. (1994) observed linear response of percent nitrogen retention to varying levels of concentrate fed at restricted levels to sheep. Also, Lallo (1996) observed similar trend with increasing energy levels fed to goats. Liu et al. (2005), on the other hand, observed curvilinear or quadratic response of percent nitrogen retention to increasing levels of concentrate supplementation. According to Gubert (1979), increase in daily energy intake resulted in linear increases in nitrogen retention in dairy cattle. However, when energy intake increases to a level where nitrogen becomes limiting, the response of nitrogen retention may be curvilinear or quadratic. The study of Hassan and Bryant (1986) however, demonstrated that not in all cases would increase in level of concentrate significantly affect percent nitrogen retention.

The nitrogen balance data of this study have also shown that the pattern of urine and faecal production as well as their associated nitrogen content was not influenced by the increase in level of concentrate supplementation. This observation does not support the findings of other researchers who observed significant effect of level of concentrate supplementation

on urine and/or faecal nitrogen output (Hassan and Bryant, 1986; Murphy *et al.,* 1994; Lallo, 1996; Liu *et al.,* 2005).

Conclusion

From the results of the study, it is obvious that supplementation of goats on basal diet of *Digitaria smutsii* hay at 2% of BW with concentrate mixture containing 4% palm oil enhanced consumption of hay, total dry matter intake and marked increase in digestibility of nutrients and nitrogen retention. It is concluded that concentrate supplementation to Red Sokoto Goats on basal diet of *Digitaria smutsii* hay at 1% of body weight may not enhance appreciable digestion of critical nutrients (DM, CP, NSC and ADF) and nitrogen retention percent.

Impact

It is desirable to supplement Red Sokoto goats with concentrate mixture to improve the utilization of poor quality basal diet. From the results of the study, supplementation of Red Sokoto goats with concentrate mixture containing 4 % palm oil at feeding level of 1% of body weight may not enhance appreciable digestion of critical nutrients (DM, CP, NSC and ADF) and nitrogen retention percent. Such concentrate mixture for our indigenous breeds of goats should be fed at 2% of body weight for optimum consumption of basal diet (grass hay), higher DM intake, greater digestibility of nutrients and more efficient nutrient utilization.

Acknowledgements

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107

PRINCIPAL COMPONENTS REGRESSION OF BODY MEASUREMENTS IN FIVE STRAINS OF LOCALLY ADAPTED CHICKENS IN NIGERIA

Adenaike A S, Akpan U and Ikeobi C O N

Department of Animal Breeding and Genetics, College of Animal Science and Livestock Production, Federal University of Agriculture Abeokuta, Ogun State, Nigeria

Abstract

This study aimed at unfolding the interdependence among the linear body measurements in chickens and to predict body weight from their orthogonal body measurements using principal component regression. Body weight and seven biometric traits that are; body length (BL), breast girth (BG), wing length (WL), wing span (WS), thigh length (TL), shank length (SL), keel length (KL) were measured on eight week old chicks comprising 53 each of Marshal (M), Marshal x naked-neck (MNk), Marshal x normal-feathered (MNm), Naked-neck (Nk) and Normal-feathered (Nm). General linear model, factors and partial least squares procedures of statistical analysis system (S.A.S 9.1) were used to compute the variations among the five ecotypes. Pearson correlations between body weight and biometric traits were positive and highly related (r = 0.614-0.937, 0.518-0.929 and 0.496-0.943, 0.411-0.959 and 0.760-0.961 in M, MNk, MNm, Nk and Nm ecotypes respctively). Only the first principal component (PC) exhibited eigenvalues greater than 1. Observed communalities ranges from 0.787 to 0.946 in M, 0.784 to 0.957 in MNk, 0.685 to 0.928 in MNm, 0.818 to 0.959 in Nk and 0.930 to 0.998 in Nm. This offered credibility to the relevance of the principal component regression. In principal component regression models, TL alone accounted for 76.21%, 62.72%, and 75.52% of the variation in BW for M, MNm and Nk respectively. The best prediction equation (R2=85.61%) for BW was obtained when BG was included in the model for M. In Nk chickens, the best prediction equation (R2=85.52%) for BW was obtained when BG was included in the model. BG alone accounted for 91.66% and 69.05% of the variation in BW for Nm and MNk respectively. Principal component regression can be used to classify independent and informative variables thereby eliminating redundant information for the purpose of reducing costs of chicken genetic programmes.

Key words: Body weight, Biometric traits, Principal component, Orthogonal, Eigenvalues and Linear measurement

LES PRINCIPALES COMPOSANTES DE REGRESSION DES MESURES corporelles chez CINQ SOUCHES DE POULETS LOCALEMENT ADAPTÉES AU NIGERIA

Résumé

Cette étude visait à développer l'interdépendance entre les mensurations linéaires chez les poulets et pour prédire le poids corporel à partir de leurs mensurations orthogonales en utilisant la principale composante de régression. Le poids corporel et sept traits biométriques qui sont : la longueur du corps (LC), le tour de poitrine (TP), la longueur des ailes (LA), l'envergure des ailes (EL), la longueur de la cuisse (LC), longueur de la jambe (LJ), la longueur de la carène (LC) mesurés sur 53 poussins de huit semaines composés chacun de Maréchal (M), de Maréchal x Cou Nu (MCN, de Maréchal x Plumes Normales (MPN), Cou Nu (CN) et les Plumes Normales (PN). Le modèle linéaire général, les facteurs et les méthodes des moindres carrés du système d'analyse statistique (SAS 9,1) ont été utilisés pour calculer les variations entre les cinq écotypes. Les corrélations de Pearson entre le poids corporel et les traits biométriques étaient positifs et très liés (r = 0,614¬-0,937, 0,518 - 0,929 et 0,496 -0,943, 0,411 - 0,959 et 0,760 - 0,961 respectivement dans les écotypes M, MCN, MPN, CN et PN). Seule la première composante principale (CP) présentait les valeurs propres supérieures à 1. Les valeurs communes observées variées de 0,787 à 0,946 dans M, de 0,784 à 0,957 en MCN, de 0,685 à 0,928 en MPN, de 0,818 à 0,959 en CN et de 0,930 au 0,998 en PN. Ceci a offert la crédibilité à la pertinence de la composante principale de régression. Dans les modèles des principales composantes de régression, la LC représentait à elle seule 76,21%, 62,72% et 75,52% de la variation dans PC, M, MPN et CN respectivement. La meilleure équation de prédiction

(R2 = 85,61%) de PC était obtenue lorsque le PC était inclus dans le modèle de M. chez les poulets CN, la meilleure équation de prédiction (R2 = 85,52%) pour PC était obtenue lorsque TP était inclus dans le modèle. Le PC représentait à lui seul 91,66% et 69,05% de la variation de PC respectivement pour PN et MCN. La principale composante de régression peut être utilisée pour classer les variables indépendantes et informatives, éliminant ainsi des informations redondantes dans le but de réduire le coût des programmes génétiques du poulet.

Mots clés : le poids corporel, les traits biométriques, les composantes principales, les orthogonales, les valeurs propres et les mesures linéaires.

Introduction

Nigeria is endowed with a number of locally adapted chickens that are important in meat and egg production. The Nigerian indigenous chickens represent a large group of unexploited genetic resource. Despite increase in the growth of the poultry industry in Nigeria utilizing exotic chicken in production, the indigenous chicken ecotypes still remain the largest source of poultry meat and eggs. Although they are less productive when compared to the exotic counterpart, indigenous chickens play a vital role in the socio-economic life of those keeping them (Alabi et al., 2012). Indigenous chickens represent a highly conserved genetic reservoir, with high level of heterozygosity, which may provide the biological material for the development of genetic stocks with improved adaptability and productivity (Ajayi et al., 2012). Also, they have potential to serve as broiler meat if they are improved on. Meanwhile, Marshal ecotype is among the strains of broiler chickens reared by farmers in Southern Nigeria. They cope fairly well with the hot season of January to March in Nigeria and reach market weight at about 8 weeks of age (Udeh and Ogbu, 2011). Understanding the interrelationships between body weight and body measurements in these birds and their crossbreds will help the farmers to predict their body weight at various ages especially in the rural areas where scales might not be readily available.

The accuracy of models used to predict body weights from linear body measurements has a colossal financial input to livestock production enterprises. Ability of livestock producers and buyers to relate linear body measurements to body weight will result in an optimum production and value-based trading system as a result of accurate predictions. This will ensure that livestock farmers are adequately rewarded rather than the middlemen and/or livestock product processors who tend to gain more profit in livestock production enterprises, especially in the rural areas of developing countries (Afolayan et al., 2006) such as Nigeria. Also, accuracy of models developed to predict body weights from linear body measurements could improve selection efficiency for growth by enabling the breeder to recognise early maturing and late maturing animals of different sizes. Linear body measurements have been used to predict body weights by several authors in many chicken breeds (Mendes, 2009; Yakubu et al., 2009; Udeh and Ogbu, 2011; Ajayi et al., 2012). The authors used different models (canonical discriminant, multiple regressions and principal components) to predict body weight in different breeds, sexes and environmental conditions. Both canonical discriminant and multiple regressions models are highly affected by high correlations (multicollinearity problem) between predictor variables. It is well known that in the presence of multicollinearity problem, the standard errors of the parameter estimates could be quite high, resulting in unstable estimates of the regression model.

Hence, the multicollinearity between predictor variables can lead to incorrect identification of the most important predictors (Sharma 1996; Thompson et al., 2001 and Hoe and Kim, 2004). Sousa et al. (2007) and Mendes (2009) reported that one of the approaches to avoid multicollinearity problem is the principal component analysis. However, principal component analysis (PCA) might not be able to account for variation due to differences in breeds. Hence, categorization of data according to breed is necessary (if its effect is significant)

to improve prediction power of the principal component analysis. Also, multivariate analyses involving the use of PCA has been reported for extensively-managed Nigerian indigenous chickens in the Northern part of Nigeria (Yakuku et al., 2009) while Ajayi et al. (2012) reported for intensively-managed Nigerian indigenous chickens and Anak Titan (an exotic chicken). However, Yakubu et al. (2009) did not account for spatially dependent correlation as a result of sampling of data in different locations and PCA carried out with spatially-dependent samples will most often result in identifying spatial correlations (Clemens *et al.,* 2008). Strong spatial correlations may completely mask linear body measurement correlations within the sample variables which PCA needed to account for. Also, Ajayi et al. (2012) used small sample size (30 naked-neck and 27 Anak Titan chickens). This might make the results to be biased. Guadagnoli and Velicer (1988) reviewed several studies that reached the conclusion that the minimum sample size should be 50. Gorsuch (1983) and Hatcher (1994) also supported ratio of the minimum value of sample size to variables as being of greater importance in PCA and recommended at least 5:1. Therefore, this study sought to increase sample size of all variables to minimum of 50 and use Principal components regression (PCR) to correct for multicollinearity among linear body measurements when fitting multiple regression models. The PCR approach involves constructing principal component (PC) and then using these components as the predictors in a linear regression model that is fit using least squares. The prime idea of PCR is to use scores rather than the original data for the regression step. This has two advantages: scores are orthogonal, so there are no problems with correlated variables, and secondly, the number of PCs taken into account usually is much lower than the number of original variables. This reduces the number of coefficients that must be estimated considerably, which in turn leads to more degrees of freedom for the estimation of errors.

Materials and Methods

Study location:
The research was conducted at the Poultry Breeding Unit of the Federal University of Agriculture, Abeokuta, (FUNAAB) located on latitude 7°10 N in Odeda Local Government Area, Ogun State, in South-Western Nigeria. The ambient temperature during the period ranged from 26.9°C in June to 27.1°C in December with average relative humidity of 80%, while the vegetative site represents an inter-phase between the tropical rainforest and the derived savannah (AGROMET, FUNAAB, 2014).

Experimental animals and their management:
A total of 265 chicks comprising 53 each of Normal-feathered, Marshal, Naked-neck Marshal x naked-neck and Marshal x normal-feathered crosses generated from Hatchery in Abeokuta were used for the study. The chicks were raised in deep litter pen for eight weeks. The birds were fed *ad libitum* on broiler starter diet from day-old to 4 weeks of age and a broiler finisher diet from 4 to 8 weeks of age. Clean drinking water was also made available to the birds all the time. All the necessary vaccines for broiler chicks were administered at the appropriate ages. The body weights of the birds were recorded on weekly basis to 8 weeks of age. The body measurements namely shank length, thigh length, drumstick length, body length, body width, breast width and wing length were measured at 8 weeks of age.

Traits measured:
Body weight (BW) (grammes) and seven linear body measurements were measured on each chicken. The body measurements (centimetres) were taken using a measuring tape except for body weight that was taken using a measuring scale in grammes. The parts measured were body length (BL), measured as the distance between the tip of the beak and the longest toe without the nail; wing length (WL), taken as the distance between the tip of the phalanges and the coracoids-humerus joint; wing span (WS), measured as the distance between the left wing tip to the right wing tip

across the back of the chicken; shank length (SL), taken as the distance from the hock joint to the tarsometatarsus; thigh length (TL) measured as the distance between the hock joint and the pelvic joint; breast girth (BG), measured as the circumference of the breast around the deepest region of the breast and keel length (KL), taken as the distance between the anterior and posterior ends of the keel.

Statistical analysis:

Means and standard errors values of body weight and body measurements of each ecotypes were obtained using the descriptive statistic of S.A.S (Version 9.1). Two-way analysis of variance was used to test the effects of genotype and sex of ecotypes on the parameters. Pearson correlation coefficients among the body measurements were calculated for each ecotypes and the correlation matrix which was the primary data required for PCA generated.

PCR is an appropriate multivariate technique to reduce the dimension of a data set consisting of a large number of interrelated variables, while retaining as much as possible the variation present in the data set (Sharma, 1996; Özkan and Mendes, 2004). This is achieved by transforming a set of original variables to a new set of variables, the principal components which are ordered so that the first few retain most of the variation present in all of the original variables (Joliffe, 2002).

Principal component analysis is a method for transforming the variables in a multivariate data set $x_1, x_2, ---, x_p$, into new variables, $y_1, y_2, ---, y_p$ which are uncorrelated with each other and account for decreasing proportions of the total variance of the original variables defined as:

$$y_1 = a_{11} x_1 + a_{12} x_2 + ------------------ + a_{1p} x_p$$
$$y_2 = a_2 x_1 + a_{22} x_2 + -------------------- + a_{2p} x_p$$
$$y_p = a_{p1} x_1 + a_{p2} x_2 + ------------------ + a_{pp} x_p$$

The aim of the Varimax rotation is to maximize the sum of variances of a_{ij}^2 quadratic weight.

The stepwise variable selection multiple regression procedure was used to obtain models for predicting body weight from body measurements (a) and from established principal components (b).

$$BW = a + B_i x_i + --------- B_k x_k ----------- (a)$$
$$BW = a + B_i PC_i + -------- B_k PC_k ---------- (b)$$

where; BW is the body weight, a is the regression intercept, B1 is the ith partial regression coefficient of the i-th linear body measurement, X_i or the ith principal component. Anti-image correlations and Barlett's Test of Sphericity were computed to test the validity of the factor analysis of the data sets. The appropriateness of the factor analysis was further tested using communalities and ratio of cases to variables. Components were extracted until some stopping criteria is encountered or until p components were formed. The weights used to create the principal components are the eigenvectors of the characteristics equation:
$(R - \lambda_1 I) a = 0$

Where R is the correlation matrix, the λ_1 are the eigenvalues (the variances of the components). The eigenvalues were obtained by solving $(R - \lambda_1 I)a = 0$ for λ_1. Cumulative proportion variance was employed in determining the number of principal components to extract. The overall reliability of the factor solution was tested using Chronbach's Alpha. The means, correlation, factor, PLS and regression procedures of S.A.S 9.1 statistical package were used for the principal component regression analysis.

Results and Discussion

Table 1 shows the means, standard errors and coefficients of variation of body weight and body measurements of three ecotypes and their two crossbred of chickens at 8 weeks of age. Genotype had significant effect (P<0.001) on all the biometric traits with Marshal chickens having significantly highest means compared to the four other ecotypes (Table 1). Marshal, Marshal x normal-feathered and Marshal x naked-neck attained average body weight of 790.06, 626.34 and 611.03g respectively which were superior to normal-

Table 1: Means, standard errors (SE) and coefficients of variation (CV) for body weight (kg) and body measurements (cm) of five Nigerian ecotype chickens according to genotype.

	Marshal		Marshal x naked		Marshal Normal		Naked-neck		Normal	
	Mean ± SE	CV	Mean ± SE	CV	Mean ± SE	CV	Mean ± SE	CV	Mean ± SE	CV
Body weight	790.06±22.67[a]	19.59	611.03±20.09[b]	22.44	626.34±18.96[b]	23.84	494.62±18.42[c]	22.82	472.24±21.78[c]	32.49
Body length	14.53±0.17[a]	8.29	12.77±0.18[c]	9.79	13.47±0.13[b]	7.63	12.46±0.21[cd]	10.55	12.17±0.24[d]	13.47
Breast girth	19.02±0.22[a]	8.06	16.83±0.26[b]	10.83	17.16±0.18[b]	8.08	15.07±0.20[c]	8.38	15.07±0.30[c]	13.86
Width length	17.81±0.22[a]	8.58	16.20±0.26[b]	11.19	16.68±0.15[b]	6.98	15.53±0.21[c]	8.43	15.26±0.26[c]	11.55
Wing span	38.82±0.45[a]	8.04	34.11±0.58[b]	11.66	36.49±0.32[b]	7.01	33.09±0.45[cd]	8.41	32.63±0.74[d]	15.47
Thigh length	15.09±0.16[a]	7.59	13.88±0.18[b]	8.91	13.86±0.13[b]	7.85	13.41±0.19[bc]	8.67	13.17±0.21[c]	10.84
Shank length	11.83±0.17[a]	9.96	10.61±0.16[b]	10.53	10.99±0.12[b]	8.88	10.08±0.19[c]	11.96	9.84±0.19[c]	13.59
Keel length	7.99±0.11[a]	9.35	6.90±0.09[b]	9.05	7.27±0.07[c]	8.19	6.44±0.10[d]	10.02	6.19±0.13[d]	15.37

feathered (Nm) (472.24g) and naked-neck (494.62g) at 8 weeks of age. This observation attested to previous reports that Nigerian indigenous chickens were light breeds (Peters, 2000 and Adeleke *et al.,* 2011), but suggests the suitability of Marshal chickens for crossbreeding programmes which when mated with indigenous ecotypes will eventually improve the growth and carcass trait potentials of Nigerian indigenous chickens. The observable differences between body weight of naked-neck and normal-feathered chickens were similar to the findings of Patra et al. (2002) who reported that naked-neck chickens had heavier body weight compared to their Normal-feathered counterparts. However, the higher average body weight observed in naked-neck chickens compared to normal-feathered chickens was contrasted to that observed by Gunn (2008) and Ajayi et al. (2012) who reported better performance of normal-feathered chickens in comparison to naked-neck chickens. Also, male birds had higher values for all linear body measurements compared to their female counterparts (Table 2). This result is in agreement with the findings of earlier researchers (Peters *et al.,* 2006; and Ajayi *et al.,* 2012). This observed dimorphism might be attributed to the differential sex hormonal effects on growth. Table 3 shows the coefficient of correlations of body weight and body measurements of the chicken ecotypes. The correlation coefficients ranged from 0.614 to 0.937, 0.518 to 0.929 and 0.496 to 0.943, 0.411 to 0.959 and 0.760 to 0.961 in Marshal (M), Marshal x naked-neck (MNk), Marshal x normal-feathered (MNm), naked-neck (Nk) and normal-feathered (Nm) ecotypes respectively. Relationships between body weight and all the body measurements were positive and significant (p<0.001) in the five chicken ecotypes. Highest positive correlations were recorded between wing span and wing length in M, MNm and Nk while shank length and thigh length had highest positive correlations in MNk and Nm ecotypes. Shank length and body length had lowest positive relationship in M. Meanwhile, lowest positive correlations were recorded between body length and wing length (0.518) in MNk, body

Table 2: Means, standard errors (SE) and coefficients of variation (CV) for body weight (kg) and body measurements (cm) of five Nigerian ecotype chickens according to sex.

Traits	Male		Female	
	Mean ± SE	CV %	Mean ± SE	CV %
Body weight	643.82±16.85[a]	30.37	553.90±15.03[b]	27.47
Body length	13.39±0.13[a]	12.32	12.76±0.14[b]	10.34
Breast girth	17.09±0.19[a]	13.13	16.17±0.19[b]	12.15
Wing length	16.60±0.15[a]	11.07	15.99±0.15[b]	9.98
Wing span	35.96±0.34[a]	13.06	34.10±0.40[b]	10.20
Thigh length	14.29±0.12[a]	9.50	13.47±0.11[b]	9.26
Shank length	11.01±0.11[a]	12.74	10.34±0.12[b]	11.44
Keel length	7.15±0.08[a]	14.12	6.76±0.09[b]	12.29

Table 3: Pearson correlations among body weight and linear body measurements of five Nigerian ecotype chickens weight and linear body traits of Marshal (first upper diagonal), Marshal x naked-neck (first-middle upper diagonal) Marshal x normal (second-middle upper diagonal), naked-neck (first lower diagonal) and normal-feathered chickens (second lower diagonal).

	BW	BL	BG	WL	WS	TL	SL	KL
BW	I	0.774***	0.869***	0.754***	0.795***	0.873***	0.769***	0.696***
BL	0.721***	I	0.787***	0.659***	0.686***	0.713***	0.614***	0.726***
BG	0.831***	0.706***	I	0.748***	0.786***	0.774***	0.757***	0.729***
WL	0.635***	0.518***	0.565***	I	0.937***	0.828***	0.925***	0.831***
WS	0.707***	0.679***	0.734***	0.645***	I	0.854***	0.942***	0.819***
TL	0.799***	0.837***	0.837***	0.582***	0.802***	I	0.856***	0.704***
SL	0.766***	0.829***	0.768***	0.562***	0.755***	0.929***	I	0.804***
KL	0.794***	0.745***	0.805***	0.623***	0.763***	0.854***	0.818***	I
BW	I	0.567***	0.718***	0.778***	0.771***	0.792***	0.724***	0.770***
BL	0.856***	I	0.547***	0.643***	0.615***	0.639***	0.496***	0.511***
BG	0.869***	0.741***	I	0.688***	0.708***	0.695***	0.537***	0.690***
WL	0.868***	0.825***	0.822***	I	0.943***	0.872***	0.848***	0.752***
WS	0.837***	0.775***	0.799***	0.959***	I	0.885***	0.851***	0.779***
TL	0.787***	0.716***	0.781***	0.813***	0.772***	I	0.828***	0.723***
SL	0.760***	0.767***	0.614***	0.733***	0.699***	0.411***	I	0.721***
KL	0.821***	0.781***	0.778***	0.796***	0.766***	0.681***	0.751***	I
BW	I	0.941***	0.957***	0.909***	0.760***	0.909***	0.946***	0.935***
BL		I	0.923***	0.919***	0.779***	0.917***	0.928***	0.932***
BG			I	0.923***	0.763***	0.920***	0.950***	0.929***
WL				I	0.816***	0.954***	0.949***	0.903***
WS					I	0.788***	0.767***	0.767***
TL						I	0.961***	0.915***
SL							I	0.942***
KL								I

BW body weight, BL body length, BG breast girth, WL wing length, WS wing span, TL thigh length, SL shank length, KL keel length
***P<0.001*

length and shank length (0.496) in MNm, shank length and thigh length (0.411) in Nk and wing span and body weight (0.760) in Nm. The lowest positive correlations recorded between body weight and other linear body measurements was observed between body weight and body length (0.567) in MNm. In all the five chicken ecotypes, body weight had highly positive correlations with all the linear body measurements. These values revealed the pattern of correlations among the traits and high positive correlations among most of the linear body measurements with body weight in all the ecotypes may be useful as selection criterion. Positive correlations of traits suggest that the traits are under the same gene action (pleiotropy) (Yakubu *et al.,* 2009) and selection for a trait may lead to a correlated response in the other trait. Although only the first principal component (PC) exhibited eigenvalues greater than 1, observed communalities, which represent the proportion of the variance in the original variables that is accounted for by the factor solution ranged from 0.787 to 0.946 in M, 0.784 to 0.957 in MNk, 0.685 to 0.928 in MNm, 0.818 to 0.959 in Nk and 0.930 to 0.998 in Nm (Table 4). This offered credibility to the relevance of the principal component regression. PC1 accounted for the largest variance (81.74%, 77.44%, 76.02%, 78.95%, and 90.57%) for M, MNk, MNm, Nk and Nm respectively. This had been the usual trend in studies that involved PCA as stated by earlier researchers (Mendes, 2009; Yakubu *et al.,* 2009; Udeh and Ogbu, 2011 and Ajayi *et al.,* 2012). The best loading of each trait is indicated by a bolded number for each PCs in each ecotypes. PC1 had high positive loadings on all biometric traits ranged from 0.811 to 0.954 in M, 0.734 to 0.983 in MNk, 0.724 to 0.966 in MNm, 0.797 to 0.959 in Nk and 0.848 to 0.977 in Nm. This implies an increase in any of the traits will results to correlated increase in the other traits. Based on PCA criteria, seven linear body measurements were reduced to three components. Combined PC1, PC2 and PC3 accounted for 93.82%, 90.43%, 91.33%, 92.13% and 96.90% of the total variance for M, MNk, MNm, Nk and Nm respectively. Higher combined PC1, PC2 and PC3 values were

recorded for M and Nm in this study compared to report Udeh and Ogbu, (2011) who reported 74.76% in M at 8 weeks old and Ajayi *et al.,* (2012) who reported 89.78% in Nm. However, Ajayi *et al.,* (2012) reported higher value (94.83%) for combined PC1, PC2 and PC3 in Nk compared to this study. The three principal components (PC1, PC2 and PC3) obtained for each ecotype could be useful in assessing animals for breeding and selection purposes especially in the crossbred ecotypes. Table 5 illustrates the percent variation accounted for by PCs and cross validation for the number of extracted factors of the linear body measurements of chickens. Only one PC is retained according to the PRESS in MNk, MNm and Nk. The seven traits were collapsed into a single measure and the percent of the variance explained in the model was 77.44%, 76.02% and 78.95% in MNk, MNm and Nk respectively. Needless to say, this is adequate. If all seven PCs are treated as independent variables (no variable reduction is used), the percent of variance explained becomes 100%, but the model might be over-fitted. Meanwhile, three components should be retained according to the PRESS in M and Nm. PCR suggested number of the proper number of PCs based on the PRESS statistics and variance explained in the model effects. Principal component factor score coefficients for M, MNk, MNm, Nk and Nm chickens were generated and used instead of the original interdependent linear body measurements in predicting the BW of each ecotypes. The interdependent original linear body measurements and their independent principal component factor scores were used for the prediction of BW (Table 6). TL alone accounted for 76.21%, 62.72%, and 75.52% of the variation in BW for M, MNm and Nk respectively. However, the best prediction equation (R^2=85.61%) for BW was obtained when BG was included in the model for M. In Nk chickens, the best prediction equation (R^2=85.52%) for BW was obtained when BG was included in the model. Also, In MNm chickens, the best prediction equation (R^2=70.88%) for BW was obtained when KL was included in the model. BG alone accounted for 91.66% and 69.05% of the variation in BW

Table 4: Eigenvalues and share of total variance along with factor loadings after rotation and communalities of the linear body measurements of chickens

Traits	PC1	PC2	PC3	Communality
Marshal				
Marshal				
BL	0.811	0.535	0.042	0.946
BG	0.880	0.280	-0.152	0.853
WL	0.942	-0.231	0.062	0.939
WS	0.954	-0.190	-0.013	0.950
TL	0.907	-0.040	-0.315	0.824
SL	0.938	-0.283	-0.038	0.958
KL	0.887	0.020	0.424	0.787
Eigen values	5.722	0.536	0.309	
Percentage of total variance %	81.74	7.66	4.42	
Marshal x naked-neck				
BL	0.892	-0.225	0.349	0.809
BG	0.907	-0.064	-0.312	0.786
WL	0.734	0.637	0.166	0.957
WS	0.897	0.138	-0.182	0.784
TL	0.983	-0.155	0.008	0.941
SL	0.952	-0.179	0.123	0.893
KL	0.940	-0.012	-0.116	0.837
Eigen values	5.421	0.589	0.319	
Percentage of total variance %	77.44	8.42	4.57	
Marshal x normal-feathered				
BL	0.724	0.620	-0.233	0.898
BG	0.801	0.235	0.415	0.685
WL	0.959	-0.083	-0.088	0.909
WS	0.966	-0.117	-0.042	0.928
TL	0.941	-0.048	-0.079	0.871
SL	0.882	-0.335	-0.172	0.880
KL	0.859	-0.120	0.218	0.739
Eigen values	5.322	0.588	0.438	
Percentage of total variance %	76.02	8.41	6.9	

Table 5: Percent variation accounted for by Principal components and Cross validation for the number of extracted factors of the linear body measurements of chickens.

Number of Extracted factors	Model effects		Dependent variables		Prob >PRESS
	Current	Total	Current	Total	
Marshal					
1	81.739	81.739	76.171	76.171	0.0001
2	7.660	89.400	3.191	79.361	0.006
3	4.415	93.815	5.672	85.033	0.031
Marshal x naked-neck					
1	77.442	77.442	73.095	73.095	0.004
Marshal x normal-feathered					
1	76.023	76.023	70.916	70.916	0.0001
Naked-neck					
1	78.950	78.950	87.041	87.041	0.0001
Normal-feathered					
1	90.569	90.569	91.413	91.413	.0001
2	4.632	95.201	1.128	92.542	0.01
3	1.701	96.902	1.024	93.566	0.05

Table 6: Stepwise multiple regression of body weight on original body measurements and on their principal component (PC) factor scores in chickens.

Variables	Model	SE	R²
Marshal chickens			
Original body measurements as explanatory variables			
Thigh length	BW= -981.656+117.564TL	9.180	76.21
breast girth and thigh length	BW= -981.656+48.676BG+117.564TL	12.304	85.61
Orthogonal traits as independent variables			
	BW=785.130+56.116PC1	3.838	76.17
	BW=785.130+56.116PC1-65.886PC3	16.514	81.84
	BW=785.130+56.116PC1+37.516PC2-65.886PC3	12.538	85.03
Marshal x naked-neck chickens			
Original body measurements as explanatory variables			
breast girth	BW= -447.878+63.141BG	6.233	69.05
breast girth and thigh length	BW= -626.199+41.4716BG+78.505TL	28.733	73.45
Orthogonal traits as independent variables			
	BW=620.395+51.130PC1	4.573	73.09
Marshal x normal-feathered chickens			
Original body measurements as explanatory variables			
thigh length	BW= -886.690+109.185TL	10.777	62.72

Variables	Model	SE	R²
breast girth and keel length	BW= -1072.933+67.933TL+104.214KL	25.411	70.88
Orthogonal traits as independent variables			
	BW=631.333+54.945PC1	4.505	70.92
Naked-neck chickens			
Original body measurements as explanatory variables			
breast girth	BW= -671.302+77.303TL	7.439	75.52
breast girth and thigh length	BW= -704.931+40.285BL+46.221BG	8.311	85.52
Orthogonal traits as independent variables			
	BW=490.972+44.469PC1	2.900	87.04
Normal-feathered chickens			
Original body measurements as explanatory variables			
breast girth	BW= -565.974+68.558BG	3.116	91.66
breast girth and thigh length	BW= -609.395+34.963BL+43.185BG	8.938	93.85
Orthogonal traits as independent variables			
	BW=454.652+56.102PC1	2.592	91.41
	BW=454.652+56.102PC1-27.560PC2	10.805	92.54
	BW=454.652+56.102PC1-27.560PC2+43.339PC3	16.759	93.57

for Nm and MNk respectively. The high association between BW and BG in Nm might be attributed to large deposits of bones and muscles in breast region of the birds (Ajayi *et al.*, 2012). The proportion of the explained variance increased to 93.85% when body length was included in the model. In MNk ecotype, the proportion of the explained variance was further increased to 70.88% when KL was included in the model. Meanwhile, PC1 explained 76.17%, 73.09%, 70.92%, 87.04% and 91.41% of the total variability in BW in M, MNk, MNm, Nk and Nm respectively. However, a combination of PC1, PC2 and PC3 led to a significant improvement in the proportion of variance explained (R2=85.03%) in M ecotype while PC1, PC2 and PC3 led to substantial improvement in the percentage of variance explained (R2=93.57%) in Nm ecotype. The use of principal component scores (orthogonal traits) gave an improved and more reliable assessment of body weight since it was able to remove multicollinearity, a problem associated with the use of interdependent original body measurements (Yakubu *et al.*, 2009). Principal component regression can be used to classify independent and informative variables thereby eliminating redundant information for the purpose of reducing costs of chickens" genetic programmes.

Conclusions

PCR has a tendency to do well when the first few principal components are sufficient to explain most of the variation in the predictors as well as the relationship with the response. Hence, in this study the PCR method explored the interdependence in the original biometric traits of Marshal, Marshal x naked-neck, Marshal x normal-feathered, Naked-neck and Normal-feathered chickens. The use of independent orthogonal indices (PC1, PC2 and PC3) was more appropriate than the use of the original interrelated linear type traits for predicting the body weight of chickens. This is because multicollinearity of two or more interdependent original body measurements could lead to unstable regression coefficients and over-fitting of the model, hence inaccurate interpretation of the results. The subsequent principal components derived for each ecotypes could assist in selection and breeding programmes of the five ecotypes especially in

the upgraded indigenous chickens with Marshal for broiler purposes.

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119

PATHOLOGICAL STUDY OF FEMALE REPRODUCTIVE ORGANS OF LOCAL ZEBUS IN ADAMAWA REGION

Kouamo J*, Meyoufey B and Zoli A P
School of Veterinary Medicine and Sciences. The University of Ngaoundere. PO BOX 454, Ngaoundere, Cameroon.

Abstract

Genital tracts of female zebus (n=501) collected from Ngaoundere Municipal Slaughterhouse (NMSH) were examined with respect to breed, age, body condition score (BCS) and physiological status. Out of these specimens, the pregnancy rate was 20.4%. A total of 292 (58.28%) specimens had abnormalities. Maximum pathological conditions were observed in the ovaries (39.6%), followed by those in the uterus (15.4%), oviduct (2.8%) and vulvo-vagina (0.6%). Pathological conditions observed in the ovaries included anoestrus (25.2%), repeat breeding (8.4%), ovarian cysts (3.8%), double and multiple ovulation (1.2%), oophoritis (0.4%), ovarobursal adhesions (0.4%) and ovarian abscess (0.2%) whereas, those in the uterus included mucometra (7.8%), metritis (5.6%), hydrometra (1.6%) and lymphosarcoma (0.4%). Oviduct abnormalities include parovarian cysts (1.4), hydrosalpinx (0.6), lymphosarcoma (0.4), salpingitis (0.2%) and double oviduct (0.2). A total of 54 female zebus (10.78%) had at least two abnormalities. The ovarian cyst-anoestrus (3.39%), mucometra - anoestrus (2.20%) and metritis - anoestrus (1.20%) associations were the most observed. The thin cows (body condition score 1-2) aged 4 to 8 years old were more predisposed to genital pathologies. Results of this study indicated that lesions of the female reproductive system represent a significant source of infertility.

Keywords: zebu, ovaries, oviducts, uterus, abnormalities, Adamawa.

ETUDE DES PATHOLOGIES DES ORGANES REPRODUCTEURS FEMELLES DES ZÉBUS LOCAUX DANS LA RÉGION DE L'ADAMAOUA

Résumé

Les tractus génitaux (n=501) des femelles zébus collectés à l'abattoir municipal de Ngaoundéré ont été examinés en fonction de la race, de l'âge, de la note d'état corporel et de l'état physiologique. Un taux de gestation de 20,4% a été observé chez les animaux examinés. Un total de 292 (58,28%) spécimens a présenté des anormalités. Les conditions pathologiques les plus élevées ont été diagnostiquées au niveau des ovaires (39,6%), suivies de l'utérus (15,4%), des oviductes (2,8%) et des portions vaginale et vulvaire (0,6%). Les pathologies ovariennes étaient l'anoestrus (25,2%), le repeat breeding (8,4%), les kystes ovariens (3,8%), les double et multiple ovulations (1,2%), l'oophorite (0,4%), les adhésions des bourses ovariennes (0,4%) et les abcès ovariens (0,2%) tandis que celles affectant l'utérus étaient le mucomètre (7,8%), les métrites (5,6%), l'hydromètre (1,6%) et le lymphosarcome (0,4%). Les anormalités de l'oviducte incluaient les kystes para-ovariens (1,4), l'hydrosalpinx (0,6), le lymphosarcome (0,4), la salpingite (0,2%) et le double oviducte (0,2). Un total de 54 femelles zébus (10,78%) présentaient au moins deux pathologies concomitantes. Les associations anoestrus-kystes ovariens (3,39%), mucomètre - anoestrus (2,20%) et métrites - anoestrus (1,20%) étaient les plus observées. Les animaux maigres (note d'état corporel 1-2) âgés de 4 à 8 ans étaient prédisposés aux pathologies génitales. Les résultats indiquent que les pathologies du tractus reproducteur des femelles zébus locaux représentent une source significative d'infertilité.

Mots-clés: zébus, ovaires, oviductes, utérus, anormalités, Adamaoua.

Corresponding author email: justinkouamo@yahoo.fr

Introduction

In Cameroon, cattle rearing (estimated at 7 million) represents 16% of national agricultural production (Ebangi *et al.,* 2002). The main local zebu breeds are Gudali, White Fulani (Akou), Red Fulani (Djafoun) and Bokolo. The region of Adamawa alone has 38% of cattle (MINEPIA, 2003), with Gudali as the major breed. Local breeds are characterized by low productivity. Genetics, husbandry, health and reproductive problems have previously been identified as factors responsible for the low cattle productivity (Ebangi *et al.,* 2011). In the last decade, dairy cow fertility has declined significantly (Talib Ali and Faraidoon Ameen, 2014). Like humans, livestock animals including cows, suffer from infertility or sub-fertility, which lowers their lifetime productivity and reduces the number of offspring that could be obtained. The prevalence of this problem coupled with the desire of people to understand and subsequently control the reproductive processes has led to the development of novel reproductive biotechnologies (Rahman *et al.,* 2008), which are also known as assisted reproductive techniques (ARTs). The applications of these ARTs such as artificial insemination, embryo transfer, estrus synchronization, superovulation and in vitro production of embryos in cow enable to increase the rate of genetic progress, reduce generation interval, enhance production, improve the management of infertile/sub-fertile animal and eliminate reproductive diseases (Deuleuze *et al.,* 2009). However, the results across sub-Saharan Africa and Cameroon in particular are still low (Kouamo et al., 2009, 2014). Ignorance of the prevalence of pathologies of the genital tract could also constitute a factor responsible for the failure rate of biotechnology of reproduction. Indeed, determining the prevalence of diseases of the genital tract is an essential first step to identify the individual factors responsible for infertility and propose specific recommendations as possible to farming conditions encountered (Nguyen and Hanzen, 2014).

Investigation of bovine reproductive abnormalities based on a survey of abattoir specimens provides a great deal of information on prevalence of reproductive disorders and their incidence (Fathalla *et al.,* 2000). Reproductive failure occurs in many different forms, as failure to return to heat, repeat breeding, sometimes, even severe disorders may be difficult to detect clinically. Hence, abattoirs are a good source for studying pathological lesions of animal reproductive organs causing subfertility or even sterility, for providing information on prevalence of reproductive disorders and infectious diseases, for supporting clinicians in the application of the most suitable diagnostic and therapeutic approach (Palmieria *et al.,* 2011). Several studies on pathological conditions of reproductive organs of exotic cows have been done during last few decades. But similar studies on Cameroonian indigenous zebus have not been made so far which is of utmost importance. Pathological conditions of ovary seriously interfere with normal functions of the entire reproductive tract, consequently, influencing the reproductive potential of the animal. The present study was designed to study the abnormalities of bovine reproductive organs based on locally slaughtered zebus. Specific objectives were to characterize the slaughtered cows, to determine the prevalence and risk factors of reproductive tract diseases.

Materials and Methods

Study Area

The study was conducted using samples collected at the NMSH and analyzed at the veterinary laboratory of IRAD-Wakwa Regional Center in Adamawa region of Cameroon. The cattle slaughtered at the NMSH were from the Vina Division (76.8%) and Mayo Rey Division (23.2%). Ngaoundere is situated between Latitude 7°19'39N and Longitude 13°35'4E and have an average annual rainfall of 1496.7 mm. The temperatures varied from 15.2°C to 29°C with an average humidity of 58.2%.

Characteristics of animals

A total of 501 local zebus of different breeds [Gudali (359), White Fulani (91), Red Fulani (34) and Bokolo (17) based on phenotypic

criteria as described by Lhoste (1991)] were randomly selected for this study. The body condition score (BCS) and age (years) have been determined as described by Natumanya et al. (2008) and Moussa Garba et al. (2013), respectively. The pregnancy status has been determined by the presence of a fetus.

Examination of the genital tract

After transportation of bovine reproductive tracts in Wakwa, each specimen included ovaries, oviducts, uterine horns, cervices, vagina and vulva was grossly examined carefully in the laboratory in order to determine the nature of the reproductive abnormality and its location in the tract (Assey *et al.,* 1998). Lesions were diagnosed, evaluated and then photographed. Some pathological conditions portions were also dissected. The presence of ovarian follicles, ovarian cysts and any abnormalities of ovaries, uterine tubes and vagina were checked grossly.

The diameter and color of each corpus luteum (CL) permits to classify them into three types as described by Nguyen and Hanzen (2013): hemorrhagic CL or CL1 (diameter less than 2 cm, reddish color), diestrus CL or CL2 (diameter between 2 and 3 cm, yellowish or orange) and decreased CL or CL3 (diameter less than 1 cm and whitish color). Ovarian cyst was considered as an anovulatory follicle with a diameter greater than 24 mm (Hanzen et al., 2008). The classification of types of anoestrus was based on the presence of follicles, CL and / or cysts on the ovaries (Hanzen *et al.,* 2012; Peter *et al.,* 2009). Thus, six types of anoestrus were identified: type 0 (absence on both ovaries, follicle with diameter ≥ 2 mm, CL and cysts); type I (presence on one ovary, follicles of diameter between 2 and 7 mm with the absence in both ovaries CL and cysts); type II (presence on one ovary, at least one follicle of diameter > 7 mm and follicles between 2 and 7 mm in the absence of both ovaries CL and cysts); type III (presence on one ovary, ovarian cyst in the presence or not of follicles diameter > 7mm but in the absence on both ovaries CL1 and CL2); type IV (presence on one ovary, a CL2 in the presence or not of follicles ≥ 8 mm diameter and the presence of a pyometra); type V or of

pregnancy (presence on one ovary a CL2 in the presence or not of follicles of diameter > 7 mm and the presence of an embryo or fetus in the uterus). The repeat breeding, parovarian cysts and mucometra were determined as described by Bah et al. (2010), Riasat et al. (2013) and Herenda (1987), respectively.

Statistical analysis

Data were analyzed using SPSS (Statistical Package for Social Sciences) version 20. The analysis of variance and Duncan's test statistics were used to analyze appropriate data sets. Differences were significant at P < 0.05.

Results

Characterization of slaughtered animals

BCS and average age (minimum-maximum) of the animals were 3.14 ± 0.04 (1-5) and 6.62 ± 0.11 years old (2-15), respectively. Of the 501 female zebus examined, 102 (20.4%) were pregnant. Out of 1002 ovaries examined, 535 (53.4%) carried a CL: 296 (29.6%) and 239 (23, 9%) on the right and left ovaries respectively. The CL1, 2 and 3 were 26 (2.6%), 229 (22.9%) and 280 (27.9%), respectively.

Prevalence of genital abnormalities

In gross examination, out of 501 reproductive tracts, 58.28% presented abnormalities (table 1). Out of the 19 identified cases of ovarian cysts, one case of luteal cyst was observed versus 18 of follicular cysts. 14/19 ovarian cysts were diagnosed on the right ovary. 4/6 cases of double and multiple ovulation were identified on the right ovary. All cases of oophoritis and hydrosalpinx were observed on the right ovary. Only one case (0.2%) of double bilateral oviduct was identified. The mucometra was the most common disease of the uterus (7.8%) and 2 cases (0.4%) of uterine lymphosarcoma were observed.

Concomitant pathologies of the genital tract

Out of the 292 cows, 54 (10.78%) had at least two concomitant genital diseases (table 2). The ovarian cyst-anoestrus (3.39%), anoestrus-mucometra (2.20%) and anoestrus-metritis

Table 1: Prevalence of reproductive abnormalities in local zebus.

Location and disease condition	Number of specimens	Percentage (%)
Ovarian pathologies	**156**	**39.6 %**
Anoestrus	126	25.2
Type 0	2	0.4
Type I	18	3.6
Type II	86	17.2
Type III	17	3.4
Type IV	3	0.6
Ovarian cysts	19	3.8
Double and multiple ovulation	6	1.2
Oophoritis	2	0.4
Ovarobursal adhesions	2	0.4
Ovarian abscess	1	0.2
Repeat breeding	42	8.4 %
Oviduct pathologies	**14**	**2.8 %**
Salpingitis	1	0.2
Hydrosalpinx	3	0.6
Double oviducts	1	0.2
Lymphosarcoma	2	0.4
Parovarian cysts	7	1.4
Uterine pathologies	**77**	**15.4 %**
Metritis	28	5.6
Acute	7	1.4
Chronic (+pyometra)	21	4.2
Hydrometra	8	1.6
Mucometra	39	7.8
Lymphosarcoma	2	0.4
Vulvovaginal pathologies	**3**	**0.6 %**
Vaginitis	2	0.4
Vaginal prolapsus	1	0.2
Total	**292**	**58.4 %**

(1.20%) associations were the most observed. Only 4 cows presented a combination of three concomitant pathologies (table 2).

Prevalence of reproductive abnormalities based on breed, BCS, age and physiological status

Table 3 presented the pathological conditions observed in the genital tract of female zebu based on breed, BCS, age and physiological status. The Gudalis were more affected with anoestrus types I and II compared to other breeds ($p < 0.05$). The thin cows (BCS 1 and 2) showed ($p < 0.05$) more anoestrus type III (1.6%) and ovarian cyst (1.6%). The cows aged 4 to 8 years presented more anoestrus type III. However, no young cow presented any metritis. Only non-pregnant cow presented pathological cases (table 3).

Table 2: Concomitant pathologies of the genital tract of local zebu.

Disease condition	Number of specimens	Percentage (%)
An+ Oc+ Rb- Me- Mu+ Hy- Pc- Dmo-	1	0.20
An + Oc - Rb+ Me+ Mu- Hy- Pc - Dmo -	1	0.20
An + Oc + Rb- Me- Mu- Hy+ Pc - Dmo -	1	0.20
An + Oc - Rb- Me- Mu+ Hy- Pc + Dmo -	1	0.20
An + Oc + Rb- Me- Mu- Hy- Pc - Dmo -	17	3.39
An + Oc - Rb- Me- Mu+ Hy- Pc - Dmo -	11	2.20
An + Oc - Rb- Me+ Mu- Hy- Pc - Dmo -	6	1.20
An + Oc - Rb- Me- Mu- Hy+ Pc - Dmo -	3	0.60
An + Oc - Rb- Me- Mu- Hy- Pc + Dmo -	2	0.40
An - Oc - Rb- Me+ Mu- Hy- Pc - Dmo +	1	0.20
An - Oc - Rb- Me- Mu+ Hy- Pc + Dmo -	1	0.20
An - Oc - Rb- Me- Mu- Hy+ Pc + Dmo -	1	0.20
An - Oc - Rb+ Me- Mu+ Hy- Pc - Dmo -	3	0.60
An - Oc - Rb+ Me+ Mu- Hy- Pc - Dmo -	5	1.00
Total	**54**	**10.78%**

An = anoestrus, Oc = ovarian cyst, Rb = repeat breeding, Me = metritis, Mu = mucometra, Hy = hydrometra, Pa = parovarian cyst, Dmo = double and multiple ovulation, (-) = absent and (+) = present.

Discussion

Characterization of slaughtered cows

The average ages of cows were similar to those reported by Bah et al. (2010) and Kouamo et al. (2014) in the same study environment. The average BCS of animal (3.14) would be linked to the period of the study. In fact, it was conducted in rainy season during which feed supplies were not lacking. The percentage of pregnant animals observed (20.4%) represented a huge economic loss and was different from many authors who worked in slaughterhouses: 16.6%, 22%, 37.3%, 52.2%, 7.5% and 4.9% obtained by Tchoumboue (1988), Ndi et al. (1993), Bah et al. (2010), Kouamo et al. (2014), Moussa Garba et al. (2013) and Nguyen and Hanzen (2013), respectively. The difference could be related to the size of the sample which varies with respect to studies, environmental stress, reproductive stage (reformed, in activity or in depletion) and diseases of the genital tract.

Abnormalities of the reproductive organs

Determining the prevalence of diseases of the genital tract of animals is necessarily a first step to improve fertility through reproductive biotechnologies. This study revealed that the overall prevalence (58.28%) of abnormalities of the genital tract of local zebu slaughtered at NMSH was higher than those reported by Abalti et al. (2006), Bah et al. (2010), Simenew et al. (2011) and Berhanu et al. (2013) who reported a prevalence of 36.9%, 39.9%, 22.3% and 39.1%, respectively; but lower than that obtained by Herenda (1987), 62%. The difference may be due to several factors such as breed, number of animals studied, but also to their geographical origin, health and / or nutritional status. Indeed, Lewis (1997) reported that lack of sanitary precautions in artificial breeding of cows might predispose them to a variety of specific and nonspecific microorganisms.

When a dairy cow is not observed in estrus by 60 days post-partum, the condition is defined as post-partum anoestrus. It includes cyclic and non-cyclic cows. The prevalence of pathological anoestrus (type 0, I, II, III and IV) was similar to that reported by Bah et al. (2010). According to the latter, the existence of abundant economically important diseases in the rainy season, justifies the high

Table 3: Pathological conditions observed in the genital tract of female zebu based on breed, BCS, age and physiological status.

Factors		Type0	TypeI	TypeII	TypeIII	TypeIV	Ovarian cyst (n=18)	DM Ovulation (n=6)	Repeat breeding (n=42)	Metritis (n=28)	Mucometra (n=39)	Hydrometra (n=8)	Parovarien cyst (n=7)
Breed	Gudali	0.4a	2.0a	4.7a	2.2a	0.4a	2.6a	1.2a	5.8a	4.0a	5.4a	1.2a	1.2a
	Akou	0.0a	0.8a	2.2a,b	0.6a	0.2a	0.6a	0.0a	1.8a	1.4a	1.6a	0.4a	0.2a
	Djafoun	0.0a	0.2a,b	0.6a,b	0.6a	0.0a	0.6a	0.0a	0.6a	0.2a	0.8a	0.0a	0.0a
	Bokolo	0.0a	0.6b	0.0b	0.0a	0.0a	0.0a	0.0a	0.2a	0.0a	0.0a	0.0a	0.0a
BCS	Thin (1-2)	0.2a	0.0a	4.0a	1.6a	0.2a	1.6a	0.0a	1.4a	1.8a	2.0a	0.6a	0.2a
	Good (3)	0.0a	1.0a	6.6a	0.8b	0.4a	1.2b	0.4a	3.8a	2.6a	3.4a	0.4a	0.6a
	Fat (4-5)	0.2a	1.8a	6.4a	1.0a,b	0.0a	1.0a,b	0.8a	3.2a	1.2a	2.4a	0.6a	0.6a
Age (years)	< 4	0.2a	0.8a	5.0b	0.6b	0.0a	0.6a	0.2a	0.4a	0.0b	0.8a,b	0.2a	0.1a
	4-8	0.2a	2.4a	11.8a	2.8a,b	0.6a	3.0a	0.6a	6.0a	4.2a,b	4.0a	1.4a	1.0a
	> 8	0.0a	0.4a	0.4c	0.0a	0.0a	0.2a	0.4a	2.0a	1.4a	3.0b	0.0a	0.3a
Physiological status	Pregnant	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	2.2a	0.0a	0.0a	0.0a	0.2a
	Non pregnant	0.4a	3.6b	17.2b	3.4b	0.6a	3.8b	1.2a	6.2a	5.6b	7.8b	1.6a	1.2a

a,b percentages in a column with different superscripts are significant at p <0.05. DM = double and multiple.

proportion of cows in anestrus. However, it is less than that obtained by Nguyen and Hanzen (2013), 39.7%. These authors linked the high prevalence to dietary deficiency. Indeed, the nutritional deficiency and the low energy supply are the major causes of ovarian inactivity or anoestrus (Butler and Smith, 1989). Thin (BCS 1 and 2) animals aged 4 to 8 years old were significantly affected by anoestrus type III. This would explain the major impact of feed intake on follicular growth with non-return of heat in cows. The Gudali breed was more predisposed to anoestrus types I and II when compared other breeds and may explain either their susceptibility to anoestrus or their representation in the studied sample size.

Cystic ovarian disease is the most important pathologic causes of irregular estrus cycles. An insufficient pre-ovulatory LH-surge or an inopportune timing of the LH-surge is thought to be the principal cause of ovarian cyst disease. The prevalence of ovarian cysts (3.8%) was comparable to those obtained by Chaudhari and Paul-Bokko (2000), Abalti et al. (2006) and Moussa Garba et al. (2013); but less than those reported by several authors [10%, Herenda, (1987); 7%, Fathalla et al. (2000); 13.9%, Feyissa and Bekana (2000); 7%, Abdul (2013)] and higher than 2.72% reported by Riasat et al. (2006). Noakes et al. (2002) suggested that the breed, age, level of milk production and feeding were the factors that influenced the prevalence of ovarian cysts in cattle. Previous studies have also shown that incidence of cystic ovarian disease is maximal in high-yielding dairy cattle, 5-6 years old and 30-45 days post-partum (Garverick, 1999). Indeed, the preferential period of occurrence of ovarian cysts is during the phase of increased milk production, correlated with a significant deficit of energy accompanied by a mobilization of fat reserves. Hepatic metabolism is then increased, causing a greater elimination of estradiol and progesterone which explains the origin of the increased occurrence of ovarian cysts. A high progesterone level in milk or plasma is indicative of a luteal cyst. Hence the high prevalence of ovarian cysts in thin animals (BCS 1 and 2) in this study, reflecting the important aspect of nutritional factor include

□ carotene deficiency and phyto-oestrogens in the development of ovarian cysts via its impact on follicular growth. The right ovary carried more cysts (14 of 19) compared to the left as reported by Tanabe and Brofee (1982), explaining the high metabolic activity on the right ovary.

Double and multiple ovulation is due to a co-dominance of two or several follicles consecutive to a hormonal disorders. The prevalence of double and multiple ovulations (1.2%) was lower than those obtained by Lopez-Gatius and Hunter (2005) in dairy cows (15.5 - 26.6%). This variation may be due to the level of dairy production which represents a risk factor of this pathology.

Parovarian cysts were considered as a small cysts (diameter between 2 to 5 mm) attached to mesovarium and mesosalpinx (20). They can be colored by melanin, or calcified. In literature review, the prevalence varied from 0.4% to 2.3% (Abalti *et al.,* 2000 ; Chaudhari and Paul-Bokko, 2000 ; Fathalla *et al.,* 2000 ; Feyissa and Bekana, 2000 ; Riasat *et al.,* 2006 ; Berhanu *et al.,* 2013; Abdul, 2013 ; Moussa Garba *et al.,* 2013). A higher prevalence (15.4%) has been reported by Kunbhar et al. (2003). They were frequently attached to the mesovarium in the present study. Congenital parovarian cysts have been described in cow.

The prevalence of repeat breeding (8.4%) was higher than that reported by Bah et al. (2010), 6.1%. The low sample size and poor health status of animals of study may have influenced the percentage of affected animals. Thus Simenew et al. (2011) have shown that subclinical genital tract infections are potential causes of repeat breeding. Factors such as breed, BCS, age and physiological status did not influence the prevalence of repeat breeding in this study. However, studies conducted by Zobel et al. (2011) and Tadegegne et al. (2012) established relationship between breed, age and repeat breeding. According to these authors, crossbreeds and heifers were more predisposed to repeat breeding. Other authors have also noted the role of age and nutritional deficiencies in the appearance of this pathology (Ibrahim *et al.,* 2011; Teferi *et al.,* 2011).

Metritis cases (5.6%) were similar to the results reported by Bah et al. (2010); but lower than those reported in the European dairy breeds (53%; Gilbert et al., 2005; 23.6%; Gautam *et al.,* 2010). The difference may be due not only to the breed effect, but also to microbial infections, postpartum complications, farming conditions and age of the animals. Indeed, microbial agents play a key role in the development of metritis. Poor farming conditions and postpartum complications promote by ascending infection, the appearance of this abnormality. Cows aged 4 to 8 years old were predisposed to metritis. Previous studies reported the relationship between age and prevalence of metritis (Abdul, 2013). Thus, Smith and Risco (2002) got less postpartum metritis in cows aged 2 to 4 years old than those over 7 years old. In sub Saharan Africa, cows enter into reproduction very late (more than 4 years).

The prevalence of mucometra was higher than that obtained by Herenda (1987), 5%. Indeed, this author conducted his study with dairy breeds that seemed to be more predisposed at the onset of reproductive disorders due to excessive energy loss following high milk production. Older cows aged 4 to 8 years old were the most predisposed to mucometra. The prevalence of hydrometra (1.6%) was higher than that reported by Herenda (1987). This variation may be related to the effect of age. Indeed, the study of Herenda (1987) had as target, the heifers.

Abnormalities of vulvo-vagina were observed in three specimens. In adult cows, vaginitis may occur due to infection from the environment and can easily lead to endometritis.

Concomitant genital diseases

The prevalence of concomitant genital diseases (10.78%) in a single cow was comparable to those reported by many authors. Herenda (1987) reported 57 cases of cows that had both ovarian cyst and mucometra. Moreover, Rogério et al. (2008) showed that the prevalence of uterine inflammation was higher in cows with repeat breeding. The combination of the anoestrus-metritis has also been reported as a cause of infertility in cow (Hanzen, 2005). Anoestrus is one of the possible symptoms of cystic ovarian disease. Luteal cysts are associated with anoestrus; however, differentiation between follicular and luteal cysts on the basis of behavior is not possible. The prevalence of associated disease of genital tract suggested that combined treatments should be realized before any practice of reproductive biotechnologies.

Conclusion

Genital tract abnormalities represent a major source of infertility of local zebus in Cameroon. The treatment of these various diseases should be a prerequisite for any genetic improvement program of the local bovine population.

Acknowledgments

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PREVALENCE AND DEMOGRAPHIC DISTRIBUTION OF CANINE RABIES IN PLATEAU STATE, NIGERIA, 2004 – 2009

Bolajoko Muhammad-Bashir[1, 2], Ahmed Mohammed Sani[2], Okewole Philip Ademola[2], Kumbish Peterside[2], Muhammad Maryam[2] and Jenna Fyfe[1]

[1]College of Medicine and Veterinary Medicine, University of Edinburgh, EH16 4SB
[2]National Veterinary Research Institute, P.M.B. 01, Vom, Plateau State, Nigeria

Abstract

Rabies, a neglected tropical disease, is one of the most fatal diseases. Around 55,000 people die from rabies annually with over 99% of these deaths occurring in Africa and Asia.

A retrospective study of rabies cases was carried out in Plateau state, Nigeria, 2004 – 2009. Cases reported to the central diagnostic laboratory (CDL) of the national veterinary research institute, Vom, Nigeria were investigated. Head samples from cats and dogs were received by CDL for rabies diagnosis; the majority (98%) of the samples were from dogs. ArcMap 10 (ESRI, Redlands, CA) was used to produce choropleth maps to present the geographical distribution of the prevalence of canine rabies in Plateau state. IBM SPSS version 20.0 was used to run the paired samples t-test and odd ratio.

The risk of developing rabies cases was found to be higher amongst patients with previous history of dog-bites. No consistent month-wise seasonal patterns of canine rabies was identified. Geographical distributions of cases in the state revealed concentration of disease on the plateaux in each year of the study. This study revealed that factors such as poor vaccination coverage of owned dogs, high population of stray dogs and/or low confinement of domestic dogs and lack of revision and enforcement of regulations/ laws for impoundment and elimination of stray dogs are responsible for the observed canine rabies situation in the state. These problems were discussed and recommendations were suggested.

Keywords: Canine-rabies, rabies control, rabies-demography, Plateau state, Nigeria.

LA PREVALENCE ET LA DISTRIBUTION DEMOGRAPHIQUE DE LA RAGE CANINE DANS L'ETAT DU PLATEAU AU NIGERIA DE 2004 A 2009

Résumé

La rage est la maladie tropicale négligée la plus mortelle. Environ 55.000 personnes meurent de la rage chaque année avec plus de 99% de décès survenus en Afrique et en Asie.

Une étude rétrospective des cas de rage a été réalisée dans l'état du Plateau au Nigeria de 2004 à 2009. Les cas signalés ont été étudiés au laboratoire central de diagnostic (LCD) de l'Institut national de recherche vétérinaire, Vom, au Nigeria. Des échantillons prélevés sur la tête des chats et chiens étaient reçus par le LCD pour le diagnostic de la rage ; la majorité (98%) des échantillons était prélevée sur des chiens. ArcMap 10 (ESRI, Redlands, CA) était utilisé pour produire des cartes choroplèthes pour présenter la répartition géographique de la prévalence de la rage canine dans l'Etat du Plateau. Le risque de développement des cas de rage était plus élevé chez les patients ayant des antécédents de morsures de chien. Aucun modèle saisonnier cohérent de la rage canine n'a été identifié. Chaque année de l'étude, les distributions géographiques des cas dans l'Etat ont révélé une concentration de la maladie sur les plateaux. Cette étude a révélé que les facteurs tels que la faible couverture vaccinale des chiens dépendants d'un propriétaire, la haute population de chiens errants et / ou le faible confinement des chiens domestiques, l'absence de révision, l'application des règlements / lois pour la retenue et l'élimination des chiens errants sont responsables de la situation de la rage canine observée dans l'Etat. Ces problèmes ont été débattus et les recommandations suggérées.

Mots-clés : la rage canine, la lutte contre la rage, la rage-démographique, l'Etat du Plateau, le Nigeria.

Corresponding author email: mbbolajoko@gmail.com

Introduction

Rabies is one of the most feared diseases throughout human history with the highest human case-fatality proportion of any infectious disease (Anderson *et al.,* 1981; Rupprecht *et al.,* 2002). It is estimated that 55,000 people die from rabies annually and over 99% of these deaths occur in Africa and Asia (Knobel *et al.,* 2005). Rabies has remained endemic in Nigeria as one of the most important neglected disease of public health concern in the country (Adedeji *et al.,* 2010).

Rabies is a zoonotic infection and the most common route of human infection is via a dog bite (Bata *et al.,* 2011). The disease has been eliminated from domestic dog populations in Western Europe and North America (Fooks, 2007) but the same cannot be said for developing countries (Cleaveland *et al.,* 2007; Hampson *et al.,* 2009). In developing nations where dogs are principal hosts and vector of rabies, the risk is high for spill-over infections into human populations (Lai *et al.,* 2005; Sudarshan *et al.,* 2007).

This study aimed to determine the prevalence of canine rabies across the seventeen local government areas (LGA) of Plateau state, Nigeria in a six-year study period 2004 -2009. This information was used to identify any seasonality of infection and describe the geographic distribution of canine rabies across the state; with a view to identifying and assessing the risk factors in the state.

Materials and methods

The study area of the Plateau state of Nigeria is one of the largest states in the country, almost centrally located between latitude 80°24'N and longitude 80°32' and 100°38' east (Plateau, 2010 (see Figure 1a); Bata *et al.,* 2011). Plateau state has a high altitude ranging from approximately 1,200 to a peak of 1,829 metres above sea level; the state has a near temperate climate with an average temperature of between 18 and 22°C (Plateau, 2010; Bata *et al.,* 2011). The mean annual rainfall varies from 131.75 cm to 146 cm and the highest rainfall is recorded during the wet months of July and August (Bata *et al.,* 2011).

Administratively, the state is divided into 17 LGA (Figure 1b). The LGA include Barkin Ladi, Jos South, Jos North, Jos East, Wase, Bassa, Pankshin, Shendam, Kanam, Kanke, Quampam, Riyom, Mangu, Bokkos, Langtang North, Langtang South and Mikang (Plateau, 2010; Bata *et al.,* 2011).

The central diagnostic laboratory (CDL) of the NVRI is the only OIE-recognised diagnostic laboratory for animal rabies in the state ((NVRI Annual Report, 2011; 2012). The data reviewed in this study comprised of historical CDL records of rabies cases or suspected cases submitted for diagnosis between 2004 and 2009. The positive cases in the sourced data set were based on the Direct Fluorescent Antibody Test - DFAT detection methods (NVRI Annual Report, 2011; 2012). IBM SPSS version 20.0 was used to run Pearson correlation (2-tailed) and odds ratio. The Pearson correlation was done to assess the nature of relationship between the variations in the reported number of rabies cases between and amongst years, 2004-2009.

The laboratory confirmed canine rabies cases for each LGA of the state (2004-2009) was also reduced into alphanumerical tables for introduction into geographic information systems (GIS). A GIS-polygon map was provided by the National Centre for Remote Sensing (NCRS), Jos, Nigeria. ArcMap 10 (ESRI, Redlands, CA) was used to produce choropleth maps to present the geographical distribution of the prevalence of canine rabies in Plateau state.

Results

A total of 1038 samples (1019 dogs, 13 cats and 6 cattle) were submitted to the CDL between 2004 – 2009, 537 samples tested positive for rabies and 501 samples tested negative. 52% (n=532) of the submitted dog samples were positive for rabies. Dogs accounted for 99% of the total laboratory-confirmed positive samples for rabies (Table 1).

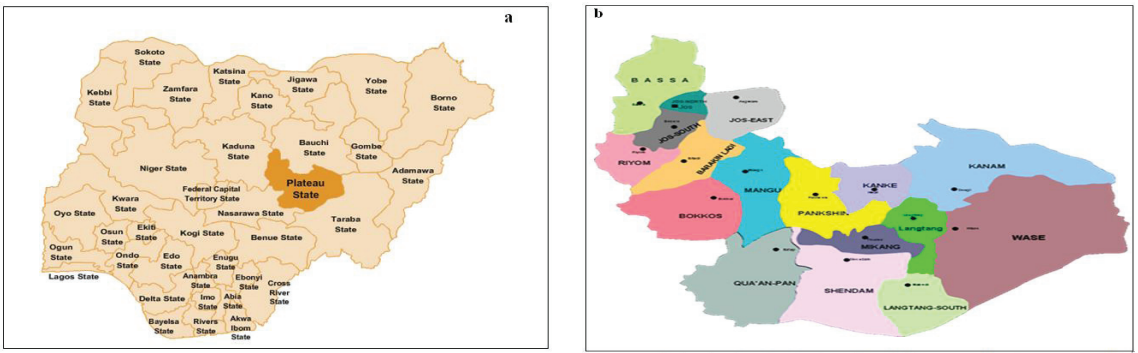In approximately 90% of all the samples submitted for laboratory diagnosis of

Figure 1: Map of Nigeria and Plateau state.

a: Map of Nigeria showing the location of all states with Plateau state highlighted in light brown colour. Source: NAIJ, 2011 – 2014: http://news.naij.com/55967.html b: An annotated map of Plateau state-Nigeria, with illustration of all the seventeen (17) LGA. Source: Zaccheus Onumba Dibiaezue Memorial Libraries – The Free Library. http://zodml.org/Nigeria/Geography/ Plateau%20State/

rabies during the study period the cases had history of dog bites. 63% of the cases were dogs bitten by suspected rabid dogs; 27% of cases were human injury-dog-bites; 7% were cat injury-dog-bites and 3% were cattle injury-dog-bites. The likelihood of rabies development amongst animals with a recorded dog bite was approximately 7.49 times higher than those with no recorded dog bite (odd ratio 7.49, 95% confidence interval: 6.82-8.66).

Approximately 33% of the dog samples reviewed during the study period were vaccinated against rabies on annual basis. In this study, only 4.2% (n=43) of the total dog samples had up-to-date vaccination status; 13.3% of which were confirmed positive for rabies. The majority of submitted samples had either expired vaccination status, 34.1% (n=347) or no history of vaccination, 61.7% (629) (Table 2). Furthermore, 37.2% of those cases with no previous vaccination history were stray dogs and 62.8% were owned dogs (Table 3). Owned dogs make up 59.5% of the laboratory confirmed rabies cases and stray dogs contribute 40.5% to the total canine rabies cases (Table 4).

Review of the annual variation of laboratory-confirmed rabies cases in the study period revealed that 2007 had the least (61 = 46.2%) and 2009 had the highest (121 = 59.3%). A gradual increase in the number of submitted rabies samples is observed from 2004 to 2006;

and then in both 2007 and 2008, the number of submitted samples dropped by approximately 50% of the number of reported cases in 2006 (Table 1). The paired samples t-test analysis revealed significant difference in the mean annual reported rabies samples between 2006-2007 ($t(23) = -0.218, P<0.05$). In 2009, the number of rabies samples reported to the CDL was double that of 2008. The paired samples t-test analysis between 2008-2009 ($t(23) = 0.008, P<0.01$).

The choropleth maps showing the geographical spread of samples submitted for rabies testing reveal that there is uneven distribution of the samples for rabies across the LGA of the state in each year (Figures 3 and 4). Consistently, in each year during the study period, the submitted samples for rabies testing are higher in LGA located on the Plateaux: Jos South, Jos North, Barkin Ladi, Bokkos and Mangu local government areas. Riyom, Jos East, Pankshin and Quampam have undulating number of submitted samples for rabies diagnosis, from low to high. Most of the LGA particularly those that are not on the Plateaux had no laboratory-confirmed rabies cases.

Table 1: Proportion and percent positive brain of animals diagnosed for rabies in Plateau state, Nigeria (2004 – 2009)

Year	Dogs		Cats		Cattle	
	No of brain samples examined	No (%) of positives	No of brain samples examined	No (%) of positives	No of brain samples examined	No (%) of positives
2004	177	92 (51.98)	3	1 (33.33)	2	1 (50)
2005	182	95 (52.2)	0	0 (0)	0	0 (0)
2006	208	99 (47.6)	5	1 (20)	1	1 (100)
2007	132	61 (46.2)	1	0 (0)	1	0 (0)
2008	116	64 (55.2)	0	0 (0)	0	0 (0)
2009	204	121(59.3)	4	1 (25)	2	0 (0)
Total	**1019**	**532(52.2)**	**13**	**3 (23)**	**6**	**2 (33.33)**
Total Positives (537)	**-**	**532(99.07)**	**-**	**3 (0.56)**	**-**	**2 (0.37)**

Table 2: Proportion and percent of vaccination history records amongst dogs examined for rabies in Plateau state, Nigeria (2004 – 2009)

Vaccination history at the time of case report	Number of dogs or brain samples submitted.	Percentage (%)
Up-to-date vaccination	43	4.2
Expired vaccination	347	34.1
No vaccination history	629	61.7
Total	**1019**	**100**

Table 3: The distribution of the proportion and percent of vaccination history records between owned and stray dogs examined for canine rabies in Plateau state, Nigeria (2004 – 2009)

Dog ownership	History of vaccination at the time of case report		Percentage (%)	
	History is available	No history	History is available	No history
Owned	211	395	54.1	62.8
Stray	179	234	45.9	37.2
Total	**390**	**629**	**100**	**100**

Table 4: The distribution of the proportion and percent of rabid dogs that tested positive for rabies between owned and stray dogs examined for rabies in Plateau state, Nigeria (2004 – 2009)

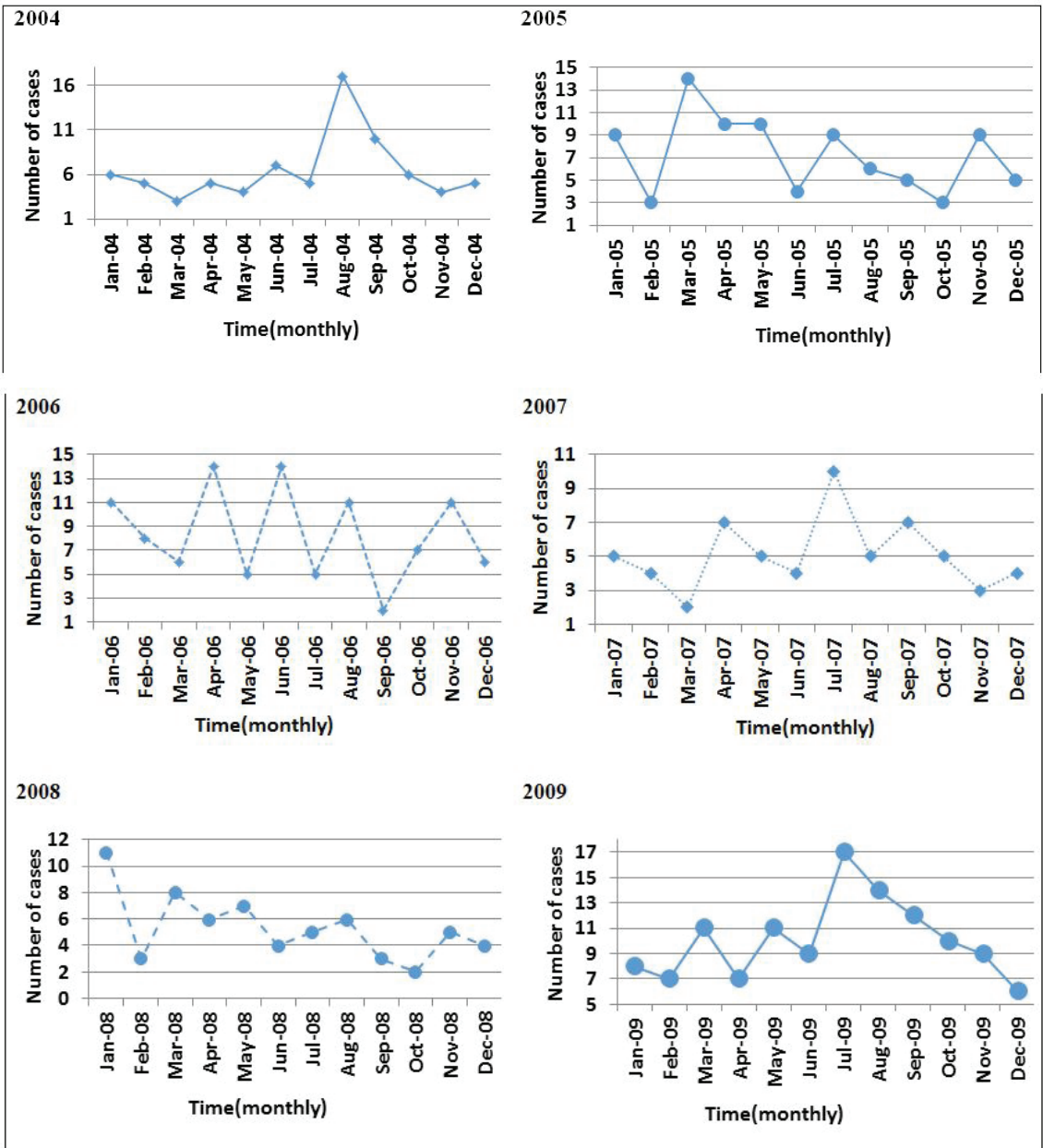| Dog ownership	Number of rabid dogs	Percentage (%)
Owned	606	59.5
Stray	413	40.5
Total	**1019**	**100**

Figure 2: Seasonal distribution of laboratory-confirmed canine rabies in Plateau state, Nigeria in each year of the six-year study period, 2004 - 2009

Discussion

The prevalence of laboratory-confirmed canine rabies 2004 – 2009; corroborates Bata's (2011) reports of canine rabies in the state. Bata (2011) attributed this situation to the fact that the state has a large population of dogs (Adedeji et al., 2010). The high prevalence of canine rabies could be a result of the suitability of the prevailing cool weather conditions of the state for breeding and keeping of exotic breeds of dogs. It is also worth noting that the state hosts the largest commercial dog market in West Africa, situated in Kanke LGA (Bata et al., 2011).

This study suggests that dogs are responsible for the maintenance and transmission of rabies across the state; as such it represents a major source of fatal animal and human rabies cases. Idachaba, 2009, revealed that between 2004 and 2007, an average of 14-15 human dog-bites from suspected rabid dogs were recorded across Plateau state. Another study in the state, on post-exposure prophylaxis (PEP) treatment for humans, reported that a total of 305 patients were presented to the hospital for PEP treatment within a period of two years, 2010 – 2012 (Ekong et al., 2012). Findings of this present study and those of Idachaba, 2009 and Ekong et al., 2012 support the conclusion that canine rabies is well established in Plateau state. Furthermore, taking a cue from the recommendations of Hampson (2009) and Lembo (2010); the low prevalence of rabies in other livestock and domestic animals in comparison to the state's dog population, suggests spill-overs of infection from dogs. We can therefore, concluded that the state's dog population may serve as the reservoirs of rabies and source of infections in other species and human population of the state (Ahmed 2000; Aghahowa and Ogbevoen 2010; Bata 2011).

Although the available data and the laboratory confirmed rabies cases cannot be said to be a complete and true representation of actual rabies scenario in the state, it still provides an approximate picture of the seasonality and demographic distribution of canine rabies across the LGA state. The under-reporting of rabies and its implications have been previously reported in Plateau state (Bata et al., 2011), and other states of the federation (Taiwo et al., 1998; Ahmed et al., 2000; Adedeji et al., 2010; Aghahowa and Ogbevoen, 2010; Bata, 2011). The concentration of the canine rabies cases on the Jos-Plateaux suggests that there could be variable(s) responsible for the observed pattern of canine rabies distribution in the state. Two factors that are suggested that could be responsible for this pattern: the first is the cooler environmental conditions on the Plateaux, which might explain the concentration of cases amongst LGA that are located on the Jos-Plateau (Bata 2011). Secondly, the location of the national veterinary research institute, Vom in the Jos South LGA; might be responsible for the increased number of reported cases in the surrounding LGA that are closer to Jos South on the Plateaux. There is need for future study to assess if there is/ are any role(s) that climate (together with any confounding modulations such as distance of LGAs from the NVRI or cultural practices of the people) play in the observed distribution of canine rabies across the state.

Moreover, the concentration of canine rabies in and around the state's capital - Jos (which comprise of Jos South, Jos East and Jos North) suggests that urban rabies could be a major problem in the state; or as implied by Adedeji (2010), it may be because urban populations are more likely to report cases.

Based on the available data for this study, there is poor responsibility and practice of vaccinating dogs against rabies amongst dog owners. The low rate of anti-rabies vaccination of domestic dogs in the state is consistent with the findings of Bata (2011) and Abubakar and Bakari (2012). If this situation is allowed to continue without any urgent mitigation plan to address issues responsible for low vaccination practice among dog owners, rabies will become a significant threat to the welfare of the general public of the state (Adedeji et al., 2010; Bata et al., 2011). The unpublished data from a participatory rural appraisal study carried out in some of the LGA of the state by Bolajoko and others in 2011; revealed that
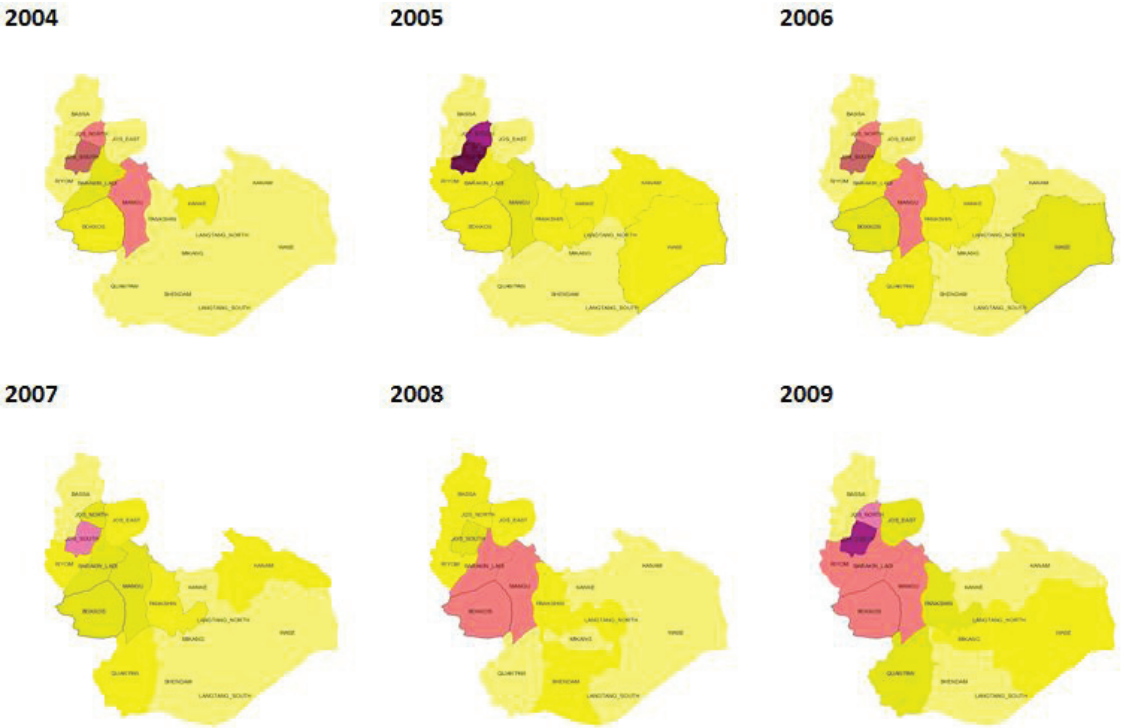
Figure 3: Choropleth disease maps showing geographical distribution of laboratory-confirmed canine rabies in the seventeen (17) local government areas (LGA) of Plateau state, Nigeria, 2004 to 2009
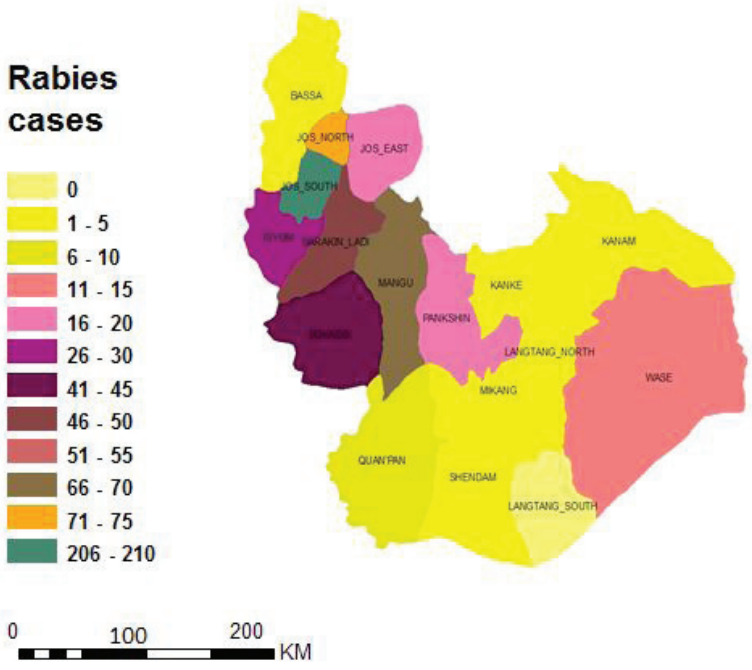

Figure 4: Choropleth maps showing geographical distribution of total laboratory-confirmed canine rabies in LGA of Plateau state, Nigeria in the six-year study period, from 2004-2009

the low rate of vaccination is largely due to the fact that money is being charged for anti-rabies vaccination of dogs in the state. This is a view also shared by Lembo (2010), the money charged makes it difficult to afford for most of the low-income and rural populace that form the majority of dog owners in the state.

Conclusion

The findings of this study reiterate the results of previous studies that canine rabies is well established in Plateau state. This may be attributed to variables of factors that include poor vaccination coverage of owned dogs, high population of stray dogs and/or low confinement of domestic dogs and lack of revision and enforcement of regulations/ laws for impoundment and elimination of stray dogs. Conclusively, even though, the available data from the CDL record for this study is an underestimation of the true canine rabies scenario in the state; there is every indication of a continued persistence of canine rabies in the state.

Recommendations for sustainable and cost-effective anti-canine rabies campaign in Plateau state-Nigeria

Considerations of the situations outlined in this study have led to the following suggestions:.

- First and foremost, the awareness of the health burden and socioeconomic impacts of canine rabies and dog-bite-injuries to both animals and humans must be raised among policy-makers and the general public.
- A census of the state's domestic dog population must be determined as this will provide informed objective(s) for sustainable canine rabies control.
- Canine rabies control via dog vaccination campaign will be better sustained and successful if a one health approach is adopted. (Lembo *et al.,* 2010).
- Adequate infrastructures such as well-organised surveillance networks and diagnostic capabilities canine- and human-rabies surveillance must be put in place.

This is very important for monitoring and evaluation of any vaccination campaign.

Conflicting interests

The authors declare that they have no conflicting interests.

Authors' contributions

MB; conceived the study, collated, analysed, interpreted the data, and prepared the draft manuscript. JF; supervised and guided the analysis, interpretation of data and helped to draft the manuscript. MS, PA, M, PR; conceived the study, provided technical and administrative support and helped to draft the manuscript. All authors have read and approved the submitted version of the manuscript.

Acknowledgements

Impact

Rabies, a neglected tropical disease, is one of the most fatal diseases. Around 55,000 people die from rabies annually with over 99% of these deaths occurring in Africa and Asia.

This study evaluates and quantifies the prevalence and demographic distribution of canine rabies across Plateau state, Nigeria. Outcome of this study reveals canine rabies as a major public health problem in the state. Furthermore we were able to identify possible factors responsible for the observed prevalence level of the disease. They include poor vaccination coverage of owned dogs, high population of stray dogs and/or low confinement of domestic dogs and lack of revision and enforcement of laws for

impoundment and elimination of stray dogs.

These problems were considered; which led to the suggestions of the following recommendations: First and foremost, the awareness of the health burden and socioeconomic impacts of canine rabies and dog-bite-injuries to both animals and humans must be raised among policy-makers and the general public. A census of the state's domestic dog population must be determined as this will provide informed objective(s) for sustainable canine rabies control. Canine rabies control via dog vaccination campaign will be better sustained and successful if a one health approach is adopted. Adequate infrastructures such as well-organised surveillance networks and diagnostic capabilities canine- and human-rabies surveillance must be put in place. This is very important for monitoring and evaluation of any vaccination campaign.

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139

EMERGENCE OF NEW DELHI METALO-β- LACTAMASE (NDM-1) - PRODUCING MULTIDRUG RESISTANT GRAM NEGATIVE BACTERIA FROM POULTRY IN NIGERIA.

Ogunleye A O[1]*Ajuwape A T P[1] and Carlson S A[2]
[1]Department of Veterinary Microbiology and Parasitology, University of Ibadan, Nigeria.
[2]Department of Biomedical Sciences, Iowa State University of Science and Technology, Ames, USA.

Abstract:

The New Delhi metallo-beta- lactamase (NDM-1) gene is an emerging well acknowledged public health threats among human and animal pathogens worldwide. Since its first description in 2009, a lot of animal and human associated reports have been documented around the world, including some parts of Africa. There is however a dearth of information on it in Nigeria, particularly from food animals.

The current work therefore screened 55 Gram negative bacteria isolated from cloaca swabs of both healthy and sick birds from commercial poultry houses in Nsukka, Enugu State, Nigeria: that were multi drug resistant and particularly resistant to 3 of or all of, cefepime, ceftazidime, ceftriaxone and amikacin for the presence of bla NDM-1 gene in a PCR assay. The Gram negative bacteria were .identified by conventional bacteriological procedures and with16s RNA PCR method. In all, 7.3% (4/55) of the pathogens including; 2 Proteus mirabilis, 1 Pseudomonas aeruginosa and 1 Enterobacter cloacae were positive for the gene.

The result shows that poultry could be a possible source of spreading the public health important gene to other animal and human pathogen in Nigeria, thus constituting a serious public health hazard in terms of treatment failures in animal and human, should there be transmission of the gene. A nationwide surveillance is advocated for the purpose of instituting a well scientific informed preventive measures.

L'ÉMERGENCE DU GÈNE MÉTALLO-BÊTA- LACTAMASE (MDN-1) DE NEW DELHI – PRODUCTEUR CHEZ LA VOLAILLE AU NIGERIA DE LA BACTÉRIE « GRAM NÉGATIF » RÉSISTANT À PLUSIEURS MÉDICAMENTS.

Résumé

Le gène métallo-bêta- lactamase (MDN-1) de New Delhi est une menace de santé publique émergente reconnu parmi les agents pathogènes humains et les animaux dans le monde entier. Depuis sa première description en 2009, plusieurs cas humains et animaux ont été documentés dans le monde entier, y compris certaines régions d'Afrique. Il y a cependant un manque d'information sur la menace au Nigeria, en particulier les animaux destinés à l'alimentation. Les travaux en cours ont par conséquent sélectionné 55 bactéries Gram négatives isolées à partir des prélèvements de cloaque des oiseaux sains et malades des maisons commerciales de volailles à Nsukka, l'État d'Enugu au Nigeria qui étaient résistants à plusieurs médicaments et particulièrement résistants à 3 ou tous ces médicaments le céfépime, le ceftazidime, l'amikacine ceftriaxone pour la présence du gène bla MDN-1 dans un essai PCR. Les bactéries à Gram négatif étaient identifiées par des procédures bactériologiques classiques et avec la méthode 16s RNA PCR. Au total, 7,3% (4/55) des agents pathogènes, y compris 2 Proteus mirabilis, 1 Pseudomonas aeruginosa et Enterobacter cloacae 1 étaient positifs. Le résultat montre que la volaille pourrait être une source potentielle de propagation de gènes importants de santé publique à d'autres pathogènes animaux et humains au Nigeria, constituant ainsi un risque grave de santé publique en termes de défaillances de traitement chez l'animal et l'humain, lors de la transmission du gène. Une surveillance nationale est préconisée dans le but d'instaurer une des mesures préventives éclairées et scientifiques.

Corresponding author e-mail: peculiarj@yahoo.com, ao.ogunleye@mail.ui.edu.ng.

Introduction:

Carbapenems are very important group of antibiotics, because they are most of the times regarded as last-resort antibiotic option for treatments of infections caused by multidrug-resistant Enterobacteriaceae (Nordmann *et al.,* 2012). However, for more than a decade running, there has been public health concern due to reports of emergence of isolates capable of hydrolyzing carbapenems along with most beta-lactam antibiotics, thereby making them ineffective as preferred treatment option when need arose(Nordmann *et al.,* 2012). There are 3 types of- lactamases that inactivate the carbapenems: the KPC types, the metallo-beta-lactamases (MBLs), and the oxacillinases (Livermore *et al.,* 2006). The 2 most common types of MBL found in Enterobacteriaceae are the VIM and IMP types (Cornaglia *et al.,* 2007). By way of traditional classification, carbapenemases have been assigned to Ambler classes A, B and D, however, KPC, VIM, IMP type and OXA-48 enzymes particularly exhibit worldwide dissemination and prevalence (Nordmann *et al.,* 2012; Patel *et al.,* 2013). In 2009, a novel class B metallo-beta-lactamase (MBL), called the New Delhi metallo-beta-lactamase-1 (NDM-1), was described, from a Klebsiella. pneumoniae and Escherichia coli isolated from a Swedish patient who had earlier received medical care in India (Yong *et al.,* 2009; Kumarasamy *et al.,* 2010). New Delhi metallo-beta-lactamase (NDM-1) Among 55 Gram negative bacteria isolated from cloaca swab, aztreonam and is usually expressed in multidrug or pandrug-resistant isolates (Kumarasamy *et al.,* 2010). Up to nine minor variants of NDM-1 (NDM-2 to -10) have been identified (http: //www.lahey. org/studies/).

The gene is commonly found among Enterobacteriaceae species and some other Gram-negatives bacteria like Vibrio cholerae, Pseudomonas species and Acinetobacter baumannii (Kumarasamy *et al.,* 2010; Darley *et al.,* 2012; Flateau *et al.,* 2012; Decousser *et al.,* 2013). Although, chromosomal location has been identified for it in certain NDM bearing bacteria isolates, nevertheless, the NDM carbapenemase has been primarily linked to multiple separate acquisition events, mediated by plasmids of various sizes belonging to palette of incompatibility groups like: IncF, IncA/C, IncL/M, IncH, IncN and IncX3(Kumarasamy *et al.,* 2010; Hishinuma *et al.,* 2013; Villa *et al.,* 2012; Poirel *et al.,* 2011; Sonnevend *et al.,* 2013; Johnson *et al.,* 2013). Bacteria carrying it is therefore of great public health threat in terms of transfer of resistance factors to other bacteria isolates both of human and animal origin.

Following its initial report, it was subsequently recognized as an emerging mechanism of resistance in multiple species of Enterobacteriaceae in the United Kingdom(Patel *et al.,* 2013; Yong et al., 2009; Health Protection Agency, 2009; Walsh and Toleman, 2012; Nordmann *et al.,* 2011), India and Pakistan (Nordmann , e*t al.,* 2011), across Europe (Struelens et al., 2010); United States (Anonymous *et al.,* 2010);Netherlands(Leverstein-Van Hall *et al.,* 2010); Australia (Poirel *et al.,* 2010); Canada, France and the Sultanate of Oman(Poirel e*t al.,* 2010).

In the continent of Africa, four metallo-beta-lactamases (NDM, VIM, IMP and DIM) had been reported among Enterobacteriaceae and NDM or VIM variants had been reported in at least one country from each African regions (Manenzhe et al., 2014). The IMP had been identified in Morocco, Tunisia and Tanzania (Chouchani *et al.,* 2011; Barguigua e*t al.,* 2012; Barguigua *et al.,* 2013; Mushi *et al.,* 2014), and DIM-1 in Sierra Leone (Leski *et al.,* 2013). Most of the VIM-producing Enterobacteriaceae from these Africa region were recovered from adult patients hospitalized in intensive care units or surgery wards(Barguigua *et al.,* 2013; Abdelaziz *et al.,* 2013; Dimude *et al.,* 2013; Ktari *et al.,*2006; Poirel *et al.,*2013; Peirano *et al.,* 2012).

Likewise, VIM-4-producing K. pneumoniae isolates were recovered from an outbreak in a Tunisian University hospital (Ktari et al., 2006).

NDM-1 producing Klebsiella pneumoniae had also been reported in Kenya and Morocco (Poirel *et al.,* 2011; Poirel e*t al.,* 2011). Also from Mulago, Uganda, there was a

report of both phenotypic/ genotypic evidence of cabapenmase producing enterobacteria, and the most prevalent gene from the enterobacteria was blaVIM (21, 10.7%) followed by bla OXA-48(19, 9.7%), bla IMP(12, 6.1%), bla KPC(16, 5.1%) and blaNDM- (5, 2.6%) (Okoche *et al.,* 2015).

In Nigeria, there has been a report of phenotypical evidence of clinical enterobacteria isolates from two hospitals in the Northern zone of Nigeria to cabapenase and metalo beta lactamase from modified Hodges test and EDTA Disc synergy test (Yusuf *et al.,* 2012).Also in Lagos State South West Nigeria, 2 strains of Klebsiella species isolated from hospital and community subjects that were susceptible to imipenem were MBL producer (Enwuru *et al.,* 2011). More recently, also in Lagos State, Nigeria, Raji and co researchers reported a high prevalence of enterobacteriaceae isolates carrying bla CTX-M-15 in a tertiary teaching hospital (Raji *et al.,* 2015) Likewise, a multidrug resistant Acinetobacter baumanni was reported as OXA-23 carbapenemase producer in Nigeria(Olaitan *et al.,* 2013).

To the best of our knowledge, there has not been any report on Gram negative bacteria bearing NDM-1gene from multidrug resistant isolates from poultry in Nigeria. In the current work, we therefore used a universal primers to screen for the presence of plasmid borne NDM-1 gene among 54 Gram negative bacteria isolated from cloaca swabs of both healthy and sick birds from commercial poultry houses from Nsukka, Enugu State, Nigeria in a PCR assay. The isolates were selected based on their multidrug resistant status, particularly to cefepime, ceftazidime, ceftriaxone and amikacin.

Materials and Methods

Bacteria isolates:

The bacteria screened for the presence of NDM 1 genes included 55 Gram negative bacteria isolated from cloaca swabs of both healthy and sick birds from commercial poultry houses from Nsukka, Enugu State, Nigeria according to standard bacteriological procedures (Barrow and Feltham, 2004; Garcia and Isenberg, 2007). Their identities were

further confirmed with Oxoid Microbact GNB 24E® (MB24E) and accompanying computer software package (Oxoid Microbact®) 2000 version 2.03 according to the manufacturers procedures and through 16S ribosomal RNA PCR identification procedure. They were screened for presence of NDM 1 gene based on their resistance to at least three of or all of the following antibiotics: ceftazidime, ceftriaxone, amikacin and cefepime at 32µg/mL breakpoint. The isolates comprised of 35 Pseudomonas aeruginosa, 19 Proteus mirabilis and 1 Enterobacter cloacae totalling 55 isolates.

16S RNA Identification of the Gram negative bacteria

The 16S ribosomal RNA identification of the 55 isolates were performed as previously described by Weisburg and his co-workers (Weisburg *et al.,* 1991) with some modification. Chromosomal DNA were produced from the 55 isolates by heating the LB broth cultures at 99^0C for 15minutes. A 100µl of the boiled isolates were mixed with equal volume of PCR grade water, 1 µl of the mixture was used as DNA template in a 50 µl reaction. The DNA was amplified using QS PCR reagents(New England Bio labs) containing 1µM of fD2= 5'AGATTTGATCATGGCTCAG3' and rP1 = 5'ACGGCTACCTTGTTACGACTT3', including 10 µl QS buffer, 1 µl dNTPs, 0.25 µl fD1, 0.25 µl rP1, 0.5 µl QS enzyme, 10 µl QS enhancer and 27 µl PCR water, using the PCR programme: initial denaturation step at 98^0C for 30 seconds, followed by 35 cycles of DNA denaturation at 98^0C for 10 seconds, primers annealing at 55^0C for 30seconds, primers extension at 72^0C for 1minutes 15seconds followed by final extension at 72^0C for 7minutes. The amplified products were resolved with precasted E- gel in an Electrophoresis unit (Life Technologies).

The amplified products were purified with Qiagen kits and sequenced at Iowa State University DNA sequencing facilities (Ames, IA, USA). The identities of the sequenced products were analysed by using BLASTN 2.2.31+ as described by Zhang and co-workers (Zhang *et al.,* 2000).

Determination of Resistance to ceftazidime, ceftriaxone, cefepime and amikacin

The isolates were grown aerobically in breakpoint concentrations of 32µg/mL each for ceftazidime, ceftriaxone, amikacin, and cefepime (all from SIGMA- ALDRICH) according to standard method (CLSI, 2009). Resistance was ascribed if flocculent growth was observed after 16h of aerobic growth at 37⁰C.

NDM- 1 gene screening

The 55 Gram negative bacteria shown in Table 1 were screened for the presence of bla NDM-1 by PCR as earlier described with some modifications [Chen *et al.,* 2011].All the isolates were screened with primers NDM- 1 F (5′ -ATGGAATTGCCCAATATTAT-3′) and NDM-1 R-(5′ -TCAGCGCAGCTTGTCGGCCA-3′). Chromosomal DNA were produced from the 55 isolates by heating the LB broth cultures at 99⁰C for 15minutes. A 100µl of the boiled isolates were mixed with equal volume of PCR grade water, 1 µl of the mixture was used as DNA template in a 50 µl reaction.The DNA was amplified using QS PCR reagents(New England Bio lab) containing 1µM of NDM- 1 F(5′-ATGGAATTGCCCAATATTAT-3′) and NDM-1 R-(5′ -TCAGCGCAGCTTGTCGGCCA-3′), including 10 µl QS buffer, 1 µl dNTPs, 0.25 µl NDM 1- F, 0.25 µl NDM-1 R, 0.5 µl QS enzyme, 10 µl QS enhancer and 27 µl PCR water, using the PCR protocol: 98⁰C for 30 seconds, 35 cycles of 98⁰C for 10seconds, 55⁰C for 30seconds, 72⁰C for 1minute 15seconds and 72⁰C for 7minute. The synthesized DNA manufactured with the Oligosequence shown in Figure 2 was used as positive control. The amplified products were resolved with precast E- gel in an Electrophoresis unit (Life Technologies).

Results:

Of all the 55 Gram negative bacteria screened for the presence of NDM-1 gene, 7.3% (4/55) including; 2 Proteus mirabilis, 1 Pseudomonas aeruginosa and 1 Enterobacter cloacae carried the gene (Table 2). Table 1 showed the resistance patterns of the Gram negative bacteria screened. They had 96.4% resistance (53/55) for each of the four antibiotics: ceftazidime, ceftriaxone, amikacin and cefepime.As shown in table 2 four isolates F77nlf, RO43, F1, F42nlf and as shown in Figures 1- as shown in figure 1 with lane M loaded with DNA ladder with lanes M loaded with DNA ladder, and lanes 34, 35,36 and 40 with positive as shown in figure 1 with lane M loaded with DNA ladder controlled synthesized GenscriptR, F 77nlf, RO 43 and F1 were respectively positive for NDM-1 screening with the expected band of about 814bp.

Discussion:

Since the first description of the NDM-1 producing pathogen in 2009 (Yong *et al.,* 2009, Kumarasamy *et al.,* 2010), its emergence has been one of the prominent carbapenemase producing enterobacteria in most parts of the world, and it is usually associated with serious public health concern(Nordmann *et al.,* 2011; Bushnell *et al.,* 2013; Johnson *et al.,* 2013). The fact that NDM-1 harbouring bacteria also carries resistance to virtually all other available classes of antimicrobials (pan resistance) including; fluoroquinolones, trimethoprim/sulfamethoxazole and aminoglycosides contributes in no measure to its public health concerns (Kumarasamy et al., 2010 ; Rogers et al.,2013).Since there are usually some challenges in getting an alternative effective treatment option, infection due to bacteria harbouring it can lead to death, as earlier reported for a case involving bloodstream infection in Greece (Voulgari *et al.,* 2014). Early detection and surveillance for it, is therefore imperative so as to identify any pathogen carrying it and to put in place stringent infection control to prevent its transmission.

From the current study, 7.3% (4/55) of the Gram negative bacteria of poultry origin from eastern part of Nigeria, screened for the presence of the plasmid borne NDM-1 gene were found positive.This finding signifies a very great public health concern because of the characteristics and tendencies of organism bearing it. For instance it has been documented that the gene encoding NDM-1 is located on

Table 1: Antibiotic sensitivity patterns of Gram negative bacteria from poultry to four antibiotics used as indicators for possible presence of NDM-1 gene.

Isolate	16s RNA identity	Source	ceftaz	Ceftria	amik	Cefep
E 319nlf	*P.aeruginosa*	poultry E	R	R	R	R
F4nlf	*P.aeruginosa*	poultry E	R	R	R	R
XI 19NLF	*P.aeruginosa*	poultry E	R	R	R	R
XI5nlf	*P.aeruginosa*	poultry E	R	R	R	R
D24nlf	*P.aeruginosa*	poultry E	R	R	R	R
E11nlf	*P.aeruginosa*	poultry E	R	R	R	R
E312	*P.aeruginosa*	poultry E	R	R	R	R
E32nlf	*P.aeruginosa*	poultry E	R	R	R	R
F51nlf	*P.aeruginosa*	poultry E	R	R	R	R
E210	*P.aeruginosa*	poultry E	R	R	R	R
RO16	*P.aeruginosa*	poultry E	R	R	R	R
F30nlf	*P.aeruginosa*	poultry E	R	R	R	R
RO 37	*P.aeruginosa*	poultry E	R	R	R	R
E31	*P.aeruginosa*	poultry E	R	R	R	R
KO 49	*P.aeruginosa*	poultry E	S	S	S	R
F12	*P.aeruginosa*	poultry E	R	R	R	R
RO28nlf	*P.aeruginosa*	poultry E	R	R	R	R
RO17	*P.aeruginosa*	poultry E	R	R	R	R
RO 8	*P.aeruginosa*	poultry E	R	R	R	R
`F36nlf	*P.aeruginosa*	poultry E	R	R	R	R
O17	*P.aeruginosa*	poultry E	R	R	R	R
E310	*P.aeruginosa*	poultry E	R	R	R	R
XIII7	*P.aeruginosa*	poultry E	R	R	R	R
O12	*P.aeruginosa*	poultry E	R	R	R	R
RO 42	*P.aeruginosa*	poultry E	R	R	R	R
F44	*P.aeruginosa*	poultry E	R	R	R	R
F49	*P.aeruginosa*	poultry E	R	R	R	R
XII6nlf	*P.aeruginosa*	poultry E	R	R	R	R
RO 50nlf	*P.aeruginosa*	poultry E	R	R	R	R
F45nlf	*P.aeruginosa*	poultry E	R	R	R	R
F 16	*P.aeruginosa*	poultry E	R	R	R	R
XI10nlf	*P.aeruginosa*	poultry E	R	R	R	R
XIII 1nlf	*P.aeruginosa*	poultry E	R	R	R	R
RO48	*P.aeruginosa*	poultry E	R	R	R	R
XIII 8	*P.aeruginosa*	poultry E	R	R	R	R
RO 43	Ent cloacae	Poultry E	R	R	R	R
H24nlf	Proteus mirabilis	Poultry E	R	R	R	R

Isolate	16s RNA identity	Source	ceftaz	Ceftria	amik	Cefep
E313	Proteus mirabilis	Poultry E	R	R	R	R
XII 11	Proteus mirabilis	Poultry E	R	R	R	R
F42nlf	Proteus mirabilis	Poultry E	R	R	R	R
XII7nlf	Proteus mirabilis	Poultry E	R	R	R	R
FI3nlf	Proteus mirabilis	Poultry E	R	R	R	R
F2	Proteus mirabilis	Poultry E	R	R	R	R
RO39	Proteus mirabilis	Poultry E	R	R	R	R
RO 23	Proteus mirabilis	Poultry E	R	R	R	R
RO 22	Proteus mirabilis	Poultry E	R	R	R	R
RO44 nlf	Proteus mirabilis	Poultry E	R	R	R	R
XI 4nlf	Proteus mirabilis	Poultry E	R	R	R	R
RO 21NLF	Proteus mirabilis	Poultry E	R	R	R	R
F17nlf	Proteus mirabilis	Poultry E	R	R	R	R
E39nlf	Proteus mirabilis	Poultry E	R	R	R	R
E316	Proteus mirabilis	Poultry E	R	R	R	R
E36	Proteus mirabilis	Poultry E	R	R	R	R
F40nlf	Proteus mirabilis	Poultry E	R	R	R	S
O117LF	Proteus mirabilis	Poultry E	R	R	R	R

P. aeruginosa = Pseudomonas aeruginosa; Ent cloacae = Enterobacter cloacae; ceftaz = Ceftazidime; Ceftria = ceftriaxone; amik = amikacin; Cefep = cefepime; R = resistant; S = sensitive; poultry E = poultry from Eastern, Nigeria

Table 2: NDM-1 gene positive Gram negative bacteria from poultry

Isolate	16s RNA identity	Source	ceftaz	Ceftria	amik	Cefep
F77nlf	P mirab	Poultry E	R	R	R	R
RO 43	Ent cloacae	Poultry E	R	R	R	R
F1	P aerug	Poultry E	R	R	R	R
F 42nlf	P mirab	Poultry E	R	R	R	R

P mirab = Proteus mirabilis; Ent cloacae = Enterobacter cloacae; P aerug = Pseudomonas aeruginosa; ceftaz = Ceftazidime; Ceftria = ceftriaxone;amik = amikacin;   Cefep = cefepime; R = resistant; S = sensitive; poultry E = poultry from Eastern, Nigeria
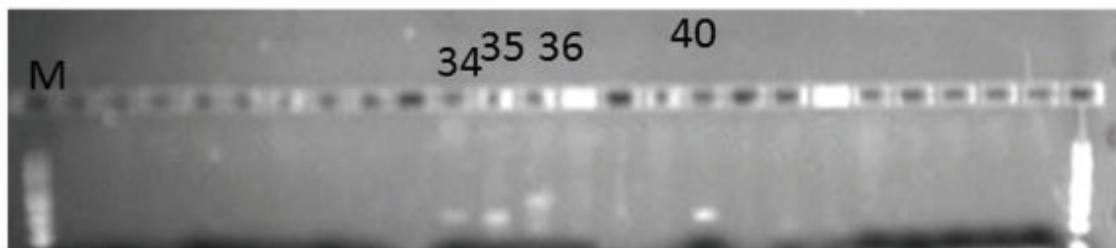
Figure 1: NDM-1 screening for Gram negative bacteria from poultry. Lower gel: lane M, DNA ladder; lane 34, GenscriptR NDM-1 positive control ; lane 35, F77nlf; lane 36, RO 43; lane 40, F1.

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ATGGAATTGCCCAATATTATGCACCCGGTCGCGAAGCTGAGCACCGCATTAGCCGCTGCATTGATG
CTGAGCGGGTGCATGCCCGGTGAAATCCGCCCGACGATTGGCCAGCAAATGGAAACTGGCGACCA
ACGGTTTGGCGATCTGGTTTTCCGCCAGCTCGCACCGAATGTCTGGCAGCACACTTCCTATCTCGA
CATGCCGGGTTTCGGGGCAGTCGCTTCCAACGGTTTGATCGTCAGGGATGGCGGCCGCGTGCTGGT
GGTCGATACCGCCTGGACCGATGACCAGACCGCCCAGATCCTCAACTGGATCAAGCAGGAGATCA
ACCTGCCGGTCGCGCTGGCGGTGGTGACTCACGCGCATCAGGACAAGATGGGCGGTATGGACGCG
CTGCATGCGGCGGGGATTGCGACTTATGCCAATGCGTTGTCGAACCAGCTTGCCCCGCAAGAGGG
GATGGTTGCGGCGCAACACAGCCTGACTTTCGCCGCCAATGGCTGGGTCGAACCAGCAACCGCGC
CCAACTTTGGCCCGCTCAAGGTATTTTACCCCGGCCCCGGCCACACCAGTGACAATATCACCGTTG
GGATCGACGGCACCGACATCGCTTTTGGTGGCTGCCTGATCAAGGACAGCAAGGCCAAGTCGCTC
GGCAATCTCGGTGATGCCCGACACTGAGCACTACGCCGCGTCAGCGCGCGCGTTTGGTGCGGCGTTC
CCCAAGGCCAGCATGATCGTGATGAGCCATTCCGCCCCCGATAGCCGCGCCGCAATCACTCATAC
GGCCCGCATGGCCGACAAGCTGCGCTGA
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Figure 2: Oligosequence of the positive control by Genscript[R]

a large 180kb resistance – conferring genetic element: which can easily be transferred to other enterobacteriaceae; contained alongside other resistant determinants such as gene encoding resistance to other broad spectrum beta- lactamases (CMY-4); for gene inactivating erythromycin; ciprofloxacin; rifampicin and chloramphenicol (Robert and Moellering, 2010). Apart from these pan-resistance genetic tendencies, the genetic element can also encode an efflux pump that can further activate additional antimicrobial resistance and promotes transcription of genes present in the genetic elements (Yong *et al.,* 2009).

Based on the aforementioned characteristics of NDM-1 bearing pathogen, its presence in Gram negative bacteria from poultry in Nigeria, carries the public health implication of its possible spread to humans, since poultry and poultry products contributes largely to the source of animal protein in Nigeria. They are potential source of transferring the drug resistant traits to other human and animal pathogens. If this happens, it will come along with grave implications in terms of jeopardizing choice of effective antibiotics treatment and possible treatment failures. A national surveillance for this antibiotic resistant determinant trait among food animal is thus imperative, so as to institute a well-informed control measures regarding its possible spread in food animals and human.

Iowa State University of Science and Technology, Ames, Iowa State, USA.

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PERFORMANCE AND BLOOD INDICES OF GROWING RABBITS FED DIETS CONTAINING SHRIMP WASTE MEAL AS PARTIAL SUBSTITUTES FOR SOYBEAN MEAL.

Okorodudu A[1,2], Oluwatosin O O[1,2], Fafiolu A O[1,2], Obadire F O[3], Njoku C P[1], Togunde O M[1].

[1]World Bank Africa Centre of Excellence in Agricultural Development and Sustainable Environment
[2]College of Animal Science and Livestock Production, Federal University of Agriculture, PMB 2240 Abeokuta, Ogun State, Nigeria.
[3]Department of Animal Science, Federal University Jigawa, PMB 7156 Duste, Jigawa State.

Abstract

A total of 96 growing rabbits were used in an experiment to determine the performance and blood indices of rabbits fed shrimp waste meal (SWM) as partial substitutes for soybean meal (SBM). Rabbits were allotted on weight equalization basis to 4 treatment groups having 4 replicates of 6 rabbits each. Four iso-caloric (2240kcal/Kg) and iso-proteinous (15% CP) diets (NRC, 1977) were formulated at 0g/kg (0%), 66.3g/kg (33%), 132.7g/kg (66%) and 201.1g/kg (100%) SWM inclusion levels replacing SBM. Feed and water were offered ad-libitum during the 8 weeks feeding trial. Data on performance were taken and at the 8th week, blood samples collected were assayed for haematology and serum metabolites. Data analysis was done using ANOVA in a Completely Randomized Design. Weight gain and feed conversion ratio indicated that rabbits on 132.7g SWM/kg diet performed better than the control and other substitution levels with those on 201.1g SWM/kg diet having the lowest performance indices. Feed cost per kilogram diet reduced with increasing levels of SWM; also feed cost per unit weight gain. Rabbits fed 132.7g SWM/kg had higher (cubic) mean values for total protein, albumin and globulin and lower ($p<0.05$) values for urea and creatinine than those on the other diets. There was no significant difference on all haematological indices assayed attributable to the SWM inclusion. It was concluded that SWM can be used as partial substitutes for soybean meal in the diets of growing rabbits up to 66% (132.7g/kg) without adversely affecting their performance, haematology and key serum metabolites.

Keywords: Shrimp waste meal; Soybean; Rabbits; Performance; Blood.

PERFORMANCE ET PROFIL SANGUINS DE LAPINS NOURRIS À DES RÉGIMES ALIMENTAIRES CONTENANT DE LA FARINE DE DÉCHETS DE CREVETTES EN SUBSTITUTION PARTIELLE DU TOURTEAU DE SOJA.

Résumé

96 lapins en croissance ont été utilisés dans une expérience pour déterminer les performances et le profil sanguin des lapins nourris de déchets de farine de crevettes (DFC) comme des substituts partiels de tourteau de soja (TS). Les lapins étaient attribués sur la base de poids égal à 4 groupes de traitement comportant 4 répétitions de 6 lapins chacun. Quatre régimes iso-caloriques (2240kcal / Kg) et iso-protéique (15% CP) (NRC, 1977) ont été formulés à 0g / kg (0%), 66.3g / kg (33%), 132.7g / kg (66 %) et 201.1g / kg (100%) des niveaux d'inclusion DFC remplaçant le TS. La nourriture et l'eau étaient offertes à volonté pendant les 8 semaines d'essai d'alimentation. Les données sur la performance étaient prises et à la 8ème semaine, les échantillons de sang prélevés étaient analysés pour l'hématologie et les sérums métabolites. Le gain de poids et le taux de conversion des aliments indiquaient que les lapins soumis au régime de 132.7g / kg DFC donnaient de meilleurs résultats que le contrôle et les autres niveaux de substitution avec ceux de 201.1g DFC / kg d'aliments ayant les indices de performance les plus bas. Le coût du régime des aliments par kilogramme réduisait avec l'augmentation des niveaux de DFC ; ainsi que le coût d'alimentation par unité de gain de poids. Les lapins nourris avec 132.7g DFC / kg avaient des valeurs moyennes plus élevées (cubes) en protéines totales, d'albumine et de globuline et (p <0,05) des valeurs plus faibles pour l'urée et de la créatinine que celles des autres régimes. Il n'y avait pas de différence significative sur tous les indices

Corresponding author: Okorodudu, A. +2348064273135: +2348074715873

hématologiques testés attribuable à l'inclusion de DFC. Il a été conclu que le DFC peut être utilisé comme substituts partiels pour la farine de soja dans l'alimentation des lapins en croissance jusqu'à 66% (132.7g / kg) sans affecter négativement leur performance, l'hématologie et les métabolites sériques clés.

Mots-clés : les déchets de farine de crevettes ; le tourteau de Soja ; les Lapins ; la performance ; le sang.

Introduction

High cost of conventional feed ingredients especially soybean meal which supply protein in livestock feed formulation which can be attributed to seasonal availability and competition with man has greatly affected the production of monogastric animals such as poultry and rabbits. Thus, there is the need for animal nutritionists to find alternative protein sources that are relatively cheap and not in competition with man. One of such alternative feed ingredients is sun-dried shrimp waste meal (SWM) which is basically the dried, milled waste from the shrimp processing industry consisting of the head, appendages and the exoskeleton of shrimps processed and packaged for both local and export markets (Fanimo *et al.,* 2004). SWM is highly palatable and of a pleasant aroma. It is particularly rich in lysine which makes it an ideal supplement to cereals; although the presence of chitin (found in the exoskeleton) which is a carbohydrate with a structure similar to cellulose has reduced its use in livestock (especially monogastric) feed formulation (Oduguwa *et al.,* 2004). Rabbits being pseudo-ruminants with a level of microbial degradative activity in their enlarged caecum may be able to utilize SWM. This study sought to determine the feeding value of shrimp waste meal and its attendant effects on performance characteristics, haematology, and some key serum metabolites of growing rabbits.

Material and Methods

Experimental site

This experiment was carried out at the Rabbit unit, Directorate of University Farms (DUFARMS), Federal University of Agriculture Abeokuta, Ogun State, Nigeria. The farm is geographically located on Latitude70, 10'Nand Longitude 30, 2^{0}E; it is 76m above sea level. Abeokuta lies within the tropical rainforest vegetation zone in South Western Nigeria, South of the Sahara with annual rainfall of 103mm, relative humidity of 82% and the temperature ranges from 26 -36^0 with a yearly average of 34^0 (Google Earth, 2014).

Shrimp Waste Meal

Fresh shrimp waste was sourced from a shrimp processing industry located in Lagos, South West Nigeria and was immediately sun-dried. Sun drying was done by spreading the shrimp waste thinly on a concrete slab for three consecutive days during the daytime. The length of drying period was about 8 hours per day. The resulting dried shrimp waste (≤ 10% moisture content) was ground to pass through 2mm sieve using a hammer mill.

Experimental diets

Four iso-caloric (2240kcal/Kg) and iso-proteinous (15% CP): (1, 2, 3 and 4) diets (NRC, 1977) were formulated at 0g/kg (0%), 66.3g/kg (33%), 132.7g/kg (66%) and 201.1g/kg (100%) SWM inclusion levels replacing SBM in that order. Other conventional feed ingredients such as maize, wheat offal, rice husk, fish meal, limestone, premix, bone meal and common salt were included in the formulation of the experimental diets (Table 1).

Experimental rabbits, design and management

The rabbits used for this experiment were averagely 8 weeks old, of no particular breed but from the same source and within weight range of 450g – 520g. A total of 96 growing rabbits were allotted based on weight equalization into 4 treatment groups having 4 replicates of 6 rabbits each.

The rabbits were housed, 2 per cell in two tier wire mesh hutches with 4 cells each large enough to give room for concrete feeders and drinkers, movement and exercise. They were offered respective experimental diets and

clean drinking water ad-libitum throughout the 8 weeks of the feeding trial.

Data collection

Performance

Weekly live weight was taken and recorded every Saturday morning before feeding. Feed intake was determined by subtracting leftovers from feed offered on a daily basis to get daily feed intake. Other performance characteristic such as the feed conversion ratio (FCR), protein intake and protein efficiency ratio (PER) were calculated based on the weight gain and feed intake. Cost of individual feed ingredients was used in calculating the cost of each diet and thus the cost per kilogram weight gained.

Blood

At the end of the feeding trail, 12 rabbits per treatment (3 per replicate) were randomly selected and bled before the morning feeding; blood was drawn into ethylene diamine tetra acetate(EDTA)bottles and another sample into plain bottles. The heparinized blood samples were centrifuged at 1006xg for 10 minutes and serum separated and stored at -10^0 until analysed.Measurements taken includepacked cell volume (PCV), haemoglobin (Hb), white blood cell (WBC), red blood cell (RBC), total protein, albumin, globulin, urea and creatinine. Haemoglobin concentration was measured in fresh EDTA anti-coagulant samples using the Sahl's (acid haematin) method.The PCV was determined usingWintrobe's microhaematocrit kit. RBC and WBC were determined using an improved Neubauer haemocytometer as described by Baker and Silverton (1985).

Total serum protein was determined using the Biuret method as described by Kohn and Allen (1995). Albumin was determined using Bromocresol Green (BCG) method. Serum creatinine (SC) was determined using the principle of Jaffe reaction as described by Bonsnes and Tausslay (1945) while the serum uric acid (SUA) was measured using the Kit (Quinica Clinica, Spain) as described by Wootton (1964).

Analytical techniques

Chemical

Test ingredient (SWM) and samples of experimental diets were analyzed for their proximate constituents using the methods of the AOAC (1990). Gross energy was determined using a Gallenkamp oxygen bomb calorimeter and Chitin extraction was done using the method described by ICES (2000). SWM sample was weighed and placed in a tared centrifuge tube and was successively treated with 1N HCL for 3 minutes in a bath of boiling water, then 4N NaOH for 20 minutes in the water bath; this was done to remove all organic components apart from chitin, between each step the sample was centrifuged and the supernant decanted. Residues of sample was rinsed into a crucible using distilled water and dried at 60°C for 24 hours. The pure chitin content was calculated as the mass difference between the dry mass the remains after incineration at 500°C for some hours.

Statistical Analysis

The experimental design was a Completely Randomized Design. Data were analyzed by analysis of variance using General Linear Models (GLM) procedures (SAS Institute, 1999). Duncan's Multiple Range Test was used to separate significant means ($p < 0.05$) with differences among treatments (Duncan, 1955).Polynomial analysis was done to determine linear, quadratic or cubic trends for SWM inclusion.

Results

Proximate analysis of shrimp waste meal on dry matter basis is as shown on Table 2. Percentage crude protein is 39.35%, crude fibre is 8. 58%, ether extract is 1.47, ash, 28.99, and 10% chitin.

Table 3 shows the performance characteristics of growing rabbits fed SWM as partial substitutes for soybean meal. Final weight of rabbits fed 201.1g SWM/kg(diet 4) was significantly ($p<0.05$) lower than those of rabbits fed diets 2 (66.3g/kg) and 3 (132.7g/ kg) which had similar mean values as those fed

Table 1: Gross composition of experimental diets (g/kg)

Ingredients	Shrimp waste meal substitution levels (%) for SBM			
	0	**33**	**66**	**100**
Maize	270.0	270.0	270.0	270.0
Soybean meal	160.0	84.0	42.0	0.0
Shrimp waste meal	0.0	66.3	132.7	201.1
Wheat offal	330.0	330.0	330.0	330.0
Rice husk	170.0	170.0	170.0	170.0
Fish meal	10.0	10.0	10.0	10.0
Limestone	25.0	25.0	25.0	25.0
*Trace mineral Premix	5.0	5.0	5.0	5.0
Bone meal	25.0	25.0	25.0	25.0
Salt	5.0	5.0	5.0	5.0
Total	1000	1000	1000	1000
Determined analyses (g/kg)				
Gross energy (kcal/kg)	2235.00	2232.73	2261.65	2269.97
Crude protein	200.60	133.80	155.40	155.50
Ether extract	18.00	19.30	17.90	23.00
Crude fibre	106.10	114.00	114.70	110.70
Ash	121.80	103.60	94.60	106.40
Dry matter	872.20	853.10	873.10	878.50

*Trace/mineral premix contains Vit. A- 4,000,000.00IU; Vit. D3- 800,000.00IU; Vit. E- 9,200.00mg; Vit. K- 800.00mg; Thiamin (B1)- 720mg; Riboflavin (B2)- 2,000.00mg; Pyrodixine (B2)- 1,200.00mg; Vit. B12- 6.00mg; Biotin- 24.00mg; Niacin- 11,000.00gm; Panthothenic acid- 300.00gm; Chlorine chloride- 120,000.00gm; Iron- 8,000.00gm; Manganese- 16,000.00gm; Copper- 1,200.00gm; Zinc- 12,000.00gm; Cobalt- 80.00mg; Iodine- 400.00gm; Selenium- 80.00gm; Antioxidants- 500.00mg. *Trace/mineral premix contains Vit. A- 4,000,000.00IU; Vit. D3- 800,000.00IU; Vit. E- 9,200.00mg; Vit. K- 800.00mg; Thiamin (B1)- 720mg; Riboflavin (B2)- 2,000.00mg; Pyrodixine (B2)- 1,200.00mg; Vit. B12- 6.00mg; Biotin- 24.00mg; Niacin- 11,000.00gm; Panthothenic acid- 300.00gm; Chlorine chloride- 120,000.00gm; Iron- 8,000.00gm; Manganese- 16,000.00gm; Copper- 1,200.00gm; Zinc- 12,000.00gm; Cobalt- 80.00mg; Iodine- 400.00gm; Selenium- 80.00gm; Antioxidants- 500.00mg.

Table 2: Proximate Analysis of Shrimp Waste Meal (% Dry matter)

Constituent	Percentage composition
Crude protein	39.35
Crude fibre	8.58
Ether extract	1.47
Ash	28.99
Nitrogen free extract	3.08
Chitin	10.00
Calcium	15.00
Phosphorus	0.95

Table 3: Performance Characteristics of Growing Rabbits Fed Diets containing SWM as Partial Substitutes for SBM

Measurements	Shrimp waste meal substitution levels (g/kg) for SBM								
	0%	33%	66%	100%	SEM	P-Value	L	Q	C
Initial weight (g)	516.67	483.33	466.67	483.33	12.50	0.6140	NS	NS	NS
Final weight (g)	1322.33[a]	1319.33[a]	1398.67[a]	997.33[b]	48.01	0.0001	*	**	**
Total weight (g)	805.67[ab]	769.00[b2]	923.67[a]	514.00[c]	48.01	0.0005	NS	*	***
Daily weight gain (g)	14.39[ab]	13.73[b]	16.49[a]	9.18[c]	0.86	0.0005	NS	*	***
Feed intake (g)	60.10[a]	55.09[b]	59.01[a]	51.83[c]	1.01	<0.0001	*	*	***
Cost/Kg feed (N)	60.45[a]	50.19[b]	44.52[c]	38.85[d]	2.41	<0.0001	***	***	***
Cost/weight gain (N)	48.70[a]	38.60[b]	41.12[b]	19.97[c]	3.30	<0.0001	***	**	***
Protein intake (g)	12.06[a]	7.37[d]	9.17[b]	8.06[c]	0.54	<0.0001	*	**	***
PER	1.19[b]	1.86[a]	1.80[a]	1.14[b]	0.11	0.0005	NS	***	**
FCR	4.19[b]	4.07[b]	3.58[b]	5.66[a]	0.25	0.0007	NS	**	**

[a,b,c,d] *Means on the same row with different superscript were significantly different (P<0.05): SEM = Standard Error of Means: NS = Not Significant: L: Q: C = Linear, Quadratic, Cubic.*

Table 4: Haematology and Serum Metabolites ofGrowing Rabbits Fed Diets containing SWM as Partial Substitutes for SBM

Measurements	Shrimp waste meal substitution levels (g/kg) for SBM								
	0%	33%	66%	100%	SEM	P-Value	L	Q	C
Initial weight (g)	516.67	483.33	466.67	483.33	12.50	0.6140	NS	NS	NS
Final weight (g)	1322.33[a]	1319.33[a]	1398.67[a]	997.33[b]	48.01	0.0001	*	**	**
Total weight (g)	805.67[ab]	769.00[b2]	923.67[a]	514.00[c]	48.01	0.0005	NS	*	***
Daily weight gain (g)	14.39[ab]	13.73[b]	16.49[a]	9.18[c]	0.86	0.0005	NS	*	***
Feed intake (g)	60.10[a]	55.09[b]	59.01[a]	51.83[c]	1.01	<0.0001	*	*	***
Cost/Kg feed (N)	60.45[a]	50.19[b]	44.52[c]	38.85[d]	2.41	<0.0001	***	***	***
Cost/weight gain (N)	48.70[a]	38.60[b]	41.12[b]	19.97[c]	3.30	<0.0001	***	**	***
Protein intake (g)	12.06[a]	7.37[d]	9.17[b]	8.06[c]	0.54	<0.0001	*	**	***
PER	1.19[b]	1.86[a]	1.80[a]	1.14[b]	0.11	0.0005	NS	***	**
FCR	4.19[b]	4.07[b]	3.58[b]	5.66[a]	0.25	0.0007	NS	**	**

[a,b,c] *Means on the same row with different superscript were significantly different (P<0.05): SEM = Standard Error of Means: NS = Not Significant: L: Q: C = Linear, Quadratic, Cubic*

the control diet. Total weight gain was higher (cubic) in rabbits fed 132.7g/kg (diet 3)and lowest in those fed 201.1g/kg (diet 4), daily weight gain followed the same trend as total weight gain. Rabbits on the control diet and diet 3 had similar feed intake values significantly higher than those on the other diets. Cost per kilogram feed was highest in the control diet and reduced with increasing inclusion levels of shrimp waste meal in the diets. Rabbits fed control diet had significantly highest cost per kilogram weight gained, while those fed 201.1g SWM/kg (diet 4) had the lowest cost per kilogram weight gain. Protein intake by rabbits fed control diet was significantly higher than those fed SWM diets with those on diet 4 having the lowest protein intake. Mean values for protein efficiency ratio for rabbits on diets 2 and 3 were higher (quadratic) than those on diet 1 and 2. Feed conversion ratio (FCR) was worst (quadratic and linear) in rabbits fed diet 4 compared to those on the other diets with rabbits fed diet 3 having the best FCR.

Haematology and serum metabolites of growing rabbits fed diets containing SWM as partial substitutes for SBM is as shown on Table 4. No significant difference was observed in all the measurements assayed for haematology attributable to the inclusion of SWM in the diets of growing rabbits, while all measurements determined for serum biochemistry were significantly (p<0.05) affected by the inclusion of SWM in the diets of growing rabbits. Total protein was highest (cubic) in rabbits fed diet 3 and lowest in those fed diet 2 with those fed the diets 1 and 4 having statistically similar values in-between. Albumin content ranged between 32.55g/L in rabbits fed diet 4 to 48.25g/L in those fed diet 3. Rabbits fed diets 3 and 4 had significantly higher mean values for globulin than those fed diets 1 and 2. Those fed the control diet had higher mean values for urea, followed by those on diets 2 and 3, while those on diet 4 had the lowest mean values. Creatinine value was higher (quadratic and linear) in rabbits fed the control diet as compared to those fed the other experimental diets.

Discussion

Proximate composition of SWM

The crude protein of SWM evaluated in this experiment is in agreement with 39.4% reported by Fanimo et al.(2000) but lower than 50.89% reported by Rosenfeld et al. (1997). According to Oduguwa et al. (2005), these differences may be attributed to a number of factors including variation in composition of the waste, processing methods and storage conditions. The authors specifically noted that sun-drying; the commonest processing method for SWM in tropical countries is quite slow and this allows for compositional changes that led to reduced quantity and quality of protein in the final product, they opined that prompt processing of SWM preferably in a tunnel dryer at a low heat (≤80^{0}C) will give best results (Oduguwa *et al.,* 2004). Fanimo et al. (2000; 2004), reported CF in SWM as 12.3% which is higher than the value obtained in this study. The high ash content is an indication that SWM is very rich in minerals and are comparable to those in other animal protein sources like fish meal.

Performance and cost analysis

Partial substitution of SBM with SWM in the diets of growing rabbits significantly (p<0.05) influenced all the performance indices measured. Rabbits fed diet 3 (132.7g SWM/Kg) had significantly higher weight gain even though they had similar values for total feed intake as those fed the control diet which had significantly higher mean values for protein intake but lower protein efficiency ratio. This translated to a better feed conversion ratio for rabbits on diet 3 and thus higher mean value for weight gain than other treatments. Those fed diet 4 where SWM completely replaced SBM, generally performed significantly lower than those on other treatments. This could be attributed to the reduced voluntary feed intake which partly may be due to bulkiness, reduction in nutrient digestion and absorption as a result of an increase in the proportion of chitin in the diets. Chitin has been reported to be nearly indigestible (Gohl, 1975) and thus its low utilization (Austin, *et al.,* 1981).

Cost per kilogram feed was highest for the control diet and reduced with increasing inclusion levels of SWM in the diet because SWM is cheaper than SBM; cost per kilogram weight gain followed the same trend. Thus, inclusion of SWM up to 66% replacing SBM in the diet of growing rabbits reduced the cost of production and improved weight gain.

Blood metabolites

Dietary contents influenced the blood profile of rabbits (Etim *et al.,* 2014). Esonu et al. (2001) reported that haematological constituents reflect the physiological responsiveness of the animal to its internal and external environments which includes feed and feeding.

Haematological indices of growing rabbits fed SWM showed no significant difference for all measurements taken. Values obtained from this study are higher than those reported by Olabanji et al. (2007) but are within normal ranges reported by Medirabbit, (2007) as 33 to 50% for PCV, 3.8 – 7.9x106/

mm3for RBC and 9.40 – 17.90g/dl for Hb. Isaac et al. (2013) stated that packed cell volume is involved in the transport of oxygen and absorbed nutrients

The implication of the above is that all dietary treatments including SWM diets did not elicit detectable deleterious effects on the physiology of the experimental animals. Furthermore, haematological measurements have often times been associated with health indices and have diagnostic significance in routine clinical evaluation of the state of health of animals (Toghyani *et al.,* 2010). The fact that the PCV, RBC, Hb and WBC were similar for all animals on the experimental diets and within normal range gives a plausible reasoning that the animals were apparently in a stable health condition throughout the feeding trial. All indices assayed for serum metabolites were significantly influenced by SWM. Rabbits fed diet 3 had significantly ($p<0.05$) higher mean values for total protein, an indication that animals were able to digest and efficiently absorb the amino acids present in the diet compared to those on the other diets. Rabbits fed the control diet had significantly higher values for urea and creatinine, although within normal ranges. There was probably a complimentary effect when the SWM and SBM protein were fed that led to better protein utilization by the animals concerned. High serum urea levels have been shown to indicate possible impairment of protein utilization in an animals' body (Eggum, 1970). Total protein values were lower than 8.17 – 8.90g/100ml but those of albumin were within 3.93 – 4.27g/100ml while mean values for urea were lower compared to 56 – 58.33mg/100ml reported by Ahemen et al. (2013) in an experiment where rabbits were fed water spinach (Ipomoea aquatic) leaf meal.

It was concluded that feeding SWM at the level of 132.7 g/kg (66%) in practical diets for growing rabbits gave best results in terms of growth and feed utilization. Furthermore, there was no detectable adverse effects on the physiology and health of the rabbits as measured by selected haematological and serum metabolites.

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PERFORMANCE CHARACTERISTICS OF GROWING RABBITS FED DIET BASED ON A NON-CONVENTIONAL INGREDIENT

Ojebiyi, O.O., Onifade O.E. and Aboderin O.J.

Department of Animal Nutrition and Biotechnology, Ladoke Akintola University of Technology, P.M.B. 4000, Ogbomoso, Oyo State, Nigeria.

Abstract

A feeding trial using twenty four cross bred 8-9 weeks old rabbits was conducted to investigate the effect of feeding cerelac waste - CW (a by-product of the infant food industry considered as waste) on the performance and organ characteristics of growing rabbits. Three experimental diets were formulated with diet 1 serving as the control. Diets 2 and 3 had CW included at 25 and 50% respectively. The rabbits were randomly allocated into 3 treatment (after weight balancing) groups of 8 rabbits each and the groups were assigned randomly to the three diets with each rabbit serving as a replicate in a Complete Randomized Design experiment. The experiment lasted for 8 weeks. Dietary treatments had no significant (p>0.05) effect on the average daily gain, which was 11.3, 11.6, 11.3 g/d for the control, 25 and 50% inclusion respectively. In addition, the feed conversion rate was not significantly (p>0.05) affected by the inclusion of CW, which were 6.64, 6.24 and 6.45 for the control, 25 and 50% inclusion of CW respectively. However, the feed cost per kg as well as feed cost per kg weight gain decreased linearly with increasing level of CW. The relative organ weights showed no significant differences (p>0.05) across the dietary treatments. In conclusion, 50% CW can be included in growing rabbit diets without negative effect on performance.

Keywords: Cerelac waste, daily weight gain, growing rabbit, haematological parameters, relative organ weight.

LES CARACTERISTIQUES DE LA PERFORMANCE DES REGIMES ALIMENTAIRES A BASE D'UN INGREDIENT NON CONVENTIONNEL DES LAPINS EN CROISSANCE

Résumé

Un essai d'alimentation utilisant vingt-quatre descendances croisées de lapins âgés de 7-8 semaines a été réalisé pour étudier l'effet de l'alimentation des déchets de Cérélac - DC (un sous-produit de l'industrie des aliments pour nourrissons considéré comme des déchets) sur les caractéristiques de la performance et des organes de lapins en croissance. Trois régimes expérimentaux avaient été formulés avec le régime 1 servant de témoin. Les régimes 2 et 3 contenaient les DC à 25 et 50% respectivement. Les lapins avaient été répartis au hasard en 3 groupes de traitement (après le poids d'équilibrage) de 8 lapins chacun et les groupes étaient assignés au hasard à trois régimes avec chaque lapin servant de répétition dans un design expérimental complètement aléatoire. L'expérience a duré pendant 8 semaines. Les traitements alimentaires n'avaient aucun (p> 0,05) effet significatif sur le gain moyen quotidien qui était de 11,3, 11,6 et 11,3 g / j pour le contrôle, 25 et 50% d'inclusion respectivement. En outre, le taux de conversion des aliments pour animaux n'était pas significativement (p> 0,05) affecté par l'inclusion des DC, qui étaient de 6,64, 6,24 et 6,45 pour le témoin, respectivement de 25 et 50% d'inclusion de DC. Cependant, le coût d'alimentation par kg ainsi que le coût d'alimentation par kg de gain de poids diminuaient de façon linéaire avec le niveau croissant de DC. Les poids relatifs des organes n'avaient montré aucune différence significative (p> 0,05) à travers les traitements alimentaires. En conclusion, 50% de DC peuvent être inclus dans la croissance des régimes de lapin sans effet négatif sur la performance.

Mots-clés : les déchets de Cérélac, le gain de poids quotidien, le lapin en croissance, les paramètres hématologiques, le poids relatif des organes.

Corresponding author email: segunojebiyi@gmail.com

Introduction

Rabbit production is a veritable alternative in meeting the protein need of the growing population especially the developing countries. According to Adama (2008), the population of rabbits in Nigeria is estimated to be 1.7 million. Rabbit meat is a source of healthful food as it is low in cholesterol and good source of protein for coronary heart patients (Hernandez, 2004). One of the major constraints to commercial rabbit production is the shortage of cheap quality feed stuffs. This is because feed accounts for more than 70% of the total cost of production (Jiya *et al.*, 2013). Consequently profitability in rearing rabbit in Nigeria as in other developing countries requires a combination of varied but relatively available and cheaper resource input. Reduction in feeding cost which is the highest operational cost (Ojebiyi *et al.*, 2009) will go a long way in increasing the expected profit of a rabbit farmer.

According to FAO (2012), balance nutrition contributes to improving animal output as well as reducing cost of production and emission of greenhouse gases per unit of animal product. The global price of feed ingredients such as maize, wheat, fish meal and soybean meal has increased by 169, 118, 186 and 108% respectively in the last decade (Index Mundi, 2013). A key to sustainable productivity in livestock is the efficient use of available feed resource base through a quest for novel feed resources, particularly those not competing with human food (Wadhwa and Bakshi 2013). With the growing food industry, there are a lot of wastes which are not eaten directly by man but are great potential as animal feed ingredient. Although there has been extensive researches into the use of alternative non-conventional ingredients in rabbit diets, there is paucity of information on the possible use of cerelac waste in rabbit diets. Cerelac waste is a waste in the production process of infant food. The present study is designed to investigate the possibility of using cerelac waste in growing rabbit's diets.

Materials and Methods

Location:

The study was carried out at the Rabbitry unit of the Teaching and Research Farm, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria. Ogbomoso is in the derived savannah zone of Nigeria. The coordinates of the location has been described in Ojebiyi et al 2014.

Collection and Processing of Test Ingredient:

The cerelac waste (CW) was collected from an infant food manufacturing factory at Industrial Estate Agbara Ogun state, Nigeria. The semi.-solid waste was spread thinly on a clean water proof material and sun-dried while turning at intervals to prevent spoilage until about 12% moisture content was achieved. The dried material was then milled, and a sample was analyzed for chemical composition.

Preparation of experimental diets:

The dried CW was mixed with other ingredients to formulate three experimental diets as presented in Table 1.

Experimental Animals and Management

Twenty-four cross-bred rabbits of between 8-9 weeks old were used for the study. The rabbits were allowed seven days adjustment period on the control diet after which the rabbits were weight balanced such that the initial average ranged between 829-833 g and assigned into 3 groups of 8 rabbits per treatment with relative equal mean weights each serving as a replicate in complete randomized design experiment.

The rabbits were individually housed in metal cages measuring 45 × 35 × 45cm. The drinking and feeding troughs were made of earthen pot re-enforced with cement to prevent tipping over by rabbits. Under each cage were placed removable trays for easy cleaning; the feeders and drinking troughs also were removable types. A total of 100 g of feed divided into two rations of 50 g in the morning at 8:00 hour and 50 g in the evening at 16:00 hour were supplied to each rabbit per day. Orts were collected and weighed the following

morning in order to determine feed intake. Water was provided *ad libitum*.

The rabbits were weighed at the start of the experiment and thereafter they were weighed weekly to determine weight gain. Records of actual feed intake and weight changes were kept for further analyses. The experiment lasted for 8 weeks. At the end of the experiment, rabbits were starved overnight, stunned and bled for carcass analysis. The rabbit were immediately eviscerated and the internal organs were carefully dissected and weighed. The organ weights were expressed as percentage of the carcass weight while the dressed carcass was weighed and expressed as a percentage of the live weight.

Chemical Analysis

Representative samples of the test ingredient as well as the experimental diets were analyzed for their chemical constituents using the method of AOAC (2005).

Statistical Analysis

All data collected were subjected to analysis of variance (ANOVA) using the General Linear Model of SAS (2005). Means was compared using Duncan Multiple Range test of the same statistical package.

Results and Discussion

Table 2 shows the proximate composition of experimental diets and cerelac waste. The percentage crude protein (16.58%), crude fat (5.85%) as well as the crude fibre (3.71%) of CW are higher than that of conventional maize. This may be due to the primary ingredients (high quality maize + dehulled soybean)

The composition of the experimental diets indicated that the diets were balanced in nutrients recommended for growing rabbits (Cheeke, 1984) and falls within the range reported by Fielding (1991) for breeding does and Lebas *et al.,* (1986) for fattening rabbits.

The performance characteristics of rabbits fed diets containing cerelac waste is presented in Table 3. The values obtained for all parameters measured except feed:gain, feed cost/kg and feed cost/kg gain were not significantly (P>0.05) affected by dietary treatments.

Table 1: Gross Composition of the Experimental diets

Ingredients	Control 0% CW	Diet 2 25% CW	Diet 3 50% CW
Maize	35.00	26.25	17.50
Cerelac waste	0.00	8.75	17.50
Soybean meal	17.00	13.00	12.00
Palm kernel meal	6.00	6.00	5.00
Rice husk	23.00	23.00	21.00
Corn bran	5.00	9.00	13.00
Fixed ingredients	14.00	14.00	14.00
Total	100.00	100.00	100.00
Cost /kg (□)	67.03	62.09	60.66

¶*Fixed ingredients: fish meal (72%) 2.00kg, brewers spent grain 8.00kg, bone meal 3.00kg, lysine 0.25kg, methionine 0.25kg, premix 0.25kg, salt 0.5kg*

*Premix Composition per kg Diet: Vitamin A 10,000,000. iu Vitamin D3 2,000,000.iu, Vitamin E 20,000mg, Vitamin K33 2,000mg, Vitamin B, 3,000mg Vitamin 83 5,000mg, Niacin 45,000mg, Calcium pantothenate 10,000mg, Vitamin B6 , 4,000mg, Vitamin B12 20,000mg, choline chloride 300,000mg, Folk Acid 1000mg, Biotin 50mg, Manganese (Mn) 300,000mg, Iron (Fe) 120,000mg, Zinc (Zn) 80,000mg,opper (Cu) 8,500mg, iodine (I) 1,500mg, cobalt (Co) 300mg, Selenium (Se) 120mg, Anti-Oxidant 120,000mg.

One Naira is Equivalent to 315 Dollars

Table 2: Proximate Composition of the cerelac waste and experimental diets fed to growing rabbits

Parameters	Cerelac Waste inclusion (%)			Cerelac waste
	0	25	50	
Dry matter (%)	88.85	89.12	88.12	92.72
Crude protein (%)	16.07	16.08	16.65	16.58
Crude fibre (%)	11.14	10.68	10.32	3.71
Crude fat (%)	3.68	3.61	3.65	5.85
Ash (%)	7.13	7.39	7.71	3.71
NFE (%)	55.65	55.36	55.32	60.87

NFE = Nitrogen free extract = 100- (% crude protein + % crude fibre + % moisture + % crude fat + % ash).

Table 3: Growth Performance of Rabbit Fed Varying Levels of Cerelac Waste

Parameters	Cerelac waste inclusion (%)			SEM	P-Value
	0	25	50		
Initial weight(g)	829	833	832	17.0	0.12
Final weight(g)	1463	1485	1463	53.8	0.12
Daily weight gain (g)	11.3	11.6	11.3	0.80	0.08
Daily feed intake (g)	75.3	72.7	72.6	0.74	0.09
Feed:gain ratio	6.64[a]	6.24[b]	6.45[a]	0.57	0.02
Feed cost/kg (□)	67.0[a]	62.1[b]	60.7[c]	0.57	0.03
Feed cost/kg gain (□/kg)	445.1[a]	387.4[b]	391.1[b]	35.5	0.02

[abc] Means along the same row with different superscript are significantly different (P<0.05)

Table 4: Effect of varying levels of cerelac waste on carcass and organ characteristics of growing rabbits

Parameters	Cerelac waste inclusion (%)			SEM	P-Value
	0	25	50		
Live weight (g)	1463	1485	1463	57.1	1.00
Carcass weight (g)	833	844	855	53.3	0.06
Dressing percentage (%)	56.5	57.3	58.0	2.18	0.06
Relative organ weights (% of carcass weight)					
Kidney	1.13	1.13	1.10	0.13	0.18
Lungs	0.98	0.97	0.95	0.12	0.27
Heart	0.32	0.33	0.34	0.28	0.22
Spleen	0.21	0.25	0.22	0.26	0.12
Liver	4.34	4.38	4.31	0.49	0.06

The result of carcass and organ characteristics as affected by dietary treatment is presented in Table 4. The results obtained shows that there were no significant (P>0.05) differences in the carcass and organ characteristics of growing rabbits fed cerelac waste diets.

As the level of cerelac waste in the diets increased the feed cost/kg reduced. The highest cost/kg weight gain (□445.01) was recorded in the control diet. This was due to the higher cost of maize at the time of the study, compared with the lower cost of cerelac waste. Thus, the incorporation of cerelac waste in the diet of growing rabbits lowered the feed

cost and hence reduced the cost of production. This agree with Agunbiade et al. (2002) who reported savings in feed cost achieved as a result of using dry cassava peel in rabbit diets. However, this result contradicts the findings of Whyte and Wadak (2002) who observed increase in the cost per unit weight gain with increased level of sweet potato in poultry and rabbit diets.

The inclusion of cerelac waste did not have effect on relative organ weight. The results obtained in this study bear credence to the safety of cerelac waste as a potential ingredient for rabbit feeding.

Conclusion

Cerelac waste had beneficial effect in reducing the cost/kg of feed and of weight gain and did not affect performance negatively. It is concluded that cerelac waste can be included in the diet of growing rabbits at up to 50% inclusion level.

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RESPONSE OF RABBITS TO VARYING LEVELS OF CASSAVA AND LEUCAENA LEUCOCEPHALA LEAF MEAL DIETS

Fasae O A*, Oladeji B O, Onabekun, B A, Fasae O C and Odiakaose, N E

Department of Animal Production and Health, Federal University of Agriculture, P.M.B 2240, Abeokuta, Nigeria

Abstract

An experiment was carried out to determine the performance, haematology, carcass characteristics and sensory evaluation of meat from rabbits (n = 30) fed varying levels of cassava and Leucaena leaf meal diets. Rabbits were randomly allocated to five dietary treatments of 0, 10, 20, 30 and 40% CLM replaced with LLM for treatments 1 to 5, respectively. Results showed that rabbits fed dietary treatment 30%CLM and 10%LLM had the highest (p<0.05) feed intake (94.49±3.63g/day), body weight gain (12.65±0.62g/day), with those fed 40%LLM having the lowest values of 58.37g/day and 5.22±0.62g/day, respectively. Best (p<0.05) nutrient digestibility values were observed in 30%CLM and 10%LLM diets. Data on carcass characteristics shows significant (p<0.05) differences across dietary treatment. Dressing percentage ranged from 52.28% to 56.49% with rabbits fed 30% CLM having the highest (p<0.05). The sensory properties of the meat samples from loin of rabbits ranked the same (p>0.05) across dietary treatments. There were no significant differences (P<0.05) among groups for the haematological parameters, except for mean cell haemoglobin and mean cell haemoglobin concentration. It was therefore concluded that 30% of cassava and 10% Leucaena leucocephala leaf meals can be best incorporated in the diet of rabbits to achieved optimum production without any adverse effects on the animals.

Keywords: Rabbits, cassava, Leucaena, leaf meal, performance, haematology, carcass.

LA REACTION DES LAPINS A DES NIVEAUX DIFFERENTS DES REGIMES A LA FARINE DE FEUILLE DE MANIOC ET DE LEUCAENA LEUCOCEPHALA

Résumé

Une expérience a été effectuée pour déterminer les performances, l'hématologie, les caractéristiques de la carcasse et l'évaluation sensorielle de la viande de lapins (n = 30) alimentés à des niveaux différents de régime de farine de feuille de manioc et de Leucaena. Les lapins ont été répartis au hasard en cinq traitements alimentaires de 0, 10, 20, 30 et 40% de FFM remplacés par la FLL respectivement pour les traitements 1 à 5. Les résultats avaient montré que les lapins nourris au traitement diététique de 30% FFM et 10% de FLL avaient la ration alimentaire la plus élevé (p <0,05) (94.49 ± 3.63g / jour), le gain de poids corporel de (12,65 ± 0,62 g / jour), avec ceux nourris avec 40% de FLL ayant les valeurs les plus basses de 58.37g / jour et 5,22 ± 0,62 g / jour, respectivement. Les meilleures (p <0,05) valeurs de digestibilité des nutriments avaient été observées dans les régimes de 30% de FFM et 10% de FLL. Les données sur les caractéristiques de la carcasse montrent (p <0,05) des différences significatives tout au long du traitement. Le rendement de carcasse variait de 52,28% à 56,49%, avec des lapins nourris avec 30% de FFM ayant la plus forte (p <0,05). Les propriétés sensorielles des échantillons de viande de longe de lapins étaient les mêmes (p> 0,05) à travers les traitements diététiques. Il n'y avait pas de différence significative (P <0,05) entre les groupes pour les paramètres hématologiques, à l'exception de l'hémoglobine cellulaire moyenne et la concentration en hémoglobine cellulaire moyenne. Il a donc été conclu que 30% de farine de feuille de manioc et 10% de farine de Leucaena leucocephala peuvent être mieux intégrées dans l'alimentation des lapins à une production optimale obtenue sans aucun effet indésirable sur les animaux.

Mots clés : les lapins, la farine de feuilles, le manioc, la leucaena, la performance, l'hématologie, la carcasse

*Corresponding author email: *animalexp@yahoo.co.uk*

Introduction

Rabbits have potential as a meat producing animal in the tropics due to the characteristics such as small body size, short generation interval, rapid growth rate and ability to utilise forages or agricultural by-products. The increase in human population over the last decades has influenced the need towards uncovering the nutritional worth and exploitation of crop residues and agro-industrial waste, unsuitable for human consumption, which may be suitable for utilization in rabbit nutrition especially in Nigeria where raising of rabbit plays an increasingly important role for small farmers (Shaahu *et al.,* 2014). Rabbits can subsist on inexpensive diets based on forages under small-scale farm conditions (Ruiz-Feria 1998). Cheeke (1986) stated that rabbits can be sustained on diets comprised entirely of forages and agricultural by-products, which are the cheapest form of animal feed available, providing essential vitamin value for rabbit roughage thereby greatly economizing the amount of concentrate fed.

Cassava and Leucaena leucocephala forage have been widely used as a palatable fodder for livestock in the tropics (Onwudike, 1995, Fasae *et al.,* 2009). The leaf meals have been shown to serve as sources of proteins, vitamins, minerals as well as carotenoids for non-ruminants (Budi *et al.,* 1990; Okonkwo et al, 2010). Though, both leaves contains anti-nutritional factors that has been implicated to be toxic to livestock and could limit its usage in the raw state, they have been reported to be minimized through various processing methods such as sun-drying and fermentation (Caplice and Fitzgerald 1999; Adekojo *et al.,* 2014). This study therefore evaluates the effect of feeding varying levels of cassava and Leucaena leaf meal in the diets of rabbits on performance, haematology, carcass characteristics and meat sensory evaluation.

Materials and methods

Experimental site, animals and management

The experiment was carried out at the rabbitary unit of the Directorate of University Farms, Federal University of Agriculture, Abeokuta, Ogun state, Nigeria. Thirty (30) growing rabbits of 10 weeks old, sourced from a reputable farm were subjected to 5 dietary treatments of 6 replicates each made up of varying inclusion levels of cassava leaf meal (CLM) as replacement for Leucaena leucocephala leaf meal (LLM) at 0%, 10%, 20%, 30%, 40%, respectively. The rabbits were placed individually in separate cages and managed under these experimental conditions for a period of 14 weeks.

The leaves of Leucaena leucocephala and cassava were sourced from an established plot within the University and neighbouring communities, chopped into smaller sizes and air dried for 5 days before incorporation into the concentrate diet comprising of maize (30%), groundnut cake (11%), wheat offals (18%), with bone meal, salt, premix, lysine and methionine at 0.20%, respectively. The concentrate diets were fed to the rabbits at 0800 and 1600hours daily with fresh water given *ad libitum.*

Data collection

Data were on feed supply and refusal from each experimental animals to calculate the feed intake, computed as the difference between feed offered and feed refusal. A weighing balance calibrated in grams was used to measure the weight of the rabbits on weekly basis.

For the digestibility trials, three rabbits per replicate were transferred to a metabolism cage and fed the experimental diets *ad libitum* for five days to enable the rabbits get used to the environment, while faecal collection was carried out each day for another 7 days, oven dried at 600C, weighed and bulked for proximate analysis.

Haematological and biochemical analysis

Blood samples, about 4 ml per animal were collected from the experimental rabbits via ear vein puncture at the end of the feeding trial and analyzed for the packed cell volume, red blood cells and white blood cells. The red blood cells, white blood cells haemoglobin concentrations were measured using the Wintrobes Microhaematocrit,

improved Neubauerhaemocytometer and Cyanomethaemoglobin methods, respectively (Coles, 1986), while mean corpuscular haemoglobin levels were calculated according to Bush (1991). Similarly, serum biochemical constituents' namely albumin, globulin, total protein, glucose, cholesterol and blood urea were analyzed using commercially available analytical kits.

Carcass characteristics and sensory evaluation

Three rabbits each were selected randomly from each replicate group, starved for 12 hours so as to empty their gastro intestinal tract and to reduce the variability in body weight due to intestinal content. Prior to slaughtering the rabbits were weighed, stunned, singed and eviscerated as described (Omojola and Adesehinwa, 2006). The carcass weight, empty body weight, and dressing percentage was determined and recorded, using a sensitive electronic weighing scale. Individual weights was noted for each rabbit and then expressed as percentage of live weight. Determination of bled weight was by the difference between slaughter weight and hot carcass weight. Dressing percentage was determined by dividing the hot carcass weight by the live weight and multiplied by hundred.

In evaluating the sensory properties, samples of meat were cut from the loin of each of the carcass, coded in labelled polythene bags and cooked for 30 minutes in a water bath to a temperature of 650C. Ten panellists were trained and sequentially served bite size portions of the cooked meat to rate the meat samples on a 9-point hedonic scale for colour, flavour, tenderness, juiciness and overall acceptability (AMSA 1978). The meat pH was measured with pH meter.

Chemical Analysis

The dry matter, crude protein, crude fibre, ash, and ether extract contents of feed and faecal samples were analysed (AOAC, 1995).

Statistical analysis

Data generated was subjected to analysis of variance in a completely randomized design using (SAS, 2000) while significant differences was done using Duncan multiple range test (Duncan, 1955).

Results and discussion

The proximate composition of the experimental diets fed to rabbits is shown in Table 1. The results showed considerable amounts of crude protein (CP) and crude fibre (CF) in the CLM and LLM with LLM values having a higher CP compared CLM. The CP content of 23.05% reported for LLM is within the range of 20.26 - 24.9% earlier reported for LLM (Raharjo *et al.,* 1986; Safwat *et al.,* 2015), while CLM is lower than 23.0 – 24.68% reported by Adegbola and Okonkwo, (2002); Khang and Wiktorsson, (2005). The variation could be attributed to the age at harvesting as well as processing effects on leaf meal. The chemical compositions of the experimental diets showed that they were adequate to meet the nutrient requirements of the growing rabbits (Aduku, 2005).

The performance characteristics of rabbits fed varying levels of CLM with LLM are presented in Table 2. Results on feed intake shows a decrease (p<0.05) with an increase with the inclusion of LLM in the diets, which is similar to earlier observations in rabbits fed Leucaena diets (Awosanya, and Akinyode, 2000, Fayemi *et al.,* 2011,Adedeji *et al.,* 2013).The dry matter intake of the rabbits ranged from 58.31 to 94.49 g/day/rabbit with the highest (P<0.05) obtained in rabbits fed 30%CLM, which within the range of 40-90g/day/rabbit previously reported in rabbits fed cassava and Leucaena leaf diets (Iyeghe-Erakpotobor *et al.,* 2006, Olorunsanya *et al.,* 2007, Adekojo *et al.,* 2014).

Results on weight gain (g/day) showed a decreased (P<0.05) with an increase in the inclusion of LLM in the diets. Values varied (P<0.05) across dietary treatments ranging from 5.22 to 12.65g/day with rabbits fed 30%CLM having the best (P<0.05) weight gain (g/day) and least values observed in rabbits fed 40%LLM.The marked depression in weight gain of rabbits with higher LLM inclusion was similar to the finding of Makinde, *et al.,* (2015). However, values obtained in this study corroborate range

values of 4.99 to 16.1g/day observed in rabbits fed cassava by products and Leucaena based diets (Adejumo, 2006). Feed conversion ratio in rabbits across the dietary treatments ranged from 7.57 to 11.43 with the best observed in rabbits fed 30%CLM, suggesting its efficient utilization. The poor feed conversion ratio observed in higher levels of LLM inclusion could be attributed to poor nutrient utilization by the rabbits. Mtenga and Laswai, (1994) reported low growth rate and feed utilisation in rabbits fed on 30% of Leucaena leaf blended meal. Moreover, the reduced performance in rabbits fed higher concentration of LLM could be associated to the effects of anti-nutritional factors at these levels acting as an appetite depressant thereby resulting in poor palatability and consequent decrease in feed intake and in poor performance (Awosanya and Akinyode, 2000).

Table 3 shows the digestibility coefficient of diets containing varying levels of CLM and LLM fed to rabbits. The digestibility of nutrients differed (P < 0.05) across dietary treatments. DM and crude protein digestibility decreased (P < 0.05) with an increase in LLM inclusion in the diets. Budi et al, (1990) reported negative effects of anti-nutritional factor present at those levels.

The digestibility values for crude fibre were lower than other nutrients corroborating the reports of Leng, (2008) that rabbits are less efficient in digesting fibre compared to ruminants. Cheeke (1986) reported that fibre is poorly digested in rabbits because it is rapidly propelled through the colon and excreted as hard faeces. The decrease in crude fibre digestibility with increasing fibre in the diets has been previously reported (Adegbola and Okonkwo, 2002).

The results of the haematological and serum indices of rabbits fed the experimental diets are presented in Table 4. There were no significant differences (P>0.05) among groups for the haematological parameters with the exception of Mean cell haemoglobin and Mean cell haemoglobin concentration. However, all the parameters monitored were within normal range for rabbits (Mitruka and Rawnsley, 1977) which is an indication that the diets did not show any adverse effect on these parameters during the experiment.

Table 1: Proximate composition (%) of experimental diets fed to rabbits.

Parameters	0%CLM 40%LLM	10%CLM 30%LLM	20%CLM 20%LLM	30%CLM 10%LLM	40%CLM 0%LLM	CLM	LLM
Dry matter	88.99	87.10	87.96	75.16	86.00	80.34	89.55
Crude protein	23.21	23.02	22.57	22.16	21.66	19.68	23.05
Crude fibre	3.11	3.21	3.51	3.68	3.78	4.98	3.92
Ether extract	13.26	12.23	12.86	12.23	12.48	7.93	8.49
Ash	5.67	5.29	5.31	4.91	5.12	6.01	7.31

CLM: Cassava leaf meal, LLM: Leucaena leucocephala leaf meal

Table 2: Performance indices of rabbits fed varying levels of cassava and Leucaena leucocephala leaf meals.

Parameters	0%CLM 40%LLM	10%CLM 30%LLM	20%CLM 20%LLM	30%CLM 10%LLM	40%CLM 0%LLM	SEM
Initial weight(g)	790.23	780.44	790.30	796.06	770.87	19.03
Final weight (g)	1156.56[c]	1241.80[c]	1586.34[ab]	1682.78[a]	1499.66[b]	44.10
Weight gain(g/day)	5.22[c]	6.59[c]	10.09[b]	12.65[a]	10.41[b]	0.62
Feed intake(g/day)	58.37[c]	68.64[bc]	76.68[b]	94.49[a]	93.37[a]	3.63
Feed conversion ratio	11.43[a]	10.43[ab]	7.83[bc]	7.57[c]	9.06[abc]	0.46

[abc] Means on the same row with different superscripts were significantly different (P<0.05)
CLM: Cassava leaf meal, LLM: Leucaena leucocephala leaf meal.

Table 3: Nutrient digestibility (%) of cassava and Leucaena leucocephala leaf meal diets in rabbits

Parameters	0%CLM 40%LLM	10%CLM 30%LLM	20%CLM 20%LLM	30%CLM 10%LLM	40%CLM 0%LLM	SEM
Dry matter	60.36[c]	66.34[bc]	75.43[ab]	78.95[a]	75.58[ab]	2.31
Crude protein	57.94[d]	58.79[d]	75.57[b]	79.94[a]	68.17[c]	2.67
Crude fibre	60.74[c]	63.59[bc]	65.84[b]	70.96[a]	65.80[b]	1.04
Ether extract	54.39[c]	62.58[b]	65.86[a]	65.11[a]	56.18[bc]	1.56
Ash	62.57[c]	69.25[b]	63.04[c]	73.69[a]	70.21[ab]	1.25

[abc] *Means on the same row with different superscripts were significantly different (P<0.05)*
CLM: Cassava leaf meal, LLM: Leucaena leucocephala leaf meal.

Table 4: Haematological and serum indices of rabbits fed cassava and Leucaena leaf meal diets

Parameters	0%CLM 40%LLM	10%CLM 30%LLM	20%CLM 20%LLM	30%CLM 10%LLM	40%CLM 0%LLM	SEM
Haematological parameters						
Packed cell volume (%)	33.83	41.16	34.16	45.33	37.33	1.82
Haemoglobin (g/dl)	11.26	13.73	11.36	15.10	12.46	0.60
Red blood cell (x106/ul)	5.33	5.00	5.86	5.30	5.43	0.19
White blood cell (x103/ul)	0.85	0.63	1.36	1.08	0.78	0.10
Mean cell haemoglobin (g/dl)	2.03[b]	2.82a	1.67b	2.85a	2.36a	0.16
Mean cell haemoglobin conc. (g/dl)	33.16[b]	33.16[b]	33.17[b]	33.10[b]	34.38[a]	0.47
Serum parameters						
Total protein (g/dl)	4.56	5.10	4.60	5.40	5.26	0.12
Albumin (g/dl)	2.93[a]	2.86[a]	2.10[b]	3.14[a]	3.26[a]	0.12
Globulin (g/dl)	1.63[c]	2.24[b]	2.50[a]	2.26[b]	2.00[b]	0.09
Glucose (mg/dl)	69.70[b]	100.93[a]	108.56[a]	98.23[a]	97.36[a]	3.95
Cholesterol (mg/dl)	58.33[b]	55.66[b]	67.66[a]	56.00[b]	70.33[a]	1.91
Aspartate aminotransferase (u/l)	48.33	57.66	61.33	51.00	52.66	1.90
Alanine aminotransferase. (u/l)	31.00	31.33	31.66	36.33	29.00	1.19

[abc] *Means on the same row with different superscripts were significantly different (P<0.05)*
CLM: Cassava leaf meal, LLM: Leucaena leucocephala leaf meal.

Moreover, all parameters observed for serum biochemical parameters showed significant (P< 0.05) variation across treatment groups except for total protein, Aspartate aminotransferase and Alanine aminotransferase, however, they were all within the normal range for rabbits   (Jenkins, 1993). The values for albumin of 2.10g/dl-3.26g/dl obtained in this study indicates nutritional adequacy of the dietary proteins for rabbits. The low globulin values in rabbits fed 0% CLM and 40% LLM (1.63g/dl) compared to other treatments, is an indicative of low immunity and poor resistance to disease in these animals (Burke, 1994). The increase in glucose level observed in CLM and LLM supplemented diets may not pose any problem as they were within the normal range for healthy rabbits. Nevertheless, the elevated glucose levels in rabbits may be due to various stress factors such as stress at blood collection and housing conditions. (Jenkins, 2008).

The effect of dietary levels of CLM and LLM on the carcass characteristics of rabbits are presented on Table 5. All parameters measured were affected (p<0.05) by the dietary treatments. The results revealed that the live weight of rabbits after the feeding trial increased (p<0.05) with increasing level

of CLM in the diets, which subsequently had effect on the bled weight, hot carcass weight, and dressed weight with rabbits fed diet with 30%CLM inclusion having highest (p<0.05) values. This supports the reports of Apata *et al.,* (1999) who recorded a similar result of higher increase in live weight, carcass yield, carcass weight with increasing cassava waste inclusion level in rabbit's diet. The dressing percentages ranged from 52.28% to 56.49% across treatments and are similar to the ranges of 50.7 to 59.5%, earlier reported for rabbits (Ani, 2006, Sobayo *et al.,* 2008). However, retail cuts like the shoulder, loin, rack and back were improved (p<0.05) in rabbits fed higher levels of CLM diets.

Organ weights differed (p<0.05) across the dietary treatments. The relative weight of GIT decreased (p<0.05) with inclusion of CLM in the diet. The significant (P<0.05) effects observed on the weight of the lung, liver and kidney of rabbits fed higher levels of LLM agrees with the report of Fayemi et al, (2011) that observed abnormalities in the liver of rabbits fed Leucaena leucocephala leaves after post-mortem examinations, which may be as a result of the possible effects of the anti-nutritional factors in LLM on the organ parts. Ahamefule et al, (2006) confirmed that the weight of some internal organs like kidney and liver may be used in animal feeding experiments as evidence of toxicity.

Table 5: Carcass characteristics of rabbits fed varying levels of cassava and Leucaena leucocephala leaf meal diets

Parameters	0%CLM 40%LLM	10%CLM 30%LLM	20%CLM 20%LLM	30%CLM 10%LLM	40%CLM 0%LLM	SEM
Live weight (g)	1150.00[d]	1230.00[c]	1580.00[b]	1610.00[a]	1460.00[b]	44.99
Bled weight (g)	1079.50[d]	1211.00[c]	1522.00[b]	1577.00[a]	1413.00[b]	47.43
Hot carcass weight (g)	1140.00[d]	1237.00[c]	1460.00[b]	1617.50[a]	1472.00[b]	47.22
Dress weight (g)	589.50[b]	637.50[b]	856.00[ab]	909.00[a]	816.50[ab]	54.99
Dressing percentage (%)	51.26[b]	51.83[b]	54.18[ab]	56.49[a]	55.92[a]	2.87
Retail-cuts (% of live weight)						
Shoulder	14.89[c]	16.31[ab]	15.98[b]	17.13[a]	16.16[ab]	0.23
Rack	18.11[b]	17.06[b]	18.09[b]	20.99[a]	21.50[a]	0.56
Leg	1.80[b]	1.80[b]	1.92[a]	1.73[c]	1.60[d]	0.03
Loin	7.57[c]	7.87[c]	13.44[b]	14.50[a]	14.20[a]	0.84
Back	12.17[c]	14.93[b]	17.46[a]	16.67[a]	16.45[a]	0.52
Head	9.17[a]	8.63[ab]	7.82[b]	7.85[b]	7.35[b]	0.33
Neck	0.80[c]	1.00[bc]	1.54[a]	1.40[ab]	1.28[bc]	0.11
Organ parts						
Gastro intestinal tract	23.21[a]	20.09[b]	19.47[b]	18.67[b]	18.01[b]	1.18
Liver	1.69[a]	1.64[a]	1.54[b]	1.55[b]	1.49[c]	0.03
Heart	0.20[ab]	0.18[ab]	0.16[b]	0.18[ab]	0.22[a]	0.01
Lung	0.80[a]	0.66[b]	0.85[a]	0.55[c]	0.42[d]	0.04
Kidney	0.76[a]	0.77[a]	0.54[b]	0.53[b]	0.32[c]	0.04
Spleen	0.08[a]	0.07[a]	0.06[a]	0.03[b]	0.03[b]	0.01

[abc] *Means on the same row with different superscripts were significantly different (P<0.05)*
CLM: Cassava leaf meal, LLM: Leucaena leucocephala leaf meal.

Table 6: Sensory evaluation of meat from loin of rabbits fed Leucaena leucocephala and cassava leaf meal diets.

Parameters	0%CLM 40%LLM	10%CLM 30%LLM	20%CLM 20%LLM	30%CLM 10%LLM	40%CLM 0%LLM	SEM
Flavour	6.30	6.25	6.03	6.13	6.15	0.09
Colour	6.30	6.70	6.20	6.52	6.48	0.09
Tenderness	6.35	6.52	6.55	7.20	6.63	0.13
Juiciness	6.10	6.02	6.57	6.30	6.45	0.10
Overall acceptability	6.23	6.55	6.50	6.88	6.95	0.15
pH	7.05	7.00	7.05	7.10	6.95	0.03

Mean values in the same row with the same superscripts are not significantly (P>0.05) different.
LLM - Leucaena leucocephala leaf meal, CLM - Cassava leaf meal

The mean scores for the sensory evaluation of meat from rabbits fed the experimental diets are presented in Table 6. The sensory quality attributes shows that the rabbit meat is well acceptable (p>0.05) to the consumers irrespective of the dietary inclusion of CLM and LLM as all the palatability criteria ranked the same (p>0.05) and was not adjudged below average by the taste panel.

Conclusion

This study showed a good performance of rabbits fed on combination of cassava and Leucaena leucocephala leaf meal concentrate. However, Leucaena leucocephala and cassava leaf meals can best be incorporated at 10 and 30%, respectively in the diets of rabbits for efficient utilization and optimum performance without any adverse effect.

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ANTIBIOTIC RESISTANCE OF *STAPHYLOCOCCUS AUREUS* ISOLATED FROM FRESH COW MILK IN SETTLED FULANI HERDS IN KADUNA STATE, NIGERIA

Umaru G A[1,2]*, Kwaga J K P[2], Bello M[2], Raji M A[3] and Maitala Y S[2]

[1]*Department of Animal Health, College of Agriculture, P.M.B.1025, Jalingo, Taraba State, Nigeria,
[2]Faculty of Veterinary Medicine, Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria, Nigeria.
[3]Faculty of Veterinary Medicine, Department of Veterinary Microbiology, Ahmadu Bello University, Zaria, Nigeria.

Abstract

Three Hundred and Sixty fresh cow milk samples were collected from settled Fulani herds in Kaduna State and examined for *S. aureus* and their antibiotic resistance. Fifty five samples (15.3%) were positive for *S. aureus*. The occurrence of *S. aureus* was statistically significant (P<0.005) based on locations. Statistical analysis showed that all the *S. aureus* (n=55) were coagulase and DNase positive while 40.0% (n=22) produced alpha haemolysin, 45.5% (n=25) produced beta haemolysin and 14.5% (n=8) produced gamma haemolysin. The resistance pattern of the 55 *S. aureus* isolates showed that all the isolates were resistant to two or more antibiotics. Multidrug resistance was detected in 96.4% of the isolates. All the *S. aureus* were resistant to penicillin 55 (100.0%) rates of resistance to ampicillin (90.9%; n=50), tetracycline (81.8%; n=45) and erythromycin (75.5%; n=41). High frequencies of resistance were recorded against vancomycin (61.8%; n=34), nalidixic acid (40.0%; n=22), streptomycin (32.7%; n=18), oxacillin and cefoxitin (29.1%; n=16), trimethoprim (27.3%; 15) and gentamicin (23.7%; n=13). Chi square test showed that significant (P<0.05) number were susceptible to ciprofloxacin (92.7%; n=51), gentamicin (70.9%; n=39), oxacillin (70.9%; n=39), chloramphenicol (65.5%; n=36) and streptomycin (67.7%; n=37). The MIC results for oxacillin and vancomycin showed values > 256 µg for all resistant strains showed no zone of inhibition along the entire length and MIC was read as greater than the highest value on the strip (256). The multiple drug resistance indices revealed that 96.4% of the *S. aureus* strains were resistant to 3 or more antibiotics tested. The study demonstrated that cow milk in the study areas are contaminated with resistant strains of *S. aureus* of animal and human biotypes and can serve as means of spread to humans through the consumption of raw milk and milk products. This can be eliminated through improving the general herd hygiene, proper management practices and proper milking hygiene.

Key words: antibiotic resistance, Staphylococcus aureus, cow milk, settled Fulani herds, MIC, Haemolysin, Kaduna State

LA RESISTANCE AUX ANTIBIOTIQUES DU *STAPHYLOCOCCUS AUREUS* ISOLES DU LAIT FRAIS DE VACHE DANS LES TROUPEAUX PEULS INSTALLES DANS L'ETAT DE KADUNA AU NIGERIA

Résumé

Trois cent soixante échantillons de lait de vache frais ont été prélevés dans des troupeaux peuls installés dans l'Etat de Kaduna et examinés pour les *S. aureus* et leur résistance aux antibiotiques. Cinquante-cinq échantillons (15,3%) étaient positifs de *S. aureus*. L'apparition de *S. aureus* était statistiquement significative (P <0,005) sur la base des emplacements. L'analyse statistique a montré que tous les *S. aureus* (n = 55) étaient à coagulase et DNase positifs, tandis que 40,0% (n = 22) avait produit l'alpha hémolysine, 45,5% (n = 25) avait produit la bêta hémolysine et 14,5% (n = 8) avait produit l'hémolysine gamma. Le profil de résistance des 55 isolats de *S. aureus* a montré que tous les isolats étaient résistants à deux ou plusieurs antibiotiques. La résistance à plusieurs médicaments avait été détectée dans 96,4% des isolats. Toutes les *S. aureus* étaient résistantes à la pénicilline 55 (100,0%), aux taux de résistance à l'ampicilline (90,9% ; n = 50), à la tétracycline (81,8% ; n = 45) et de l'érythromycine (75,5% ; n = 41). Des fréquences élevées de résistance ont été enregistrées à l'encontre de la vancomycine (61,8% ; n = 34), l'acide nalidixique (40,0% ;

n = 22), la streptomycine (32,7% ; n = 18), l'oxacilline et la céfoxitine (29,1% ; n = 16), triméthoprime (27,3% ; 15) et de la gentamicine (23,7% ; n = 13). Le test du « khi carré » a montré qu'un nombre significatif (P <0,05) était sensible à la ciprofloxacine (92,7% ; n = 51), la gentamicine (70,9% ; n = 39), l'oxacilline (70,9% ; n = 39), le chloramphénicol (65,5% ; n = 36) et la streptomycine (67,7% ; n = 37). Les résultats de CMI pour l'oxacilline et la vancomycine ont montré des valeurs> 256 ug pour toutes les souches résistantes, et a également montré qu'il n'y avait aucune zone d'inhibition sur toute la longueur et que le CMI était lu comme supérieure à la valeur la plus élevée sur la bande (256). Les multiples indices de résistance aux médicaments ont révélé que 96,4% des souches de *S. aureus* étaient résistantes à 3 ou plusieurs antibiotiques testés. L'étude avait démontré que le lait de vache dans les zones d'étude était contaminé par des souches résistantes de *S. aureus* de biotypes animales et humains et peut servir comme moyen de propagation à l'homme par la consommation de lait et de produits laitiers crus. Cela peut être éliminé par l'amélioration générale de l'hygiène du troupeau, des pratiques de gestion et une bonne hygiène de traite appropriée.

Mots clés : la résistance aux antibiotiques, le Staphylococcus aureus, le lait de vache, les troupeaux Peuls installés, MIC, l'hémolysine, l'État de Kaduna

Introduction

Livestock are raised throughout the sub-Saharan Africa and serve as the main sources of meat, milk, bye-products for the teeming industries, as draft power and income generation. One of such livestock is cattle with a population of 13 million (Junaidu *et al.,* 2011), and reared in almost all parts of Nigeria with highest concentration in the Northern parts in small and medium scale units and largely owned by the Fulani. The major limiting factor to cattle production is diseases which have a negative socio-economic impact such as poor growth, abortion, decreased milk yield and reduced income generation (Ameh *et al.,* 1999; Shittu *et al.,* 2012).

Milk is locally produced by the Fulanis and widely consumed in many African countries, including Nigeria (Akinyele *et al.,* 1999). The milk is sold to both rural and urban people as food and accounts for 16% of the total value of all food products produced from livestock in sub-Saharan Africa (F.A.O., 1986; Uzeh *et al.,* 2006). However, milk and its products are excellent medium for the growth of several micro-organisms, associated with several disease conditions notably staphylococcosis, salmonellosis, brucellosis, tuberculosis, shigellosis, cholera and host of others (Jawatz *et al.,* 1991; Fagundes *et al.,* 2010; Umaru *et al.,* 2012). Contamination of milk with *S. aureus* occurs usually during milking process which depends on the sanitary condition of the environment, equipment and personnel. Contamination can also occur by systemic infection of the udder through the teat canal (Kalsoom *et al.,* 2004; Daka *et al.,* 2012).

Staphylococcus aureus (*S. aureus*) is a major human pathogen causing a wide range of diseases, such as abscesses, osteomyelitis, necrotizing pneumonia, infective endocarditis, toxic shock syndrome (TSS), bacteraemia, septic arthritis, wound infections, pyogenic lesions, and sepsis (Alghaithy *et al.,* 2000; Cheesbrough, 2002). The organism is a major cause of nosocomial and community-acquired infections and diseases (Alghaithy *et al.,* 2000; Rong-Hwa *et al.,* 2010). In dairy cattle, *S. aureus* is frequently associated with abscesses, severe toxic shock syndrome and subclinical mastitis resulting in milk contamination (Kalsoom *et al.,* 2004; Fagundes *et al.,* 2010; Daka *et al.,* 2012).

S. aureus is one of the bacteria that readily acquires resistance to new drugs and therefore poses threat to medical and veterinary professions (Daka *et al.,* 2012). Many strains of *S. aureus* carry a wide variety of multi-drug resistant genes on plasmids, chromosomes, transposons or on gene cassettes that are incorporated into integrons (Daka *et al.,* 2012). Staphylococcal resistance to penicillin is mediated by penicillinase (a form of β-lactamase) production: an enzyme which breaks down the β-lactam ring of the penicillin molecule. Resistance to methicillin is mediated via the mec operon, part of the staphylococcal cassette chromosome mec

(SCCmec). Resistance is conferred by the mec A gene, which codes for an altered penicillin-binding protein (PBP2a or PBP2') that has a lower affinity for binding β-lactams (penicillins, cephalosporins and carbapenems).

S. aureus remains one of the major pathogens of public health concern globally, and ranks the third most important pathogen in the world among the reported food borne pathogens (Souza *et al.,* 2010; Principato *et al.,* 2014). This is due to production of staphylococcal enterotoxins (SEs) that cause food poisoning if food containing the toxin is ingested (Laba and Udonsek, 2013). The symptoms of staphylococcal food poisoning are mainly abdominal cramps, nausea, vomiting, sometimes followed by diarrhea (never diarrhea alone).

Although, the occurrence of *S. aureus* including antibiotic resistant strains in raw milk, fermented milk and other milk products have been studied and documented across the globe including Nigeria (Kalsoom *et al.,* 2004; Nnadi, 2006; Fagundes *et al.,* 2010; Daka *et al.,* 2012; Laba and Udonsek, 2013; Maduka *et al.,* 2013; Maduka *et al.,* 2014), information is still needed on the current situation in raw cow milk in Kaduna State, Nigeria. Assessing the occurrence of *S. aureus* and the antibiotic resistant patterns may provide data on the milking and entire herd hygiene, and therefore the safety of the milk. The aim of this study was to determine the prevalence of *S. aureus* in raw cow milk from settled Fulani herds in Kaduna State, Nigeria and their antibiotic resistant patterns.

Materials and Methods

Study area

This research was conducted in Kaduna State, Nigeria, which is located at the centre of Northern Nigeria with the coordinate's 10°31'N 7°26'E10.517°N 7.433°E. The state shares boundaries with Niger state to the west, Zamfara, Katsina and Kano states to the north, Bauchi and Plateau states to the east and FCT Abuja and Nasarawa states to the south. Kaduna State is located in the savanna zone, which is characterized by short wet season and long dry season. Agriculture is the main stay of the economy with about 80% of the people engaging in farming. Another major occupation of the people is animal rearing, namely cattle, sheep, goats and pigs (Kaduna State, 2010). The cattle population of the state as at 1990 was 1, 006, 634 with an annual estimated increase of 1.5%.

Study design and Sample collection

Cohort study was formed by identifying and selecting five (5) herds from each of the selected six Local Government Areas namely: Giwa, Kaduna South, Igabi, Lere, Sabon-Gari and Zaria. Each of the cohort herds was visited during milking time, where five (5) ml of milk was taken aseptically by cleaning the teat end using 70% alcohol moistened swabs and allowed to dry. The first streams (2-5 ml) of the milk samples were discarded. Twelve (12) milk samples were collected from each of five (5) cohort herds making a total of sixty (60) from each Local Government Area. Hence a total of 360 milk samples were collected during the study period.

All the samples collected were placed on ice and transported to the Bacterial Zoonoses Laboratory, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria for standard laboratory procedures.

Isolation of Staphylococcus aureus

One (1) ml of milk was suspended in 10 ml Tryptone Soya Broth (TSB, Oxoid) containing 6.5% sodium chloride and incubated for 24 hours at 37°C for selective enrichment of staphylococci. Enrichment cultures and milk samples was then streaked on to the surface of the Baird Parker medium supplemented with egg yolk and potassium tellurite which was prepared according to the manufacturer's instruction to obtain discrete colonies according to the standard protocol for testing of foods for *Staphylococcus aureus* (Tamagnini *et al.,* 2006). The plates were then incubated aerobically for 24 hours at 37°C, and finally observed for growth (Tamagnini *et al.,* 2006). Typical coagulase-positive *S. aureus* colonies will appear as black, grey or white and will be surrounded by an opaque halo of precipitation

which signifies the coagulase reaction (O' Brien *et al.,* 2009). All the isolates were then inoculated onto the nutrient agar slants and stored in a refrigerator at 4°C for further analysis and characterization.

All the *S. aureus* isolates were identified using colony morphology, gram staining, catalase, coagulase, motility, DNase, pigmentation and haemolysis tests (Normano, 2005; Tagmanini *et al.,* 2006) and confirmed further using Microbact™ Staphylococcal identification system 12S (Oxoid, Basingstoke, UK).

Antimicrobial Susceptibility Testing

All confirmed *S. aureus* were tested for resistance to a panel of 12 antibiotics using the disc diffusion method recommended by the Clinical and Laboratory Standards Institute (CLSI, 2011). The antibiotics and their concentrations (Oxoid Basingstoke, United Kingdom) were as follows: amoxicillin (30 µg), ampicillin (30 µg), cefoxitin (30 µg), chloramphenicol (12 µg), ciprofloxacin (5 µg), erythromycin (5 µg), gentamicin (10 µg), Nalidixic acid (30 µg), oxacillin (1 µg), penicillin (10 µg), tetracycline (30 µg), trimethoprim (5 µg), streptomycin () and vancomycin (30 µg). Three well-isolated colonies were selected from a nutrient agar plate culture, transferred to Brain Heart Infusion Broth (BHI) and then incubated at 350 C for 6 hours until the turbidity of the 0.5 McFarland standards was achieved (CLSI, 2011). A sterile cotton swab stick was dipped into inoculum suspension and swabbed several times on the dried surface of the Mueller-Hinton Agar (Ryan and Ray, 2004). The antibiotic discs were then applied using the disk dispenser (Oxoid Basingstoke, United Kingdom). The plates were allowed to dry and incubated at 35°C for 24 hours. Zones of inhibition were observed in some plates and the diameters measured to the nearest whole millimeter using a ruler. The sizes of the zones of inhibition were interpreted by comparing with the normal breakpoints (CLSI, 2011). *S. aureus* (ATCC 33591 strain) positive control was obtained from a known methicillin resistant Staphylococcus aureus.

Minimum Inhibitory Concentration (MIC) of the antibiotics

The minimum inhibitory concentration (MIC) values oxacillin and vancomycin to the isolates was determined by the standard broth micro dilution method (CLSI 2011) using a commercially available test (Oxoid Basingstoke, United Kingdom).

Multiple antibiotic resistance index (MARI)

The multiple resistance indexes were determined for each *S. aureus* isolates by dividing the number of antibiotics to which the isolate is resistant to by the total number of antibiotics tested (Olayinka and Olayinka, 2003).

Data Analysis

Data obtained from the study were analyzed statistically using Statistical Package for Social Sciences (SPSS) Version 16 software and presented in the form of frequencies, percentages and tables. Chi-square and Fisher's exact tests at 5% level of confidence were used for all comparisons and determination of significance. A P value < 0.05 was considered significant for all comparisons.

Results

Of the 360 cow milk samples examined, 55 (15.3%) were positive for *S. aureus*. The highest isolation of *S. aureus* was from Lere L.G.A. (26.7%) followed by Kaduna South (18.3%) while the least was from Giwa L.G.A. (5.0%) (P<0.005) (Table 1).

Statistical analysis showed that significant (P<0.05) number of the *S. aureus* 55 (100.0%) were coagulase positive using rabbit plasma, 22 (40.0%) produced alpha haemolysin, 25 (45.5%) produced beta haemolysin whereas 8 (14.5%) produced gamma haemolysin respectively (Table 2). The results also revealed that all (n= 55) of the *S. aureus* were DNase positive and fermented Mannitol whereas 32 (58.2%) showed pigmentation on Mannitol salt agar (Table 2).

Table 1: Prevalence of staphylococci in cow milk in settled Fulani herds, Kaduna State, Nigeria.

Location	No. of milk examined	No. (%) positive for S. aureus	No. (%) positive for CONS
Giwa	60	3 (5.0)	0 (0.0)
Kaduna South	60	11 (18.3)	2 (3.3)
Igabi	60	7 (11.7)	2 (3.3)
Lere	60	16 (26.7)	5 (6.3)
Sabon-Gari	60	10 (16.7)	1 (1.7)
Zaria	60	8 (13.3)	2 (3.3)
Total	**360**	**55 (15.3)**	**12 (3.3)**

Key: CONS= Coagulase negative staphylococci

Table 2: Haemolytic and biochemical characteristics of *S. aureus* isolates from cow milk in settled Fulani herds, Kaduna State, Nigeria (N=55).

Test	No. positive	% positive
Alpha haemolysis	22	40.0
Beta haemolysis	25	45.5
Coagulase	55	100.0
DNase	55	100.0
Gama haemolysis	8	14.5
Mannitol fermentation	55	100.0
Pigmentation	32	58.2

Table 3: Antibiotic susceptibility of *S. aureus* isolated from cow milk in Fulani herds, Kaduna State, Nigeria (N=55).

Antibiotic	No. (%) susceptible	No. (%) intermediate resistant	No. (%) resistant
Amoxicillin	8 (14.5)	0 (0.0)	47 (85.5)
Ampicillin	5 (9.1)	0 (0.0)	50 (90.9)
Cefoxitin	39 (70.9)	0 (0.0)	16 (29.1)
Chloramphenicol	36 (65.5)	7 (12.7)	12 (21.8)
Ciprofloxacin	51 (92.7)	2 (3.6)	2 (3.6)
Erythromycin	13 (23.6)	1 (1.8)	41 (75.5)
Gentamicin	39 (70.9)	3 (5.5)	13 (23.7)
Nalidicic acid	33 (60.0)	0 (0.0)	22 (40.0)
Oxacillin	39 (70.9)	0 (0.0)	16 (29.1)
Penicillin	0 (0.0)	0 (0.0)	55 (100.0)
Tetracycline	10 (18.2)	0 (0.0)	45 (81.8)
Trimethoprim	32 (58.2)	8 (14.5)	15 (27.3)
Streptomycin	37 (67.3)	0 (0.0)	18 (32.7)
Vancomycin	21 (38.2)	0 (0.0)	34 (61.8)

Table 4: Multiple antibiotic resistance indexes (MARI) of *S. aureus* isolated from cow milk in settled Fulani herds, Kaduna, State, Nigeria

MAR Index	No. of isolates	% isolates
0.2	2	3.6
0.3	1	1.8
0.4	14	25.5
0.5	13	23.6
0.6	19	34.5
0.7	4	7.3
0.8	1	1.8
0.9	1	1.8
Total	**55**	**100.0**
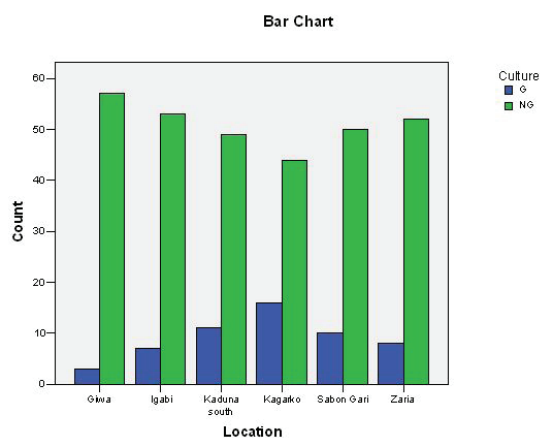
Figure 1: Occurrence of *S. aureus* according to the location

The resistance patterns of the 55 *S. aureus* isolates showed that all the isolates were resistant to two or more antibiotics (Table 3). Two isolates (3.6%) were each resistant/intermediate resistance to 2 antibiotics and one isolate (1.8%) was each resistant/intermediate resistance to 8 and 9 antibiotics. Multidrug resistance was detected in 96.4% of the *S. aureus* isolates. A number of the *S. aureus* isolates were resistant to commonly used antibiotics like penicillin 55 (100.0%), ampicillin 50 (90.9%), tetracycline 45 (81.8%) and erythromycin 41 (75.5%). High resistance rates were also recorded against vancomycin 34 (61.8%), nalidixic acid 22 (40.0%), streptomycin 18 (32.7%), oxacillin and cefoxitin 16 (29.1%), trimethoprim 15 (27.3%) and gentamicin 13 (23.7%) (Table 3). Chi square test showed that significant (P<0.05) number of the isolates were susceptible to ciprofloxacin 51 (92.7%), gentamicin 39 (70.9%), oxacillin 39 (70.9%), chloramphenicol 36 (65.5%) and streptomycin 37 (67.7%) Table 3). The MIC results showed no zone of inhibition along the entire length, the MIC was read as greater than the highest value on the strip (256).

The multiple drug resistance indexes of the *S. aureus* strains are shown in Table 4 and defined as resistant of an isolate to 3 or more antibiotics. The results revealed that 53 representing 96.4% of the *S. aureus* strains were resistant to 3 or more antibiotics tested, 4 strains were resistant to 7 antibiotics while 1 isolate each was resistant to 8 and 9 antibiotics respectively. Only 3 of the isolates were resistant to less than 4 antibiotics.

Discussion

Milk is a good medium for the growth and multiplication of several bacteria among which staphylococci ranks the first (Alien *et al.*, 2012). Contamination of milk by staphylococci is normally through infection of the mammary gland via teat canal, milkers, human handlers, milking equipment and from the environment (Alien *et al.*, 2012). The contamination and presence of staphylococci are of public health significance due largely to their role in food poisoning.

The presence of antibiotic resistant *S. aureus* in cow milk, milk products and other foods have been studied and documented in

Nigeria and elsewhere (Kalsoom *et al.,* 2004; Nnadi, 2006; Fagundes *et al.,* 2010; Daka *et al.,* 2012; Alien *et al.,* 2012; Umaru *et al.,* 2012; Laba and Udonsek, 2013; Maduka *et al.,* 2013; Maduka *et al.,* 2014). In this study, out of the 360 cow milk samples examined, 55 were positive for *S. aureus* giving a prevalence of 15.3% (Table 1). This is in line with previous studies in which 12.6% and 13.2% prevalence of *S. aureus* were respectively detected in milk samples in Zaria and Kaduna, Nigeria and in Iran (Umaru *et al.,* 2012; Alien *et al.,* 2012). However, the situation was different in Damaturu, Yobe State, where Nnadi (2006) reported a prevalence of 45% of *S. aureus* in milk samples. The results is also different from those reported in Sao Paulo, Brazil and Hawassa area, South Ethiopia, where Fagundes et al. (2010) and Daka et al. (2012) reported a prevalence of 6.7% and 48.8% of *S. aureus* in milk samples respectively. The differences could be largely due to differences in type of husbandry system, the breeds of the cattle, the sanitary conditions and the milking procedures. In the present study, the traditional hand milking was the method used in the entire herds sampled and where no hygienic measures such as teat dipping and disinfection of utensils, containers and personnel is practiced prior to milking procedures. In several cases, untreated ground water was used to wash the utensils, containers and hands of the milkers. This may probably have resulted in cross contamination and which contributed to the high occurrence of *S. aureus* in the milk samples. Therefore, improving the general herd hygiene, milking environment, containers and utensils can reduce the contamination of milk by *S. aureus* and possibly prevent its spread to other animals and human population.

In this study, it was observed that all the *S. aureus* isolates coagulated rabbit plasma and produced DNase, while significant number produced beta haemolysin (45.5%) and alpha haemolysin (40.0%) and relatively small percentage produced gamma haemolysin (14.5%). Such results show the potential of virulence and pathogenicity of these isolates and therefore of serious public health concern. It can be inferred from this study that most of the *S. aureus* isolates are of human biotypes,

since they are known to produce alpha haemolysin which are more toxigenic than the animal biotypes which are known to produce beta haemolysin and less toxigenic. It can also be concluded from this study that the contamination of the cow milk may have been from both human and animal sources. This contamination of milk by alpha and beta haemolytic *S. aureus* have been reported by Umoh (1989) in Fura-da-nono in Zaria, Nigeria and Oranusi et al. (2006) in food contact surfaces and foods prepared by families in Zaria, Nigeria.

The antibiotic resistance profiles of the 55 *S. aureus* isolates showed that all the isolates were resistant to two or more antibiotics. The highest resistance was recorded against penicillin (100.0%), ampicillin (90.9%), amoxicillin (85.5%), tetracycline (81.8%) and erythromycin (75.5%). This report is in line with our previous studies and those of others in which resistance to β-lactam antibiotics such penicillin, ampicillin, tetracycline and oxacillin were prevalent. (Umaru *et al.,* 2012; Alien *et al.,* 2012; Daka *et al.,* 2012). In our precious study involving fresh and fermented milk in Kaduna and Zaria, Nigeria, the highest rate of resistance was recorded against penicillin (100%), oxacillin (46.8%), and amoxicillin (44.7%) while the lowest was demonstrated against amikacin (2.1%), chloramphenicol (4.3%) and sulphamethoxazole/trimethoprim (6.4 %). Alien et al. (2012) showed in their study on bovine, sheep and goat raw milk in Iran that resistance to ampicillin was the most common (54.3%), followed by oxacillin (28.3%), tetracycline (26.1%), penicillin G (23.3%) and erythromycin (23.9%). Also Daka et al. (2012) in their study observed resistance in penicillin G (67.9%), ampicillin (70.9%), oxacillin (60.3%), amoxicillin (30.9%) and erythromycin (32.1%). The high rate of resistance to penicillin, ampicillin, amoxicillin and tetracycline in the present study may be due to abuse, misuse and mismanagement of these drugs because they are readily available and in common use in both human and veterinary medicine in the study areas. What is of great concern is the high number of the isolates resistant to erythromycin (75.5%). Erythromycin is not

frequently used in veterinary medicine in this area, and so high resistance could be due to transfer of genes carried on plasmid between different strains of bacteria between strains of bacteria (Olayinka and Olayinka, 2003; Alien *et al.,* 2012).

The multiple drug resistance of the *S. aureus* strains defined as resistance of an isolate to 3 or more antibiotics revealed that 53 representing 96.4% of the *S. aureus* strains were resistant to 3 or ore antibiotics, 4 strains were resistant to 7 antibiotics while 1 isolate each was resistant to 8 and 9 antibiotics respectively. Only 2 of the isolates were resistant to less than 3 antibiotics. This observation is of serious concern and may mean that the sources of milk contamination in the study areas may be reservoirs of multidrug resistant *S. aureus*. The abuse and indiscriminate use of antibiotic in animals and also their use in animal husbandry should be discouraged because resistant pathogens such as *S. aureus* can be transmitted to humans through the consumption of foods contaminated by such resistant pathogens (Alien *et al.,* 2012)

The present study revealed that cow milk in the study areas are contaminated with resistant strains of *S. aureus* which were considered as animal and human biotypes and can serve as means of subsequent spread to humans through the consumption and handling of raw milk. This risk can be eliminated through improving the general herd hygiene, proper management practices, proper milking hygiene and pasteurization of milk.

Acknowledgements

Impact

S. aureus is normal inhabitants of both humans and animals; therefore, the *S. aureus* contamination of the fresh cow milk resulted from cross-contamination from both humans and animals due largely to poor personal, milking and environmental hygiene and sanitation among famers and milk handlers in the study areas. This present study suggests that consumption of fresh raw cow milk by poses high risk of transmission of antibiotic resistant *S. aureus* and possible infection to the consumers. This study recommends;

i.   That human should desist from the consumption of fresh raw milk.
ii. That all cow milk for human consumption should be properly pasteurized to eliminate all the *S. aureus*.
iii. That the Fulani herdsmen and milkers should be educated on proper personal and environmental hygiene to reduce contamination of milk by *S. aureus*.
iv. That teat and mammary gland area should be disinfected prior to milking procedures.
v.   That human-milk and animal-milk contact should be maintained properly.
vi. That the use of antibiotics in human and veterinary medicine should be controlled and regulated so as to reduced chances of abuse and sub standardization and prevent acquiring resistance by *S. aureus*.

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183

SHORT COMMUNICATION

LIPOSARCOMA IN A MALE ALSATIAN DOG IN IBADAN, OYO STATE, NIGERIA - A CASE REPORT

Tijani M O[1], Adejumobi O A[2*], Oyebanji V O[1], Emikpe B O[1] and Omobowale O T[2]

[1]Department of Veterinary Pathology, Faculty of Veterinary Medicine, University of Ibadan, Nigeria.
[2]Department of Veterinary Medicine, Faculty of Veterinary Medicine, University of Ibadan, Nigeria.

Introduction:

Liposarcoma is a malignant tumour of adipocytes and its occurrence is rare in domestic animals (Meuten, 2002). A few foreign authors have reported the occurrence of this neoplasm in dogs (Messick and Radin, 1989; Vascellari et al., 2004; Hobert et al., 2013). Currently, there is a dearth of information on the occurrence and pathology of this tumour in dogs in Nigeria. Liposarcoma is locally invasive and can occur on the flanks and the limbs and occasionally in body cavities (Dobson and Lascelles, 2011). The aetiology of liposarcoma is uncertain, however it has been reported to be associated with foreign body (microchip) implant in a dog (Vascellari et al., 2004). Grossly, the masses are firm, poorly demarcated, locally invasive and posess a low metastatic potential (Kudnig and Séguin, 2012; Withrow, Vail & Page, 2013). The most common sites of metastasis include lungs, spleen, liver and bone (Withrow, Vail & Page, 2013). Histological subtypes of liposarcoma include myxoid, round cell, well-differentiated and pleomorphic (Kilpatrick et al., 1996). Here we describe a case of well differentiated liposarcoma in a six year-old male Alsatian dog.

Case History, Diagnosis and Discussion

A six year-old male Alsatian dog was presented to the Small Animal Clinic of the Veterinary Teaching Hospital, University of Ibadan with complaint of anorexia, hind limb deficit and overextension of the distal hind limb. A day after presentation, the dog was found to have bilateral hind limb paresis. At presentation, the dog's rectal temperature was 40.7°C. The dog also had severely pale mucous membranes. Serum chemistry and haematology revealed the following: mild azotaemia (Creatinine- 2.2mg/dl; BUN-40 mg/dl), mild elevation of liver enzymes (ALT-135µL, ALP-128µL, AST- 11µL), mild hyperalbuminaemia (3.0g/dl). Neurobion was administered. However, this intervention did not lead to an improvement in the dog's condition as it was found dead on the fifth day after it was first presented to the small animal clinic for medical attention.

At necropsy, there was a small, subcutaneous, irregular pendulous mass (6x5x5cm) in the ventral midline of the carcass about 5 cm caudal to the xiphoid sternum. The cut surface of the mass was yellowish brown, firm, lobulated (Plate 1) and haemorrhagic. Blood was dripping from the dog's nostrils. The oral and ocular mucous membranes were severely pale and the trachea contained moderate amount of blood stained mucus. The lungs were markedly congested and had multifocal, 0.3-0.5cm diameter, cream coloured, slightly raised foci .The thoracic cavity and pericardium contained 5mls and 10 mls of blood respectively. There was diffuse yellowish discolouration of the liver. Other gross lesions observed included rough, pitted kidneys, multifocal ecchymotic haemorrhages in the spleen, blood tinged watery intestinal contents and cutaneous decubital ulcers. 0.5cm thick sections of the subcutaneous tumour, lungs, liver and kidneys were fixed in 10% neutral buffered formalin for 24 hours and routinely processed for histology and stained with haematoxylin and eosin. Some of the sections were also stained with Sudan black.

Corresponding author email: muyenko@yahoo.com

Histologically, the subcutaneous mass was irregular and lobulated, consisting of sheets of loosely arranged pleomorphic cells trapped in delicate fibrovascular stroma. The atypical cells were pleomorphic with shapes ranging from round to oval to polygonal with distinct cell margins and scanty to abundant eosinophilic cytoplasm. Many of the cells posess a single, large, cytoplasmic vacuole with peripherally displaced nucleus conferring a signet ring appearance on each cell (Plate 2). Nuclear shapes varied from round to oval to irregular. Generally, nuclei were euchromatic and each posessed one to two basophilic nucleoli. There were also numerous, large, multinucleated cells (Inset, plate 2) often with one to multiple, large cytoplasmic vacuoles, scattered throughout the tumour stroma. Occasionally, there were large, irregular bizarre cells with very large, irregular, basophilic nucleus. Mitotic figures were occasionally present in some fields. There were numerous random, foci of coagulative necrosis of the tumour cells. The histological features including the morphology of constituent cells of the pulmonary nodules were similar to those found in the subcutaneous mass (Plate 3). The cytoplasm of most of the cells in the Sudan black stained sections were stained black indicating the presence of fat (Plate 4). Histologically, the kidneys and liver showed severe chronic non-suppurative tubulointerstitial nephritis and severe, chronic, diffuse hepatitis, with diffuse hepatocellular cord atrophy and moderate biliary hyperplasia respectively.

The subcutaneous and pulmonary tumours in the present case were diagnosed as well differentiated liposarcoma based on the histological findings of predominant atypical lipocytes with 'signet ring' appearance, numerous multinucleated giant cells and occasional mitoses. Well differentiated liposarcoma is defined as a locally aggressive malignant mesenchymal tumour made up either entirely or in part of a mature adipocytic proliferation showing significant variation in cell size and at least focal nuclear atypia in both adipocytes and stromal cells (Dei Tos and Pedeutour, 2002). The gross and histological findings from the subcutaneous and pulmonary tumours in this case are to a large extent in agreement with those reported for canine liposarcoma (Wang et al., 2005, Piseddu et al., 2011). The aetiology of the neoplasm in this case cannot be easily ascertained however, the aetiology of liposarcoma is largely unknown (Withrow, Vail & Page, 2013), but it has been reported to arise de novo (Messick and Radin, 1989) especially subcutaneously, often at the site of a microchip implant in a dog (Vascellari et al., 2004).

Prognosis of this tumour is essentially dependent on its anatomical location as neoplasms located in surgically manageable soft tissue do not recur following complete excision while those occurring in deep anatomic sites such as peritoneum and mediastinum tend to recur repeatedly often resulting in the death of the patient possibly as a result of uncontrolled local effects or metastasis (Dei Tos and Pedeutour, 2002). Taking these details into consideration, the prognosis of the neoplasm in the present case can be regarded as poor due to the fact that the neoplasm was also found in the lungs.

The cause of death in the present case was most likely not due to the tumour (liposarcoma), rather it could be attributed to an undetermined intercurrent disease. This is due to the fact that, other gross and histological findings not suggestive of liposarcoma were also observed. These lesions included severe chronic lymphoplasmacytic tubulointerstitial nephritis and severe, chronic, diffuse hepatitis, with diffuse hepatocellular cord atrophy and moderate biliary hyperplasia. The aetiology of these other lesions were not determined, however canine leptospirosis has been associated with lymphoplasmacytic tubulointerstitial nephritis (Prescott et al., 1991).

There is currently a dearth of information concerning the incidence, prevalence and pathology of liposarcoma in dogs in Nigeria. It is therefore pertinent that further research be done to study the incidence and pathology of this neoplasm in dogs in Nigeria with a view to determining the aetiology and formulation of effective preventive measures.
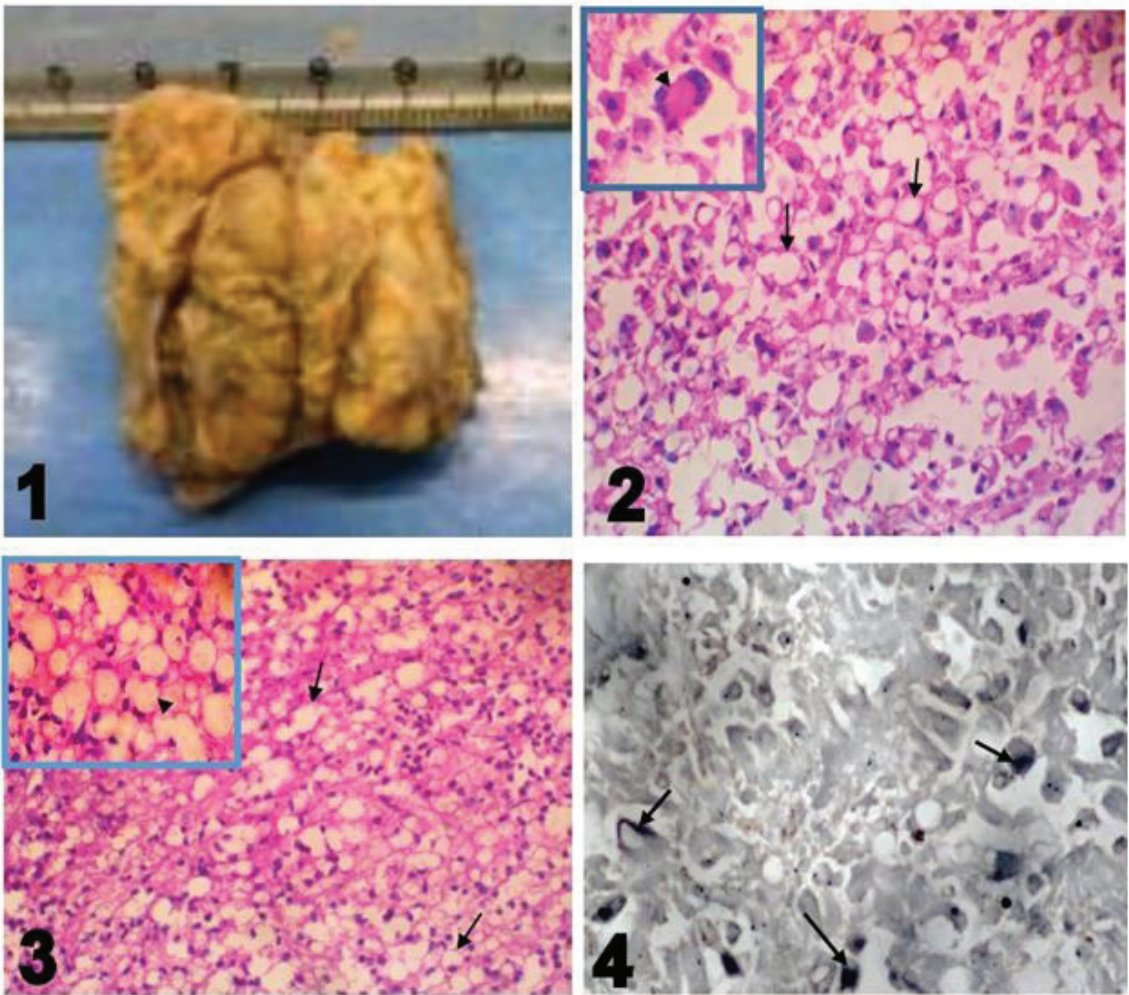
Plate 1: Cut surface of formalin fixed, subcutaneous tumour. The surface is yellowish brown and lobulated.

Plate 2: Photomicrograph of the subcutaneous mass showing numerous atypical lipocytes. Each neoplastic lipocytes has a single large cytoplasmic vacuole with peripherally displaced nucleus (arrows). The inset shows a multinucleated giant cell. H&E, x400

Plate 3: Photomicrograph of a pulmonary nodule showing sheets of atypical lipocytes each possessing a single large cytoplasmic vacuole and peripherally displaced nucleus (arrows). Inset shows a higher magnification (x1000) of the neoplasm with an atypical lipocytes indicated by the arrow head. H&E, x400

Plate 4: Photomicrograph of Sudan black stained section of the subcutaneous mass showing positive staining of the cytoplasm of the neoplastic cells for fat (arrows). The cytoplasmic fat accumulations are stained black. X400, Sudan black

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

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

The Editor in Chief
January 2013

Aims and scope

The Bulletin of Animal Health and Production in Africa (BAHPA) of the African Union Interafrican Bureau for Animal Resources (AU-IBAR) is a scientific journal which publishes articles on research relevant to animal health and production including wildlife and fisheries contributing to the human wellbeing, food security, poverty alleviation and sustainable development in Africa. The bulletin disseminates technical recommendations on animal health and production to stakeholders, including policy makers, researchers and scientists in member states. The Bulletin is the African voice on animal resources issues specific to Africa.

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.

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7. Results should be presented clearly and concisely, in a non-

repetitive way. Subheadings may be accepted.
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- *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.
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- *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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