

ISSN 0378 – 9721

Volume 67 No. 1

March / Mars 2019

African Union
Inter-African Bureau for Animal Resources

Bulletin of
Animal Health and Production
in Africa



Bulletin de la
Santé et de la Production Animales
en Afrique

Union Africaine
Bureau interafricain des Ressources Animales

ISSN 0378 - 9721

INTER-AFRICAN BUREAU FOR ANIMAL RESOURCES
BUREAU INTERAFRICAIN DES RESSOURCES ANIMALES
P.O Box 30786, NAIROBI, KENYA

BULLETIN

March
2019
Mars

Volume 67

No. 1

AFRICAN UNION
UNION AFRICAINE

**IBAR PUBLICATION
PUBLICATION DU BIRA**

**BULLETIN OF ANIMAL HEALTH AND PRODUCTION IN AFRICA
BULLETIN DE LA SANTE ET DE LA PRODUCTION ANIMALES EN
AFRIQUE**

A Quarterly journal of Original Articles and Abstracts in English and French

Annual subscription: US\$ 100.00

ISSN 0378-9721

Revue trimestrielle contenant des articles originaux et des résumés d'études en anglais
et en français

Abonnement pour un an : 100\$

1. AN ASSESSMENT OF COMMUNICATION CHANNELS UTILIZED BY POULTRY FARMERS TO ACCESS INFORMATION ON RAISING VILLAGE CHICKENS IN BAUCHI STATE, NIGERIA. <i>Sule A G, Abdu P A, Kabir J and Balami A G.....</i>	7
2. BLOOD PARAMETERS OF LACTATING SOWS AND THEIR PIGLETS FED FORTIFIED ENSILED CASSAVA ROOT-LEAF BLENDS. <i>Amos A T, Idowu O M O, Oso A O, Adeleye O O, Obembe O R, Adediran A J and Amos A O.....</i>	13
3. COMPARATIVE SURVIVABILITY AND FERTILITY POTENTIALS OF OVINE Spermatozoa STORED IN EGG YOLK CITRATE AND MIXED VEGETATIVE EXTENDERS AT ROOM TEMPERATURE. <i>Oloye A A, Omitoogun B A, Ajadi R A, Ola-Davies O E and Oyeyemi M O.....</i>	27
4. PARASITIC CONDITIONS OF CATS IN OSUN STATE, SOUTHWEST NIGERIA: RECORDS FROM THE HOSPITALS (2006-2015). <i>Ola-Fadunsin S D, Rabiun M, Ganiyu I A and Hussain K.....</i>	37
5. RESPONSE OF RABBIT DOES AND KITS TO DIFFERENT WEANING AGES. <i>Ayo-Ajasa O Y, Egbeyale L T, Ekunseitan D A, Busari M O, Bajegbo Y O, Fatkus Q O and Oyekanmi O O.....</i>	45
6. GENETIC VARIATION OF LEPTIN GENE IN THREE NIGERIAN CATTLE BREEDS. <i>Ogunnwa B E, Adenaike A S, Bello-Ibiyemi A A, Fafiolu A O and Ikeobi C O N.....</i>	57
7. HIGH QUALITY CASSAVA PEEL (HQCP) AS A REPLACEMENT FOR MAIZE IN THE DIET OF AFRICAN CATFISH (<i>Clarias gariepinus</i>) JUVENILES. <i>Orisasona O, Olaegbe A T, Olanrewaju A N and Kareem O K.....</i>	67
8. HYGIENIC PRACTICES AND MICROBIOLOGICAL QUALITY OF MILK FROM PERI-URBAN DAIRY FARMERS AND BULKING CENTRES IN LILONGWE, MALAWI. <i>Richard Fredrick Dickson Banda, Joseph Wafula Matofari and John Masani Nduko.....</i>	79
9. FIRST FINDINGS ON THE PREVALENCE AND INTENSITY OF GASTROINTESTINAL PARASITES IN CAMEROONIAN ZEBUS RAISED ON A LARGE-SCALE DAIRY FARM. <i>Sandrine Dicko Patouma, Arouna Njayou Ngapanya, Christelle Domngang Noche, Alain Kouam Simo, Philippe Vignoles and Félicité Flore Djuikwo Teukeng.....</i>	91
10. STRATIFIED LIVESTOCK PRODUCTION ADDS VALUE TO PASTORAL CATTLE: EVIDENCE FROM THE DRYLANDS OF KENYA. <i>Bulle Hallo Dabasso, Oliver Vivian Wasonga, Patrick Irungu and Brigitte Kaufmann.....</i>	101
11. PLANNING FOR RATIONAL USE OF ANTIBIOTICS IN TREATMENT OF BOVINE MASTITIS IN RWANDA. <i>Biryomumaisho S, Musanayire Y, I Kiiza D and Kanyandekwe C.....</i>	115
12. PREVALENCE OF <i>HAEMONCHUS CONTORTUS</i> IN KALAHARI RED GOATS REARED IN THE SOUTH-WEST NIGERIA. <i>Okwelum N, Akinkuotu O A, Shittu O O and Oluwatosin B O.....</i>	125

AN ASSESSMENT OF COMMUNICATION CHANNELS UTILIZED BY POULTRY FARMERS TO ACCESS INFORMATION ON RAISING VILLAGE CHICKENS IN BAUCHI STATE, NIGERIA

Sule A G¹, Abdu P A², Kabir J³ and Balami A G⁴

¹Department of Veterinary Public Health and Preventive Medicine, PMB 1069, University of Maiduguri, Nigeria. suleadamu@yahoo.com; 2348029805011.

²Department of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria.

³Department of Veterinary Public Health and Preventive Medicine Ahmadu Bello University Zaria, Nigeria.

⁴Department of Veterinary Medicine, University of Maiduguri, Nigeria.

Abstract

An assessment of communication channels utilized by farmers to access information on raising village chickens was conducted using an open ended questionnaire among 72 farmers drawn from nine communities in Bauchi State. All the participants were selected based on their ownership of more than 20 chickens and interest in participation in the proposed study during a focus group discussion held among village chicken farmers, poultry traders and community leaders in each of the nine participating communities. The demographic characteristics of respondents revealed that most of them, 47/72 (65.3%) were solely farmers who were mostly females, 40/72 (55.6%) of over 20 years of age (77.8%) and married or widowed, 71/72 (98.6%). The majority of these respondents had received some formal education, 37/72 (51.4%) and had over 5 years of experience in raising village chickens, 55/72 (77.8%). A combination of Radio and community knowledge were identified by most respondents (32%) as their major sources of information on village chicken production. Radio (29%) was the second and the single most important channel of information while school, was the least reported source of agricultural information (3%). The study highlighted the need to add and network more channels of information to effectively reach farmers with information that will lead to improvements in village chicken production.

Key words: communication channels, access to information, village chickens, farmers, sustainable development goals, Bauchi State, Nigeria

Introduction

Village chickens, the most dominant and numerous of village poultry could be employed in the attainment of sustainable development goals (SDGs) of elimination of hunger, poverty and the empowerment of women due to their availability, affordability, low requirement for land and capital inputs (Dolberg 2003). They provide an opportunity for farmers to climb the economic ladder through the sale of excess chickens to buy sheep or goats which in turn can be sold to buy a cow which has the ability to bring prestige and a rise in the social status of farmers in their communities (Dolberg, 2003; Copland and Alders, 2009).

A major limitation to the use of village chicken towards the attainment of SDGs is high chicken mortality due to disease, poor nutrition and environmental stress that are associated with the traditional ways of raising village chickens (Sonaiya 2009; Bell 2009). These constraints tend to discourage and limit farmers from investing more capital into village chicken production.

To overcome these challenges will require communicating research information to farmers on better ways of raising these chickens. Of particular importance is the access to information by village chicken farmers on selection of good breeds, survivability of chicks, disease control, hygiene, good record keeping, good housing, provision of perches and nests, provision of clean water, feed supplementation, the use of lime or disinfectant to wash houses, separation of adult from young chicks, separation of sick from healthy birds, the treatment of sick ones, the burial or burning of dead birds, sales of non productive chickens and vaccination against prevailing diseases which researchers of village chickens (CTA, 2007; Riise *et al.*, 2004; Sonaiya, 2009) have considered to be critical for bringing about improvement in the traditional ways of raising chickens.

But, access to information has been observed to be limited among people living in rural communities when compared to those living in the urban communities and

especially, among women and children who mostly raise these chickens (FAO 2014). To raise awareness on practices that could improve village chicken production among farmers required an assessment of the current channels of communications available to them in order to identify gaps that needed to be addressed to reach farmers with information that has the likelihood of transforming their traditional practice of raising chickens which has been associated with high chicken mortality (Sonaiya, 2009). The aim of this work was to identify communication channels available to village chicken farmers and their implications on bringing improvements to village chicken production.

Materials and Methods

Study area

This study was carried out in Bauchi State, Nigeria (Figure 1). The State occupies a land mass of 48,382 sq km that is located within latitudes 7°52'N and 8°56'N and longitudes 7°25'E and 9°37'E on the Bauchi plateau and shares boundary with Kaduna, Benue, Yobe, Gombe, Plateau, Taraba, Kano and Jigawa States (INEC, 2008). The State is characterized by dry and wet seasons and a savannah woodland vegetation. The state has twenty Local Government Areas (LGAs), a human population of 5,515,300 (INEC, 2008) whose occupation is mainly farming and a village poultry population of about 5,832,750 (Adene and Oguntade, 2006).

Sample size

The study was conducted among 72 village chicken farmers who were drawn from nine (9) communities in Bauchi State with eight farmers being selected from each community during a focus group discussion that mainly comprised of community leaders, farmers, poultry traders and community animal health workers. Each farmer was selected based on his ownership of more than 20 chickens. A questionnaire on channels of information available to the village chicken farmers was administered to each of the

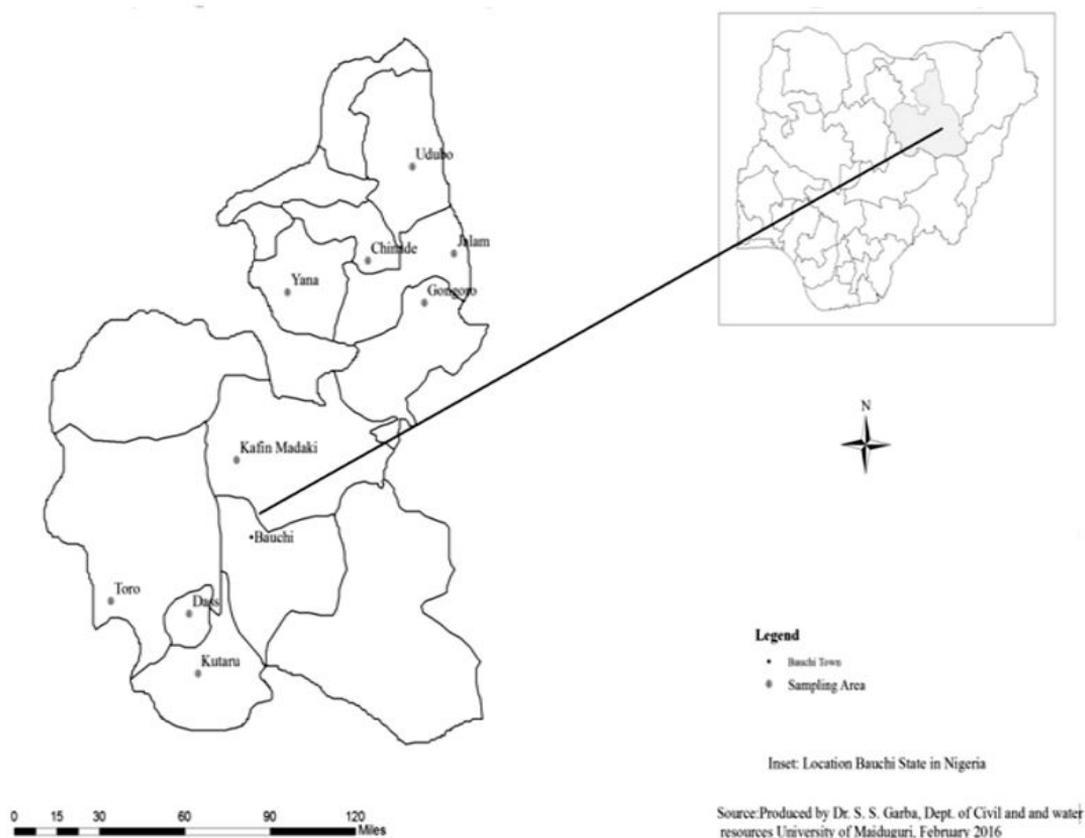


Figure 1: Map of Bauchi State, Nigeria showing communities where this study was conducted.

farmers representing 72 households (HHs). The questionnaire was administered through an interview format.

Data analyses

Data was analyzed using simple percentages and presented in a pie chart.

Results

The demographic characteristics of the respondents (Table 1) revealed that most of them were farmers 47/72 (65.3%) who were mostly females 40/72 (55.6). 56/72 (77.8%), over 20 years of age (77.8%) and married or widowed 71/72 (97.6%). Most of the respondents had received some formal education 37/72 (51.4%) and most of them 55/72 (77.8%) had more than 5 years experience of keeping village chickens. A combination of Radio and community were used by most respondents (32%) (Figure

2) to access information on village chicken production. 29% of the respondents had identified Radio as the single most important channel of information and school as the least reported source of agricultural information (3%). Extension services which are critical for transformation of the practices of raising village chickens were reported by only 7% of the respondents as a channel of information for village chicken production.

Discussion

The fact that a combination of Radio and community knowledge were recognized by most respondents as their major sources of information, implies that information received from radio could easily be disseminated when members of the community are encouraged to share information gained from such programmes with their friends or relations.

Table 1: Demographic characteristics of respondents who answered questionnaire on management of village chickens in Bauchi State, Nigeria.

Demography		Number of responses *(n = 72)	%
Occupation	Farmer	47	(65.3)
	Civil servant	7	(9.70)
	Trader, artisan and others	18	(25.0)
Gender	Male	32	(44.4)
	Female	40	(55.6)
Age	<20 years	1	(1.40)
	>20 years	71	(98.4)
Marital status	Single	1	(1.40)
	Married or widowed	71	(98.6)
Education	Non western or Informal	35	(48.6)
	Formal	37	(51.4)
Experience in chicken Rearing	<5 years	16	(22.2)
	>5 years	56	(77.8)

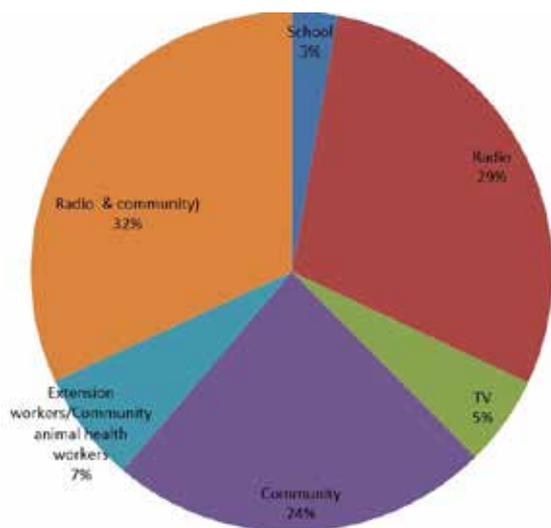


Figure 2: Distribution of communication channels utilized by farmers to access information on raising village chickens

It also implies the need for a combination of approaches to reach village chicken farmers with information on good practices of raising village chickens.

The reporting of Radio by most respondents as the single major source of their information may be related to the low cost of acquiring and maintaining a radio and the

ease with which listening to radio broadcasts programme can be combined with other activities such as household chores. Appropriate radio programmes could thus play a vital role in the dissemination of valuable research information that could bring improvement in village chicken production in Bauchi State. This finding agrees with the work of Assam *et al.* (2012) who found radio to be a good source of information on highly pathogenic avian influenza for farmers in Kaduna State, Nigeria.

Yet, caution must be exercised in the use of Radio as the sole means of passing agricultural information. Combining household chores with listening to aired programmes may often result in the loss of vital information that otherwise, could bring a change in the practice of raising these chickens. Also, Radio as an example of media is only suited for spreading short messages and stimulating interest about issues or events (Hoffmann *et al.*, 2009) and not for conveying vast information that will lead to improvements in practices of raising chickens.

The recognition of the Community as a channel of information for village chicken production in this study is commendable provided members of the community are

sharing the right information. But, high chicken mortality associated with existing village chicken production in Bauchi State seems to suggest the need for better information to be made available to farmers and if possible with practical demonstrations on best practices by an extension agent.

That only 5.9% of respondents obtained information from extension workers corroborates the work of Sonaiya (2009) who reported that less than 5% of village chicken farmers had access to extension agents. The result implies that there is limited contact between extension workers and farmers for effective demonstration and learning of practical activities like debeaking and vaccination against important diseases to take place. According to Hoffmann *et al.* (2009) extension services are so critical for transformation of agricultural production that they cannot be substituted by the use of media like radio which in this study was found to be high. It becomes very important that Bauchi State employs and deploys more extension or community animal health workers in order to reach farmers with information, advice and the practical demonstration of new techniques on one to one or group basis in relaxed informal settings.

The finding that schools were the least source of agricultural information for respondents suggests either absent or non active demonstration animal farms in community Primary and possibly Secondary schools. The presence of such farms will go a long way in stimulating interest in animal production in the lives of students who in the future could challenge traditional ways of raising village chickens by demanding the use of better ways to improve village chicken production.

Erratic electricity supply in most rural communities is the most likely a reason why Television was not recognized by most respondents as a channel of agricultural information. There is also the possibility that most of these respondents view Television for entertainment purposes rather than for educational purposes.

That other channels of communications

including live presentations, drama, videos and posters were not reported by respondents as a source of agricultural information offers the possibility of their use as additional channels to reach farmers with agricultural information. Although posters were used as channels of communication for raising awareness for the containment of avian influenza outbreaks in Nigeria, their was not remembered nor reported as a source of communication of information on village chicken production.

Similarly, respondents did not report social media platforms such as the internet and handsets as channels of agricultural information. These are most likely used for social activities and could also play a great role in village chicken production in the future.

Conclusions and recommendations

The major channels of communication utilised by respondents to access information on raising village chickens were Radio and community knowledge. The study recommends that the Bauchi State Government employ more extension service or community animal health workers as well as incorporate and network other channels of communication to effectively reach farmers with information and practices that will bring about improvement in village chicken production.

Acknowledgements

The Director and staff of the Department of Veterinary Services Bauchi State for their permission and assistance in conducting this study in Bauchi State.

References

Assam A, Abdu PA, Tabe-Ntui LN, 2012. Local poultry farmers' media use, access, and understanding of highly pathogenic avian influenza communication material in Nigeria. *Bull Anim Prod health Afr*, 60: 92-103.

Copland JW and Alders R G. 2009. The comparative advantage of village or small holder poultry in

rural development. In: Alders RG, Spadbrow P.B., Young, M.P. and Sonaiya, F. (Eds). Proceedings of International Conference held in Dar es Salaam, Tanzania 5-7 October 2005.

Dolberg F, 2003. Review of household poultry production as a tool in poverty reduction with focus on Bangladesh and India. Pro-poor Livestock Policy Initiative. PPLPI wkg Paper, No.6. FAO.34 (2003) Retrieved from <http://www.fao.org/ag/againfo/projects/en/pplpi/docarc/wp6.pdf>. 11/14/2015 at 3:50 PM

Hoffman V, Gerster-Bentaya M, Christinck A, Lemma M, 2009. Use of extension aid and media In: Rural Extension Volume 1: Basic issues and concepts (3rd Edn). Margraf Publishers, GmbH, Pp. 151-164.

Riise JC, Permin A, McAinsh CV and Fredricksen L, 2004. Keeping Village Poultry: A Technical Manual on Small Scale Production. Network for Small Holder Poultry Development, Copenhagen, Denmark, Pp. 1-89.

Sonaiya EB, 2009. Fifteen years of family poultry research and development at Obafemi Awolowo University, Nigeria. In: Alders R.G., Spradbrow P.B. and Young M.P. (eds) 2009. Village chickens, poverty alleviation and the sustainable control of Newcastle disease. Proceedings of an International Conference held in Dar es Salaam, Tanzania, 5-7 October 2005. ACIAR Proceedings No. 131, Pp. 235.

The ACP-EU Technical Centre for Agricultural and Rural Cooperation (CTA), (2007). Improved Practices in Rearing Indigenous Chickens. CTA Practical Guide Series, No. 4, Pp. 1-6.

BLOOD PARAMETERS OF LACTATING SOWS AND THEIR PIGLETS FED FORTIFIED ENSILED CASSAVA ROOT-LEAF BLENDS.

Amos A T¹, Idowu O M O¹, Oso A O¹, Adeleye O O², Obembe O R¹, Adediran A J¹ and Amos A O²

¹Department of Animal Nutrition, Federal University of Agriculture, Abeokuta, Nigeria.

²Department of Animal Production and Health, Federal University of Agriculture, Abeokuta, Nigeria.

Abstract

This study examined the effects of feeding lactating sows with Ensiled Cassava Root-Leaf Blends (ECRLB) as a replacement for maize on the haematological and serum biochemical parameters of both the dams and their suckling piglets. Cassava roots and leaves were mixed at 70:30, ensiled and then fortified as follows; ECRLB1 (ECRLB with no additive), ECRLB2 (ECRLB + amino acids; methionine and lysine), ECRLB3 (ECRLB + Allzyme), ECRLB 4 (ECRLB + amino acids and Allzyme). These four were used as a replacement for maize at 30% in the sow's diet, while a Maize Based Diet (MBD) served as the control to have a total of five experimental diets. Twenty lactating sows (Large White x Landrace) were randomly allocated to the 5 dietary treatments in a completely randomized design with four sows per treatment, each sow being a replicate. The sows were fed the experimental diets. The study lasted for 6 weeks lactating period. At the end of the experiment, blood samples were collected from both the lactating sows and their corresponding suckling piglets for the determination of haematological and biochemical indices. The results showed that the haematological and biochemical parameters of both the sows and their piglets fell within the recommended normal ranges. Blood parameters from sows fed fortified ECRLB (2-4) and their piglets had comparable results with those from the control diet (MBD) while those from sows fed ECRLB without additive (ECRLB1) showed poor haematological and biochemical indices. Results obtained indicated that the piglets did not suffer from anaemia. There were no detrimental effects of feeding fortified ECRLB in respect of the haematology and serum biochemistry of the experimental animals. It was then concluded that fortified ensiled cassava root- leaf blends be used to replace maize in the diets of sows since it shows no negative effects on the blood parameters.

Keywords: Lactating sows, suckling piglets, ensiled cassava root-leaf blends, haematological and serum biochemical parameters.

Introduction

The lactating phase of pig production is very critical since it determines the level of nutrition and maternal immunity conferred on the farrowed piglets. This is because the quality of sow milk depends largely on the dietary intake of the sows which will invariably affect the nutrients passed on to the piglets. Therefore, focusing on the sows/piglets nutrition is not out of place since healthy piglets are among the main purposes or target of pig production.

Dietary contents have been shown to affect the blood profiles of healthy animals (Odunsi *et al.*, 1999; Kurtoglu *et al.*, 2005). The blood transports nutrients to different parts of the body including the mammary glands, therefore whatever affects the blood such as nutrition will certainly affect the entire body adversely or moderately in terms of growth, health, maintenance and reproduction (Oke *et al.*, 2007). Nutritional studies involving blood examination can be used to monitor flock health towards improving the quality of animal production. Both haematological and biochemical blood components are influenced by the quality and quantity of feed and also the level of antinutritional elements or factors present in the feed (Akinmutimi, 2004). Biochemical and haematological components of blood are also valuable in monitoring feed toxicity especially with feed constituents that affect the formation of blood (Oyawaye and Ogunkunle, 1998).

Cassava tubers and leaves have been used as potential sources of energy in pig diets (Uthai and Sukanya, 2005). Pioneering studies have highlighted on the suitability of cassava tuberous meal for swine feeding and its potential as a good substitute for maize for all classes of pigs (Adesehinwa *et al.*, 1998; Sauvart *et al.*, 2004; Nnadi *et al.*, 2010). However the utilization of cassava tubers and leaves is limited (among other limitations) by its cyanogenic glycosides; lotaustralin and linamarin (Smith, 1998; Cardoso *et al.*, 2005). Both compounds are hydrogen cyanide derivatives which have been shown to be toxic to livestock (McDonald *et al.*, 1995) and therefore limits the use of

cassava in its raw state as feed for livestock (Smith, 1998) especially non ruminants.

The process of ensiling has been found to reduce not only the cyanide content of cassava products but also increases feed efficiency and improved performance of pigs (Kil and Stein, 2010). Bearing this in mind and other nutritional imbalances of feeding cassava products to monogastrics, efforts were made in this study to compensate for these nutritional imbalances by fortifying the diets with necessary additives (amino acids and enzymes). This study seeks to investigate the effects of replacing maize with fortified ensiled cassava root-leaf blends on the haematology and biochemistry parameters of lactating sows and their suckling piglets.

Materials and methods

Preparation of silage of cassava roots and leaves

Based on the outcome of a previous experiment, cassava root and leaf blend was prepared in ratio of 70:30 roots and leaves respectively. Fresh cassava roots were harvested from demonstration plots at the Federal University of Agriculture (FUNAAB), Abeokuta, Nigeria. The roots were washed in water to remove the adhering dirt and soil and then grated in a grating machine. The grated cassava pulp were then packed in Hessian bags and screw-pressed for 24 hours to reduce the moisture content. The cassava leaf biomass (a mixture of leaves, petioles and stalk) remaining after harvesting of cassava roots were collected, was chopped and wilted for 24 hours to reduce the moisture content in order to facilitate fermentation. The dewatered cassava pulp was then mixed together with the wilted leaf biomass at the ratio of 70:30. The mixture was then poured rapidly in nylon bags and pressed intermittently to express air out of the bags. The nylon bags with a capacity of 25kg were filled to three-quarters, tied and kept in sealed air tight plastic silos for 21 days before use. The product obtained, the ensiled cassava roots-leaves blend (ECRLB) was then used in feeding the experimental animals.

Chemical composition of the Ensiled product Ground samples of the dried ECRLB were used for the determination of its proximate constituents (AOAC, 1990). The cyanide content (Egan *et al.*, 1998), tannin, phytate and oxalate (Pearson, 1976), mineral content (Onimawo, 2005) and gross energy (Adiatic bomb calorimeter, Parr Instrument Company, Moline, IL, USA) were determined according to standard procedures.

Location of the feeding trial

The experiment was carried out at the piggery unit of the Directorate of University Farms (DUFARMS) of FUNAAB, Nigeria. The area lies on latitude 7°13'N and longitude 3°25'E, 76m above sea level and is located in the tropical rainforest vegetation zone with an average temperature of 34.7°C. Experimental Diets

ECRLB was supplemented with additives to compensate for the nutritional imbalances of cassava roots and leaves as follows: ECRLB1 (ECRLB + no additive), ECRLB2 (ECRLB + methionine and lysine at 25g/ton), ECRLB3 (ECRLB + Allzyme® SSF at 300g/ton), ECRLB4 (ECRLB + methionine and lysine + Allzyme® SSF at 25g and 300g/ton respectively). Previously formulated ECRLB (1-4) were incorporated to replace maize at 30% in the experimental diets of the gilts to make four test diets while a maize based diet (MBD) served as the control diet making a total of five experimental diets. The experimental diets were formulated to meet the NRC (2012) requirement standard (Table 2).

Experimental animals, design and management

A total number of twenty cross bred (Large White x Landrace) lactating sows with similar farrowing records (± 3 days) were selected from the university farms and assigned for this study. Sows were randomly allocated to one of five dietary treatments in a completely randomized design with four sows per treatment, each sow representing a replicate. The experiment lasted for six weeks lactating period. The sows were fed the experimental diets with compositions presented in Table

2. The sows were fed according to their daily requirements (NRC, 2012) and water was freely available to the sows and piglets throughout the experimental period. Daily routine practices were strictly adhered to.

Data collection

At the end of the experiment (6 weeks), blood was collected from both the sows and their piglets before feeding in the morning. Ten (10) mls of blood were collected from each of the sows (4 per treatment) through the jugular vein while 5mls of blood were similarly collected from the piglets (6 per treatment) for haematological and biochemical analyses. Adequate measures were taken to minimize pain or discomfort on the part of the experimental animals. Haematological parameters were estimated in whole blood just after bleeding using standard procedures (Jain, 1986), for haemoglobin (Hb), red blood cells (RBC), packed cell volume (PCV) and white blood cells (WBC) as described by Mafuvadze and Erlwanger (2007) and Tripathi *et al.*, (2008). The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated from values of RBC, PCV and Hb. Blood samples for biochemical assays were allowed to clot before centrifuging to obtain serum that was used in the determination of some metabolites using standard chemical procedures for: urea (Wooton, 1964), serum protein, albumin and globulin (Varley *et al.*, 1980), creatinine (Pousnes and Tausssky, 1945). The serum cholesterol was estimated using the enzymatic colorimetric method in accordance with the instructions in the Randox diagnostic cholesterol kit. The cyanide in the serum was analysed using the methods outlined by ISO (1975).

Statistical analysis

The data generated were subjected to one-way analysis of variance in a completely randomized design using SAS, 2000. The means for treatment showing significant differences were compared using Tukey's test.

Results

The results of the chemical composition of ECRLB are presented in Table 1 while the percentage composition of the experimental diets are presented in Table 2. Table 3 shows the haematological and serum biochemistry parameters of sows fed diets containing ECRLB. The result showed that significant differences ($P<0.05$) existed among all the parameters assayed. Similar ($P>0.05$) values of PCV (45.60, 45.80, 44.70 and 45.00)%, RBC (4.87, 4.88, 4.83 and 4.84) $\times 10^{12}/L$, Hb (12.00, 122.00, 121.00 and 121.30)g/L, WBC (12.60, 12.70, 12.90 and 12.20) $\times 10^9/L$, MCH (29.10, 29.15, 29.10 and 29.26) pg, and MPV (9.50, 9.40, 9.51 and 9.60) fL were observed for sows fed MBD, ECRLB2,

Table 1: Chemical composition of ensiled cassava root-leaf blends

Parameters	Values
Proximate composition	
Dry matter (%)	90.00
Crude protein (%)	10.12
Ether extract (%)	11.00
Ash (%)	2.80
Nitrogen free extract (%)	49.78
Crude fibre (%)	19.00
Gross energy (Kcal/kg)	4180.95
Mineral content (mg/l)	
Calcium	42.43
Phosphorus	0.43
Magnesium	26.59
Manganese	0.75
Iron	22.24
Copper	1.18
Zinc	120.15
Sodium	22.03
Potassium	61.05
Antinutritional factors	
Hydrocyanide (mg/kg)	0.014
Tannin (%)	0.005
Phytate (%)	0.018
Oxalate (%)	0.007

ECRLB3 and ECRLB4 respectively. MCHC values for MBD and ECRLB2 were higher when compared to other treatments. Sows fed with MBD, ECRLB2 and ECRLB4 recorded the highest ($P<0.05$) blood platelet volumes. Sows fed ECRLB1 recorded the least ($P<0.05$) concentration of PCV (43.20) %, RBC (4.21) $\times 10^{12}/L$, Hb (106.30) g/L, MCH (28.65) pg and MPV (8.63) fL, however, the highest ($P<0.05$) concentrations of WBC (13.90) $\times 10^9/L$ were recorded for the ECRLB1 group. Significant differences ($P<0.05$) existed among serum biochemical values in the various treatment groups. In the total serum protein content, MBD (8.00) g/dL was significantly different ($P<0.05$) when compared to ECRLB1 (6.00) g/dL and ECRLB3 (7.60) g/dL. No significant differences ($P>0.05$) were however observed when MBD (8.00) g/dL, ECRLB2 (8.90) g/dL and ECRLB4 (8.34) g/dL were compared. Similar ($P>0.05$) albumin contents were observed for MBD, ECRLB2 and ECRLB4; (4.5, 4.3 and 4.34) g/dL, these values were significantly higher ($P<0.05$) compared to those of ECRLB1 and ECRLB 3 (3.40 and 3.90) g/dL respectively. A higher ($P<0.05$) concentration of globulin was observed for ECRLB 2 (4.60) g/dL when compared to other treatment groups, while those of ECRLB 1 (2.60) g/dL recorded the least ($P<0.05$). All treatments with cassava root-leaf blends had lower ($P<0.05$) cholesterol contents when compared with the control group. Triglyceride concentration concentrations of ECRLB 1-4 (87.80, 93.00, 96.00 and 98.00) mg/dL were significantly higher ($P<0.05$) compared to those of MBD (83mg/dL). The same trend of significance ($P<0.05$) was observed in respect to glucose with those fed MBD (76.00) mg/dL recording the least ($P<0.05$) value compared to others fed ECRLB1-4: (ECRLB1; 90.00 mg/dL, ECRLB2; 92.00 mg/dL, ECRLB 3; 98.00 mg/dL and ECRLB 4; 102 mg/dL). The urea concentrations of MBD (8.40 mg/dL) and ECRLB2 (8.60 mg/dL) were similar and represented the least ($P<0.05$) compared to other treatment groups. Similar ($P>0.05$) creatinine concentration were observed for MBD and ECRLB 2, 3 and 4; (0.94, 0.80, 0.90 and 0.95 mg/dL) respectively while ECRLB1

Table 2: Percentage composition of sowss diets.

Ingredients	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
ECRLB1	--	30.00	--	--	--
ECRLB2	--	--	30.00	--	--
ECRLB3	--	--	--	30.00	--
ECRLB4	--	--	--	--	30.00
Maize	30.00	--	--	--	--
Brewers dry grain	13.00	13.00	13.00	13.00	13.00
Soya bean meal	13.00	13.00	13.00	13.00	13.00
Wheat offal	5.00	5.00	5.00	5.00	5.00
Palm kernel cake	34.00	34.00	34.00	34.00	34.00
Palm oil	1.00	1.00	1.00	1.00	1.00
Limestone	1.73	1.73	1.73	1.73	1.73
Di-calcium phosphate	1.00	1.00	1.00	1.00	1.00
Lysine	0.90	0.90	0.90	0.90	0.90
Methionine	0.16	0.16	0.16	0.16	0.16
*Premix	0.15	0.15	0.15	0.15	0.15
Salt	0.24	0.24	0.24	0.24	0.24
Total	100.00	100.00	100.00	100.00	100.00
Chemical composition					
Metabolizable energy (Kcal/kg)	3000.54	3103.75	3103.75	3103.75	3103.75
Crude protein (%)	16.35	17.20	17.20	17.20	17.20
Calcium (%)	0.75	0.82	0.82	0.82	0.82
Phosphorus (%)	0.50	0.55	0.55	0.55	0.55
Lysine (%)	0.62	0.65	0.65	0.65	0.65
Methionine (%)	0.30	0.41	0.41	0.41	0.41

*Premix: Vitamin A: 4000000IU, Vit D3: 2500000IU, Vit E: 40000mg, Vit K3: 800mg, Vit B3: 3000mg, Vit B2: 6000mg, Vit B6: 5000mg, Vit B12: 25mg, Niacin: 6000mg, Pantothenic Acid: 20000mg, Folic Acid: 800mg, Biotin: 8mg, Manganese: 300000mg, Iron: 80000mg, Zinc: 20000mg, Copper: 25mg, Cobalt: 80mg, Iodine: 400mg, Selenium: 40mg, Chlorine: 800000mg. ECRLB1: (ECRLB with no additive), ECRLB2: (ECRLB + amino acids), ECRLB3: (ECRLB + Allzyme), ECRLB4: (ECRLB + amino acids + Allzyme).

recorded the highest ($P < 0.05$) concentration (1.20 mg/dL) compared to other treatment groups. Similar ($P > 0.05$) serum thiocyanate concentration were recorded for ECRLB 1-4 (0.50, 0.43 and 0.48 mg/dL) respectively.

Table 4 shows the haematological and biochemical parameters of piglets from sows fed the experimental diets. PCV, MCHC and serum thiocyanate were not significantly ($P > 0.05$) affected by the dietary treatments. Piglets from sows fed ECRLB1 recorded the least RBC and Hb values while the rest treatment groups showed higher ($P < 0.05$) values of RBC

and Hb. Similar ($P > 0.05$) WBC counts were observed among ECRLB 2-4 (13.00, 13.15 and 13.00) $\times 10^9/L$ and MBD (13.10) $\times 10^9/L$. MCV concentration showed similar ($P > 0.05$) MBD value (59.75) fL with ECRLB 2-4 (58.15, 58.20 and 58.55) fL respectively. MCH concentration recorded highest concentration of MBD (20.80) pg compared other treatment groups (15.04, 17.50, 18.55 and 18.40) pg. With respect to platelets (PLT), MBD recorded similar and the highest ($P < 0.05$) concentrations (261.00) $\times 10^9/L$ with ECRLB3 (263.75) $\times 10^9/L$ and ECRLB4 (264.50) $\times 10^9/L$ compared to others.

Table 3: Haematological and serum biochemical indices of lactating sows fed ensiled cassava root-leaf blends

Indices	TREATMENTS					SEM	P.value
	MBD	ECRLB1	ECRLB2	ECRLB3	ECRLB4		
Paced cell volume (%)	45.60 ^a	43.20 ^b	45.80 ^a	44.70 ^a	45.00 ^a	1.218	0.012
Red blood cells (x1012/L)	4.87 ^a	4.21 ^b	4.88 ^a	4.83 ^a	4.84 ^a	0.197	0.010
Haemoglobin (g/L)	120.00 ^a	106.30 ^b	122.00 ^a	121.00 ^a	121.30 ^a	2.347	0.018
White blood cells (x109/L)	12.60 ^b	13.90 ^a	12.70 ^b	12.90 ^b	12.20 ^b	0.987	0.022
Mean cell haemoglobin (pg)	29.10 ^a	28.65 ^b	29.15 ^a	29.10 ^a	29.26 ^a	0.323	0.010
Mean cell haemoglobin concentration (g/dl)	39.50 ^b	40.15 ^{ab}	39.70 ^b	41.20 ^a	40.30 ^{ab}	2.969	0.038
Mean cell volume (fL)	73.60 ^a	71.90 ^b	73.20 ^a	72.60 ^b	72.80 ^b	0.434	0.029
Platelet (x109/L)	364.00 ^a	301.50 ^c	367.00 ^a	361.00 ^b	364.00 ^a	3.620	0.016
Mean platelet volume (fL)	9.50 ^a	8.63 ^b	9.40 ^a	9.51 ^a	9.60 ^a	0.210	0.023
Total serum protein (g/dl)	8.00 ^{ab}	6.00 ^c	8.90 ^a	7.60 ^b	8.34 ^{ab}	0.373	0.030
Albumin (g/dl)	4.50 ^a	3.40 ^c	4.30 ^a	3.90 ^b	4.34 ^a	0.320	0.047
Globulin (g/dl)	3.50 ^c	2.60 ^d	4.60 ^a	3.70 ^c	4.00 ^b	0.199	0.038
Cholesterol (mg/dl)	128.00 ^a	118.05 ^c	108.30 ^b	109.25 ^b	108.00 ^b	5.394	0.044
Triglycerides (mg/dl)	83.00 ^d	87.80 ^c	93.00 ^b	96.00 ^a	98.00 ^a	1.338	0.041
Glucose (mg/dl)	76.00 ^d	90.00 ^{cd}	92.00 ^c	98.00 ^b	102.00 ^a	2.376	0.040
Urea (mg/dl)	8.40 ^c	12.20 ^a	8.60 ^c	9.05 ^b	9.00 ^b	0.435	0.033
Creatinine (mg/dl)	0.94 ^b	1.20 ^a	0.80 ^b	0.90 ^b	0.95 ^b	0.155	0.047
Thiocyanate (mg/dl)	0.38 ^c	0.66 ^a	0.50 ^b	0.43 ^b	0.48 ^b	0.099	0.032

^{a, b, c, d} values within the same row with different superscripts differ significantly ($P < 0.05$). MBD: maize based diet, ECRLB1: (ECRLB with no additive), ECRLB2: (ECRLB + amino acids), ECRLB3: (ECRLB + Allzyme), ECRLB4: (ECRLB + amino acids + Allzyme).

Similar MPV values were observed for MBD (8.35) fL and ECRLB 2-4 (8.15, 8.25 and 8.28) fL. The serum biochemical parameters revealed that the serum total protein in piglets from sows fed ECRLB2 and ECRLB 4 (7.85 and 7.45) g/dL were similar and represented the highest ($P < 0.05$) compared to other treatment groups. The albumin values of 4.85 g/dL and 4.60 g/dL observed for ECRLB2 and 4 respectively was significantly different from those observed with MBD, ECRLB1 and ECRLB 3; (4.03, 3.15 and 4.10) g/dL. Globulin values shows similar values among MBD, ECRLB2 and ECRLB3 values (3.00, 3.00 and 3.10) g/dL. These values were significantly higher ($P < 0.05$) than the rest of the groups. Lower ($P < 0.05$) cholesterol values were observed for piglets from sows fed

ECRLB (2-4; 112.00, 112.50 and 111.00) mg/dL when compared to MBD (130.50)mg/dL and ECRLB1 (118.00)mg/dL. In contrast, higher ($P < 0.05$) triglycerides (96.00, 97.00 and 98.50) mg/dL and glucose (84.50, 85.50 and 86.50) mg/dL concentrations were observed for ECRLB (2-4) compared to other groups. Similar urea concentrations were observed for MBD (10.70) mg/dL and ECRLB1 (10.95) mg/dL. In respect to creatinine, ECRLB1 recorded the highest ($P < 0.05$) significant value of 1.45mg/dL, while ECRLB2 recorded the least ($P < 0.05$) concentration (0.75) mg/dL compared to other treatment groups.

Table 4: Haematological and serum biochemical indices of piglets

Indices	TREATMENTS					SEM	P.value
	MBD	ECRLB1	ECRLB2	ECRLB3	ECRLB4		
Paced cell volume (%)	49.20	48.00	49.50	49.55	50.15	1.760	0.260
Red blood cells (x1012/L)	7.75 ^a	6.31 ^b	7.98 ^a	7.67 ^a	7.70 ^a	0.510	0.007
Haemoglobin (g/L)	12.25 ^a	10.17 ^b	12.95 ^a	12.55 ^a	12.45 ^a	2.560	0.182
White blood cells (x109/L)	13.10 ^b	14.03 ^a	13.00 ^b	13.15 ^b	13.00 ^b	1.322	0.047
Mean cell haemoglobin (pg)	59.75 ^a	56.08 ^b	58.15 ^a	58.2 ^a	58.55 ^a	3.170	0.000
Mean cell haemoglobin concentration (g/dl)	20.80 ^a	15.04 ^c	17.50 ^c	18.45 ^b	18.40 ^b	0.809	0.000
Mean cell volume (fl)	26.50	24.45	26.10	26.50	26.60	1.878	0.620
Platelet (x109/L)	261.00 ^a	242.00 ^c	258.00 ^b	263.75 ^a	264.50 ^a	22.62	0.000
Mean platelet volume (fl)	8.35 ^a	6.25 ^b	8.15 ^a	8.25 ^a	8.28 ^a	0.281	0.045
Total serum protein (g/dl)	7.03 ^b	5.85 ^c	7.85 ^a	7.20 ^b	7.45 ^a	0.468	0.015
Albumin (g/dl)	4.03 ^b	3.15 ^c	4.85 ^a	4.10 ^{bc}	4.60 ^a	0.464	0.047
Globulin (g/dl)	3.00 ^a	2.70 ^b	3.00 ^a	3.10 ^a	2.85 ^b	0.288	0.019
Cholesterol (mg/dl)	130.50 ^a	118.00 ^b	112.00 ^c	112.50 ^c	111.00 ^c	6.32	0.031
Triglycerides (mg/dl)	84.00 ^b	85.50 ^b	96.00 ^a	97.00 ^a	98.50 ^a	3.208	0.042
Glucose (mg/dl)	77.50 ^c	80.50 ^b	84.50 ^a	85.50 ^a	86.50 ^a	2.47	0.046
Urea (mg/dl)	10.70 ^a	10.95 ^a	9.15 ^b	9.45 ^b	9.40 ^b	0.24	0.048
Creatinine (mg/dl)	1.05 ^b	1.45 ^a	0.75 ^c	0.90 ^b	0.93 ^b	0.181	0.011
Thiocyanate (mg/dl)	0.34	0.45	0.42	0.44	0.40	0.502	0.094

^{a, b, c, d} values within the same row with different superscripts differ significantly ($P < 0.05$). MBD: maize based diet, ECRLB1: (ECRLB with no additive), ECRLB2: (ECRLB + amino acids), ECRLB3: (ECRLB + Allzyme), ECRLB4: (ECRLB + amino acids + Allzyme).

Discussion

Diets have been established to have measurable effects on blood components and the latter in turn are widely used in nutritional evaluation and survey of animals (Church *et al.*, 1984). A readily available and fast means of assessing clinical and nutritional health status of animals on feeding trials may be the use of blood analysis because the ingestion of dietary components have measurable effects on blood composition (Church *et al.*, 1984; Maxwell *et al.*, 1990). The haematological results showed that the parameters fell within the normal range for lactating sows as supported by Zvorc *et al.*, (2006). According to Togun *et al.* (2007), when haematological values fall within the

normal range established for the animal, it is an indication that diets did not show any adverse effects on the animals during the experimental period. PCV, Hb, RBC and WBC were normally preponderant for maize based diets above other diets and obviously implied better nourishments (Irekhore *et al.*, 2015). Similar values of RBC, PCV, Hb and WBC observed in this study with sows fed MBD which is the control diet and those fed fortified ECRLB (2-4) indicated the nutritional adequacy of the test ingredients. Packed cell volume (PCV), red blood cells (RBC) and haemoglobin (Hb) are strongly correlated (Jain, 1986). RBC serve as a carrier of Hb, PCV is the percentage of RBC in the blood (Purves *et al.*, 2003), while Hb is responsible for the transport of iron containing

oxygen metallo-protein in the RBC of all vertebrates except the fish family (Maton *et al.*, 1993). They are involved in the transportation of oxygen and absorbed nutrients to the tissues as well as transport of carbon dioxide out of the body of animals (Isaac *et al.*, 2013). Thus, a reduced level affects the respiratory and digestion processes in the body of the animal. The trend of RBC, PCV and Hb reported in this study is not consistent with those of Akinfala and Tewe (2001) who recorded higher PCV, RBC and Hb concentrations in the control group of growing pigs fed whole cassava plants. Enzyme supplemented cassava based diets have been reported to increase PCV in growing pigs (Adesehinwa *et al.*, 2011) and Hb and RBC counts in broiler chickens (Udoyong *et al.*, 2010). Normal WBC exhibited among all the treatment groups suggest adequate defenses against infectious agents (Kaneko, 1989) and enhance adaptability to local environmental and prevalent disease conditions (Isaac *et al.*, 2013). This is probably due to the adequate protein in the diets as reported by Jain (1986) that nutritional deficiency particularly that of protein reduces most haematological and serum parameters. Mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) indicate blood level conditions. A lower level than the recommended level is an indication of anaemia (Aster, 2004). The MCH and MCHC of sows fed MBD and fortified ECRLB falls within the normal range (Zvorc *et al.*, 2006) and suggested an absence of hypochromasia because under this condition, MCHC is lower than normal (Olafadehan, 2011). In addition, the normal PCV indicated the absence of normocytic anaemia which was reported to be characterized by a normal MCV and MCH but only detected by a decreased number of RBC or PCV (Coles, 1986). Blood platelets are implicated in blood clotting, low platelet concentrations suggest that the process of clot formation (blood clotting) will be prolonged resulting in excessive loss of blood in the case of injury. The blood platelets volumes of sows fed fortified ECRLB were similar to those of the control group indicating that the diet did not have any

negative effects on the blood clotting process.

Serum protein, albumin and globulin measures the total amount of protein in the blood (Coles, 1986). They are indicators of adequacy of protein in terms of quality and quantity in the diet (Akinfala and Tewe, 2001). These parameters show whether there is protein malnutrition, alterations in the dietary intake of protein and pattern of utilization (Eggum, 1970). Serum protein, albumin and globulin reported in this study fell within the normal range recommended for lactating sows by Verheyen *et al.* (2006). Similar serum protein levels demonstrated in sows fed MBD and those fed fortified ECRLB implies that the protein levels in the diets were able to support normal protein reserves in the pigs resulting from efficient protein utilization and performance of the sows (Orororo *et al.*, 2014). Lower serum cholesterol concentration recorded for sows fed ECRLB compared to those fed the control diet might be due to the effect of ensiling process employed in the preparation of the test ingredients. In previous studies, Chu *et al.* (2011) reported that fermented diets could have an effect on reducing cholesterol in fattening pigs. Similarly, Loh *et al.* (2003) showed a decrease in plasma cholesterol levels in the fermented product group compared to the non-fermented group in rats. Improved triglycerides and glucose levels reported for sows fed ECRLB as compared to those of the control group implies better availability of energy to the sows. Triglycerides and glucose either meet immediate energy needs in muscles or stored as fat for future energy requirements. Serum triglycerides and glucose indicates the efficiency of utilization of metabolizable energy in a given diet (Fanimu, 1991). Improved triglycerides and glucose concentrations obtained in this study with sows fed ECRLB compared with those fed maize base diets further confirms that cassava contains more digestive starch compared to maize (Promthong *et al.*, 2005). Enzyme supplementation has been reported (Unigwe *et al.*, 2016) to improve glucose availability due to its effects on fibres which would not have been ordinarily digested by monogastrics. Urea is the main nitrogenous end product arising

from the catabolism of amino acids that are not used for biosynthetic roles in mammals. Higher serum urea indicates inefficient utilization of protein (Eggum, 1970). Although, serum urea concentration of the experimental animals were within the normal limits, better serum urea concentration were displayed by sows fed amino acids fortified diet followed by those fed enzyme supplemented diet indicating that the amino acid profile of the test ingredients were balanced and efficiently utilized. Ranjan (2001) affirmed that in a diet deficient in amino acids, the available amino acids will be deaminated and hence results in an increase in the excretion of urea. An elevation of serum creatinine concentration is often used as an index of muscle catabolism and when lactating sows are fed nutrients below their requirements, especially for energy and protein, body protein tissue is catabolized in an attempt to supply the nutrients to maintain milk production (Etieme *et al.*, 1985). In this study, the lower serum creatinine concentration displayed by groups fed fortified ECRLB were comparable to those of the control group indicating that there was no wasting or catabolism of muscle tissues and that the animals were not surviving at the expense of the body reserves (Ahamefule *et al.*, 2006). This also suggests that the residual cyanide in the ensiled products did not interfere with the nutrient utilization. It suffices to note that poor haematological and serum biochemical indices demonstrated by sows fed ECRLB without any supplement in this study strongly indicates that the test ingredients is better utilized when fortified.

Although there is paucity of information on serum and haematological parameters of piglets nursed by sows fed cassava based diets, nevertheless, the haematological and serum profile of piglets reported in this study falls within the normal range reported by Yeom *et al.* (2012). The blood profile of piglets from sows fed fortified ECRLB had comparable results with those from sows fed the control diets. This is not surprising as the blood profile of their dams also followed the same pattern. The influence of nutrition during the suckling period on piglets' metabolism are well

described (Holub 1982, 1990, 1994). During the suckling period, the dams' milk was the main source of nutrition for the piglets, indicating that the lactating sows' diet does not only influence her own blood components but also that of the suckling piglets. Hartmann and Holmes (1989) adjudged that nutrients from the blood are synthesized by the epithelial cells in the alveoli into milk components and transported into the alveolar lumen where milk is produced. PCV and other haematological parameters are useful aids to prognosis and may reveal adverse conditions even when the animals did not display obvious clinical signs of ill health (Eze *et al.*, 2010). The fact that the haematological traits of the piglets did not decline below normal values implies that the anti-nutritional factors which may be present in small quantities in the diets of the dams did not influence the haematological parameters of the piglets negatively. Cyanide has been reported to have great affinity for metals such as copper and iron, making them unavailable for absorption, thereby reducing the haemoglobin count and effective transportation of oxygen and carbohydrate (Akinfala and Tewe, 2001).

Anaemia in piglets is an important factor in piglets' survival and growth. MCV is a good indicator of anaemia. In anemic piglets, the cells become microcytic and the MCV will decrease (Jain, 1993). According to Wallach and Kanaan (2003), large platelets are common in anemic animals. Lower PCV, Hb, MCH and MCV are also said to be indicators of hypochromic microcytic anaemia. However, these were not pronounced in this study as the haematological values concerned fell within the recommended range indicating that the piglets did not suffer from anaemia. Normal WBC values in piglets suggests adequate resistance against infectious conditions, stress, allergy, parasitism and chronic tissue damage (Eggum, 1989). The serum protein indicators (total protein, albumin and globulin) of piglets from sows fed the control diet and those from piglets fed the test diets indicated that the quality and quantity of protein in their diets was sufficient for their growth and development as the values did not fall below the normal range. Protein has been documented

to be extremely important to piglets so much so that the sow prioritizes protein content in colostrum and milk if the protein content in the feed is scarce by metabolizing body reserves (King *et al.*, 1996). Improved serum triglycerides and glucose in piglets from sows fed fortified ECRLB compared with those from the control diets implied more energy fuel for these piglets. Rajman *et al.* (2006) reported that elevation in serum glucose concentration is linked with dietary energy supply

The fall in blood cholesterol level of piglets from sows fed the experimental diets compared to those fed on the control diets suggested that ECRLB could be used to produce animal products with low cholesterol content. Low and insignificant values of serum thiocyanate recorded by the piglets further confirmed that the processing method employed in the preparation of the test diets was able to drastically reduce the cyanide content to a non-lethal level as portrayed by the piglets.

Conclusion and Recommendation

From the findings in this study, it is obvious that feeding fortified ensiled cassava root-leaf blends had no adverse effects on the health status of the lactating sows and their suckling piglets taking into consideration the results of the haematological and serum metabolites of both the lactating sows and the suckling piglets. Fortified ensiled cassava root-leaf blend is therefore recommended as a total replacement for maize in the diets of lactating sows.

Impact

The study was conducted to determine the effect of feeding Ensiled Cassava Root-Leaf Blend (ECRLB) at 70:30 as a total replacement for maize on the blood parameters of lactating sows and their suckling piglets. The blend was further fortified with amino acids, enzyme and a combination of amino acids and enzymes. The results of the serum and haematological parameters revealed that there was no detrimental effect on the experimental

animals fed fortified diets and that they had comparable results with those fed maize based diet which served as the control diet. Fortified ECRLB diets were recommended as a total replacement of maize in swine diet.

References

- Adesehinwa A O K, Dafwang I I, Ogunmodede B K and Tegbe T S B. 1998. A review of utilization of some agro-industrial by-products in pig rations. *Nigerian Journal of Agricultural Extension*, 11(1-2):50-64.
- Adesehinwa A O K, Obi O O, Makanjuola B A, Oluwole O O and Adesina M A. 2011. Growing pigs fed cassava peel based diet supplemented with or without Farmazyme® 3000 proenx: Effect on growth, carcass and blood parameters. *African Journal of Biotechnology*, 10 (14): 2791-2796.
- Ahamefule F O, Eduok G O, Usman A, Amaefule K U, Obua B E and Oguike S A. 2006. Blood biochemistry and haematology of weaner rabbits fed sundried, ensiled and fermented cassava peel based diets. *Pakistan Journal of Nutrition*, 5 (3): 248-253.
- Akinfala E O and Tewe O O. 2001. Utilization of whole cassava plant in the diets of growing pigs in the tropics” *Livestock Research for Rural Development*. 13:5-12.
- Akinmutimi A H. 2004. Evaluation of sword bean (*Canavalia gladiata*) as an alternative feed resources for broiler chickens, Unpublished PhD thesis, Department of Animal Production, Michael Okpara University of Agriculture, Umudike, Nigeria.
- Aster J C. 2004. Anaemia of diminished erythropoiesis. In *Pathologic Basis of Disease* Kumar, V., Abbas, A.K. Fausto, N., Robbins, S.L. and Cotran, R.S. Eds., Robbins and Cotran Saunders Co. Philadelphia pp:638-649.
- Borin K, Lindberg J E and Ogle R B. 2005. Effect of variety and preservation method of cassava leaves on diet digestibility by indigenous and improved pigs. *Animal Science*, 80: 319-324.
- Bousness RW and Tausky A A. 1945. The calorimetric determination of creatinine by the Jaffe reaction. *Journal of Biological Chemistry*, 158: 789-800.

- Cardoso A P, Mirione E, Ernesto M, Massaza F, Cliff J, Haque R M and Bradbury J H. 2005. Cassava roots to remove cyanogens. In the Processing of the 2005 conference. *Journal of Food Composition and Analysis*, 18:451-460.
- Chu G M, Yang B S, Kim H Y, Kim J H, Ha J H, Kim C H, Lee S D and Song Y M. 2011. Effects of supplemental fermented agro by-products diet on the growth performances, blood characteristics and carcass traits in fattening pigs. *Asian-Australian Journal of Animal Science* (24): 1464-1472.
- Church J P, Judd J J, Young C W, Kebay J L and Kim W W. 1984. Relationship among dietary constituents and specific serum clinical components of subjects eating self-selected. *American Journal of Clinical Nutrition* 40: 1338-1344.
- Coles E H, 1986. *Veterinary Clinical Pathology*. 4th Edition Published by W.B.
- Eggum B O, 1989. Biochemical and methodological principles. In *Protein metabolism in farm animals, evaluation, digestion, absorption and metabolism*, Eds., Block A D, Eggum B O, Low A G, Simon O and Zebrowske T. Oxford Science Publications, London. pp. 152.
- Eggum R O, 1970. The protein quality of cassava leaves. *British Journal of Nutrition* 24:761-769.
- Etienne M, Noblet J and Desmoulin B. 1985. Mobilisation des réserves corporelles chez la truie primipare en lactation *Reprod. Nutr. Dev.* 25:341-344
- Eze J I, Onunkwo J I, Shoyinka S V O, Chah F K, Ngene A A, Okolinta N, Nwanta J A and Onyenwe I W. 2010. Haematological profiles of pigs raised under intensive management system in South-Eastern Nigeria. *Nigerian Veterinary Journal*, 31 (2): 115-123.
- Fanim O A, 1991. Substitution of soyabean meal and animal byproducts for fish meal in pigs rations. Unpublished PhD thesis Animal Science Department, University of Ibadan, Ibadan, Nigeria.
- FAO/WHO. 1991. Joint FAO/WHO food standards programme. In: *Codex Alimentarius Commission XII (suppl. 4)*. Rome, Italy: FAO.
- Holub A, 1982. Functional development and food intake of piglets. *Acta Vet Brno Suppl* 5: 5-12
- Holub A, 1990. Effect of rearing practices on the water consumption by piglets in the first eight weeks after birth. *Acta Vet Brno* 59: 129-137.
- Holub A, 1994. Prasnice a selata - nutriční interakce. (In Czech, Sows and piglets - nutrition interactions.) *Vet Med Praha* 39: 117-132.
- Irekhore O T, Adeyemi O M, Idowu O M O, Akinola O S and Bello K O. 2015. Growth Performance, Haematological Indices and Cost Benefits of Growing Pigs Fed Cassava Peel Meal Diets Supplemented With Allzyme® SSF. *International Journal of Applied Agricultural and Apicultural Research*. IJAAAR 11 (1 and 2): 51-59.
- Isaac L J, Abah G, Akpan B and Ekaette I U. 2013. Haematological properties of different breeds and sexes of rabbits. In the Proceedings of the 18th Annual Conference of Animal Science Association of Nigeria, pp 24-27.
- ISO, 1975. International standard organization determination of glycosidic cyanide by argentometric titration 2164L.
- Iyayi E A and Tewe O O. 1992 Effect of protein deficiency on utilization of cassava peel by growing pigs. In: *Cassava as livestock feed in Africa*. Proceedings of the I.I.T.A./I.L.L.A./University of Ibadan Workshop on the potential utilization of cassava as livestock feed in Nigeria, pp54-57.
- Jain N C, 1993. *Blood loss or hemorrhagic anemias. Essentials of veterinary hematology*. Lea and Febiger, Philadelphia.
- Jain N C, 1986. *Schalms Veterinary Hematology*, 4th edition, (ed. N.C. Jain), Philadelphia: Lea and Febiger, pp.20-86.
- Kaneko J J, 1989. *Clinical biochemistry of domestic animals*. 4th Edition. Academic Press, Inc. New York.
- Kil D Y. and Stein H H. 2010. Invited review: management and feeding strategies to ameliorate the impact of removing antibiotic growth promoters from diets fed to weanling pigs. *Canadian Journal of Animal Science*, 90:447-60.

- King R H, Pettigrew J E, McNamara J P, McMurtry J P, Henderson T L, Hathaway M R and Sower A F. 1996. The effect of exogenous prolactin on lactation performance of first-litter sows given protein-deficient diets during the first pregnancy. *Animal Reproduction Science*. 41: 37-50.
- Kurtoglu F, Kurtoglu V, Celik I, Kececi I. and Nizamlioglu M. 2005. Effect of dietary boron supplementation on some biochemical parameters, peripheral blood lymphocytes, splenic plasma cells and bone characteristics of broiler chicks given diets with adequate or inadequate cholecalciferol (vitamin D) content. *British Poultry Science*, 46:87-96.
- Loh T C, Foo H L, Tan S.H, Goh Y M, Shukriyah H M and Kufli C N. 2003. Effects of fermented products on performance, faecal pH, Enterobacteriaceae and lactic acid bacteria counts and interrelationships, and plasma cholesterol concentration in rats. *Journal of Animal Feed Science*, 12: 633.
- Mafuvadze B and Erlwanger K H. 2007. The effect of EDTA, heparin and storage on the erythrocyte osmotic fragility, plasma osmolarity and haematocrit of adult ostriches (*Struthio camelus*). *Veterinarski Arhiv.*, 77:427-434.
- Maton A, Hopkins R L J, McLaughlin C W, Johnson S, Warner C W, LaHart D and Wright J D. 1993. *Human Biology and Health*. Englewood Cliffs, New Jersey, USA: Prentice Hall.
- Maxwell M A, Robertson G W. and Spences S M C. 1990. Comparison Of Haematological Value In Restricted Feeds And Ad libitum Fed Domestic Fowls. *Red Blood Cell Characteristics*. *British Poultry Science*. 31: 407 – 413.
- National Research Council (NRC) 2012. *Nutrient Requirements of Swine*. 11th revised edition. National Academy Press, Washington, DC.
- Nnadi PA, Omeke B C and Okpe G C. 2010. Growth and reproductive performances of weaner pigs fed maize replaced cassava diet. *Animal Research International* 7(3): 1257-1263
- Odunsi A A, Ojifade A A and Babatunde G M. 1999. Response of broiler chicks to virginmycin and dietary protein concentration in the humid tropics. *Archives of Zoo technology*, 48 (183):317-325.
- Oke U K, Herbert U, Ebuzeome C O and Nwachukwu E N. 2007. Effect of genotype on the haematology of Nigerian local chickens in a humid tropical environment. In the Proceedings of the 32nd Annual Conference of Nigeria Society for Animal Production
- Olafadehan O A. 2011. Haematological parameters, serum constituents and organ development of growing rabbits as affected by feeding processed cassava peels. *Animal Nutrition and Feed Technology*, 11:41-51.
- Orororo O C, Tonukari N J, Awwioroko J O. and Ezedom T. 2014. Effect of Supplementation of Animal Feed with Dried Cassava (*Manihot esculenta*) Peels, and Stems of *Vernonia amygdalina* and *Pennisetum purpureum* on Some Biochemical Parameters in Pigs. *Nigerian Society for Experimental Biology* (14):4.
- Oyawoye B M and Ogunkunle H N. 2004. Biochemical and haematological reference values in normal experimental animals. New York, Masson, pp: 212-218.
- Phuc B H N, Lindberg J E, Ogle B. and Thomke S. 2001. Determination of the nutritive value of tropical biomass products for monogastrics using rats. Part 2. Effects of drying temperature, ensiling and level of inclusion of cassava leaves and sweet potato vines. *Asian-Australian Journal of Animal Science*, 14: 994-1002.
- Promthong S, 2005. Comparative studies on physiological, histological and microbial properties in the digestive tract of broilers fed cassava versus corn diets. Unpublished PhD thesis, Kasetsart University, Bangkok, Thailand.
- Purves W K, Sadava D, Orians G H and Heller H C. 2003. *Life: The science of Biology* 7:54.
- Rajman M, Jurani M, Lamosova D, Macajova M, Sedlackova M, Kostal L, Jesova D and Vyboh P. 2006: The effects of feed restriction on plasma biochemistry in growing meat type chickens (*Gallus gallus*). *Comparative Biochemistry and Physiology* 145: 363–371.
- Ranjhan S K, 2001. *Animal Nutrition in the Tropics*. 5th ed, Vikas Publishing House, PVT, LTD, New Delhi, India, pp: 576.

- Sauvant D, Perez J M and Tran G. 2004. Tables INRA-AFZ de composition et de valeur nutritive des materes premieres destinees aux animaux d'elevage, pp: 304.
- Smith O B, 1988. A review of ruminant responses to cassava-based diets Cassava as livestock feed in Africa. In the Proceedings of the IITA/ILCA/ University of Ibadan. Workshop on the Potential Utilization of Cassava as Livestock Feed in Africa. International Institute of Tropical Agriculture. Ibadan, Nigeria.
- Tewe O O, 1997. Sustainability and Development Paradigms from Nigerian Livestock Industry". Inaugural Lecture, University of Ibadan Nigeria.
- Togun V A, Oseni B S A, Ogundipe J A, Arewa T R, Hameed A A, Ajonijebu D C, Oyeniran A, Nwosisi I and Mustapha F. 2007. Effects of chronic lead administration on the haematological parameters of rabbit – a preliminary study. In the Proceedings of the 41st Conference of the Agricultural Society of Nigeria, Tokyo Hong Kong: pp 481-486.
- Tripathi M K, Mondal D. and Karin S A. 2008. Growth, haematology, blood constituents and immunological status of lambs fed graded levels of damaged wheat as substitute of maize. *Journal of Animal Physiology and Animal Nutrition*, 92: 75 – 85.
- Udoyong A O, Kibon A, Yahaya S M, Yakubu B, Augustine C and Isaac L. 2010. Haematological Responses and Serum Biochemistry of Broiler Chicken Fed Graded Levels of Enzyme (Maxigrain) Supplemented Cassava Peel Meal (CPM) Based Diets. *Global Journal of Biotechnology and Biochemistry*, 5(2): 116-119.
- Unigwe C R, Marire BN, Omeke C O, Abonyi F O, Oladipo T A and Adebayo D M. 2016. Values in normal experimental animals. New York, Masson, pp: 212-218.
- Varley H, Van E and Kass I. 1980. *Practical clinical Chemistry*, New York, Inter Science Publishers Inc, pp: 197-240.
- Verheyen A J M, Maes D G D, Mateusen B, Deprez P, Janssens G P J, Lange D. and Counotte, G. 2006. Serum biochemical reference values for gestating and lactating sows. *Elsevier Veterinary Journal*.
- Wallach J and Kanaan S. 2003. Interpretation of laboratory tests. *Interpretação de exames laboratoriais*. Medsi, Rio de Janeiro
- Wootton T D. 1964. *Micro analysis in medical biochemistry*. 4th edition. Churchill Ltd, London, pp: 214.
- Yeom S C, Cho S Y, Park C.G and Lee W J. 2012. Analysis of reference interval and age-related changes in serum biochemistry and hematology in the specific pathogen free miniature pig. *Korean Association of Laboratory Animal Science, Laboratory Animal Research*; 28(4).
- Zvorc Z, Mrljak V, Susic V and Gotal J P. 2006. Haematological and biochemical parameters during pregnancy and lactation in sows. *Veterinarski Arhivos*, 76 (3); 245-253

COMPARATIVE SURVIVABILITY AND FERTILITY POTENTIALS OF OVINE Spermatozoa STORED IN EGG YOLK CITRATE AND MIXED VEGETATIVE EXTENDERS AT ROOM TEMPERATURE.

*Oloye A A¹, Omitoogun B A¹, Ajadi R A², Ola-Davies O E³ and Oyeyemi M O⁴

¹Department of Veterinary Public Health and Reproduction, College of Veterinary Medicine, Federal University of Agriculture Abeokuta, Ogun State

²Department of Veterinary Medicine and Surgery, College of Veterinary Medicine Federal University of Agriculture Abeokuta, Ogun State

³Department of Veterinary Physiology and Biochemistry, Faculty of Veterinary Medicine University of Ibadan, Oyo State

⁴Department of Theriogenology, Faculty of Veterinary Medicine University of Ibadan, Oyo State.

Abstract

Proper semen extension is essential for successful artificial insemination and increased livestock production thereby helping in bridging the imbalance between livestock production and the high demand for animal protein in the developing world. Eight healthy multiparous non gravid West African Dwarf (WAD) ewes and two sexually matured rams intensively managed on grass, fed concentrate and water (ad libitum) were used in this study. Three diluents prepared using standard procedures were tested as extenders. Two were mixtures of 10% pawpaw juice and 90% coconut milk citrate (PIC9) and 30% pawpaw juice and 70% coconut milk citrate (P3C7). The third diluent (Standard Egg-yolk citrate) served as a control. Oestrus was synchronised in all the ewes by two intramuscular injections of 5mg PGF₂ α seven days apart. Semen collection, evaluation and extension using the three diluents were carried out by standard methods. Artificial inseminations, using semen extended with the better of the two test diluents (PIC9) and egg-yolk citrate (EYC) at 6 hours post extension were carried out. Conception was monitored using a portable ultrasound machine. At three, four, five and six hours post extension, P3C7 (64.00 \pm 1.41, 52.80 \pm 1.16, 41.00 \pm 0.71, 31.60 \pm 0.68 respectively) had significantly ($p < 0.05$) low motility score compared to PIC9 (71.20 \pm 0.86, 52.80 \pm 1.28, 52.80 \pm 1.28, 44.60 \pm 1.21 respectively) and EYC (76.00 \pm 1.14, 69.00 \pm 1.30, 61.40 \pm 0.75, 49.20 \pm 0.86 respectively). The EYC and PIC9 ewes both recorded 50% conception rates. In conclusion, a mixture of 10% pawpaw juice and 90% coconut milk-citrate was as effective as EYC and could be optimally used as an extender for ram semen stored at room temperature for up-to 6 hours

Introduction

Inefficiency in reproduction has been the costly and limiting constraint to animal production (Campbell *et al.*, 2003; Imasuen and Otoikhian, 2006) resulting in a great imbalance between livestock production and the high demand for animal protein needed to nourish the expanding population in developing countries like Nigeria (Ibe, 2004). The panacea is adoption of assisted reproduction techniques which help to enhance reproduction. Assisted Reproduction Techniques is defined as a direct or indirect artificial manipulation of the reproduction of a livestock herd for increase in livestock productivity (Al-Merestani *et al.*, 2003). It comprises of modern reproductive tools of which oestrus synchronization is primary as it provides a model for the secondary reproductive tools such as semen extension, artificial insemination, oocyte transfer and embryo collection and transfer (Al-Merestani *et al.*, 2003).

Oestrus synchronization is concerned with the manipulation of either the luteal or the follicular phase of the oestrous cycle. Synchronization of oestrus in animals serves as a model to supercharge animal production and is indeed one of the techniques being used in this era of Assisted Reproductive Technologies (ART) (Jordan, 2005). There are several routes of administration of these biologically active agents and several types of synchronization scheme combinations. Agents successfully used by some researchers in ewes have been Gonadotropin Releasing Hormone (GnRH), Prostaglandin F₂ α (PGF₂ α) (Ataman and Aköz, 2006) and intravaginal devices impregnated with progesterone or synthetic progestagen (Karaca *et al.*, 2009).

To meet the needs of artificial insemination, many diluents known as extenders have been used for extension. An extender is the aqueous solution used to increase the volume of the semen while the functional characteristics and the fertility rate of the spermatozoa are preserved (Salamon and Maxwell, 2006). Among the extenders that have been used by some workers are the

standard egg yolk, coconut milk and pawpaw juice. Over the years, extenders have improved from the simplest salt and sugar solutions used by Russians as early as 1914 (Geoffrey *et al.*, 1992) to the more advanced Tris Skimmed Milk and Egg yolk-citrate diluents (Sinha *et al.*, 1991). Sule (1996), using coconut milk citrate diluent indicated that semen of bucks extended in this diluent at 28°C would have to be used for artificial insemination within 3-4 hours post dilution to obtain an appreciable motility and hence a good conception rate. Also, Oloye *et al.* (2008), working on 80% coconut milk citrate concluded that sperm motility could be maintained at 66% for 2 hours and at 8% at 6 hours post extension. Ajala *et al.* (2010) working with a graded mixture of pawpaw juice and egg yolk concluded that semen could be extended with pawpaw juice for a maximum period of 72 hours stored at 5°C.

The fertility rate from inseminating with a particular extended semen is mainly measured with conception rate (Wang *et al.*, 1997). Diluents also keep a check on the contamination of the medium and protect semen from microbial growth. Liquid extended semen produces a higher conception rate with a relatively less number of sperm cells (Anzar *et al.*, 2003). Examples include egg yolk- phosphate (Phillips *et al.*, 1940), skim milk (Almquist *et al.*, 1962) and orange juice (Bonadonna *et al.*, 1962). This study evaluated the conception rate following artificial insemination of West African dwarf ewes with egg yolk citrate extended semen and a graded mixture of pawpaw juice – coconut milk extended semen at room temperature

Materials and Methods

Experimental Animals and management

Eight apparently healthy multiparous West African Dwarf (WAD) ewes of mean age 1.63 \pm 0.26 years and two sexually mature rams of mean age 2.05 \pm 0.25 years were used. The ewes, randomly grouped into two (of four ewes per group) and rams were all treated prophylactically and parenterally with Ivermectin (1ml/50 kg body weight,

Kepromec®, kepro B.V., Holland), multi-vitamin (1ml/5kg body weight, kepro B.V., Holland) and Cypermethrin 0.5% (50mg/kg, Pour on®, Kepro B.V., Holland). The animals were managed intensively in a clean, well-ventilated wooden pen, fed with feed concentrate and grasses and served clean water ad libitum. Ethical approval for the experiment was obtained from the ethical committee of the College of Veterinary Medicine, Federal University of Agriculture Abeokuta, Nigeria.

Preparation of 2.9% sodium citrate buffer

2.9 grams of sodium citrate was added up to make 100ml of distilled water. It was thoroughly stirred until the solute was completely dissolved in the solvent.

Preparation of media

Pawpaw juice: fresh ripe pawpaw fruit was rinsed with water and the edible part was carefully removed using a clean knife. It was cut into small pieces and then blended with a clean blender. It was then sieved and the juice was collected into a clean beaker.

Coconut milk: fresh coconut fruit was cracked and the edible part was carefully removed with a clean knife. The edible portion was cut into small pieces, grated using a clean grater and its milk was squeezed out into a clean beaker using a sterile sieve. The milk was then centrifuged at 1000rev/min for 15mins after which the coconut milk was carefully sucked up from under the topmost oily layer using a sterile pipette and collected into clean sample bottles.

Diluents: The Pawpaw juice and Coconut milk were mixed at a proportion of 10%: 90%, and 30%:70%. To each mixture was added freshly prepared sodium citrate at a proportion of 80% sodium citrate to 20% mixture leading to the constitution of two diluents (PIC9 and P3C7 respectively). *Penicillin-Streptomycin* (1000 µl/ml) was thereafter added to each of the diluents. Five aliquots (5ml) of each diluent were constituted.

Egg yolk-citrate diluent: Sterilised fresh egg was crack-opened at the tip using a clean knife and the albumen carefully separated from the egg yolk. The egg yolk was collected into a beaker to which prepared sodium citrate buffer solution was added at a ratio of 20% of egg yolk to 80% of sodium citrate. This mixture was thoroughly mixed to form a homogenous mixture.. *Penicillin-Streptomycin* (1000 µl/ml) was then added to the mixture. Five aliquots of 5ml were constituted.

Semen collection and Evaluation

Semen collection was done aseptically by the Electro-ejaculation method from the two mature rams (Noakes *et al.*, 2001). The ejaculate was collected into a clean insulated graduated semen collection tube, through a funnel held by an assistant. Semen evaluation was done as promptly as possible post collection as described by Rodriguez-Martinez and Barth (2007) for qualitative and quantitative parameters.

Semen Volume: The volume of semen collected was measured using a graduated collection tube.

pH Evaluation: The pH of the diluents was measured using a digital pH meter

Individual Motility: Using a dropping pipette, a drop of semen was placed on the warm slide, two drops of sodium citrate buffer were added, and a cover slide was placed and the slide was examined under x40 magnification using a light microscope. The motility estimate was done by taking estimates from four different apexes of the angle and finding the average.

Sperm Concentration: Neubauer haemocytometer was used to determine the sperm concentration using the method described by Zemjanis, (1970)

Sperm Morphology: The morphology of the spermatozoa was evaluated using Eosin-Nigrosin stain as described by Zemjanis (1970)

Extension and Storage

1 drop of the collected semen was added to the five aliquots of each of the three constituted diluents ((PIC9, P3C7 and EYC) at a dilution ratio of 37.5:1 (Oyeyemi *et al.*, 2010) at room temperature and semen evaluation was done at 0,1,2,3,4,5,6 and 24 hours post-extension.

Extended Semen Evaluation

Evaluation was done as described above (Rodriguez-Martinez and Barth, 2007). The pawpaw juice-coconut milk diluent that gave the better semen parameter scores of the two that were evaluated was noted for subsequent use for artificial insemination alongside the EYC extended semen.

Estrus Synchronization Protocol

Oestrus was synchronised in all the eight multiparous experimental ewes by injecting twice 5mg PGF2 α (Lutalyse®; Pharmacia & Upjohn) seven days apart as described by Leigh *et al.*, (2010). The following pointers to the elicitation of oestrus were monitored: standing heat, vigorous tail twitching, reddening of the vulva, serous vulvar discharges and mounting among pen mates.

Artificial Insemination

After adequate restraint of the ewes, 2ml of freshly collected and extended ejaculate sample containing 107 sperm cells was slowly introduced into the cervix of each ewe at 6 hours post extension using an insemination catheter guided by a small ruminant speculum (Ajala *et al.*, 1997). EYC ewes (n=4) were inseminated with EYC- extended semen evaluated at 6 hours. The remaining four ewes were inseminated with PIC9 (the better of the two pawpaw juice-coconut milk diluents) - extended semen evaluated at 6 hours

Pregnancy Diagnosis

Ewes were subjected to ultrasonography scan for confirmation of pregnancy using a portable ultrasound machine Kaixin KX2000® with a 3.5MHz transabdominal transducer at day 48 post insemination

Statistical Analysis

Descriptive statistical analysis was used. The Mean and standard error of the mean were calculated for motility, concentration, percentage morphological abnormalities (Steele, 1996). Conception rate was expressed in percentages and was calculated as the percentage of inseminations that resulted in pregnancy

(Conception rate = number of ewes that conceived / number exposed to A.I. x 100)

Differences of means were compared using one-way Analysis of Variance (ANOVA). Tukey multiple comparison was used to separate significant mean scores where appropriate. All statistical analysis was performed using SPSS 17.0 software (SPSS Inc., Chicago IL., USA). A p value less than 0.05 was considered significant.

Results

Vital parameters including rectal temperature, heart rate, respiratory rate and pulse rate of the experimental rams fell within the normal range (Table 1). The mean weight of the eight WAD ewes was 21.75 \pm 0.88 kg, while their mean age was 1.63 \pm 0.26 year. The mean weight of the two rams was 24.50 \pm 1.5 kg while their mean age was 2.05 \pm 0.25 years (Table 1). The mean scrotal circumference of the rams used was 23.25 \pm 0.35cm while mean ejaculate volume in five collections was 0.60 \pm 0.10 ml (Table 2). The semen colour observed varied from a homogenous milky to creamy white fluid. The mean pre-extended motility of the spermatozoa was 91.40 \pm 1.03% with a concentration of 216.00 \pm 22.94 $\times 10^6$ spermatozoa per ml and morphological

abnormalities of $28.33 \pm 4.00\%$ (Table 2). The pH means of the diluents PIC9, P3C7 and Egg yolk were 6.09 ± 0.02 , 6.00 ± 0.11 and 6.10 ± 0.04 while their mean after sodium citrate was added were 6.62 ± 0.09 , 6.60 ± 0.24 and 7.06 ± 0.09 respectively (Table 3).

At zero hour post extension, there were no significant differences in the spermatozoa motility scores in all the diluents ($p > 0.05$) (Fig 1)

At one hour, two hours and twenty four hours post extension, Egg Yolk citrate (EYC) (88.20 ± 0.97 , 82.40 ± 0.68 and 6.60 ± 1.03) had a significantly higher motility score compared to PIC9 (82.20 ± 1.16 , 76.80 ± 1.28 and 2.60 ± 0.68) and P3C7 (79.20 ± 1.11 , 73.40 ± 0.93 and 0.80 ± 0.37), respectively at $p < 0.05$ whereas the two test diluents had no significantly different motility scores at $p > 0.05$ (Fig 1)

At three hours, four hours, five hours and six hours, P3C7 (64.00 ± 1.41 , 52.80 ± 1.16 , 41.00 ± 0.71 and 31.60 ± 0.68 , respectively) had significantly lower motility scores compared to PIC9 (71.20 ± 0.86 , 52.80 ± 1.28 , 52.80 ± 1.28 and 44.60 ± 1.21 , respectively) and EYC (76.00 ± 1.14 ,

69.00 ± 1.30 , 61.40 ± 0.75 and 49.20 ± 0.86 , respectively) at $p < 0.05$. Also at these intervals, the PIC9 motility score was significantly lower compared to EYC at $p < 0.05$ (Fig.1).

There was a progressive reduction in motility values from zero hour to twenty four hours in all the diluents being 86.80 ± 1.66 , 85.20 ± 1.77 and 88.60 ± 1.57 at zero hour for PIC9, P3C7 and EYC respectively and reduced to 2.60 ± 0.68 , 0.80 ± 0.37 and 6.60 ± 1.03 at 24 hours post extension respectively (Fig.1).

The mean percentage spermatozoa morphological abnormalities in diluents PIC9, P3C7 and EYC, six hours post extension, were $22.21 \pm 2.99\%$, $19.84 \pm 3.78\%$ and $20.29 \pm 3.47\%$ respectively, there was no significant difference ($p > 0.05$) in the abnormalities in all the diluents (Table 4).

The oestrus synchronisation success rate in all the ewes was $95 \pm 1.89\%$, mean synchronisation - oestrus onset interval was 60.00 ± 4.54 hours while mean oestrus duration was 72.00 ± 9.07 hours (Table 5).

Both PIC9 and EYC ewe groups recorded a 50% conception rate.

Table 1: Vital Parameters for the Experimental Animals (Rams)

Parameters	*Normal values	Ram 01	Ram 02	Mean \pm SEM
Age (year)	-	1.8	2.3	2.05 ± 0.25
Weight (Kg)	-	23	26	24.50 ± 1.50
Temperature ($^{\circ}$ C)	38.0-39.6	39.0	39.4	39.20 ± 0.20
Heartrate (bpm)	70-80	79	76	77.50 ± 1.50
Respiratory rate (bpm)	16-34	24	22	22.00 ± 1.00
Pulse rate (ppm)	70-90	77	74	75.00 ± 2.00

*Khan et. al. (2010)

Table 2: Mean (\pm SEM) scrotal circumference, semen volume, colour, sperm and motility concentration of the rams and morphological abnormalities

PARAMETER	MEAN \pm SEM
Scrotal circumference (cm)	23.25 ± 0.35
Semen volume (ml)	0.60 ± 0.10
Semen colour	Milky to Creamy white
Semen concentration (10 ⁶ cell/ml)	216.000 ± 22.935
Semen motility (%)	91.40 ± 1.03
Morphological abnormality (%)	28.33 ± 4.00

Table 3: Means pH values of diluents

Diluents	MEAN±SEM of p values
Sodium citrate	8.01
Coconut milk	5.93±0.13
Pawpaw juice	5.39±0.19
P1C9	6.09±0.02
P3C7	6.00±0.11
Egg-yolk	6.10±0.04
P1C9 + Na citrate	6.62±0.09
P3C7 + Na citrate	6.60±0.24
Egg-yolk +Na citrate	6.76±0.13
*Ram Semen	5.9-7.3

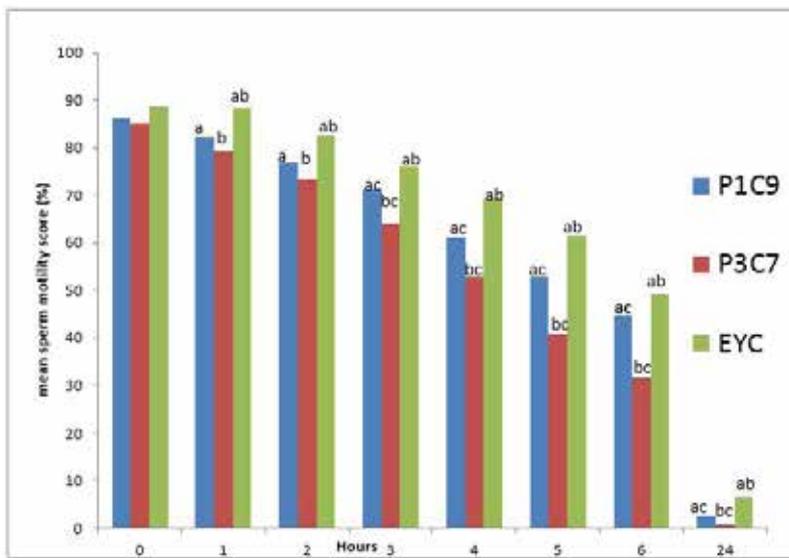
*(Source: Singh, 2005)

Table 4: Percentage mean spermatozoa morphological abnormality and progressive motility of diluents at six hours

	P ₁ C ₉	P ₃ C ₇	EYC
Percentage Morphological abnormalities	22.21±2.99	19.84±3.78	20.29±3.47
Percentage Mean progressive Motility	44.60±1.21 ^{ac}	31.60±0.68 ^{bc}	49.20±0.86 ^{ab}

Table 5: Oestrus synchronisation responses of the eight ewes

Mean Synchronisation success rate (%)	Mean Synchronisation -oestrus onset interval (Hours)	Mean oestrus duration (Hours)
95.00 ±1.89	60.00 ±4.54	72.00 ±9.07



Legends

P₁C₉- pawpaw (10 mls) + Coconut (90 mls)

P₃C₇- pawpaw (30 mls) + Coconut (70 mls)

EYC- Egg yolk citrate

Fig 1: The changes in sperm motility of ram semen in different diluents at different hours. Values with same superscript within the same hour group are significantly different at p<0.05

Discussion

The vital parameters of the experimental animals in this study were within the normal ranges showing that the animals were clinically normal (Merck manual 2010). The mean scrotal circumference obtained in this study was 23.25 ± 0.35 cm, which was in agreement with the work of Oyeyemi *et al.* (2009) who reported 23.80 ± 0.45 cm. The mean ejaculate volume collected in five trials was 0.60 ± 0.10 ml which fell within the range of 0.3-1.0 ml reported by Oyeyemi *et al.* (2009) but it was slightly lower than 0.65 ml reported by Marai *et al.* (2008). Variations observed may be due to methods of semen collection, season of the year, breed, age, body weight of animals, scrotal circumference and frequency of semen harvest which are known to affect the ejaculate volume in rams (Iheukwumere *et al.* 1990). The semen colour observed in the five collections varied from a homogenous milky to creamy white fluid which was in concordance with the findings of Moss *et al.* (1979) and Oyeyemi *et al.* (2009). The mean pH value of egg yolk citrate (7.06 ± 0.09), pawpaw juice (5.39 ± 0.19) and coconut milk (5.93 ± 0.13) in this work were slightly different from the findings of Fayomi and Oyeyemi (2010) (6.90, 5.22 and 6.06, respectively). The pH of coconut milk largely influenced the pH of diluents PIC9 and P3C7 with the pH increasing from diluents P3C7 to PIC9 as the coconut milk constituent of the diluents increased (Fayomi and Oyeyemi, 2010).

The mean pre- extended motility of the spermatozoa ($91.40 \pm 1.03\%$) fell within the range of 80-92% obtained by Hossian (2013). Also a mean pre-extended concentration of $0.22 \pm 22.94 \times 10^9$ spermatozoa per ml was within the normal range of 200 to more than 1,000 million spermatozoa/ml reported by Rodriguez-Martinez and Barth (2007).

At zero hour post extension, a slight reduction in spermatozoa motility score was observed when comparing the three diluents with the mean pre extended motility. However, there were no significant differences in all the diluents within this hour. This slight reduction was also reported by Fayomi and Oyeyemi

(2010) who worked on tomato juice citrate, pawpaw juice citrate, coconut milk citrate and egg yolk citrate. The authors attributed the pronounced reduction to a rapid pH change. The pH change in this study was not pronounced hence the reduction observed in this work could probably be related to difference in energy levels of pre and post extended semen.

At one hour and two hours post extension, EYC had a significantly higher motility score compared ($p < 0.05$) to the two test diluents. However, both test diluents at these hours had motility scores that could support fertility meaning that PIC9 and P3C7 can thrive very well with the standard EYC at these hours.

At three hours, four hours, five hours and six hours post extension, there were significant higher motility scores ($p < 0.05$) comparing PIC9 with P3C7. This showed that PIC9 was better test diluents compared to P3C7 at these hours. This could be attributed to higher constituent of coconut milk in PIC9 compared to P3C7, hence making available more energy source.

PIC9 and EYC groups, at six hours post extension, had motility scores and morphological abnormalities that met the minimum standards of 30% motility score (Schoenian, 2012, Robert and Walter 2007) and 30% morphological abnormalities (Schoenian, 2012) required for the ram. This could be attributed to favourable pH and the fat content in these diluents which could be metabolized providing an energy source (Fayomi and Oyeyemi, 2010). These motility scores recorded at six hours were higher than the 8% reported by Oloye *et al.* (2008) who used coconut milk citrate at room temperature. However, the motility scores were lower than the 60% recorded by Fayomi and Oyeyemi (2010) using coconut milk (attributable to better storage under refrigeration) but higher than 0% reported using pawpaw juice at 5°C.

There was no significant difference ($p < 0.05$) in the morphological abnormalities of all the test diluents compared with EYC at six hours. At this time, the morphological

abnormalities in all the diluents were below the value (30%) considered as standard, which conferred good fertility status on the extended semen.

At Twenty-four hours post extension, EYC and PIC9 had low motility scores but there was no significant difference ($p < 0.05$) in the motility score of all the diluents which was in contrast with the observation of Ajala *et al.* (1997) who worked with pawpaw juice. This low motility score at twenty four hours might be due to the depletion of the energy supply of the extender coupled with the environmental temperature.

This study showed that successful oestrus synchronization was achieved using prostaglandin F_{2α}. Occurrence of oestrus was 95% compared to 94% reported by Ott *et al.* 1980. According to the report of Leigh *et al.* (2010) the ewes will be in oestrus between 72-96 hours following the second injection of Lutalyse which was in concordance with the onset of oestrus in this study with mean duration of 72 hours.

The success of artificial insemination obtained in the study based on inseminating the ewes twice between 48 and 96 hours after two Lutalyse injections was good (50%). However, it has been reported (Leigh and Ajibade, 2010), that better success rate in artificial insemination is achieved by depositing semen at the bifurcation of the uterine body than at other locations such as the cervix as was done in the present study.

The conception rate result might not be solely due to the properties of the two test diluents since there are several factors that influence fertilization in assisted reproduction such as handling, storage, male factors and female factors.

The test diluent PIC9 had a motility score, percentage morphological abnormalities and conception rate close to those of the standard egg yolk citrate, which presumably, contributed to the stability observed with it. Furthermore, they both provided a medium with a pH that fell within the range (5.9-7.3) considered optimum for survivability of ram spermatozoa (Singh, 2005).

Conclusion

This work showed that a combination of pawpaw juice and coconut milk at the mixture rate of 10:90 gave motility values close to the standard egg yolk citrate within the same time interval and also compared well with EYC with regards to conception rate. Therefore, PIC9 could be recommended as an extender for ram semen stored for 6 hours at room temperature for optimal productivity

Acknowledgement

I acknowledge the technical assistance provided by Mr Akyang Philips Bassahwa and Mr Abiodun Adetomiwa of the department of Public Health and Reproduction, College of Veterinary Medicine, Federal university of Agriculture Abeokuta, Nigeria.

References

- Ajala, O.O., Oyeyemi, M.O., Akusu, M.O. and Akande, O., 1997: Survivability of caprine spermatozoa in egg-yolk citrate and pawpaw juice citrate extenders at 5oC and room temperature (28oC). In Proceedings of the 34th Annual congress of the Nigerian Veterinary Medical Association (NVMA), Nigeria. Oct: 27-31, 29-33
- Al-Merestani, M.R., Zarkawi, M. and Warden, M.F., 2003: Improving the Reproductive Efficiency, Pregnancy Diagnosis and Monitoring the Resumption of Luteal Activity in Indigenous Damascus Goats, *Reprod Domest Anim*, 38(1):36-40.
- Almquist, J. O. and Wickersham, E. W., 1962: Diluents for bovine semen. XII. Fertility and motility of spermatozoa in skim milk with various levels of glycerol and methods of glycerolization. *J Anim Sci.*, 45: 782-787.
- Anzar, M., Farooq, U., Mirza, M.A., Shahab, M. and Ahmad, N., 2003: Factors affecting the efficiency of artificial insemination in cattle and buffalo in Punjab. *Pak Vet J.*, 23: 106-113.
- Ataman, M.B. and Aköz, M. 2006: GNRH-PGF₂ and PGF₂-PGF₂ Synchronization in Akkaraman Cross-Bred Sheep in the Breeding Season. *Bull Vet*

Inst Pulawy., 50:101-104.

Bonadonna, T., Formarcoli, D. and Dozzi, G. G., 1962: The utilization of some vegetable juice for the dilution of semen. *Zoo tech and Vet.*, 16:116-118.

Campbell, J. R., Kenealy, M. D. and Campbell, K. I., 2003: The Application of Endocrinology to Selected Animals and Humans. In: *Animal Sciences: The Biology Care and Production of Domestic Animals*. 4th Edtn. Pub. McGraw-Hill co., INC NY 10020, USA: pp.196-202.

Donovan, A., Hanrahan, J. P., Lally, T., Boland, M. P., Bryne, G. P., Duffy, P., Lonergan, P., O'Neill, D. J., 2001: AI for sheep using frozen-thawed semen ISBN 1 84170 152 1: Pp. 1-43.

Fayomi, A. P., Oyeyemi, M.O., 2010: Comparative effects of different extenders on the survivability and morphological characteristics of the West African dwarf buck spermatozoa. *Proceedings of the 47th annual congress of the Nigerian Veterinary Medical Association (NVMA)*, Markurdi Nigeria,

Geoffery, H.A., David, E.N., and Harold, P. 1992. *Artificial Insemination In : Vet Reproduction and Obstetrics* sixth edition, Pp, 567

Hossain, R. R. 2013: Study of quality of native ram semen: M.V.Sc Thesis, Department of Surgery and Obstetrics, Faculty of Veterinary Science, Bangladesh Agricultural University Mymensingh. 56p

Ibe, S. N., 2004: The Role of Genetic and Livestock Breeding in Nigerian Animal Protein Self sufficiency: A case study of Day-old chicks/poults In: *Proceedings of the 9th Annual conference of Animal Science Association of Nigeria held on 13th-16th Feb 2004*; Abakaliki, Ebonyi State Nig., pp xiii-xvii.

Ihekumwumere F. C., Okere C., 1990: Effects of frequent ejaculation on semen characteristics of Nigerian Yankasa rams. *Small Rum. Res.*, 3:77 – 83.

Imasuen, J. A., Otoikhian, C. S. O., 2006: Evaluation of Growth and Reproductive Performance In Growing Boar Fed Graded Level of Dried Cocoa Pod Supplemented Rations. *J. Applied. Basic Sci.*, 4(2):139-143.

Jordan, K., M., 2005: Approaches to Improve the Ovulatory Response and Reproductive Performance

of Ewes Introduced to Rams during Seasonal Anestrus. West Virginia: West Virginia University; (2005). pp90.

Karaca F., Ataman M., B., Coyan, K., 2009: Synchronization of Estrus with Short- and Long term Progestagen Treatments and the use of GnRH Prior to Short-Term Progestagen Treatment in Ewes. *Small Rum. Res.* 81: 185–188.

Khan, C.M., Scott, L., Dana, G.A., Dave, P.A., Leo, B.J., Katherine, E.Q., et al. 2005 : *Merck's Veterinary Manual* 9th edition. Merck and Co., INC. USA, pp.2582-2583,

Leigh, O., O., Ajibade, Z. O., 2010: Conception following transcervical insemination with predetermined ejaculate concentration in synchronized West African dwarf does. *Glob Vet.* 5 (4): 239-242

Leigh, O.O., Raheem, A.K., Olugbuyiro, J.A.O., 2010: Improving the Reproductive Efficiency of the goat: vaginal cytology and vulvar biometry as predictors of estrus/breeding time in synchronized West African dwarf goat does. *Int J. Morphol.*, 28(3): 923-928.

Marai, I.F.M., El-Daraway, A.A., Fadiel, A., Abdel-Hafez, M.A.M., 2008: Reproductive performance traits as affected by heat stress and its alleviation in sheep. *Tropical and Subtropical Agroecosystems.* 8: 209 – 234.

Moss, T. A., Meltose, D.R., Reed, H.C.B., Vendeplassche, M., 1979: spermatozoa, Semen and artificial insemination, In: J.A Laing (ed.), *Fertility and Infertility in Domestic Animals*, 3rd Edition Bailliere Tindall, London, pp 262.

Noakes, D. E., Parkinson, T. J., Gray, C. W., 2001: *Artificial Insemination In: Arthur's Veterinary Reproduction and Obstetrics*, 8th edition. W.B. Saunders Company. pp764-766.

Oloye, A. A., Oyeyemi, M. O., Ajala, O. O., Akusu, M. O., 2008: Comparative study of fresh coconut milk and standard egg yolk-based semen extenders. *Tropical Veterinarian* 26 (1&2), 7-13.

Ott, R.S., Nelson, D.R. Hixon, J.E. 1980: Effect of presence of the male on initiation of estrous cycle activity of goats. *Theriogenology* 13, 183-190

- Oyeyemi, M.O., Olukole, S.G., Taiwo, B. and Adeniji, D.A. 2009: Sperm motility and viability in West African dwarf rams treated with *Euphorbia hirta*. *International Journal of Morphology*, 27(2):459-462.
- Phillips, P. H. and Lardy, H. A. 1940: A yolk-buffer pabulum for the preservation of bullsemen. *J. Dairy Sci.* 23: 399–404.
- Rodriguez – Martinez H., Barth A. D., 2007: In vitro evaluation of sperm quality related to in vivo function and fertility. In: *Reproduction in Domestic Animals Vol. I*. Edited by JI Juengel, JE Murray, M I Smith. Nottingham University Press. Nottingham U.K. pp. 39 – 54.
- Robert, S.Y., Walter, R.T., 2007: Breeding soundness examination and surgical sterilization of the ram In: *Current therapy in Large Animal Theriogenology*, Second edition, Saunders Elsevier publisher, USA. pp620-628.
- Salamon, S., Maxwell, W. M., 2006: Storage of ram semen. *Anim Reprod Sci.* 62: 77–111.
- Singh, B. K., 2005: Constituents of seminal plasma In: *Textbook of Andrology and Artificial insemination in farm animals*, Jaypee Brothers, New Delhi, India. pp 106-115.
- Sinha, S., Deka, B. C., Tamuli, M. K., Borgohain, N. 1991: Effect of extenders on quality of frozen goat semen. *Indian Journal of Animal Reprod* 12:2. 146-148
- Steele, R. G. D., Torrie, J. I. I., 1996: Principles and procedure of statistics. A biometric approach (2 edition) McGraw Hill pp. 6-15.
- Schoenian, S.: Sheep 201: A beginner's guide to raising sheep. <http://www.sheep101.info/201/ewerepro.html>. Updated December 3, 2017. Accessed April 8, 2018
- Sule, W. F., Oyeyemi, M. O., Akusu, M. O. 2007. Coconut milk as extender for West African dwarf buck spermatozoa at room temperature. *BIOKEMISTRI* 19(2): 65-67
- Wang, Y., Sharma, R.K., Agarwal, A., 1997: Effect of cryopreservation and sperm concentration on lipid peroxidation in human semen. *Urology*; 50:409– 13.
- Zemjanis R., 1970: Collection and evaluation of semen. In: *Diagnostic and therapeutic technique in animal production*. 3rd edition. The Williams and Wilkins company, Baltimore 139-180.

PARASITIC CONDITIONS OF CATS IN OSUN STATE, SOUTHWEST NIGERIA: RECORDS FROM THE HOSPITALS (2006-2015).

Ola-Fadunsin S. D., Rabiun M., Ganiyu I.A. and Hussain K.

Department of Veterinary Parasitology and Entomology, University of Ilorin, PMB 1515, Ilorin, Kwara State, Nigeria.

Abstract

Parasitism impacts negatively on the total well-being of man and animals. This study was to determine the different parasitic conditions that have been diagnosed in cats in the Veterinary hospitals of Osun State over a ten year period. During the period under review, a total of 469 cats comprising of 101 males and 368 females were presented to the hospitals. Out of these, 399 were diagnosed for helminthoses representing 85.07% (95% CI= 81.63-88.09), 168 (35.82%; 95% CI= 31.57-40.24) for flea infestation, 99 (21.11%; 95% CI= 17.59-24.98) for mange and 67 (14.29%; 95% CI= 11.34-17.68) for tick infestation. Age, sex and season were the hypothetic factors significantly ($P < 0.05$) associated with the diagnoses of helminthoses, while sex and season were significantly ($P < 0.05$) associated with flea infestation. The high prevalence of helminth infections in cats reported in this study calls for great concern as cat owners are at a risk of being infected with *Toxocara cati* (a zoonotic helminth of cats) that causes *visceral larva migrans* or human *toxocariasis* in man.

Keywords: Cats, Parasitic conditions, Retrospective study, Osun State, Nigeria

Introduction

Cats are one of the important pets of man in most parts of the world including Nigeria. Cats are kept as pets for diverse reasons and most owners keep cats for their personality and appearance (Podberscek and Blacksha 1988; Raji *et al.*, 2013). Others keep cats for rodent control but closer contact with humans is adding new dimensions of purpose (Raji *et al.*, 2013). The keeping of pets is associated with certain responsibilities such as housing, disease management and responsible pet ownership with negative consequences for public health when neglected (William *et al.*, 2002).

Cats can be infested by a wide range of parasites. Depending on the parasite species and its abundance, infestations may cause varying clinical signs in cats, from mild gastro-intestinal disorders and failure to thrive, to anemia or anorexia in more severe cases, particularly in young cats with heavy parasitic burdens (Traversa, 2012; Beugnet *et al.*, 2014). In addition, some parasites of cats have a zoonotic potential, either through close contact with parasitized animals or through exposure to a contaminated environment (Deplazes *et al.*, 2011; Beugnet *et al.*, 2014). Cats contribute significantly to contamination of the environment with their faecal droppings which are deposited in public and private places. They bury their faeces in soil, which leads to the accumulation of numerous enteric parasites in cats due to the fact that places used for defecation are often shared by several cats and this may lead to contamination of cats' paws with infective eggs (Sowemimo, 2012). Thus, this environment is a favorable habitat for parasitic infection as it protects the parasites from desiccation (Uga and Kataoka, 1995).

A major disease condition with high morbidity suffered by cats in the tropics is gastrointestinal parasitism and it has been recognized as an important public health problem in several parts of the world (Raji *et al.*, 2013).

Cats are also important hosts for many species of ectoparasites that can produce

a wide range of pathogenic effects. They are parasitized by lice, fleas, ticks and mites such as *Notoedres cati*, *Cheyletiella blakei* and *Otodectes cynotis*, which, apart from causing direct damage to the infested animal, can also infest humans (Curtis, 2012; Salant *et al.*, 2013). Mites are known to cause severe dermatitis, known as mange (Taylor *et al.*, 2007).

In Nigeria, there is little information available on the prevalence and distribution of parasites for the cat population (Sowemimo, 2012). This motivated the examination of hospital records pertaining to cat parasitic infections. Sowemimo (2012) reported on the gastrointestinal parasites of domestic cats in Ode – Irele and Oyo communities in Nigeria and Raji *et al.* (2013) documented reports on the gastrointestinal parasites of stray cats: a case study of two hospitals in Sokoto metropolis, Sokoto, Nigeria. The objective of this study was to determine the different parasitic conditions that have been diagnosed in cats in the Veterinary hospitals of Osun State, Nigeria over a ten years period.

Materials and Methods

Study area

This study was conducted in Osun State. The state is located within latitude 7° 59' N and longitude 4° 56' E in the Southwestern part of Nigeria with her administrative office is located in Osogbo. The State is characterised by a tropical wet (March – November) and dry (December – February and August) climate with a lowland tropical rain forest vegetation. Osun State is bordered in the north by Kwara State, in the east partly by Ekiti State and partly by Ondo State, in the south by Ogun State and in the west by Oyo State (Ola-Fadunsin, 2017). The mean annual rainfall, average annual temperature and annual relative humidity range from 127.8 cm to 159.8 cm, 21.1 °C to 31.9 °C and 58.7% and 79.6% respectively (National Bureau of Statistics, 2016).

Study design

A ten year retrospective evaluation from January 2006 to December 2015 was

compiled on records for cats parasitic conditions retrieved from the zonal Veterinary hospitals of the State that are located in Osogbo, Ede, Ilesa and Ikirun. The records retrieved from the database included; the date of presenting the cats to the hospital, the age, sex and the diagnoses made. The diagnosis of each parasitic condition was carried out in the hospital, based on the case history, physical examination and clinical signs. Where possible, cases were confirmed in the laboratory by faecal floatation and skin scraping using a light microscope and through the direct identification of ectoparasites using a stereomicroscope. Identification was carried out as described by Soulsby (1982).

Determination of prevalence

The prevalence of each parasitic condition of cats in the study period was calculated as the total number of the parasitic conditions diagnosed throughout the study period divided by the total number of cats presented in the hospitals throughout the period of study.

Data analysis

The retrieved data were statistically analyzed using the "Microsoft Excel 2010 and SPSS-Version 22.0" (SPSS Inc., Chicago). Descriptive statistics were conducted to estimate the prevalence using percentages in tables. The univariate analysis (chi-square) test and odds ratio with 95% confidence interval were used to determine the association between each risk factor and the parasitic condition with more than 100 cases (helminthoses and flea infestation). The odds ratios were calculated with respect to a reference category as indicated in the table. $P < 0.05$ was considered significant for the univariate analysis. Spearman's correlation was used to measure the strength of association for co-infection of parasitic conditions with $P < 0.01$ considered as the level of significance. The strength of association between parasitic conditions co-infection was measured as described by Mukaka (2012).

Results

Prevalence of parasitic conditions of cats (2006-2015)

A total of 469 cats were presented at the Veterinary hospitals visited during the period under study (2006-2015). Of the parasitic conditions reported, helminthoses was the most prevalent (399 (85.07%); 95% CI 81.63–88.09), while tick infestation was the least prevalent (67 (14.29%); 95% CI 11.34–17.68). There was a significant difference ($P < 0.05$) in the prevalence of the different parasitic conditions diagnosed (Table 1).

Risk factors associated with the occurrence of helminthoses in cats

Helminthoses was significantly higher in cats older than 1 year compared to those between 6 months and 1 year ($P < 0.05$), and those lower than 6 months although this was not significant ($P > 0.05$). Infection was significantly higher in female cats ($P < 0.05$), with the occurrence of helminthoses about four times more likely to occur in female compared to male cats. A significantly higher prevalence of the infection was recorded in the wet season ($P < 0.05$) with an odd ratio of 9.62 (4.04–27.55) (Table 2).

Risk factors associated with the occurrence of flea infestation in cats

The association between age, sex and season with the occurrence of flea infestation over the study period is presented in Table 3. Cats below 6 months of age had the highest prevalence of flea infestation compared to the other age categories. Male cats were significantly more infested with flea ($P < 0.05$) and were 5.50 times more likely to be infested compared to female cats. Flea infestation was 2.4 times more likely to be diagnosed during the dry season with the difference being significant ($P < 0.05$).

Co-infection of rabbit parasitic conditions in cats

There was a low negative correlation between flea infestation and tick infestation and it was significant. A significant low positive

correlation existed between flea infestation and mange. The correlation between tick infestation and mange was significant although negligible. There were no significant correlations between helminthoses and flea infestation, helminthoses and tick infestation and helminthoses and mange (Table 4).

Table 1: Prevalence of Parasitic conditions of cats at the Veterinary hospitals in Osun state (2006–2015).

Parasitic Conditions	Number of cases (%)	95% CI
Helminthoses	399 (85.07) ^a	81.63–88.09
Flea infestation	168 (35.82) ^b	31.57–40.24
Tick infestation	67 (14.29) ^c	11.34–17.68
Mange	99 (21.11) ^d	17.59–24.98

Different alphabetic superscripts (a,b,c) indicate significant differences (χ^2 test, $P < 0.05$) between prevalence of parasitic infestations.

Table 2: Univariate association between age, sex and season with the occurrence of helminthoses in cats presented at Osun state Veterinary hospitals (2006–2015).

Risk factors	Total cases	Cases of Helminthoses (%)	OR	95% CI	P value
Age					
< 6 months a	301	264 (87.71)	1.00		
6 months – 1 year	101	72 (71.29)	0.35	0.20–0.61	<0.01*
> 1 year	67	63 (94.03)	2.20	0.81–7.49	0.13
Sex					
Male a	101	68 (67.33)	1.00		
Female	368	331 (89.95)	4.32	2.52–7.42	<0.01*
Season					
Dry a	294	229 (77.89)	1.00		
Wet	175	170 (97.14)	9.62	4.04–27.55	<0.01*

a=Reference category; *=Significant; OR = odds ratio; CI = confidence interval.

Table 3: Univariate association between age, sex and season with the occurrence of flea infestation in cats presented at Osun state Veterinary hospitals (2006–2015).

Risk factors	Total cases	Cases of Helminthoses (%)	OR	95% CI	P value
Age					
< 6 months a	301	111 (36.88)	1.00		
6 months – 1 year	101	34 (33.66)	0.87	0.54–1.39	0.57
> 1 year	67	23 (34.33)	0.90	0.51–1.56	0.70
Sex					
Male a	368	100 (27.17)	1.00		
Female	101	68 (67.33)	5.50	3.43–8.92	<0.01*
Season					
Dry a	294	126 (42.86)	1.00		
Wet	175	42 (24.00)	0.42	0.28–0.64	<0.01*

a=Reference category; *=Significant; OR = odds ratio; CI = confidence interval.

Table 4: Spearman's correlation coefficient for the co-infection of parasitic conditions of cats at the Veterinary hospitals in Osun State (2006–2015).

	Helminthoses	Flea infestation	Tick infestation	Mange
Helminthoses	1.000			
Flea infestation	-0.040	1.000		
Tick infestation	0.070	-0.305**	1.000	
Mange	-0.040	0.344**	-0.211**	1.000

** Correlation is significant at the 0.01 level (2-tailed).

Discussion

The present study showed that the cats presented to the hospitals during the period under review were affected by four parasitic conditions with helminthoses being the most prevalent with an alarming prevalence of 85.07%. A similar high prevalence (85.5%) of helminth infections among cats was obtained in a study conducted in Ode – Irele and Oyo communities, Southwest Nigeria (Sowemimo, 2012), suggesting that helminth infection is endemic among cats in Southwestern Nigeria. Also, 80.77% prevalence has been reported among cats in Sokoto Northwest Nigeria (Raji *et al.*, 2013). In Egypt, 91.00% prevalence of helminth infections has been reported among stray cats (Khalafalla, 2011). 83.00% and 85.20% helminthoses prevalence has been reported from cats in Qatar and India by Abu-Madi *et al.* (2010) and Borthakur and Mukharjee (2011) respectively. The high prevalence recorded in our study and those of the other researchers outside Nigeria suggest that helminthoses in cats is a global issue. Sowemimo (2012) postulated that the high prevalence of helminth parasitic infections in cats is considered to be critical from the viewpoint of public health importance since some of these helminth species are responsible for several zoonotic diseases such as *visceral larva migrans* and *ocular larva migrans* caused by infection with *Toxocara cati* in humans (especially children).

Flea infestation was the most prevalent ectoparasite diagnosed in our study with 35.82% of the cats presented being infested. Similarly, flea infestation has been reported to be the most prevalent ectoparasite of cats in Florida USA (Akucewich *et al.*, 2002), Thailand

(Jittapalapong *et al.*, 2008), Albania (Xhaxhiu *et al.*, 2009), Iran (Bahrami *et al.*, 2012) and Israel (Salant *et al.*, 2013). Omonijo and Sowemimo (2017) documented the flea as the most prevalent ectoparasite of cats in their study conducted in Ekiti State, Southwest Nigeria. The dominance of flea infestations among cats in our study may be associated with the fact that environmental conditions affect the proliferation and survival of ectoparasites (especially fleas) as flea larvae development occurs in protected microhabitats that combine moderate temperatures and high relative humidity (Akucewich *et al.*, 2002). Fleas are known to possess a well-developed and much longer third pair of legs; an adaptation for jumping. This may be a reason behind its high prevalence as the parasite finds it easier to infect a large number of animals compared to other ectoparasites, because they can jump. Mites (the causative agent for mange) and ticks have been reported in cats in Nigeria, with mites being more prevalent compared to ticks (Omonijo and Sowemimo, 2017).

A higher prevalence of helminthoses was observed in cats less than 6 months old and in cats above one year. These findings corroborate reports by Sowemimo (2012), who documented that dogs below 6 months old were most infected with helminths compared to other age categories in a study conducted in southwest Nigeria and that of Raji *et al.* (2013) who reported higher prevalence of helminth infections in adult dogs compared to juveniles in a study carried out in northwest Nigeria. The high prevalence of helminth infections observed in cats less than six months old (< 6 months) may be due to the trans-mammary passage of the parasite to the kittens (Labarthe

et al., 2004), while that recorded in the older cats (>1 year) may be attributed to the hunting ability of this age group of cats (Raji et al., 2013).

In the current study, female cats were at a higher risk (4.32) of infection with helminths than male cats. This is as reported by Zibaei et al. (2007) who documented that female cats in Iran were more prone to helminth infection than their male counterparts. Our finding however is contrary to reports by Raji et al. (2013). The higher prevalence in females may be attributed to stress associated with hormonal interplays, pregnancy, parturition and lactation. Seasonal difference was significant in the diagnoses of helminth infection as more cats were diagnosed for the infection during the wet season than in the dry season. Climatic seasons are determined by the amount of rainfall, mean temperature, relative humidity, solar radiation and wind strength, which are important indices in the epidemiology of parasitic diseases in animals as these either favour or hinder the survival of parasites and their vectors, the transmission of parasitic diseases and the development of diseases in the host (Patz et al., 2000).

There was no significant difference in the prevalence of flea infestation among the age categories, although kittens (< 6 months) were more infested compared to the other categories. Kittens (< 6 months) were significantly more prone to flea infestation compared to older cats in Jerusalem, Israel (Salant et al., 2013) and northwest of Iran (Hajipour et al., 2015). The higher prevalence recorded in juvenile cats may reflect the decreased elimination of fleas as a result of less self-grooming (Salant et al., 2013).

Male cats showed a significantly higher prevalence of flea infestation compared to female cats. This finding was similar to that reported by Hajipour et al. (2015). The higher infestation of male cats with fleas may be explained by their social behaviour, which brings them into contact with a greater numbers of other cats.

Fewer diagnoses for flea infestation were made during the wet season with a significant almost four fold increase during the dry season. A higher prevalence of flea

infestation has been reported during the autumn in Israel as against the other seasons (Salant et al., 2013). Rainfall that is associated with the wet season has been considered as the most important climatic factor that negatively influences the prevalence of flea infestation in small ruminants and small mammals (Yakhchali and Hosseine, 2006; Young et al., 2015).

The low correlation between the different parasitic conditions of cats in our study shows that the disease conditions are not related in terms of etiology, transmission and vector.

Conclusion

Helminthoses, flea infestation, mange and tick infestations are the parasitic conditions that affected cats in the reviewed period. There was a significant high prevalence of helminth infection compared to other parasitic conditions reported. This shows that helminthoses is endemic among cats in Osun State, Southwest Nigeria. The high prevalence of helminth infections in cats reported in this study calls for great concern as cats owners are at a risk of infection with *Toxocara cati* (a zoonotic helminth of cats) that causes *visceral larva migrans* or human *toxocariasis* in man. Age, sex and season were the hypothetical risks associated with helminth infections and flea infestations among cats in this study.

Acknowledgments

The authors would like to thank the Director and staff of the Veterinary Division of the Ministry of Agriculture and Food Security, Osun State for granting us access to the records used for the study.

References

Abu-Madi MA, Behnke JM, Prabhaker KS, Al-Ibrahim R, Lewis JW, 2010. Intestinal helminthes of feral cat populations from urban and sub – urban districts of Qatar. *Veterinary Parasitology*, 168(3-4): 284-292.

- Akucewich LH, Philman K, Clark A, Gillespie J, Kunkle G, Nicklin CF, Greiner EC, 2002. Prevalence of ectoparasites in a population of feral cats from north central Florida during the summer. *Veterinary Parasitology*, 109: 129–139.
- Bahrani AM, Doosti A, Ahmadi-Asbchin S, 2012. Cat and dogs ectoparasite infestations in Iran and Iraq boarder line area. *World Applied Sciences Journal*, 18: 884-889.
- Beugnet F, Bourdeau P, Chalvet-Monfray K, Cozma V, Farkas R, Guillot J, Halos L, Joachim A, Losson B, Miró G, Otranto D, Renaud M, Rinaldi L, 2014. Parasites of domestic owned cats in Europe: co-infestations and risk factors. *Parasites and Vectors*, 7: 291-303.
- Borthakur SK, Mukharjee SN, 2011. Gastrointestinal helminths in stray cats (*Felis catus*) from Aizawl, Mizoram, India. *Southeast Asian Journal of Tropical Medicine and Public Health*, 42(2): 255-258.
- Curtis FC, 2012. Ectoparasites. In: *BSAVA Manual of Canine and Feline Dermatology*, 3rd Eds., Jackson H, Marsella R, BSAVA Woodrow House, Gloucester, pp: 153-163.
- Deplazes P, van Knapen F, Schweiger A, Overgaauw PA, 2011. Role of pet dogs and cats in the transmission of helminthic zoonoses in Europe, with a focus on echinococcosis and toxocarosis. *Veterinary Parasitology*, 182(1): 41–53.
- Hajipour N, Keighobadi M, Abad AMR, Golabi M, Badali A, 2015. Prevalence of flea infestation in stray cats in North West of Iran, Iran. *Biological Forum*, 7(1): 575-580.
- Jittapalapong S, Sangvaranond A, Inpankaew T, Pinyopanuwar N, Chimnoi W, Kengradomkij C, Wongnakphet S, 2008. Ectoparasites of stray cats in Bangkok Metropolitan areas, Thailand. *Kasetsart Journal (Natural Science)*, 42: 71-75.
- Khalafalla RE, 2011. A Survey Study on Gastrointestinal Parasites of Stray Cats in Northern Region of Nile Delta, Egypt. *PLoS ONE*, 6(7): 1-7.
- Labarthe N, Serrao ML, Ferreira AMR, Almeida NKO, Guerrero J, 2004. A survey of gastrointestinal helminths in cats of the metropolitan region of Rio de Janeiro, Brazil. *Veterinary Parasitology* 123: 133-139.
- Mukaka MM, 2012. Statistics corner: A guide to appropriate use of Correlation coefficient in medical research. *Malawi Medical Journal*, 24(3): 69–71.
- National Bureau of Statistics Annual Abstract of Statistics (2016). Federal Republic of Nigeria. <http://www.nigerianstat.gov.ng/pdfuploads/ANNUAL%20ABSTRACT%20VOLUME-2.pdf>
- Ola-Fadunsin SD, 2017. Prevalence, risk factors and parasitic diseases co-infection of pigs in Osun state, south-west Nigeria, 2006-2015. *Bulletin of Animal Health and Production in Africa*, 65(4): 845–53.
- Omonijo AO, Sowemimo OA, 2017. Prevalence of ectoparasites of dogs and cats in Ijero and Moba LGAs, Ekiti State, Nigeria. *Nigerian Journal of Parasitology*, 38(2): 278-283.
- Patz JA, Graczyk TK, Geller N, Vittor AY, 2000. Effects of environmental change on emerging parasitic diseases. *International Journal of Parasitology*, 30: 1395-1405.
- Podberscek A, Blacksha J, 1988. Reasons for liking and choosing a cat as a pet. *Australian Veterinary Journal*, 65: 332-333.
- Raji AA, Magaji AA, Bello MB, Lawal MD, Mamuda A, Yahaya MS, 2013. Prevalence of Gastrointestinal Parasites of Stray Cats: A Case Study of Two Hospitals in Sokoto Metropolis, Sokoto, Nigeria. *Journal of Bacteriology and Parasitology*, 4: 4:3 pages DOI: 10.4172/2155-9597.1000175.
- Salant H, Mumcuoglu KY, Baneth G, 2013. Ectoparasites in urban stray cats in Jerusalem, Israel: differences in infestation patterns of fleas, ticks and permanent ectoparasites. *Medical and Veterinary Entomology*, doi: 10.1111/mve.12032
- Soulsby E JL, 1982. *Helminths, Arthropod and Protozoa of Domesticated Animals*. 7th Eds., Bailliere: Tindall and Cassell Ltd.
- Sowemimo OA, 2012. Prevalence and intensity of gastrointestinal parasites of domestic cats in Ode – Irele and Oyo communities, Southwest Nigeria. *Journal of Parasitology and Vector Biology*, 4(1): 7-13.
- Taylor MA, Coop RL, Wall RL, 2007. *Veterinary Parasitology*. 3rd Eds., Blackwell Publishing.

Traversa D 2012. Pet roundworms and hookworms: a continuing need for global worming. *Parasites and Vectors*, 5(1): 91–110.

Uga S, Kataoka N, 1995. Measures to control *Toxocara* egg contamination in sandpits of public parts. *The American Journal of Tropical Medicine and Hygiene*, 52: 21-24.

William A, Chaudhari SUR, Atsanda NN, 2002. Prevalence of some diseases of dogs and cats at the state government Veterinary clinic in Maiduguri (Nigeria). *Pakistan Veterinary Journal*, 22(2): 56-58.

Xhaxhiu D, Kusi I, Rapti D, Visser M, Knaus M, Lindner T, 2009. Ectoparasites of dogs and cats in Albania. *Parasitology Research*, 105: 1577-1587.

Yakhchali M, Hosseine A, 2006. Prevalence and ectoparasites fauna of sheep and goats flocks in Urmia suburb, Iran. *Veterinarski Arhiv*, 76(5): 431-442.

Young HS, Dirzo R, McCauley DJ, Agwanda B, Cattaneo L, Dittmarjj K, Eckerlin RP, Fleischer RC, Helgen LE, Hintz A, Montinieri J, Zhao S, Helgen KM, 2015. Drivers of Intensity and Prevalence of Flea Parasitism on Small Mammals in East African Savanna Ecosystems. *The Journal of Parasitology*, 101(3): 327-335.

Zibaei M, Sadijadi SM, Srkari B, 2007. Prevalence of *Toxocara cati* and other intestinal helminths in stray cats in Shiraz, Iran. *Tropical Biomedicine*, 24: 39-43.

RESPONSE OF RABBIT DOES AND KITS TO DIFFERENT WEANING AGES

Ayo-Ajasa OY*, Egbeyale LT, Ekunseitan DA, Busari MO, Bajegbo YO, Fatkus QO and Oyekanmi OO.

Department of Animal Production and Health, Federal University of Agriculture, Abeokuta, Nigeria

Abstract

This study was conducted to determine the effect of weaning age on the growth performance and blood indices of rabbit does and kits. A total of thirty (30) matured rabbits comprising of twenty-four (24) rabbit does and six (6) rabbit bucks with an initial average live weight of 1,800g were used in this study. The rabbit does were assigned into four treatments with six replicates and one animal per replicate in a completely randomized design. Feed and water were supplied ad libitum throughout the experiment which lasted for ten (10) weeks. The weaning treatments were designated as T1 (Does with kits weaned at 3 weeks), T2 (Does with kits weaned at 4 weeks), T3 (Does with kits weaned at 5 weeks) and T4 (Does with kits weaned at 6 weeks). Data collected on the performance characteristics, haematological parameters and serum biochemical indices were subjected to One-way Analysis of Variance (ANOVA) in a Complete Randomized Design (CRD). The results showed that weaning age had a significant ($P < 0.05$) effect on the final weight, total feed intake and daily feed intake of the rabbit does. Does in treatment 1 had the highest final weight (2716.67g) with the least weight (1383.33g) observed in does in treatment 3. The feed intake of does in treatment 4 was the highest (8106.67g) while does in treatment 1 had the lowest feed intake (3101.00g) and also, the same trend was observed in the daily feed intake. Total weight gain was numerically highest (483.5g) in kits weaned at 5 weeks and least (423.1g) in kits weaned at 6 weeks. The results obtained on the haematological parameters showed no significant ($p > 0.05$) differences in all parameters measured in the does while Red blood cells (RBC), Basophils (BAS) and Monocytes (MON) were significantly ($p < 0.05$) affected in the kits. Serum phosphorus was highest in kits weaned at 5 weeks (17.87mg/dl). It was therefore concluded that the early weaning of litters at 3 weeks of age permitted does to reduce body energy utilization for milk production and approach body equilibrium faster than weaning at later ages and that different weaning ages had no adverse effect on the physiological status of the does and kits.

Keywords: Rabbit does, kits, weaning ages, growth performance and blood profile

*Corresponding author email: olapejuyemisi@yahoo.com

Introduction

Several attempts have been made to cushion the ever-widening protein deficiency gap (Sonaiya *et al.*, 1997) being experienced in developing countries such as Nigeria, through increased poultry production. But in recent times, the poultry industry in Nigeria has been experiencing production crises arising mainly from high feed costs which has led to the winding up of many poultry farms all over Nigeria (Mmereole, 2004). Recent outbreaks of bird flu have further worsened the protein crisis in most of the developing countries which has led to small-scale poultry farmers shifting to backyard or small scale rabbit production.

Rabbits are pseudo ruminants generally reared for their meat and for experimental purposes on farms and backyards. The advantages and potentials of rabbit production over other livestock species have been well documented worldwide (Finzi 2000; Laximi *et al.*, 2009; Odeyinka *et al.*, 2014). Lebas *et al.* (1997) stated that rabbits are reared differently in specific environments and their production has helped to improve family diets of poor rural families and also the inflow of a regular source of income. Their small body size, high prolificacy and broad feeding habits also contribute to their productivity success in smallholder farms compared to other livestock.

The optimization of the reproductive performance is one of the main factors that assure high productivity on rabbit farms (Friggens, 2003). The profitability of rabbit production as an enterprise depends on the number of rabbits kindled per doe per year and the postnatal survival of the kits. Garcia and Baselga (2002) reported that rabbit does have been successfully selected for prolificacy in recent times. They can kindle up to 8 kits or more at once. It has therefore become necessary to find ways of improving the management practices which include weaning and thus maximize the profitability of rabbit production (Mmereole, 2009).

A gradual process of introducing a mammal infant to what will be its adult diet and withdrawing the supply of its mother's

milk is referred to as weaning. Weaning kits all at once reduces the incidence of *mastitis* and other benefits such as reduced transmission of pathogens due to the restricted contact of does with their offspring, lower incidence of digestive disorders (Gidenne and Fortun-Lamothe, 2002), improvement of the condition of does and their health (Pascual, 2001; Gidenne and Fortun-Lamothe, 2002), decrease in the energy expenditure for milk synthesis and the associated systemic energy deficiency due to the shorter lactation period (Parigi-Bini and Xiccato, 1998).

Intense body energy deficit during lactation, especially in highly-productive commercial hybrids whose voluntary feed intake is often insufficient to fulfil requirements for lactation and concurrent pregnancy are very common in rabbit does (Xiccato *et al.*, 2004). Xiccato *et al.* (2001) observed a significant increase on body energy losses of reproductive rabbit does when the weaning age of their kits increased while Fernandez-Carmona *et al.* (2003) reported that age at weaning plays an important role on the growth performance of rabbit does.

Weaning in rabbits is a complex step causing dietary, environmental and psychological stress, influencing gastro-intestinal tract development, feed intake and adaptation to the weaning diet (Bhatt *et al.*, 2017). Kits are highly dependent on the output of their mother's milk and individual milk intake and body growth depend on the litter size. Until the 21st day of age the kits are solely dependent on their mother's milk and the intake of milk by the individual is affected by litter size at birth (Rommers *et al.*, 2001).

It has been reported that weaning age could affect the growth of rabbits. Early weaned kits were reported to show a lower live weight when compared to rabbits weaned at 32 days of age (Trocino *et al.*, 2001). However, Gidenne *et al.*, (2004) and Tumova *et al.*, (2006) did not prove any significant differences in live weights during the time of fattening. Also there have been conflicting reports on the effect of weaning age on mortality. While Gidenne and Fortun-Lamothe (2001, 2004) reported higher

mortality in early weaned rabbits, Trocino *et al.*, (2001) and Xiccatto *et al.*, (2003) reported that mortality was not affected by the age of weaning.

It should be noted that weaning causes some degree of stress in rabbits. This effect can be analyzed by investigating the constituents of blood that help to detect conditions of stress, which can be nutritional, environmental or physical (Aderemi, 2004). A lot of work has been carried out on the blood parameters of various domestic animals used in feeding trials on either conventional or non-conventional feedstuffs (Kral and Suchy, 2000; Ahamefule *et al.*, 2006). Despite all these studies, information on the effect of different weaning ages on rabbits' growth and physiological status is limited. Hence, this study evaluated the effect of different weaning ages on the growth performance and blood profile of rabbit does and kits.

Materials and Methods

Experimental Site

The experiment was carried out at the Rabbitry Unit of the Teaching and Research Farm Directorate, Federal University of Agriculture Abeokuta (FUNAAB), with Latitude 7°10'N, Longitude 3°2'E and altitude 76m above sea level in Odeda Local Government Area of Ogun State, Nigeria (Google Earth, 2017). The area is characterized by tropical climate with a mean annual rainfall of about 1037mm. The mean monthly ambient temperature ranges from 28°C in December to 36°C in February with a yearly average of 34°C. Relative humidity ranges from 60% in January to 94% in August with a yearly average of 82%. The vegetation represents an interphase between the tropical rainforest and the derived savannah.

Experimental Animals and Management

A total of Thirty (30) matured rabbits comprising of 24 rabbit does and 6 rabbit bucks of mixed breed with initial weights of 1800g were used in this study. Prior to arrival, the hutches were cleaned thoroughly and disinfected. On arrival, the rabbits were

given feed and water containing anti-stress medication (Maxiyield). The rabbits were allowed to acclimatize in the new environment for one week before the commencement of the experiment. The experimental animals were housed in a wooden hutch with the following dimensions: Length: 60cm, Width: 60cm, Height: 45cm for each compartment. The base of the wooden hutch was covered with wire mesh for easy removal of faeces and urine. They were supplied with clean water in concrete drinkers and fed on a commercial diet (Table 1) in concrete feeders. The animals were also fed on Tridax procumbens twice a week. The rabbit hutches were periodically cleaned to prevent a buildup of pathogens. The rabbit does were randomly selected into four (4) weaning treatments with each treatment having six (6) replicates with one animal per replicate.

Experimental Procedure

The does were mated at a mating ratio of 1:4. The does were taken to the buck hutches in the morning for mating, and the bucks were allowed to mount 3-4 times before they were withdrawn. Pregnancy tests were carried out on the does by palpating on the 14th day after service. A clean and well disinfected kindling box was provided on the 28th day for the does to make nests of fur in preparation for birth. The kits were weighed within 24 hours of birth using hand gloves and a sensitive scale. Forty eight kits from the litter obtained were allotted to four treatment groups as was the case for the does with six replicates each and two rabbit kits per replicate. The experimental treatments were:

Treatment 1 = weaning at 21 days (3 weeks)

Treatment 2 = weaning at 28 days (4 weeks)

Treatment 3 = weaning at 35 days (5 weeks)

Treatment 4 = weaning at 42 days (6 weeks)

A good hygienic environment was maintained throughout the experiment and all the necessary medications were given and

Table 1: Nutrient Composition of the Commercial Feed (As Declared)

Crude Protein (%)	16.00
Fats and Oil (%)	5.00
Crude Fibre (%)	7.00
Calcium (%)	1.60
Available Phosphorus (%)	0.45
Lysine (%)	0.75
Methionine (%)	0.36
Salt (%)	0.30
Metabolizable Energy (kcal/kg)	2450

routine management practices such as cleaning of the feeders, drinkers and sweeping of the environment were carried out. This experiment lasted 5 weeks and 6 weeks for the does and kits, respectively making a total of 11 weeks.

Data Collection

Animals in each replicate were weighed at the beginning of the experiment and subsequent weighing was done on a weekly basis to determine their weekly body weights and weight gain. Weight gain was determined as the difference in the body weight of two consecutive weighings for each replicate. Feed Intake was determined weekly through deduction of left over feed from the initial feed supplied. Feed efficiency of each animal was determined weekly as the ratio of weight gain to feed intake.

Blood collection

Blood analyses were carried out on the does and kits at the 11th and 6th weeks respectively, with three (3) animals per treatment in both cases. 5mls of blood samples were collected from the ear vein of each rabbit using syringes and hypodermic needles. Separate syringes and hypodermic needles were used for each rabbit after thorough cleaning of the ear with cotton wool soaked with xylene to dilate the ear vein. After blood collection, the ear was cleaned with methylated spirit to prevent excessive bleeding and infection. The blood samples were divided

into two sets of sterilized labeled bottles. The first set of blood samples (2.5mls) from each rabbit, was collected into a sterile sample bottle containing ethylene diamine tetra-acetic acid (EDTA) to prevent coagulation; these were used for haematological studies. The second set of blood samples was collected into sterile heparin sample bottles; these were used to determine serum parameters. The blood samples were taken to the laboratory for further analysis. Blood samples collected with EDTA were analysed for packed cell volume (PCV), white blood cells (WBC), red blood cells (RBC), neutrophils (NEUT), haemoglobin (HB), eosinophils (EOS), monocytes (MON) and lymphocyte (LYM). Samples collected in bottles without EDTA were analysed for serum total protein, globulin, albumin, cholesterol, calcium and phosphorus.

Statistical Analysis

All data collected were subjected to One-Way Analysis of Variance (ANOVA) using the General Linear Model procedure of SAS (2009). The significant differences among means were separated using Duncan's New Multiple Range Test of the statistical package at a 5% level of probability.

$$\gamma_{ijk} = \mu + \tau_i + \varepsilon_{ij}$$

Where;

γ_{ijk} = Parameter of interest (growth performance)

μ = Population Mean (overall mean)
 τ_i = Mean Effect of the *i*th weaning age
 ε_{ijk} = Random residual error

Results

The effect of different weaning ages on growth performance of rabbit does is shown in Table 2. Among all the growth parameters measured, final weight, total feed intake and daily feed intake were significantly ($P < 0.05$) affected. Does in treatment 1 recorded the heaviest ($P < 0.05$) final weights (2716.67g) while does in treatments 2 and 4 had comparable final weights and does in treatment 3 recorded the least final weights (1383.33g). The total feed intake ranged from 3101.00g to 8106.67g.

The feed intake of does in treatment 4 was found to be the highest (8106.67g) while does in treatment 1 recorded the least (3101.00g) total feed intake. A similar trend was observed in the daily feed intake of experimental animals.

Numerically, the total weight gain and daily weight gain of does in treatment 1 was observed to be the highest while does in other treatments recorded a total weight loss with does in treatment 3 having the highest total weight loss. The total body weight gain per total feed intake was observed to be positive only in does in treatment 1 while does in other treatments recorded a negative feed efficiency. Mortality of 16.67% was recorded in does in each of treatments 1 and 3.

Table 2: Growth performance of rabbit does at different weaning ages

Parameters	Treatment 1	Treatment 2	Treatment 3	Treatment 4	SEM
Initial Weight(g)	2450.00	2400.00	2200.00	2516.67	89.79
Final Weight (g)	2716.67 ^a	2333.33 ^{ab}	1383.33 ^b	2400.00 ^{ab}	220.09
Total Weight Gain (g)	266.67	-66.67	-816.67	-116.67	208.29
Daily Weight Gain (g)	9.52	-2.38	-29.17	-4.17	7.44
Total Feed Intake (g)	3101.00 ^b	4528.67 ^b	4481.00 ^b	8106.67 ^a	627.75
Daily Feed Intake (g)	110.75 ^b	161.74 ^b	160.04 ^b	289.52 ^a	22.42
Feed Efficiency	0.08	-0.01	-0.33	-0.02	0.08
Mortality (%)	16.67	0.00	16.67	0.00	0.11

^{ab} Means with different letters in a row differ significantly ($P < 0.05$)

Table 3: Growth performance of rabbit kits at different weaning ages

Parameters	Treatment 1	Treatment 2	Treatment 3	Treatment 4	SEM
Initial Weight(g)	337.40	282.60	195.70	188.70	26.20
Final Weight (g)	766.50	726.30	679.20	611.90	69.30
Total Weight Gain (g)	429.10	443.70	483.50		423.10
Daily Weight Gain (g)	9.40	11.80	13.80		12.10
Total Feed Intake (g)	797.30	1298.50	1127.90		1168.40
Daily Feed Intake (g)	22.80	37.10	28.90	33.30	4.10
Feed Efficiency	0.30	0.30	0.40	0.40	0.10
Mortality (%)	1.70	2.00	1.30	2.00	0.50

The effect of different weaning ages on growth performance of rabbit kits is shown in Table 3. All the parameters measured were not significantly ($p > 0.05$) affected. Kits weaned at 21 days recorded the highest initial weight (337.4g) with kits weaned at 28 days and 35 days weighing 282.6g and 195.7g respectively. Though non-significantly ($p > 0.05$) influenced, total weight gain was numerically highest (483.5g) in kits weaned at 35 days and least (423.1g) in kits weaned at 42 days. Total feed intake was numerically highest (1298.5g) in kits weaned at 28 days and least (797.3g) in kits weaned at 21 days. Kits weaned at 28 and 42 days recorded the same mortality (2.0%) that was higher than observed in those weaned at

21 and 35 days (1.7% and 1.3%, respectively).

The effect of different weaning ages on the haematological parameters of rabbit does with kits weaned at different ages is presented in Table 4. There was no significant ($p > 0.05$) differences observed in all the parameters measured. However, PCV values ranged from 33.00 to 37.67 with rabbit does who had their kits weaned at week 6 having the lowest PCV; Hb values ranged from 10.97 to 12.13g/dl with rabbit does that had kits weaned at week 3 having the lowest Hb value of 10.97g/dl. RBC values ranged from 5.73 to 6.27 $\times 10^{12}/L$ while the lowest WBC value of 5.30 $\times 10^9/L$ was recorded in rabbit does with kits weaned at week 3.

Table 4: Haematological parameters of rabbit does with kits weaned at different ages

Parameters	Treatment 1	Treatment 2	Treatment 3	Treatment 4	SEM
PCV (%)	34.33	37.67	34.33	33.00	1.06
Hb (g/dl)	10.97	12.13	11.23	11.27	0.40
RBC($\times 10^{12}/L$)	5.73	6.27	5.80	5.73	0.17
WBC($\times 10^3/L$)	5.30	7.93	9.30	7.73	0.67
NEUT (%)	28.67	26.67	33.00	31.33	1.80
LYM (%)	68.67	70.67	64.33	67.00	1.66
EOS (%)	0.66	0.33	0.67	0.33	1.15
BAS (%)	0.33	1.33	0.33	0.67	0.19
MON (%)	1.67	1.00	1.67	0.67	0.67

PCV-Packed cell volume, HB- Haemoglobin, RBC- Red blood cell, WBC-White blood cell, NEUT-Neutrophil, LYM-Lymphocyte, EOS-Eosinophil, BAS-Basophil, MON-Monocyte

Table 5: Shows the serum biochemical indices of rabbit does with kits weaned at different ages; no significant ($p > 0.05$) differences was found in all the parameters measured.

Parameters	Treatment 1	Treatment 2	Treatment 3	Treatment 4	SEM
T-PROT (g/dl)	6.53	7.67	7.23	5.23	0.44
ALB (g/dl)	3.23	4.33	3.47	3.23	0.20
GLO (g/dl)	3.30	3.33	3.77	2.50	0.25
CHOL (mg/dl)	62.33	74.3	75.33	59.00	4.31
GLU (mg/dl)	73.67	77.33	90.00	77.67	4.62
CAL (mg/dl)	8.90		10.47	9.83	0.56
PHOS (mg/dl)	9.17	5.50	5.97	8.13	0.77

T-PROT-Total protein, ALB-Albumin, GLO-Globulin, CHOL-Cholesterol, GLU-Glucose, CAL-Calcium, PHOS-Phosphorus.

The effect of different weaning ages on the haematological parameters of rabbit kits is presented in Table 6. There was no significant ($p>0.05$) difference found in most of the parameters measured except for the Red blood cell (RBC), Basophil (BAS) and Monocyte (MON) counts. The highest ($p<0.05$) Red blood cell value of $6.67 \times 10^{12}/L$ was recorded in kits weaned at week 4 and the lowest Red blood cell of $5.40 \times 10^{12}/L$ was recorded in kits weaned at week 3. Basophil values ranged from 0.33% to 1.67%, with kits weaned at weeks 4 and 5 having the lowest BAS values while kits weaned at week 3 had the highest ($p<0.05$) value (1.67%). Kits weaned at week 1 recorded the highest ($p<0.05$) Monocyte value (0.67%).

The serum biochemical indices of rabbit kits weaned at different ages are presented in Table 7. No significant ($p>0.05$) differences were found in all the parameters measured except for Phosphorus. The phosphorus value

ranged from 11.70 to 17.87mg/dl with the highest value of 17.87mg/dl being recorded in kits weaned at week 5 and the least value of 11.70mg/dl observed in kits weaned at week 3.

The effect of different weaning ages on the haematological parameters of rabbit kits is presented in Table 6. There was no significant ($p>0.05$) difference found in most of the parameters measured except for the Red blood cell (RBC), Basophil (BAS) and Monocyte (MON) counts. The highest ($p<0.05$) Red blood cell value of $6.67 \times 10^{12}/L$ was recorded in kits weaned at week 4 and the lowest Red blood cell of $5.40 \times 10^{12}/L$ was recorded in kits weaned at week 3. Basophil values ranged from 0.33% to 1.67%, with kits weaned at weeks 4 and 5 having the lowest BAS values while kits weaned at week 3 had the highest ($p<0.05$) value (1.67%). Kits weaned at week 1 recorded the highest ($p<0.05$) Monocyte value (0.67%).

Table 6: Haematological parameters of rabbit kits weaned at different ages

Parameters	Treatment 1	Treatment 2	Treatment 3	Treatment 4	SEM
PCV(%)	32.67	36.00	32.00	34.33	0.95
Hb(g/dl)	10.74	11.93	10.20	11.43	0.31
RBC($\times 10^{12}/L$)	5.40 ^{ab}	6.67 ^a	5.23 ^b	5.73 ^{ab}	0.23
WBC($\times 10^3/L$)	4.83	7.43	8.07	6.40	0.55
NEUT (%)	31.33	30.33	29.67	30.00	1.04
LYM (%)	66.00	68.67	67.33	69.00	0.93
EOS (%)	0.33	0.33	0.67	0.33	0.14
BAS (%)	1.67 ^a	0.33 ^b	0.33 ^b	0.67 ^{ab}	0.21
MON (%)	0.67 ^{ab}	0.33 ^b	2.00 ^a	0.00 ^b	0.30

^{a,b,c} Means in the same row with different superscripts differ significantly; PCV-Packed cell volume, HB-Haemoglobin, RBC-Red blood cell, WBC-White blood cell, NEUT-Neutrophil, LYM-Lymphocyte, EOS-Eosinophil, BAS-Basophil, MON-Monocyte

Table 7: Serum biochemical indices of rabbit kits weaned at different ages

Parameters	Treatment 1	Treatment 2	Treatment 3	Treatment 4	SEM
T-PROT(g/dl)	6.17	6.60	6.63	5.23	0.39
ALB(g/dl)	3.56	3.03	3.63	2.63	0.21
GLO(g/dl)	2.60	3.57	3.00	2.60	0.32
CHOL(mg/dl)	67.70	80.00	74.7	61.70	4.47
GLU(mg/dl)	102.00	86.00	96.7	90.30	5.45
CAL(mg/dl)	7.27	10.90	7.00	6.73	0.91
PHOS(mg/dl)	11.70 ^b	12.13 ^b	17.87 ^a	12.20 ^b	1.03

^{a,b,c} Means in the same row with different superscript differ significantly; T-PROT-Total protein, ALB-Albumin, GLO-Globulin, CHOL-Cholesterol, GLU-Glucose, CAL-Calcium, PHOS-Phosphorus

The serum biochemical indices of rabbit kits weaned at different ages are presented in Table 7. No significant ($p>0.05$) differences were found in all the parameters measured except for Phosphorus. The phosphorus value ranged from 11.70 to 17.87mg/dl with the highest value of 17.87mg/dl being recorded in kits weaned at week 5 and the least value of 11.70mg/dl observed in kits weaned at week 3.

Discussion

The highest final weights recorded with does in treatment 1 could be as a result of early weaning of the kits which allowed the does to regain the energy lost and attain higher body weights earlier than does in the other treatments. While the least weight recorded with does in treatment 3 could be due to late weaning of kits as observed by Xiccatto *et al.* (2001) who reported significant increase on body energy losses of reproductive rabbit does when the weaning age of their kits is increased. Parigi-Bini *et al.* (1992) also reported that the energy balance of lactating females is usually negative. Hence, rabbit does subjected to late weaning experience a severe energy deficit during their lactation resulting in loss of weight (Alfonso *et al.*, 2014).

The weight loss observed in this study is in accordance with the report of Xiccatto *et al.* (2004) that rabbit does are susceptible to an intense body energy deficit during lactation, especially highly-productive commercial hybrids whose voluntary feed intake is often insufficient to fulfil the requirements for lactation. The highest total and daily feed intake observed in does in treatment 4 could possibly be as a result of feeding to compensate for the energy deficit during lactation. Rabbit does in treatment 1 recorded the least total and daily feed intake which might be due to early weaning of the kits and this agrees with the findings of Xiccatto *et al.* (2004) who reported that daily feed intake in does with kits weaned early reduced due to the longer dry period. The negative feed efficiency recorded with does in treatments 2, 3 and 4 could be due to their weight losses.

The non-significant effect of different weaning ages of rabbit kits on their final weights and total weight gain could be that the early weaned kits consumed more feed to gain more energy for body growth in compensation for milk withdrawal. This result however contradicts the findings of Trocino *et al.* (2001) who observed that early weaned kits showed a lower live weight in comparison with rabbits weaned at 32 and 42 days of age.

The non-significant effect obtained on feed intake in this study is in accordance with Gidenne and Jehl (2001) and Xiccatto *et al.* (2000) who reported that weaning age did not influence feed consumption. However, it was contrary to the findings of Gallois *et al.* (2004) who observed that early weaned rabbits had a higher feed intake in comparison with rabbits weaned at 35 days of age.

Haematological parameters and serum biochemistry indices are important and reliable mediums for monitoring and evaluating the health status of an animal (Gupta *et al.*, 2007), reflect the physiological status of an animal (Iheukwumere *et al.*, 2006) and generally provide information on inflammation and the presence of stress factors (Melillo, 2007; Betancourt-Alonso *et al.*, 2011). In this study, the haematological parameters and serum biochemistry indices of the does were not influenced by the different treatments. All the haematological parameters were within the normal physiological ranges reported for rabbits (Mitruka and Rawnsley, 1977). This indicated that the different weaning ages did not have any negative effect on the blood status of the rabbit does.

The results of this study showed that in the kits, the red blood cell, basophil and monocyte counts were significantly influenced by weaning age. The values $5.23-6.67 \times 10^{12}/L$ for red blood cells obtained in this study fell within the range of $3.7-7.5 \times 10^{12}/L$ reported for healthy rabbits by Mitruka and Rawnsley (1977). According to Blood and Studdert (1999), the percentage of the volume of whole unclotted blood occupied by red blood cells is a useful prognostic indicator in dehydration or excitement when packed cell volume is

abnormally high which aids in the diagnosis of anaemia when it is low; anaemia occurs when there is reduction in RBC count in circulation, haemoglobin content and PCV. Nutrition, physical activities and volume are known to influence red blood cell counts (Swenson, 1990) and its reduction indicates anaemia. But no clinical state or anaemic condition was observed in any of the rabbits in this study.

The values for basophil counts obtained fell within the range of 1-7% reported by Research Animal Resources (RAR, 2009). The lymphocyte counts also fell within the range of 40-80% reported by Research Animal Resources (RAR, 2009) but were above the range of (53.5-65.8%) reported by Mitruka and Rawnsley (1977). A high value of serum phosphorus is an indication of soft tissue trauma and may be due to growth hormone-driven phosphate renal re-absorption as a result of the age of the animals used in the ranged experiment (Rosol and Capen, 1997).

Conclusion

This study concluded that early weaning of litters at 3 weeks of age permitted does to reduce body energy utilization for milk production and approach body equilibrium faster than weaning at later ages and that different weaning ages had no adverse effect on the physiological status of the does and kits.

References

Aderemi, F.A., 2004. Effects of replacement of wheat bran with cassava root sieviate supplemented or unsupplemented with enzyme on the haematology and serum biochemistry of pullet chicks. *Trop. Journal. Animal. Science.*, 7: 147-153.

Alfonso-Carillo, C., Garcia-Rebollar, P., De Blas C. 2014. Effect of late weaning and use of alternative cages on performance of does, suckling and fattening rabbits under extensive reproductive management. *Livestock Science*. September, volume 167; Pp 425-434.

Betancourt- Alonso M.A., Orihuela A., Aguirre V., Vazquez R., Flores I. 2011. Changes in behavioural and

physiological associated with *Taeniapisiformis* infection in rabbits (*Oryctolagus cuniculus*) that may improve early detection of sick rabbits. *World Rabbit Sci.*, 19: 21-30. Doi: 10.4995/wrs.2011.801.

Blood, D. C. and Studdert, V. P. 1999. *Saunders comprehensive veterinary dictionary*, 2nd Ed. W.B. Saunders, New York, p. 88.

Fernandez-Carmona, J., Pascual, J.J., Cervera, C., Blas, E. 2003. High-energy diets for reproductive rabbit does: effect of energy source, *Nutrition Abstract Revised*, 73: 27R-39R.

Finzi, A. 2000. Raising Rabbit for Food Security. In *proceedings of the 7th World Rabbit Congress*, Valencia, Spain 4-7 July. Volume B: 13-33.

Friggens, N.C. 2003. Body lipid reserves and the reproductive cycle: towards a better understanding. *Livestock Production Science*, 83: 209-226.

Gallois M., Gidenne T., Fortun-Lamothe L., Le Huerou-Luron I., Lalles J. P. 2004. Weaning age and development of the small intestinal mucosa in the young rabbit. *Proc. 8th World Rabbit Congress*, Puebla, Mexico, 1079-1085.

Gidenne T., Fortun-Lamothe L. 2004. Growth, health status and digestion of rabbits weaned at 23 or 32 days of age. *Proc. 8th World Rabbit Congress*, Puebla, Mexico, 1079-1085.

Gidenne, T. and Fortun-Lamothe, L. 2002. Feeding strategy for young rabbits around weaning: a review of digestive capacity and nutritional needs. *Journal of Animal Science*, 75, 169-184.

Gidenne T., Fortun-Lamonthe L. 2001. Early weaning : effect on performances and health. In: *Proc. 2nd Meeting of Workshop 3rd and 4th COST Action 848*, Godollo, Hungary, 34pp.

Gidenne, T. and Jehl, N. 2001. Bacterial biomass production in the growing rabbit according to the dietary fibre level. *Proc. 2nd Meetings of workshop 3rd and 4th COST Action 848*, Godollo, Hungary, 43.

Google Earth. 2017. Earth maps. *Onlinemapfinder.com*

- Gupta, A.R., Putra, R. C, Sani, M. Swarup, D. 2007. Haematology and Serum Biochemistry of Chital (*Axis axis*) and Barking Deit (*Muntiacus Muntiyax*) Reared in semi-captivity. *Veterinary Research Communication*, 3 1:801-808.
- Iheukwumere, F.C, Abu A.H, Ameh .M. 2006. *International Journal of Poultry Science*. 5,7,632-634.
- Kral, I. and P. Suchy, 2000. Haematological studies in adolescent breeding cocks. *Acta Veterinaria Brno*, 69: 189-194.
- Laximi, P, Ramesh G. B., Gnana, M., Ekambaram, B. and Amareswari, P. 2009. A study on the performance of fryer rabbits under different systems of rearing. *Livestock Research for Rural Development*. Volume 21, Article #118. Retrieved September 26, 2013, from <http://www.lrrd.org/lrrd21/8/laxm2111>.
- Lebas, F., Coudert, P., Rochambeau, H., and Thebault, R. G. 1997. *The Rabbit: Husbandry, Health and Production*. Food and Agriculture Organization of the United Nations, Rome, Italy. Pp. 1 – 10. Cited from: <http://www.fao.org/docrep/t1690E/t1690E00.htm>.
- Medirabbit. 2007, www.medirabbit.com/EN/Hematology/blood_chemistry.htm.
- Melillo A. 2007. Rabbit clinical pathology. *Journal. Exot. Pet Med.*, 16:135-145. Doi:10.1053/j.jepm.2007.06.002.
- Mitruka B.M., Rawnley H.M. 1977. Clinical biochemical and haematological reference values in normal and experimental animals. *Masson Publishing USA, Inc.* pp 83, 134-135.
- Mmereole, F.U.C. 2004. Cooperative haematological and serological profile of the Nigerian local and exotic chickens of southern Nigeria.
- Mmereole, F.U.C. 2009. The effects of rebreeding intervals on the performance characteristics of doe (rabbits). *Agricultura Tropica et Subtropica*. Vol: 42 (3).
- Odeyinka, S.M., Oyedele, O.J., Olosunde, A.O., Segun-Olasanmi, A.O., Asaolu, V.O., Ayeni K.S. and Onifade, C. B. 2014. Growth performance and haematological parameters of weaner rabbits fed dietary levels of soybean milk residue. *Nigerian Journal of Animal Science*. 16 (1):61-66.
- Parigi-Bini, R., Xiccato, G., Cinetto, M., Dalle-Zotte, A. 1992. Energy and protein utilization and partition in rabbit does concurrently pregnant and lactating. *Animal Production*. 55:153-162.
- Parigi-Bini, R. and Xiccato, G. 1998. Energy metabolism and requirements. In: *The Nutrition of the Rabbit*. (Edited De Blas, C., Wiseman, J.) CABI Publishing, Wallingford, UK, 103-131.
- Pascual, J.J. 2001. Recent advances on early weaning and nutrition around weaning. *Proceedings of the 2nd Meeting of workgroups 3 and 4, COST Action 848, Godollo, Hungary*, pp. 31–36.
- Research Animal Resource (RAR). 2009. Reference values for laboratory animal: normal haematological values. RAR Websites, RAR, University of Minnesota.
- Rommers, J.M., Kemp, B., Meijerhof, R. and Noordhuizen, J.P.T.M. 2001. Effect of different feeding levels during rearing and age at first insemination on body development, body composition and puberty characteristics of rabbit does. *World Rabbit Sci.*, 9. 3. 101-108.
- Rosol, T.J. and Capen, C.U. 1997. Chapter 23. In: *Kaneko J.J., Harvey J.W. and Bruss M.L. (Eds.). Clinical Biochemistry of Domestic Animals*, 5th ed., Academic Press, San Diego, California, USA, p619-702.
- SAS. 2009. Institute Incorporation. SAS Technical Report Package 234 SAS/STAT Software. The GEMOD Procedure. Release 6.09. SAS Institutes Incorporation. Cary, NC. USA.
- Sonnaiya, E.B., Atumbi, O.A., Dare, A.C. 1997. An assessment of health and production costs for small - holder poultry in the South Western Nigeria. In: *Sustainable Rural Poultry Production in Africa*. African Network for Rural Poultry Development, pp. 89–93.
- Swenson, M. J. 1990. *Duke's Physiology of domestic animals*, 9th ed. Comestock publishing Co. UK., pp. 15-40.
- Trocino, A., G. Xiccato, A. Sartori, P.I. Queaque. 2001. Effect of diet and weaning age on growth ,ceacal

fermentation and body composition of young rabbits. Proc. of the second meeting of workshop 3rd and 4th Cost Action 848, Godollo, Hungary, pp. 52-53.

Tumova, E., Zita, L., Marounek, M., Skirivanova, V. and Berladyn, A. 2006. The effect of weaning age on growth performance, nutrient digestibility and lipase activity in broiler rabbits. 3rd Rabbit Congress of the Americas, Maringa, Brasil, p.21-23.

Xicatto, G., Trocino, A. and Sartori, A. (2003). Effect of weaning diet and weaning age on growth, body composition and caecal fermentation of young rabbits. *Animal Science*, 7(1): 101-111.

Xicatto, G., Trocino A., Queque P.I., Sartori A., 2001. Effect of weaning age and parity order on reproductive performance and body balance of rabbit does. 2nd Meeting of workgroup 3 and 4 COST Action 848. Godollo Hungary.

Xicatto G., Trocino A., Sartori A., Queaque P.I. 2000. Early weaning of rabbits: effect of age and diet on weaning and post-weaning performance. In: Proc. 7th World Rabbit Congress, Valencia, Spain, C, 483-490.

GENETIC VARIATION OF LEPTIN GENE IN THREE NIGERIAN CATTLE BREEDS

Ogunnwa B E¹, Adenaike A S¹, Bello-Ibiyemi A A¹, Fafolu A O² and Ikeobi C O N¹

¹Department of Animal Breeding and Genetics. Federal University of Agriculture, Abeokuta, Nigeria.

²Department of Animal Nutrition. Federal University of Agriculture, Abeokuta, Nigeria.

Abstract

Leptin, a 16 kDa polypeptide hormone is synthesized predominantly in the adipose tissue and affects a number of processes in the body. This study evaluated the genetic variation in the leptin gene in three Nigerian cattle breeds (White Fulani, Kuri and N'Dama). A total of 45 blood samples were collected from different farms across Nigeria from which DNA was extracted and amplified. The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique was used to screen for DNA polymorphisms of the leptin gene in the DNA samples. One region of the leptin gene was amplified, a 620 bp fragment comprising part of intron 1 and exon 2. The amplified products were digested with MspI and HindIII restriction enzymes and *heterozygosity* estimates, genetic differentiation (F-statistics), genetic distances and phylogenetic relationships among the cattle breeds were determined. Analysis using MspI showed the allelic frequencies in each breed indicated that allele B in White Fulani, Kuri and N'Dama cattle with 50%, 47%, and 50% values respectively, was the most frequent. The Kuri breed had the highest observed and expected heterozygosities in the leptin locus, 56.7% and 35.2% respectively, while the White Fulani had the lowest observed homozygosity and expected *heterozygosity* at the leptin locus; 43.3% and 32.3% respectively. The Shannon's information index at the leptin locus among breeds are 46.9%, 50.8%, and 52.2% among White Fulani, Kuri and N'Dama cattle breeds respectively. Results on the inbreeding coefficient (FIS) revealed outbreeding (-0.152) with high genetic differentiation (FST) (0.573) indicating a high gene flow (Nm) rate (0.230) among breeds. The phylogenetic relationship among the three cattle breeds shows a distinct clustering for the White Fulani as a group, while the Kuri and N'Dama formed the second cluster. The divergence of the White Fulani cattle breed from the other two breeds is attributable to its zebu nature while the Kuri and Ndama clustering suggests their taurine descent.

Keywords: PCR-RFLP, Leptin gene, Nigerian cattle breeds.

Introduction

Biodiversity and specifically, genetic diversity allows for sustained genetic improvement as well as facilitation of rapid adaptation to changing breeding objectives (Shalaby *et al.*, 2016). The process of domestication has produced a rich and dynamic history, comprising of population shaping events, which have resulted in an assortment of distinct phenotypes. Variations at the DNA level contribute to the genetic characterisation of livestock populations and this may help to identify possible hybridisation events as well as past evolutionary trends (Choudhary *et al.*, 2005). The genetic variation that exists within a population for a particular trait presents opportunities for breeders to improve the animals in response to the necessary demands which cut across prolificacy, resistivity, adaptivity and conformity (Ásbjarnardóttir, 2008); although not all such traits can be improved simultaneously, and breeders will therefore have to prioritize certain traits of interest (Vinet *et al.*, 2018).

Leptin is key to maintaining the energy equilibrium by controlling feed intake and energy expenditure, as well as regulating reproductive functions and immune responses (Kulig *et al.*, 2009). In cattle, the leptin gene is located on chromosome 4, consisting of three exons and two introns. Only two out of the three exons are translated into the protein. The coding region of the leptin gene (501 nucleotides in length) is contained in exons 2 and 3, which are separated by an intron of approximately 2 kb with the leptin gene promoter region, spanning approximately 3kb (Zadworny and Kuhnlein, 1990). Leptin treatment of animals has been shown to cause body weight loss and increased energy metabolism. Therefore, it does not only cause reduced feed intake, but also potential body weight losses are enhanced due to an increased metabolic rate (Lindersoon *et al.*, 1998). The Leptin gene, previously known as the Obese (*ob*) gene, was first characterised in mice through positional cloning and shown to be conserved in various vertebrate species including cattle (Zhang *et al.*, 1994).

Several published studies have shown Leptin to accelerate the onset of puberty in rodents (Almog *et al.*, 2001), induce ovulation in eCG (equine chorionic gonadotropin)/hCG (human chorionic gonadotropin) primed rats (Roman *et al.*, 2005), stimulate aromatase protein expression and activity (Kitawaki *et al.*, 1999), increase insulin and gonadotropin-stimulated follicular progesterone, testosterone and estradiol production in a dose-dependent manner and accelerate follicular maturation by attenuating follicular atresia (Almog *et al.*, 2001). Also, there have been reports that the Leptin gene plays an important role in the regulation of feed intake, energy metabolism, growth and reproduction of cattle (Ramsay and Cranwell, 1999). Although, many polymorphic studies on the bovine Leptin gene have been reported (Lien *et al.*, 1997; Haegeman *et al.*, 2000; Choudhary *et al.*, 2005; Ásbjarnardóttir, 2008; Ahani-Azari *et al.*, 2012); there has been no report on Leptin polymorphism studies involving cattle originating from the African continent (*Bos africanus*). Thus, the focus of this investigation is to study genetic variation in the Leptin gene of the Nigerian cattle population.

Materials and Methods

Experimental site

The study was carried out at the Biotechnology Centre Laboratory of the Federal University of Agriculture, Abeokuta Nigeria. The blood samples of different breeds of cattle were collected within the south-western region of Nigeria, which includes Lagos State, Ogun State, Oyo State, Ondo State and Osun State.

Experimental animals

A total of forty-five (45) animals were used for this research comprising 15 White Fulani, 15 N'dama and 15 Kuri cattle.

DNA extraction and PCR amplification

Blood (5ml) was collected from the jugular vein of each animal into tubes containing 0.5 ml of 2.7% EDTA solution as anti-coagulant. The samples were transported

in an icebox to the laboratory where they were stored at 4°C for DNA extraction. The total blood DNA was extracted using a DNA extraction kit (NORGEN) according to the manufacturer's protocol. The primers used in the amplification of the leptin gene were designed using primer3 software, primer1F (5'-GAAACATGGTGGTCACGTGG-3') and primer1R (5'-GCTCTCTTCTCCTGTGGACA-3') to amplify a 620 bp region, spanning over a part of intron 1 and exon 2 of the bovine leptin gene. The polymerase chain reaction (PCR) was carried out for both fragments in a final volume of 25µl containing 100 M dNTPs mix, 10 pmol of primer, 2.5µl of 10x PCR assay buffer containing 1.5 mM MgCl₂, 1.0 unit of Taq DNA polymerase and 80-100 ng of the purified bovine genomic DNA. The amplification of the 599 bp region was carried out using thermal cycler PCR Hyaid Touchdown Express System (PE 9600) using the following conditions: initial denaturation for 5 minutes at 94°C followed by 35 cycles (denaturation at 94°C for 15 seconds, annealing at 62.5°C for 30 seconds and extension at 72°C for 1 minute) and a final extension at 72°C for 5 minutes.

Gel documentation and amplicon

The PCR products were separated by 1% agarose gel electrophoresis using 100 volts for one hour. The 620 bp PCR product was digested with ~10 units of MspI and HindIII restriction enzymes for 1µg of DNA, in a 10x NE buffer making a total reaction volume of 50 µl at 37°C for 15 minutes in a thermo cycler. The digested PCR products were then subjected to 1% agarose gel electrophoresis and stained with gel red.

Statistical Analyses

RFLP data was scored based on the number of bands produced in each breed. GenAlex software version 1.31 was used in the determination of gene and genotypic frequencies. The estimated gene and genotypic frequencies were subjected to an exact test to deduce possible deviation of the cattle populations from Hardy-Weinberg equilibrium.

Heterozygosity of the population (Observed (Ho) and Expected (He)) was also deduced using the software package.

Where $He = 1 - \sum p_i^2$

Genetic distances between and among cattle populations in respect to the gene of interest (leptin) was calculated using Nei's coefficient of genetic distance (Nei and Li, 1979). The dendrogram of cattle populations in relation to observed variations in the leptin gene was constructed using the Unweighted Pair Group Method with Arithmetic averages (UPGMA) generated by the software MEGA 7.0. Population differentiation and F-statistic were estimated by the POPGENE software.

Results

The digested products of Leptin with MspI and HindIII are shown in plates 1 and 2. Amplified PCR products of the bovine leptin gene genotyped A and B were digested using restriction enzyme MspI. The digested AA PCR product exhibited two fragments of 94bp and 600bp; the AB genotype exhibited four fragments at 94bp, 200bp, 500bp and 600bp, while the BB genotype exhibited three fragments of 94bp, 200bp and 500bp. Table 1 shows that the leptin/MspI locus allele A was less frequent than the B allele (0.30, 0.70), (0.29, 0.71) and (0.28, 0.72) in the White Fulani, Kuri and N'dama cattle breeds respectively. The observed frequencies of the genotypes were (0.10, 0.40 and 0.50), (0.06, 0.47 and 0.47) and (0.07, 0.43 and 0.50) for AA, AB and BB genotypes, in the white Fulani, Kuri and N'dama breeds respectively. Amplified PCR products of bovine leptin gene were digested using restriction enzymes MspI and HindIII.

The bovine leptin gene digestion with restriction enzyme HindIII produced a PCR fragment which exhibited alleles M and N in frequencies (0.41, 0.59), (0.37, 0.63) and (0.33, 0.67) for White Fulani, Kuri and N'dama breeds of cattle respectively and genotypic frequencies (0.24, 0.34, 0.42), (0.18, 0.37, 0.45), (0.20, 0.25, 0.55), (MM, MN and NN) for White Fulani, Kuri

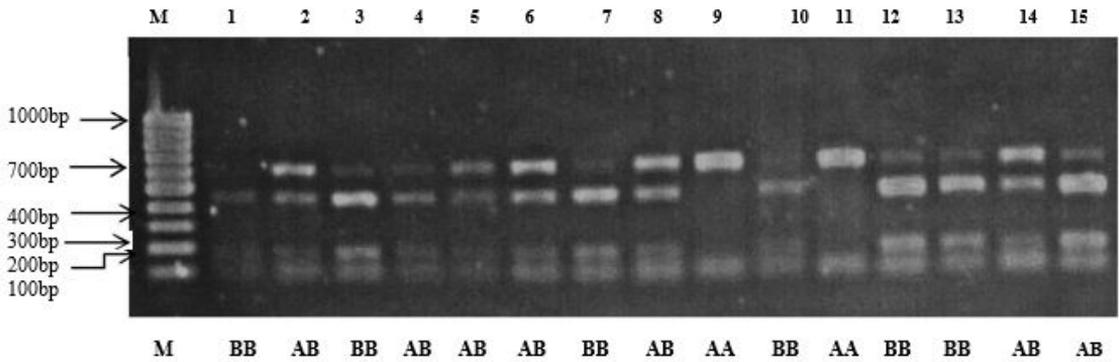


Plate 1: Showing the resolution for PCR primer I amplified products digested with MspI on 1% agarose gel.

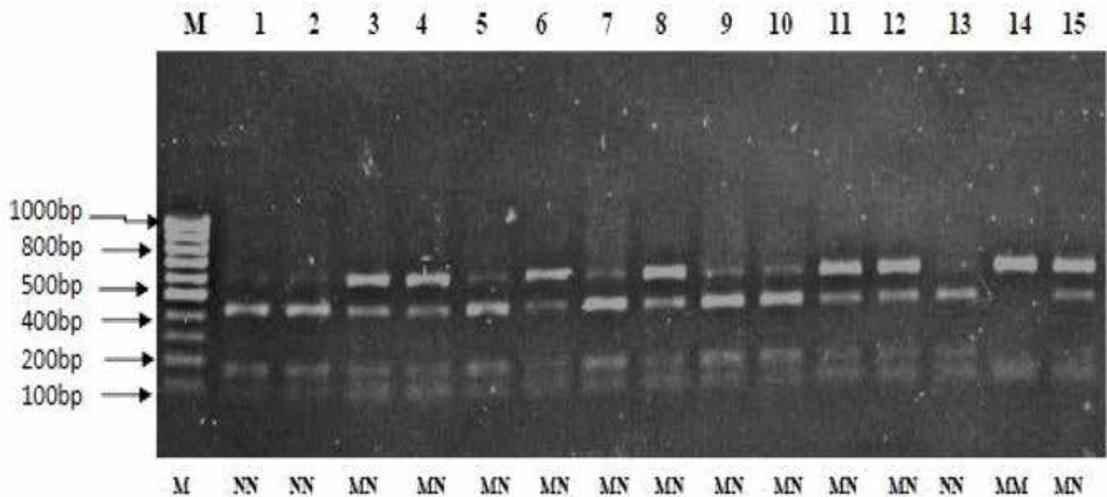


Plate 2: Resolution for PCR primer I amplified products digested with Hind III on 1% agarose.

and N'dama breeds of cattle respectively. The most frequent genotype for leptin/HindIII locus in observed population was NN for N'dama.

The observed *heterozygosity* estimates in Table 2 show the mean value for observed *heterozygosity* across the population ranged from 0.433 in White Fulani to 0.567 in Kuri breeds. The highest value of observed *heterozygosity*, (0.933) was observed in the N'dama breed with HindIII restriction enzyme while the lowest value (0.101) was observed in the Kuri for the MspI. Mean values of expected *heterozygosity* across populations ranged from 0.323 in White Fulani cattle to 0.352 in the Kuri breed. The highest value of expected *heterozygosity* (0.517) was produced by HindIII in both White Fulani and Kuri breeds of cattle, while the lowest (0.129) was produced by MspI

in the White Fulani breed.

The level of genetic diversity among the three populations as presented on Table 2 shows HO values of 43% in the White Fulani, 56% in the Kuri and 47% in the N'dama. The overall genetic diversity in the population was low, with an unbiased HE value of 32% in White Fulani compared to 35% in Kuri and N'dama. The mean Shannon index value across the population ranged from 0.469 in White Fulani to 0.522 in N'dama cattle.

The Nei's genetic distance was used to construct a dendrogram as shown in figure 1, to reveal the evolutionary relationship among the three cattle breeds using the unweighted pair-group with arithmetic means method (UPGMA). The White Fulani diverged farthest and earliest of the three breeds from their

common ancestor (0.250), while the Kuri and the N'dama were clustered together and showed a later divergence (0.220).

The genetic differentiation measure of the three Nigerian cattle breeds at the leptin gene locus were estimated using the F-statistics indices (FIS, FIT, FST) and presented in Table 3. The *heterozygosity deficit* (FIS) was -0.691 in the HindIII and 0.388 in MspI, with a mean inbreeding coefficient (-0.152 ± 0.540)

signalling outbreeding at the leptin locus among the three Nigerian cattle populations. The inbreeding coefficient of the individual relative to the total population (FIT) ranged from -0.014 to 0.844, while the genetic differentiation FST ranged from 0.401 to 0.746, with a mean genetic differentiation of 0.573 ± 0.172 . The gene flow/migrant rate ranged from 0.085 to 0.374 with a mean rate of 0.230 ± 0.144 .

Table 1: Distribution of allele and genotypic frequencies of leptin gene among three indigenous Nigerian cattle breeds.

Locus		Genotypic frequency			Allelic frequency			
Leptin								
MspI	μ	AA	AB	BB	A	B	p-value	S.E.M
White Fulani	15	0.10	0.40	0.50	0.30	0.70	0.8145	0.0032
Kuri	15	0.06	0.47	0.47	0.29	0.71	0.8145	0.0004
N'dama	15	0.07	0.43	0.50	0.28	0.72	0.8421	0.0001
HindIII		MM	MN	NN	M	N	p-value	S.E.M
White Fulani	15	0.24	0.34	0.42	0.41	0.59	0.0609	0.0001
Kuri	15	0.18	0.37	0.45	0.37	0.63	0.0517	0.0003
N'dama	15	0.20	0.25	0.55	0.33	0.67	0.0702	0.0030

Table 2: Observed and expected heterozygosities.

Breeds	Locus	N	Na	N _E	I	H _O	H _E
Leptin							
White Fulani	MspI	15	2.000	1.142	0.245	0.133	0.129
	HindIII	15	2.000	2.000	0.693	0.733	0.517
	Mean±SEM		2.000±0.000	1.571±0.429	0.469±0.224	0.433±0.300	0.323±0.194
Kuri	MspI	15	2.000	1.316	0.485	0.101	0.248
	HindIII	15	2.000	2.000	0.653	0.867	0.517
	Mean±SEM		2.000±0.000	1.605±0.386	0.508±0.183	0.567±0.367	0.352±0.075
N'dama	MspI	15	2.000	1.220	0.325	0.200	0.186
	HindIII	15	2.000	1.991	0.591	0.933	0.515
	Mean±SEM		2.000±0.167	1.611±0.174	0.522±0.082	0.478±0.168	0.351±0.164

Na = No. of different Alleles

Ne = No. of effective alleles

I = Shannon's information index

H_O = Observed Heterozygosity

H_E = Expected Heterozygosity

Table 3: F-statistics (F_{ST}, F_{IS}, and F_{IT}) for all loci and gene flow rate (N_m).

Locus	F _{IS}	F _{IT}	F _{ST}	N _m
Leptin/Mspl locus	0.388	0.844	0.746	0.085
Leptin/HindIII locus	-0.691	-0.014	0.401	0.374
Mean±SEM	-0.152 ± 0.540	0.415 ± 0.429	0.573 ± 0.172	0.230 ± 0.144

F_{IS}: Inbreeding coefficient of individual relative to the Sub-population; F_{IT}: Inbreeding coefficient of individual relative to the Total population; F_{ST}: Genetic differentiation; N_m: Gene flow rate.

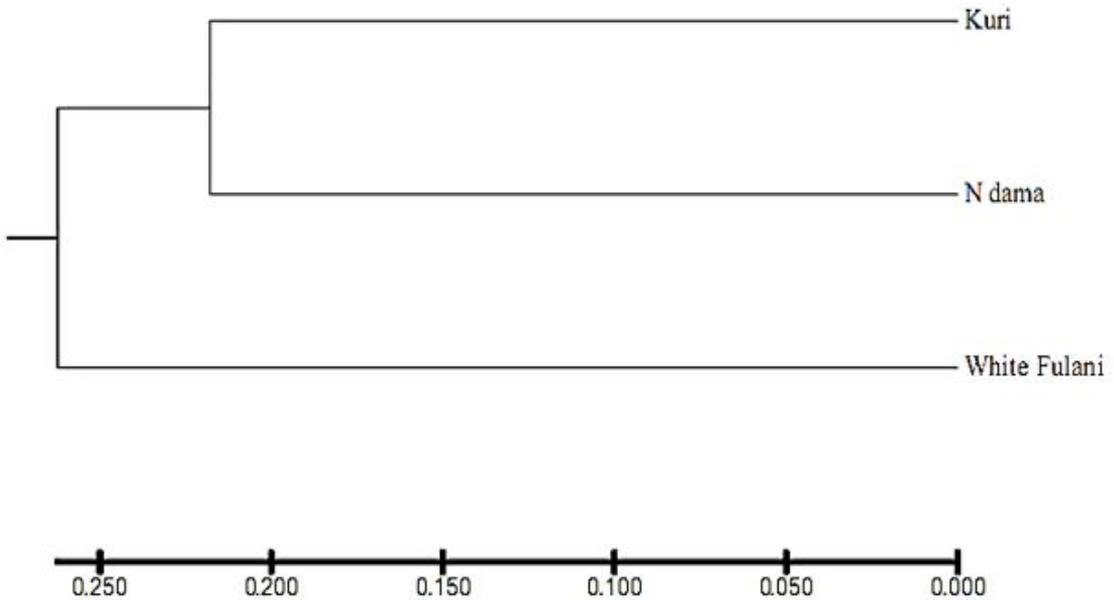


Figure 1: Phylogenetic relationship among three Nigerian indigenous cattle breeds based on Nei's genetic distance (1978) as revealed by UPGMA method.

Discussion

Genetic characterisation to assess the existing biodiversity and differences among the important cattle breeds is an essential prerequisite to facilitate conservation programmes in an effective and meaningful way (Nassiry *et al.*, 2008). Observations from this study showed that the *Leptin locus allele A* was less frequent than the *B allele*. Gibrilin *et al.* (2010) also reported a lower frequency in allele A in Holstein-Friesian cattle. The *A allele*, whose frequency range was between 0.30 and 0.28 in the three Nigerian breeds of cattle suggests that it is likely to be involved in milk performance traits. However, the range in this study is found to be lower than those reported

in earlier studies by Buchannan *et al.* (2002) and Madeja *et al.* (2004) for the leptin gene in Charolaise cattle (0.66), Simmental cattle (0.68), Brown Swiss (0.55), Polish Black and White (0.66) and Cuban buffalo (0.68). These variations in the allele frequency explain the conspicuous variation in performance between *Bos taurus*, *Bos indicus* and *Bos africanus* breeds of cattle. As reported by Carvalho *et al.* (2012), the alleles and genotypic frequencies are expected to vary between breeds and even between different populations of the same breed, considering not only the genotype/ environment interaction, but also the existence of epistatic interaction and pleiotropy of other genes.

The effectiveness of allele impact in the populations as expressed by the effective allele numbers in this study was within the expressive limits of 2.00 across all the cattle populations. This trend is in agreement with Armando and Aurora (2013) who stated that the value 2.00 is the limit of effective allele number in the dialogic genetic system. *Heterozygosity* as a population genetic parameter is the probability that any two alleles randomly selected from within the population are different.

The *heterozygosity* value is the most accurate way to measure the genetic diversity of a population, and to get an overview of the genetic variability (Madeja *et al.*, 2004). The spectrum of the most frequent alleles in the three Nigerian cattle breeds differed, while the mean range of gene diversity (HE) was in range with the values reported by Nadia *et al.* (2016) for Cuban water buffalo. The gene diversity (HE) values obtained in this study for the leptin gene were below 0.5 (50%) which suggests that the variation is low. This explanation is supported by Javanmard *et al.* (2005), who reported that *heterozygosity* values below 0.5 (50%) indicate low variation of a gene in the population. The decreased *heterozygosity* observed might probably be due to the low gene flow which led to inbreeding; and could be a potential problem at the population level. The clustering of the Kuri and the N'dama breeds could be attributable to the fact that both breeds are of taurine descent, while the White Fulani is a zebu type cattle. Also, the three Nigerian cattle breeds (White Fulani, Kuri and N'dama) were in Hardy-Weinberg equilibrium at the leptin locus. This indicates that, the effect of the forces of evolution namely; selection, migration (immigration, emigration), genetic drift, mutation is at rest in the breeding of Nigerian cattle populations, thus not warranting a change in the allele frequencies between generations.

The pairwise genetic differentiation among breeds establishes the degree at which the three Nigerian breeds of cattle admix at the population level. The White Fulani-Kuri had the highest value which indicates isolation between both breeds. The Kuri-N'dama population shows a lower value which signifies the

possibility of intermixing between both breeds. Since isolation is necessary in the genetic differentiation of a population, outbreeding at the locus (FIS) -0.152 between the cattle breeds could be an indication of increase in frequency of the heterozygote (Robertson, 1965). The mean genetic differentiation for the Nigerian adapted cattle breeds of FST (0.572) indicates a highly differentiated population of cattle with the minutest of foreign gene influx into their pool. The migrant rate estimate value $NM = 0.230$ supports this.

The White Fulani diverged farthest and earliest of the three breeds from their common ancestor (0.250), while the Kuri and the N'dama were clustered together and showed a later divergence (0.220). The divergence of the White Fulani cattle breed from the two other breeds might be attributable to the zebu nature of the breed. Genetic purity however for the White Fulani breed of cattle may have been preserved in Nigeria extensively by the resourceful activities of the Bunaji Breeders Association, an arm of the Myetti Cattle Breeders Association of Nigeria. The Kuri and N'dama however are taurine cattle which have both their origins outside Nigeria precisely in the adjacent regions of West Africa (Blench, 1998; Starkey, 1984). This allowed for a closer relationship at the leptin locus between both breeds relative to the common ancestor.

Conflict of Interest Statement

The authors declare no conflict of interest.

References

- Ahani-Azari M, Hasani S, Heidari M, Yousefi S, 2012. Genetic Polymorphism of Leptin Gene Using PCR-RFLP Method in Three Different Populations. *Slovak Journal of Animal Science*, 45:(2): 39-42.
- Almog B, Gold R, Tajima K, Dantes A, Salim K, Rubinstein M, Barkan D, Homburg R, Lessing JB, Nevo N, Gertler A, Amsterdam A, 2001. Leptin attenuates follicular apoptosis and accelerates the onset of puberty in immature rats. *Molecular Cell Endocrinology*, 183: 179-191.

- Armando C and Aurora G, 2013. Allelic Diversity and Its Implications for the Rate of Adaptation. *Genetics*, 195: 1373–1384.
- Ásbjarnardóttir MG, 2008. Genetic variation within the Icelandic cattle breed. Assessment using microsatellites and analysis of single nucleotide polymorphisms in the Leptin. Visited June 14, 2016 from https://www.agrogen.is/wp-content/uploads/2017/03/MSc_ritgerd_KYR_MGA_041208.pdf
- Blench RM, Maitlan P, De Jode A, Gherzi E, Di Domenico C, Hall S, Sambo NA, 1998. West African Dwarf Shorthorn cattle in Nigeria: History, Distribution and Productivity. In *Des Taurins au Cameroun et Nigeria*. Paris: ORSTOM/IEMVT, Maisons-Alfort. eds. C. Seignobos and E. Thys. 249-292.
- Buchanan FC, Fitzsimmons CJ, Van Kessel, AG, Thue TD, Winkelman-Sim DC, Schmutz SM, 2002. Association of a missense mutation in the bovine leptin gene with carcass fat content and leptin mRNA levels. *Journal of Genetic. Selection and Evolution*, 34: 105-116.
- Carvalho TD, Siqueira T, Junior RA, Medeiro S, 2012. Association of polymorphisms in the leptin and thyroglobulin genes with meat quality and carcass traits in beef cattle. *R. Bras Zootec.* 41: 2162 – 2168.
- Choudhary V, Pushpendra K, Tarun KB, Bharat B, Arjava, S. 2005. DNA polymorphism of Leptin gene in *Bos indicus* and *Bos taurus* cattle. *Genetics and Molecular Biology*, 28: 740-742.
- Giblin L, Butler ST, Kearney BM, Water SM, 2010. Association of bovine leptin polymorphisms with energy output and energy storage traits in progeny tests Holstein – Friesian dairy cattle sires. *BMC Genetics*. 11: 73.
- Haegeman A, Van Zevenen, A, Peelman LJ, 2000. New mutation in exon 2 of the bovine Leptin gene. *Animal Genetics*. 31(1): 79.
- Javanmard A, Asadzadeh N, Banabazi MH, Tavakolian J, 2005. The allele and genotype frequencies of bovine pituitary specific transcription factor and Leptin genes in Iranian cattle and buffalo populations using PCR-RFLP. *Iranian Journal of Biotechnology*. 3: 104-108.
- Kitawaki J, Kusuki L, Koshiba H, Tsukamoto K, Honjo H, 1999. Leptin directly stimulates aromatase activity in human luteinized granulosa cells. *Molecular Human Reproduction*, 8: 708-713.
- Kulig H, Kmie M, 2009. Association between Leptin Gene Polymorphisms and Growth Traits in Limousin Cattle. *Russian Journal of Genetics*, 45(6): 738-741.
- Lien S, Sundvold H, Klungland H, Vage DI, 1997. Two novel polymorphisms in the bovine obesity gene (OBS). *Animal Genetics*, 28: 245.
- Lindersoon M, Andersson-Eklund L, De Koning DJ, 1998. Mapping of Serum Amylase-I and Quantitative Trait Loci for Milk Production Trait to Cattle Chromosome 4. *Journal of Dairy Science*, 81: 1454-1461.
- Madeja Z, Adamowicz T, Chmurzynska A, Jankowski T, and Melonek J, 2004. Effect of Leptin Gene Polymorphisms on Breeding Value for Milk Production Traits. *Journal of Dairy Science*, 87: 3925-3927.
- Nadia MM, Elizabete C, Alina MV, Giselle P, Regina CB, Severino BPB, Odalys UR, Manoel A GF 2015. Genetic relationships between Cuban and Brazilian buffaloes (*Bubalus bubalis*) by microsatellite markers *Rev. Salud Anim.* 37(3): 152-163.
- Nassiry MR, Shahroudi FE, Moussavi AH, Sadeghi B, Javadmanesh A, 2008. The diversity of leptin gene in Iranian native, Holstein and Brown Swiss cattle. *African Journal Biotechnology*, 7(15): 2685-2687.
- Ramsay TG and Cranwell PD, 1999. A review - Leptin: A regulator of feed intake and physiology in swine. *Proceeding of the Seventh Biennial Conference of the Australasian Pig Science Association (APSA)*, 28 November - 1 December, Adelaide, Australia, pp 157-170.
- Robertson A, 1965. The interpretation of genotypic ratios in domestic animal populations. *Animal production*, 7: 319 – 324.
- Roman EA, Ricci AG, Faletti AG, 2005. Leptin enhances ovulation and attenuates the effects produced by food restriction. *Molecular and Cellular Endocrinology*. 24: 33-41.

Shalaby SIA, Heba, Hozyen F and Awasthi DK 2016. Molecular markers in farm animals: Advanced tools for evaluation of genetic diversity. Department of Animal Reproduction and Artificial Insemination, Veterinary Research Division, National Research Centre, Egypt. Research article Visited October 11, 2017 from <https://journalijcir.com/sites/default/files/issue-files/IJCIR-A-00396.pdf>.

Starkey PH, 1984. N'Dama cattle – a productive trypanotolerant breed. *World Animal Review* 50: 2–15.

Zadworny D and Kuhnlein V, 1990. The Identification of the Kappa Casein Genotype in Holstein Dairy Cattle Using Polymerase Chain Reaction. *Theor. Appl. Genet.* 80: 631-634.

Zhang Y, Proenca R, Maffe M, Barone M, Leopold L, Friedman JM, 1994. Positional cloning of the mouse obese gene and its human homologue. *Nature*, 372: 425-432.

HIGH QUALITY CASSAVA PEEL (HQCP) AS A REPLACEMENT FOR MAIZE IN THE DIET OF AFRICAN CATFISH (*CLARIAS GARIEPINUS*) JUVENILES.

Orisasona O¹, Olaegbe A T¹, Olanrewaju A N² and Kareem O K³

¹Department of Animal Sciences, Obafemi Awolowo University, Ile-Ife, Nigeria

²Federal College of Freshwater Fisheries Technology, P.M.B 1060, Maiduguri, Nigeria

³Department of Aquaculture and Fisheries Management, University of Ibadan, Nigeria.

Abstract

The high cost and scarcity of most conventional fish feed ingredients, such as maize has necessitated the search for cheap, accessible and readily available non-conventional nutrient sources. High quality cassava peel (HQCP) is a meal from processed cassava peels which hitherto, has been a major waste with attendant environmental problems. This study investigated the growth response and economic performance of using HQCP meal as a replacement for maize in the diet of *Clarias gariepinus*. The proximate composition and metabolizable energy of HQCP were determined using standard methods. Five diets (CP1, CP2, CP3, CP4 and CP5) were formulated with HQCP meal replacing maize at 0%, 25%, 50%, 75% and 100% inclusion levels, respectively. *Clarias gariepinus* (Mean weight 8.30 ± 0.12 g) were randomly distributed at a stocking density of 5.54 gL⁻¹. Diets were fed to triplicate groups of fish at 5% body weight daily for 84 days. The Mean Weight Gain (MWG), Feed Conversion Ratio (FCR), Specific Growth Rate (SGR) and Survival rate (%) were determined using standard methods. Packed Cell Volume (PCV) and White Blood Cell (WBC) were determined. The cost of feed, Profit index (PI) and Incidence of cost (IC) were also calculated. Data were subjected to ANOVA at $p < 0.05$. Crude protein, crude fibre, ash, ether extract of HQCP meal was 5.47%, 3.88%, 10.07% and 2.16 respectively, with a metabolizable energy of 2200 kcal. MWG ranged from 51.39g in CP2 to 54.09g in CP1, but showed no significant difference ($P > 0.05$) between treatments. Similarly, there was no significant difference ($P > 0.05$) in FCR and SGR due to treatments. The value of fish produced was highest (37.86) with CP1 and least (35.17) with CP2, while PI and IC values were better in fish fed HQCP diets. The growth parameters, value of fish produced and incidence of costs in this study suggested that high quality cassava peel can successfully replace maize in the practical diets of *Clarias gariepinus*.

Keywords: Cassava peel, processing, maize, *Clarias gariepinus*, economic indices, growth

Introduction

The high cost and scarcity of conventional feed ingredients such as maize, have been an impediment to sustainable aquaculture development especially in developing countries. This is mainly due to the increasing demand for this class of ingredients for human food and livestock feeds (Nwanna, 2003). According to Gabriel *et al* (2007), feed cost accounts for over 60% of the total investment in aquaculture production. This impacts on the production capacity and the profit margins of fish farmers, hence, the concerted efforts at finding alternative non-conventional nutrient sources in aqua-feed production.

Cassava (*Manihot esculenta*) is widely valued as a low-cost carbohydrate source (Hillocks, 2002). Murugan *et al* (2012) referred to it as the most important source of calories in the tropics after rice and corn, as it provides energy nourishment to over 500 million people worldwide. Its nutritional importance is derived from the roots and leaves, which make up 50% and 6%, respectively of mature plants. Cassava roots (referred to as an energy storehouse), made up of bark, peel and edible cortex, contain 80% to 90% carbohydrate on a dry weight basis (Gil and Buitrago, 2002) of which 80% is starch. The peels make up 10-13% of the roots by weight and contain 5% protein on dry matter basis and appreciable amounts of minerals (Tewe and Kasali, 1982). Nigeria is presently the highest producer of cassava in the world, with a production of over 54 million metric tonnes in 2014 (FAOSTAT, 2014). It is estimated that a tonne of cassava produces 250-300kg peels (FAO, 2001) therefore; about 13 million metric tonnes of cassava peels are produced annually in Nigeria. ILRI (2015a) reported that approximately 98% of Nigeria's cassava peels are wasted yearly. This is due to constraints associated with drying and safety concerns with regard to hydrocyanide and mycotoxins-related food poisoning. Cyanide contents ranging from 10 to 500 mg HCN equivalents/kg dry weight in roots, phytate, tannin, polyphenols, oxalate, and saponins that can reduce nutrient bioavailability have been

reported (Siritunga and Sayre, 2003; Sarkiyayi and Agar, 2010).

Attempts at utilizing cassava peel meal as an energy substitute in tilapia and catfish diets have not been encouraging. A decrease in weight gain, feed intake and feed efficiency was reported when more than 10% of cassava peel was used to replace maize in the diet of Nile tilapia (Ugwu *et al*, 2004). Similar results have been reported in other assessments with same species (Adewale, 2013; Ubuaya and Ezeronye, 2008; Tachia *et al*, 2016). Fagbenro and Arowosoge (1991), compared the suitability of meals from cassava peel, yam peel and maize shafts as energy sources in the diet of *Clarias isheriensis* and reported that the least performance was recorded in fish fed diets containing cassava peel meal. The results obtained from all these studies were attributed to the cyanide content in peels which is said to impact on palatability and feed intake thus reducing nutrient utilization (Saka and Nyirenda, 2012).

Cassava peels have been subjected to processing methods like soaking, cooking, fermentation and drying to improve its nutritional value. Of all the methods, Adesehinwa *et al* (2011) reported drying as the best method, although this may take 2-3 days in sunlight (This is however impossible during the rainy season when peels are left to rot in heaps or set on fire, resulting in pollution). Recently, cassava peels has been processed through grating, pressing, sieving and drying into a feed ingredient called High Quality Cassava Peel (HQCP) by the International Livestock Research Institute (ILRI, 2015b) for effective utilization in animal diets. This product considered as a safe, high quality and hygienic feed ingredient contains 6.7% crude protein, 8.3% crude fiber, 7.7% ash, 1.2% ether extract, 79.6% NFE and reduced HCN levels (Okike *et al.*, 2015). Considering the increased pressure on the use of maize by both increasing human population and livestock feed millers, trials on the use of HQCP as energy source in growing pigs revealed a 75% replacement of maize did not compromise animals' performance (Adesehinwa *et al*, 2016). It is important to

take advantage of this value chain improvement in fish feed production with the aim of reducing feed cost. This study is therefore aimed at determining the effect of replacing maize with HQCP as an energy source on the nutrient utilization, growth, hematological and economic performance of *C. gariepinus*.

Materials and Methods

Experimental diets

HQCP was obtained from the International Livestock Research Institute (ILRI) Ibadan. Six isonitrogenous diets were formulated using the Pearson square method, with HQCP replacing maize at 0, 25, 50, 75 and 100% levels (Diets CP1, CP2, CP3, CP4 and CP5 respectively) as shown in Table 1. All the ingredients were reduced to fine particle size in a hammer mill and the required quantities weighed out using an electronic weighing scale, and properly mixed in a Hobart A200 Mixer. Each diet was pelletized using a 2mm die mesh, with starch as binder. Pellets were sun-dried and packed into well labelled plastic bags and stored in a cool and dry place until ready to use.

Experimental Fish

Fingerlings of *C. gariepinus* obtained from a reputable fish hatchery, were transported to the Wet Lab of the Department of Animal Sciences, Obafemi Awolowo University, Nigeria. The fish were acclimatized to laboratory conditions for 14 days, during which they were fed a commercial diet (40% crude protein). After acclimatization, 20 fingerlings (Mean weight: 8.31 ± 0.01 g) were randomly stocked into each of the fifteen 30L capacity aquaria at 5.54g/L.

Complete water exchange was carried out weekly in the experimental units. Dissolved oxygen, temperature and pH were monitored throughout the feeding trial.

Feeding

Each of the five experimental diets (CP1, CP2, CP3, CP4 and CP5) was fed to triplicate groups of *C. gariepinus* at 5% body

weight given in two instalments twice daily (7.30hr - 8.00hr and 16.30hr - 17.00hr) for 84 days. Fish were weighed bi-weekly per tank using an electronic top-loading balance (OHAUS corporation model:V21PW15).

Chemical analysis and measurements

The HQCP and five experimental diets were analyzed according to procedures described by A.O.A.C (2005) for proximate compositions. The HCN in HQCP was determined using methods described by Bradbury *et al* (1991).

At the start of the feeding trial, three fish were randomly selected for initial proximate and mineral analyses. Fish were sacrificed, oven-dried and ground into powdery form for analysis as described earlier. This was repeated at the end of the trial with three fish randomly selected per treatment and analyzed also for proximate and mineral composition.

At the end of the 84 day nutritional experiment, growth and nutrient utilization parameters were determined using bi-weekly averages determined according to Castell and Tiews (1980) as follows:

Feed intake (g)

Sum of feed fed during experimental period.

Weight gain (g)

Given as Final weight of fish (W2) less Initial weight (W1)

$$\text{Weight gain (g)} = W2 - W1$$

Average daily weight gain (g)

This is given as the weight gain divided by feeding days.

$$\text{Average daily weight gain (g)} = \frac{\text{Weight gain}}{\text{days of feeding}}$$

Specific growth rate (SGR)

$$\text{Specific growth rate (SGR)} = \frac{[\text{Log}_e W2 - \text{Log}_e W1]}{[T2 - T1]} \times 100$$

Where W_2 = final weight,
 W_1 = initial weight
 \log_e = Natural logarithm

$T_2 - T_1$ = experimental period in days.

Food conversion ratio (FCR)

$$FCR = \text{Feed Intake (g)} \div \text{Weight gain (g)}$$

Protein efficiency ratio (PER)

It was calculated as:

$$PER = \text{Mean weight gain} \div \text{Protein intake}$$

Where, Protein intake = Feed intake \times % Protein in diets

Survival rate (%)

$$\text{Survival rate} = [\text{No. of fish at } T_2 \div \text{No. of fish at } T_1] \times 100$$

Blood samples were collected after tranquilizing fish with 150mg/l solution of tricaine methanesulphonate (MS-222; Sigma Chemical co. St. Louis, MO, USA) as described by Wagner *et al.* (1997), from the caudal vein of the fish using plastic syringes (2ml) and 21Swg needle into labeled bottles with Ethylenediamine tetraacetic acid (EDTA) as an anti-coagulant.

Initial samples were collected by randomly selecting three fish before the start of the experiment, while three fish were randomly selected for blood collection from each treatment at the end of the experiment. The blood samples were analyzed for Packed Cell Volume (PCV), Hemoglobin (Hb), Red Blood Cells (RBC) and White Blood Cells (WBC) as described by Johnson *et al.* (2002).

The hematocrit value was calculated based on the following formula:

$$PCV = \frac{\text{Height of packed red cells (mm)}}{\text{Height of packed red cells and plasma (mm)}} \times 100$$

Where PCV = Packed cell volume.

$$\text{Haemoglobin con } gL^{-1} = (\text{Absorbance of sample} \div \text{Absorbance of standard}) \times 100$$

Mean Corpuscular Haemoglobin Concentration $MCHC \text{ } gL^{-1} = \text{Haemoglobin \%} \div \text{Haematocrit volume \%} \times 100$

$$\text{Mean Corpuscular Haemoglobin } MCH \text{ } \mu g \text{ cell}^{-1} = \{\text{Haemoglobin (g\%)} \div \text{Erythrocyte (millions } mm^{-3})\} \times 10$$

$$\text{Mean Corpuscular Volume } MCV \text{ } \mu m^3 = \text{Haematocrit volume} \div \text{Erythrocyte (millions } mm^{-3}) \times 10$$

$$RBC \text{ (} mm^{-3} \text{)} = (N \times 5 \times 10 \times 200)$$

Where; N is the number of cells in 5 squares. 5 is the multiplication factor to give the number of cells in 1mm². 10 is the multiplication factor to bring the depth of the chamber from 0.1 to 1mm. 200 is the dilution factor.

White Blood Cell Count (WBC)

$$\text{Leucocyte } mm^{-3} = LC \times 500$$

Where, LC is the number of cells in 4mm² squares and 500 = dilution and volume correction factor.

Economic analysis

The economic analysis of feeding *C. gariepinus* with HQCP as a replacement for maize was carried out with emphasis on the profit index (P.I.) and incidence of cost (I.C) determination (Vincke, 1969) and feed cost in relation to feed conversion.

$$P.I. = \text{Value of fish produced (NGN/kg)} / \text{Cost of feed used in production (NGN/kg)}$$

$$I.C. = \text{Cost of feed used in production (NGN/kg)} / \text{Total weight of fish produced (kg)}$$

The cost of feed was based on the prevailing market prices at the feed mills, while the value of fish was based on the selling price of fish/kg (NGN700/kg) in fish markets around Ibe at the end of the experiment.

Statistical analysis

Data resulting were subjected to one-way Analysis of Variance at $p < 0.05$ (Using the Statistical Package for Social Sciences Version 20, IBM corporation, New York, USA). Duncan's Multiple Range Test (DMRT) was used to separate differences between means.

Results and Discussion

The crude protein of 5.47% for HQCP (Table 2) and metabolizable energy of 2200 kcal/kg in this study were lower than 8.75% and 3422 kcal/kg respectively reported for maize (Ape *et al.*, 2016). However, cassava peel had more ash (10.07%) and crude fibre (3.88%) than the 2.19% for ash and 2.4% for crude fibre reported for maize. The variation in the nutrient composition of maize and HQCP is responsible for the slight reduction in the crude protein and increase in the ash contents of the experimental diets with increased inclusion of HQCP as presented in table 2. The dietary fibre content ranged from 5.55% in CP3 to 6.75% in CP5 and falls within the recommended range

for fish diets (Gatlin, 2010). The HCN content of 6.21mg/kg recorded for HQCP in this present work is far below the permissible level of 100 ppm (Okike *et al.*, 2015). This is a clear indication of the effectiveness of the innovative processes of grating, dewatering, fermenting and sun-drying in the reduction of the cyanide level in cassava peel.

The final weight of the experimental fish ranged from (59.67g) in CP2 to (62.29g) in CP1 and showed no significant difference ($P > 0.05$) among treatments (Table 3). Similarly, there was no significant difference ($P > 0.05$) in the Mean weight gain with values ranging from 51.39g in CP2 to 54.09g in CP1. The feed intake was marginally high in CP1 (65.27g) and lowest in CP4 (59.08g). The protein efficiency ratio ranged from 3.11g in CP2 to 3.37g in CP4. There was no significant difference in Feed conversion ratio among the treatments. The feed intake showed a direct relationship with the mean weight gain of the experimental fish in this study. The growth performance and the nutrient utilization of *Clarias gariepinus* fed graded levels of HQCP showed no

Table 1: Gross composition of experimental diets fed to *Clarias gariepinus* juveniles for 84 days

Ingredients	Treatment				
	CP1	CP2	CP3	CP4	CP5
Fish meal	31.39	31.39	31.39	31.39	31.39
Soya bean meal	31.39	31.39	31.39	31.39	31.39
Maize	32.23	24.18	16.11	8.06	0
HQCP	0	8.05	16.12	24.17	32.23
Dicalcium phosphate	0.5	0.5	0.5	0.5	0.5
Salt	1.0	1.0	1.0	1.0	1.0
Vitamin premix	1.0	1.0	1.0	1.0	1.0
Starch	1.0	1.0	1.0	1.0	1.0
Lysine	1.0	1.0	1.0	1.0	1.0
Palm oil	0.5	0.5	0.5	0.5	0.5
Total	100	100	100	100	100
Calculated composition					
Crude Protein (%)	39.31	39.03	38.74	38.46	38.18
Fiber (%)	2.90	3.05	3.20	3.35	3.51
Fat (%)	4.38	4.23	4.11	3.98	3.85
Energy (kcal/100g)	276.97	267.06	256.92	247.10	237.27

Table 2: Proximate composition of HQCP in experimental diets

Proximate Composition (%)	HQCP	Treatment				
		CPI	CP2	CP3	CP4	CP5
Crude protein (0%)	5.47	38.89	38.86	38.72	37.76	37.65
Fat (%)	2.16	6.55	6.76	6.45	6.56	6.89
Crude fiber (%)	3.88	5.67	5.56	5.55	5.75	6.75
Ash (%)	10.07	7.42	9.02	8.77	9.97	10.31
Moisture (%)	12.32	7.06	8.06	9.35	7.59	7.38
Nitrogen free extract (%)	66.1	34.41	31.74	31.16	32.37	31.02
*HCN (mg/kg)	6.21	-	-	-	-	-

*Cyanide content only determined for High quality cassava peel meal

Table 3: Performance of *Clarias gariepinus* juvenile fed various inclusion levels of HQCP meal as replacement for maize

Parameter	Treatment					SEM
	CPI	CP2	CP3	CP4	CP5	
Initial Wgt (g)	8.22±0.02	8.27±0.02	8.29±0.00	8.26±0.01	8.30±0.02	0.01
Final Wgt (g)	62.29±0.30	59.67±2.20	60.50±0.26	60.76±0.30	61.44±1.26	0.73
Mean Weight Gain (g)	54.09±0.30	51.39±2.22	52.21±0.26	52.50±0.29	53.13±1.24	0.74
ADWG (g)	0.64±0.00	0.61±0.02	0.62±0.00	0.62±0.00	0.63±0.01	0.03
Feed Intake (g)	65.27±0.94	59.37±3.98	59.54±4.83	59.08±4.53	61.20±3.41	1.48
FCR	1.12±0.01	1.15±0.03	1.14±0.09	1.20±0.00	1.19±0.05	0.02
SGR	2.44±0.00	2.39±0.06	2.35±0.00	2.37±0.00	2.38±0.02	0.01
Survival (%)	81.33±1.33	71.66±1.33	81.33±0.66	77.66±2.33	80.00±0.00	1.09

Means with the same superscript along row are not significantly different ($P>0.05$)

MWG = Mean weight gain, ADWG= Average daily weight gain, FCR= Feed conversion ratio, SGR= Specific growth rate, PER= Protein efficiency ratio

significant difference irrespective of the level of inclusion in the diets. Similar results were reported when Carp was fed cassava root meal as a replacement for maize grain, with no detrimental effects on the final weight, final length, feed conversion ratio, condition index and survival (Lacerda et al., 2005). This differs from the results in an earlier report (Akegbejo-Samson, 1999) where reduced growth was recorded when cassava flour meal was used to replace 33-100% of maize grain in the diet of *Clarias gariepinus* fingerlings. In another study, (Dada et al., 2013), cassava peel meal was optimal at 24% level of replacement for maize in the diet of Nile tilapia. Considering the marked reduction in the HCN content of HQCP, the marginal decrease in weight gain in HQCP fed groups may be attributed to the

reduction in the digestible energy/protein ratio (DE/P) which ranged from 6.30kcal/g (CP5) to 7.12kcal/g (CPI) against the recommended 7.3-10.0kcal/g (Robinson and Li, 2007). Growth is slow with reduced DE/P ratio in the diet of fish.

The carcass composition of the experimental fish before and after the feeding trial is presented in table 4. Crude protein increased in all treatments compared to the initial value of 50.23%. Fat ranged from 6.54% in CP5 to 7.81% in CP2. The ash, phosphorus, calcium and zinc contents of the experimental fish also increased when compared with the initial values in this study. The increase in crude protein contents affirms protein synthesis in fed fish, as nutritional quality of feed ingested determines the carcass composition

Table 4: Proximate and mineral composition of fish before and after the feeding trial

Parameter	Initial	After				
		CP1	CP2	CP3	CP4	CP5
Crude protein (%)	50.23	53.45	53.28	52.87	53.82	52.34
Fat (%)	7.7	7.4	7.81	6.92	7.41	6.54
Fibre (%)	1.82	1.21	1.52	1.09	1.89	1.27
Ash (%)	10.08	11.98	10.21	11.20	10.89	10.91
Moisture (%)	9.12	8.89	9.01	8.50	8.79	9.04
NFE (%)	21.05	17.07	18.17	19.91	17.20	19.90
Phosphorus (mgg ⁻¹)	25.21	30.01	30.22	28.43	27.89	27.01
Calcium (mgg ⁻¹)	19.06	20.98	22.12	24.32	19.70	19.50
Magnesium (mgg ⁻¹)	1.91	1.07	1.24	1.09	0.98	1.03
Zinc (µgg ⁻¹)	26.00	30.21	32.01	28.73	31.03	29.11

NFE: Nitrogen Free Extract

of experimental fish (Tiamiyu *et al.*, 2015). This assertion is supported by Fountoulaki *et al.* (2003), where tissue production, protein synthesis and increased weight were said to be responsible for fish growth.

The cost of producing a kilogram of feed was highest in CPI (Control) and this value reduced with increased inclusion of HQCP in the diets (Table 5). The values of fish produced also followed this trend with the highest value observed in CPI. However, since the cost of production is mostly dependent on the quality and cost of feed and effective quantity consumed for flesh production, variables such as cost of producing a unit weight of fish (kg), Profit Index (PI) and Incidence of Cost (IC) may be more useful. In this study, PI increased with increased HQCP inclusion peaking at 75% level. There exists an inverse relationship between inclusion level and IC in this study. Therefore, when feed cost, quantity consumed and the flesh produced are considered as factors in economic analysis, HQCP would successfully replace maize as an energy source in the diet of *Clarias gariepinus*. The two indices (PI and IC) are essentially dependent on the feed cost and quantity of flesh produced, thus a reduction in feed cost accompanied with a slight reduction in the weight gained accounted for the results obtained in this study. Similar results were reported by Abu *et al.* (2009), where economic

analysis showed that cassava root meal could profitably replace 100% of maize in the diet of hybrid (*H. bidorsalis* × *C. gariepinus*) catfish fingerlings, but with an optimal level of 60% recorded. However, Orisasona *et al.* (2016) did not find any variation in the economic indices when boiled lima bean meal was used to replace soybean meal in the diet of *C. gariepinus*. However, in their study, weight gain significantly reduced at replacement levels above 50%. The quantity of feed consumed is germane to the results obtained in their study, as less feed results in reduced cost of production, but this is balanced out to an extent with the reduction in flesh production,

Packed Cell Volume values ranged from 27.67% in CPI to 31.67% in CP4 as presented in table 6 and all fell within the recommended range for fish culture (Erundu *et al.*, 1993; Fagbenro *et al.*, 1993). Increase in the PCV and RBC values in fish fed HQCP diets when compared with the control diet may not be attributed to the treatments. This is because the results of the chemical analysis of diets and growth performance did not indicate superiority of HQCP diets, which could have improved the PCV and RBC values. According to Kumar *et al.* (2010) reduction in RBC and Hb values results from poor erythrocyte maturation which may be attributed to inadequate nutritional quality. Values for WBC

Table 5: Economic analysis of diets containing varying HQCP inclusion levels fed to *Clarias gariepinus* juvenile for 84 days

PARAMETER		CPI	CP2	CP3	CP4	CP5
Ingredient	Price/Kg of ingredient	Contribution of ingredients to price of experimental diets				
Fishmeal	950	29.82	29.82	29.82	29.82	29.82
Soybean Meal	135	4.24	4.24	4.24	4.24	4.24
Maize	139	4.48	3.36	2.24	1.12	0.00
HQCP	40	0.00	0.32	0.64	0.96	1.29
DCP	350	0.17	0.17	0.17	0.17	0.17
Vitamin Premix	400	0.40	0.40	0.40	0.40	0.40
Lysine	100	0.92	0.92	0.92	0.92	0.92
Salt	920	0.10	0.10	0.10	0.10	0.10
Palm oil	100	0.05	0.05	0.05	0.05	0.05
Cost of 1kg feed		402.80	394.80	381.40	373.40	365.50
FCR		1.12	1.15	1.14	1.20	1.19
Cost of producing 1 kg of fish		450.24	454.02	434.79	448.08	434.95
Fish produced		54.09	51.39	52.21	52.50	53.13
Feed consumed		65.27	59.37	59.54	59.08	61.20
Value of fish		37.86	35.97	36.54	36.75	37.19
PI		1.44	1.53	1.61	1.66	1.66
IC		0.48	0.45	0.43	0.42	0.42

PI, Profit Index; IC, Incidence of Cost

Table 6: Haematological parameters of *Clarias gariepinus* juveniles fed graded levels of HQCP meal as replacement for maize

PARAMETERS	CPI	CP2	CP3	CP4	CP5	SEM
PCV	27.67±0.33 ^b	31.33±1.67 ^a	29.67±1.20 ^{ab}	31.67±0.33 ^b	30.00±0.00 ^{ab}	0.52
WBC	13.00±0.00 ^c	16.33±0.33 ^a	11.5±2.89 ^d	13.33±0.44 ^{bc}	14.00±0.00 ^b	0.43
RBC	1.96±0.00 ^e	2.73±0.67 ^a	2.35±0.66 ^c	2.07±0.33 ^d	2.51±0.76 ^b	0.07
Hb	7.02±0.15	6.91±0.16	6.63±0.62	6.56±0.54	6.74±0.33	0.16
MCHC (gdL ⁻¹)	25.28±0.56	22.06±0.54	22.35±2.12	21.19±2.03	22.46±1.11	0.66
MCH(pgCell ⁻¹)	35.72±0.78 ^a	25.36±0.58 ^c	28.22±2.68 ^{bc}	31.72±2.63 ^{ab}	26.85±1.33 ^{bc}	1.21
MCV (µm ³)	14.54±0.38 ^a	11.90±0.38 ^b	12.55±0.06 ^b	15.25±0.14 ^a	11.91±0.06 ^b	0.38

Means of values with same superscript across rows are not significant different ($p < 0.05$).

PCV= Packed Cell Volume, WBC= White Blood Cell, RBC= Red Blood Cell, Hb= Haemoglobin, MCHC= Mean Corpuscular Haemoglobin Concentration, MCH= Mean Corpuscular Haemoglobin, MCV= Mean Corpuscular Volume.

Table 7: Mean water quality parameters of the treatments monitored for the periods of 84 days

PARAMETER	CPI	CP2	CP3	CP4	CP5
Temperature (°C)	25.9	25.87	25.75	25.8	25.9
pH	6.6	6.77	6.84	6.85	6.84
Dissolved oxygen (mg/L)	4.9	4.8	4.6	3.7	5.05

showed significant variation ($p < 0.05$) with 13.00, 16.33, 11.50, 13.33 and 14.00 recorded for CPI, CP2, CP3, CP4 and CP5 respectively. Dada *et al* (2015) opined that increase in White Blood Cells could be attributed to microbial infection or the presence of a foreign body or antigen in the circulatory system. The results of the present study clearly show that HQCP inclusion level did not influence the WBC values obtained.

The pooled mean for water quality parameters during the experimental period are presented in table 7. Temperature, pH and dissolved oxygen were all within the range recommended for fish culture in the tropics (El-Sayed, 2006).

Conclusion

This study has revealed that High Quality Cassava Peel can successfully replace maize in the diet of *C. gariepinus* as evident in the growth, nutrient utilization and economic parameters. Thus, cassava peels that currently constitute an environmental burden, can be effectively utilized in fish diets.

References

Abu, O. M. G., Sanni, L. O. Erundu, E. S. and Akinrotimi, O. A. (2010). Economic viability of replacing maize with whole cassava root meal in the diet of hybrid cat-fish. *Agric. J.*, 5: 1-5.

Adesehinwa, A. O. K., Obi, O. O., Makanjuola, B. A., Oluwole, O. O. and Adesina, M. A. (2011). Growing pigs fed cassava peel based diet supplemented with or without Farmazyme 300 proenx: Effect on growth, carcass and blood parameters. *African Journal of Biotechnology* 10(14):2791-2796

Adesehinwa, A. O. K., Samireddypalle, A., Fatufe, A. A., Ajayi, F, Boladuro, B. and I Okike (2016). High quality cassava peel fine mash as energy source for growing pigs: effect on growth performance, cost of production and blood parameters. *Livestock Research for Rural Development* 28 (11).

Adewale, S. 2013. Growth response of Nile tilapia (*Oreochromis niloticus*) fed graded levels of sun-dried cassava peel meal. Paper presented at the 28th Annual Conference of the Fisheries Society of Nigeria, Abuja, Nigeria.

Akegbejo-Samsons, Y. (1999). The use of cassava flour as a partial substitute for yellow maize in diets for *Claris gariepinus* fingerlings. *J. Aqua. Trop.* 14(3):247-253

Ape, D. I., Nwogu, N. A, Uwakwe, E. I and Ikedinobi, C (2016). Comparative proximate analysis of maize and sorghum bought from Ogbete main market, Enugu State Nigeria. *Greener Journal of Agricultural Sciences*, 6(9):272-275

Association of Official Analytical Chemists. (2005). *Official Methods of Analysis*, 17th edition. A.O.A.C., Washington, DC, USA

Bradbury, J. H., Egan, S. V. and Lynch M. J. (1991). Analysis of cyanide in cassava using acid hydrolysis of cyanogenic glucosides. *J. Sci. Food Agric.* 55:277-90

Castell, J.D. and K.Tiewes (1980). Report of the EIFAC, IUNS and ICES working group on standardization of methodology in fish nutrition research (Hamburg, Federal Republic of Germany, 21-23 March 1979). EIFAC Technical Paper No. 36, FAO, Rome, Italy, pp: 1-24.

Dada, A.A., Adeparusi, E.O. and Malomo, E.O. (2013). Dietary utilization of different portions of sweet cassava (*Manihot palmata*) for the Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758). Book of abstracts. International Symposium on Tilapia

- in Aquaculture (ISTA 10) October 6–10 2013. Jerusalem, Israel.
- Dada, A. A., Adeparusi, E. O. and Malomo E, O (2015). Dietary utilization of different portions of sweet cassava root (*Manihot palmata*) for the Nile Tilapia *Oreochromis niloticus* (Linnaeus, 1758). *Journal of Fisheries and Aquatic Science*, 10(6):569-574
- El-Sayed, A. F. M. (2006). *Tilapia Culture*. CABI Publishing Wallingford, Oxfordshire, U.K. Pages:277
- Erondu, E. S., Nnubia, C and Nwudukwe F. O (1993). Haematological studies on four Catfish species raised in freshwater ponds in Nigeria. *Journal of Applied Ichthyol.* 9:250-256
- Fagbenro, O. A., Adedire, C. O., Owoseeni, E. A and Ayotunde, E. O (1993). Studies on the biology and aquaculture of feral Catfish *Heterobranchus bidorsalis* (Geoffrey St, Hilaire, 1809). *Trop. Zool.* 6:67-79
- Fagbenro, O.A. and Arowosoge, A.I. 1991. Replacement value of some household wastes as energy substitutes in low-cost diets for rearing catfish in southwestern Nigeria. *Bioresource Technology* 37:197–204.
- FAO (2001). *Proceedings of the Validation Forum on the Global Cassava Development Strategy*. Food and Agriculture Organization of the United Nations International Fund for Agricultural Development, Rome, 2001. Pp 65.
- Food and Agriculture Organization, FAOSTAT (2014). (Accessed, 17/05/2017)
- Fountoulaki, E., Alexis, M.N., Nengas, I. and Venon, B. (2003). Effects of dietary arachidonic acid (20:4n-6), on growth, body composition, and tissue fatty acid profile of gilthead bream fingerlings (*Sparus aurata* L.). *Aquaculture*, 225: 309-323.
- Gabriel, U.U., Akinrotimi, O.A., Anyanwu, P.E., Bekibele, D.O., and N ,Onunkwo .. 2007. The role of Dietary Phytase in formulation of least cost and less polluting fish feed for sustainable aquaculture development in Nigeria. *African journal of Agricultural Research* 2(7):279-286
- Gatlin, D. M. (2010). *Principles of fish nutrition*. New York: Southern Regional Aquaculture Center. Publication no. 5003.
- Gil, J. L. and Buitrago, A. J. A. (2002). Cassava in animal feed. In: *Cassava in the third millennium : modern production, processing, utilization and marketing*. Centro Internacional de Agricultura Tropical; p. 527-69
- Hillocks, R. J. (2002). Cassava in Africa. In: Hillocks, R. J., Thresh, J. M. and Belloti, A. C (eds). *Cassava: Biology, Production and Utilization*. CAB International, pp 41-54
- International Livestock Research Institute (2015a). *Processing African Cassava peels, potentially a billion dollar business*. News.ilri.org (Accessed 17/05/2017)
- International Livestock Research Institute (2015b). *From food waste to animal feed, cassava peels potentially big business for Nigeria women*. News.ilri.org (Accessed 15/05/2017)
- Johnson, C.W., Timmons, D.L. and P.E. Hall. (2002). *Essential Laboratory Mathematics: Concepts and Applications for the Chemical and Clinical Laboratory Technician*. Cengage Learning, Florence, KY, USA, 268pp
- Lacerda, C. H. F., Hayashi, C., Soares, C. M., Boscolo, W. R. and L. C. B., Kevata (2005). Replacement of corn *Zea mays* L. by cassava *Manihot esculenta* crants meal in grass carp *Ctenopharyngodon idella* fingerlings diets. *Acta Scientiarum-Animal Sciences*, 27(2):241-245
- Murugan, K., Yashitha, Kuppusamy, S. and Al-Sohaibani, S. (2012). Detoxification of cyanides in cassava flour by Linamarase of *Bacillus subtilis* KMO5 isolated from cassava peels. *African Journal of Biotechnology*, 11: 7232-7237.
- Nwanna, L. C. (2003). Nutritional value and digestibility of shrimp head waste meal by African Catfish *Clarias gariepinus*. *Pakistan Journal of Nutrition* 2(6): 339-345.
- Okike, I., Samireddypalle, A., Kaptoge, L., Fauquet, C., Atehnkeng, J., Bandyopadhyay, R., Kulakow, P., Duncan, A., Alabi and Blummel, M. (2015). Technical innovations for small-scale producers and households to process wet cassava peels into high quality animal feed ingredients and aflasafe™ substrate. *Practical*

- Action Publishing. www.practicalactionpublishing.org <http://dx.doi.org/10.3362/2046-1887.2015.005>
- Orisasona, O., Falaye, A. E., Ajani, E. K. and O. K. Kareem (2016). Effect of replacement of Soybean with Lima bean (*Phaseolus lunatus*) meal on growth and hematological parameters of *Clarias gariepinus*. *Journal of Aquatic Sciences* 31 (2A): 229-240
- Robinson, Ed and Li M. H. (2007). *Catfish Nutrition: Nutrient requirement*. Extension Service of Mississippi State University, cooperating with U.S. Department of Agriculture Publication 2412.
- Saka J. D. K., Nyirenda K. K., 2012 Effect of two ethnic processing technologies on reduction and composition of total and non-glucosidic cyanogens in cassava. *Food Chemistry* 130:605-609.
- Sarkiyayi, S. and Agar, T. M. (2010). Comparative analysis on the nutritional and anti-nutritional contents of the sweet and bitter cassava varieties. *Advance Journal of Food Science and Technology* 2(6). 328-334.
- Siritunga, D. and Sayre, R.T. (2003). Generation of cyanogen-free transgenic cassava. *Planta* 217(3):367–373
- Tachia, M. U., Ataguba, G. A and P. O. Abuh (2016). Replacing maize with cassava peels in diets of *Oreochromis niloticus*: evaluation of feed performance and meta-analysis. *AAC Biflux* 9(5):1001-1010
- Tiamiyu, L. O., Okomoda, V.T. and Agbese, V. E. (2015). Growth performance of *Clarias gariepinus* fingerlings fed *Citrullus lanatus* seed meal as a replacement for soybean meal. *J. Aquacult. Eng. Fish. Res.* 1 (1):49–56
- Tewe, O. O and Kasali, O. B. N. (1982). Effect of cassava peels processing on the nutrient utilization and physiopathology of the African giant rat (*Cricetomys gambianus*, Water house). *Tropical Agriculture, Trinidad*, 63:1-2
- Ubalua, A.O. and Ezeronye, O.U. 2008. Growth responses and nutritional evaluation of cassava peel based diet on tilapia (*Oreochromis niloticus*) fish fingerlings. *Journal of Food Technology* 6(5):207–213.
- Ugwu, L.L.C., Asogwa, M.O. and Mgbenka, B.O. 2004. Influence of dietary levels of cassava (*Manihot esculenta*) peel meal on feed efficiency and productive protein value of young tilapia (*Oreochromis niloticus*, Trewavas). *Journal of Sustainable Agriculture and Environment* 6(2):148–156.
- Vincke, M. (1969). *Compte-rendud'activiteanness*. Division des Recherchespiscicoles. Tenanarive, Madagascar. 56-63
- Wagner E.J., T. Je:sen, R. Arndt, M. D. Routledge and Q. Brddwisch. (1997). Effects of rearing density upon cutthroat trout hematology, hatchery performance, fin erosion and general health condition. *The progressive fish-culturist*, 59:173-187

HYGIENIC PRACTICES AND MICROBIOLOGICAL QUALITY OF MILK FROM PERI-URBAN DAIRY FARMERS AND BULKING CENTRES IN LILONGWE, MALAWI

Richard Fredrick Dickson Banda^{1,2}, Joseph Wafula Matofari¹ and John Masani Nduko¹

¹Department of Dairy and Food Science and Technology, Egerton University, P.O. Box 536-20115, Egerton Kenya

²Department of Food Science and Technology, Lilongwe University of Agriculture and Natural Resources P.O. Box 219, Lilongwe, Malawi.

Abstract

Hygiene practices at milk production and handling have an effect on levels of microbial contamination and consequently on the quality of milk produced for human consumption as well as meeting processing requirements. This study analysed the hygiene practices at peri-urban dairy farm level and at bulking centres and their influence on the microbial load along the raw milk value chain in Lilongwe, Malawi. A survey using a structured questionnaire was conducted on 256 randomly selected dairy farmers that supply milk to and belong to 6 milk bulking centres (MBCs) on the hygiene practices during milk production and handling up to the bulking centres. Additionally, raw milk samples (n=60) were aseptically collected from farmers and bulking centres and analysed for Total Viable Count (TVC), Total Coliform Count (TCC), Lactic Acid Bacteria (LAB), and Yeasts and moulds (Y/M). From the study, most farmers (91.0%) and all MBCs collect water from boreholes and most of them do not treat the water before use (66.3%-farmers; 50% MBC). All the dairy farmers clean the udder before milking with 87.8% of them using soap. Plastic containers were mostly used by farmers for milk handling (51.6%) and bulking (59.0%) and by half of the MBCs. Most farmers (81.3%) cleaned the containers using warm water while MBCs used cold water. Most farmers (94.9%) and all MBCs cleaned using soap. Additionally, most farmers (87.1%) sun-dried their containers before use and a majority (96.5%) of them sieved milk before bulking. A high percentage (73.8%) of farmers, who milked twice, stored evening milk using a water bath and delivered the milk to a bulking centre once a day in the morning. The delivery was done mostly (91.8%) within 2 hours of milking in the morning. The mean TVC were $>7 \log_{10}$ cfu/ml, CC were $>6 \log_{10}$ cfu/ml while Y/M and LAB counts were both $>5 \log_{10}$ cfu/ml for both at the farms and MBCs levels. Based on the findings of this study, the hygiene practices of the dairy farmers in Malawi do not conform to the required standards and contribute to high microbial numbers in the milk. It is imperative therefore to strengthen smallholder farmers' knowledge through extension services and training programmes on milking hygiene practices and the post-harvest handling of milk, to assure safety and minimize losses due to rejection of spoiled milk.

Key words: Lilongwe, hygiene practices, milk spoilage, Post-harvest losses, milk handling

Introduction

The dairy industry in Malawi is divided into the formal and informal sectors, with 65,027 heads of dairy animals (Revoredo-Giha *et al.*, 2013; FAOSTAT, 2014). The informal sector produces around 27,000 tons of milk in a year (Imani Development Consultants, 2004). Milk from the informal sector is mainly produced from the Malawi Zebu cattle (*Bos indicus*), a breed kept by Malawians for a long time for subsistence, prestige and insurance against drought. The formal sector, rising as a result of urbanization and industrialization, has about 4,000 dairy farmers with a dairy herd of approximately 15,000 Friesians and crossbreeds of the Malawi zebu producing about 64,747 tons of milk per annum (Sindani, 2012; FAOSTAT, 2014). The sector relies mainly on smallholder farmers, with only five large scale farms contributing to the milk supply. The informal sector sells raw milk directly to consumers, while the formal sector sells milk to milk processors through milk bulking groups (MBGs).

The dairy industry in Malawi has the potential to grow. It is promoted by the government of Malawi and non-governmental organizations through funding for research, dissemination of technologies to farmers and capacity building and development (Sindani, 2012). However, its growth is hindered by milk post-harvest losses that are estimated at 17% due to microbial contamination that causes spoilage. Economically, this post-harvest loss accounts for approximately MK 481,274, 250.00 (equivalent to USD 670,300) per annum. Sindani (2012) reported that the milk supplied by the farmers is of poor quality. This is attributed to among others, poor hygiene at the milking stage and the use of unsterile containers to collect and transport milk to MBGs (Wiggins, 2016).

Milk is processed from the farm through handling, cooling and transportation to the processing plant where it is transformed into other products. Hygienic practices along the dairy value chain are important in assuring the quality and safety of milk for consumers

and for reducing losses at production and at post-harvest handling. The presence of microorganisms in food is undesirable as they have significant effects on both the consumers and food processors. Spoilage microorganisms make food unpalatable while pathogenic microorganisms cause foodborne diseases. Pathogenic microorganisms are eliminated through pasteurization and sterilization during milk processing (Purnell *et al.*, 2012). Hygiene practices of importance include cleanliness of the animals (udder), the milking environment, milking persons and milk harvesting and storage containers (Kashongwe, 2017). The type and design of equipment coming into contact with food is also one of the critical elements in ensuring consistent safety of food (Hasting, 2012). Cleaning is the process of removing soil, food residues, 'dirt', grease or other objectionable matter from surfaces that come into contact with food, using specified detergents under specified conditions such as temperature and time of contact (Schmidt, 2012). Cleaning and sanitation procedures for food contact surfaces involve rinsing, cleaning, rinsing again and finally sanitizing. Sanitation Standard Operating Procedures (SSOP) vary from one processor to another (Goode *et al.*, 2013). Cleaning involves wetting of the surface, degradation and removal of the soils from the surface, and prohibition from re-deposition of the soils by the cleaning chemicals followed by disinfection (Gibson *et al.*, 1999). Cleaning reduces surface microorganisms, but it is not adequate to reduce their populations to the acceptable levels. Disinfection reduces 99.9% of surface microorganisms (CAC, 2009; Schmidt, 2012). The surface microbial load on the food contact surface varies from one processor to another depending on the microbial quality of the food being processed, the cleaning regimes used, and the disinfection post-cleaning (Evans *et al.*, 2004).

This study evaluated the hygiene practices of the dairy farmers and milk bulking centres and their influence on microbial loads in milk in Lilongwe, Malawi.

Materials and Methods

Study Area

The study was conducted in Lilongwe District located in the Central Region of Malawi. Lilongwe is located at the approximate latitude of 13°58'S and 33°47'E and at an altitude of between 1,300 to 1,700 meters above sea level. The mean annual temperature varies between 22°C in low altitude areas and 18°C in high altitude areas. The annual rainfall ranges from 900mm to 1500 mm (Saka *et al.*, 2003).

Sampling Procedure

A cross-sectional study was conducted in Lilongwe District, Malawi, from February 2018 to August 2018. The study used semi-structured questionnaires to evaluate the milk hygiene practices of smallholder dairy farmers (n=256), randomly selected from six milk bulking centres. The data comprised of the type of container farmers used to handle milk, type of detergents and/or sanitizers used in cleaning and sanitization, the cleaning method and procedures, the source of their water, availability and accessibility in terms of the time required to reach the milk cooling facilities. In addition, it assessed if the dairy actors had a water treatment programme in place, how many times milk was delivered to the cooling facility and how the milk was kept while awaiting delivery to the cooling facility.

Raw bulked milk samples were collected at farm gate and at milk bulking centres. A total of 60 samples (50 ml each) of raw milk were used for the microbial analysis. The milk samples were transferred into sterile screw-capped sampling bottles, which were then securely capped, clearly labelled and immediately transported on ice (4°C) to the laboratory for analysis. Swabbing was done on the milk handling containers when the containers had been cleaned and ready to be used for handling milk. Surface swabs for collecting microorganisms were done at an area of 25 cm² in three replicates using sterile cotton swab buds pre-wetted in peptone water. The swab samples were then transferred into 9 ml of 0.1% (w/v) buffered peptone water in a

screw-cap tube and stored in a cool box at 4°C. Water samples from milk production areas were taken from farmer households and the milk bulking centres.

Sample Microbial Analysis

Analysis of the samples was done at the Community Health Science Unit (CHSU) of the National Public Health (Microbiology) Reference Laboratory in Lilongwe, Malawi. For Total Viable Count (TVC), milk samples and milk-handling container surface swabs were serially diluted appropriately in buffered peptone water and the dilutions pour-plated using Nutrient agar (Oxoid, UK) and incubated at 37°C for 48 hours. For the Total Coliform Count (TCC), serial dilutions were pour-plated on MacConkey agar (Oxoid, UK), incubated at 37°C for 24 hours and typical dark red colonies on the plates were considered as coliforms and counted. For Lactic Acid Bacteria (LABs), the dilutions were pour-plated on MRS agar (Oxoid, UK) then incubated at 37°C for 48 hours. For yeasts and moulds, milk-handling container surface swabs, water and milk samples were pour-plated on Sabouraud Dextrose agar and the plates were incubated at 37°C for 72 hours, and colonies were counted and recorded.

Statistical Analyses

Data obtained from the sanitation practices were analyzed by means of general descriptive statistics and chi-square test for determination of independence using SPSS version 20 (SPSS, Inc., Chicago, IL, USA). The data obtained for the microbial counts was transformed into log₁₀. This data together were compared using Analysis of Variance (ANOVA) by the General Linear Model (GLM) of SAS version 9.1.3 (SAS Institute, Inc.; Cary, NC). Means comparisons were done using least significant differences (LSD) at P<0.05.

Results

Socio demographic characteristics of the respondents

A total of 256 smallholder dairy farmers were interviewed in this cross sectional study in the six selected milk bulking groups of Lilongwe District, Malawi. Males comprised 62.5% of the respondents while the remaining 37.5% were females of different ages and educational levels (Table 1). The respondents' average age was 43.94 ± 15.38 , which implied that most of the respondents were in the productive age group. There were 17.2% illiterate respondents, 64.5% attended school up to primary level, 17.6% attended school up to secondary level, while 0.8% attended school up to tertiary level. On average, each of the households had 2 ± 1 cows, producing an average of 12 ± 6 litres of milk per day, of which 10.3 ± 5 litres were sold while 1.66 ± 1.09 litres of the milk were consumed in the household. In this study, 75.8% of the respondents sold the milk through formal channels, 5.9% sold milk

through informal channels, and 18% used both formal and informal channels, while 0.4% used the milk for home consumption (Table 1).

Dairy Cattle Housing Characteristics

In the study area, most of the cows (54.7%) were housed in earth type floor barns and 45.3% were in barns with concrete floors. Most of the respondents (69.1%) provided beddings for their cattle while (30.9%) did not provide beddings. In the barns, 77.3% of the respondents separated the feeding areas from the sleeping areas while 22.7% did not (Table 2).

Milk Hygiene Practices

This study showed that milking was done by hand (100%), with milking frequency of twice (98.4%) or once (1.6%) a day. The dairy farmers sourced water from boreholes (91.0%), rivers (4.7%), and taps (4.3%), (Table 3) while all the milk bulking centres sourced water from boreholes (Table 4). The water was not treated before usage by 66.4% of the dairy

Table 1: Socio demographic characteristics of the dairy farmers in Lilongwe, Malawi

Parameter	Category	Frequency	Percentage (%)
Gender	Male	160	62.5
	Female	96	37.5
Average Age	43.9 ± 15.4		
Average Number of cows per household	2 ± 1		
Average number of cows milked	1		
Average liters of milk produced per household/day	12 ± 6		
Average liters of milk sold per household/day	10.3 ± 5		
Level of education	Illiterate	44	17.2
	Primary	165	64.5
	Secondary	45	17.6
	Tertiary	2	0.8
Channels of selling milk	Formal	194	75.8
	Informal	15	5.9
	Both formal and informal	46	18
	Home consumption	1	0.4

*the number after \pm is a standard deviation

Table 2: Dairy cattle housing characteristics

Parameter	Category	Frequency	Percentage (%)
Floor	Concrete	116	45.3
	Earth	140	54.7
Beddings	Available	177	69.1
	Not available	79	30.9
Feeding area	Same as sleeping area	58	22.7
	Separate from sleeping area	198	77.3

Table 3: Milk Hygiene Practices of Smallholder Dairy Farmers

Hygiene Practice	Category	N (255)	Frequency (%)
Source of water	Tap	11	4.3
	Borehole	232	91.0
	River	12	4.7
Treatment of water	No treatment	170	66.4
	Water guard	16	6.3
	Chlorine	40	15.6
	Boiling	30	11.7
Hand washing	With soap	245	95.7
	Without soap	11	4.3
	Using cold water	16	6.3
	Using Warm water	238	93
	Both cold and warm	2	0.8
Udder washing	With soap	224	87.9
	Without soap	31	12.1
	Using cold water	8	3.1
	Using warm water	247	96.5
	Both cold and warm	1	0.4
Udder drying	Yes	231	90.2
	No	25	9.8

farmers and 50% of the milk bulking centres. Approximately 11.7% of dairy farmers treated their water by boiling, 15.6% used chlorine and 6.3% used Water guard (Table 3). The bulking centres treated their water by using Water guard and chlorine (Table 4). All the dairy farmers washed their hands and the udders of the cows before milking. The hands were washed with soap (95.7%) and without soap (4.3%), while using cold water (93%) warm (6.3%) and, both cold and warm 0.8%) (Table

3). The udders were cleaned with detergents by 87.9% of the respondents while 12.1% did not use a detergent. The udders were cleaned using warm water (96.5%), cold water (3.1%) or both warm and cold water (0.4%). Most of the dairy farmers (90.2%) dried the udder after cleaning using a separate towel when more than one cow was milked while 9.8% did not dry the udder after cleaning (Table 3). The MBCs conducted various quality tests prior to the acceptance of the milk (Table 4). These

tests included density, sourness, organoleptic, temperature, and fat and rezasurin tests.

Milk Equipment and Milk Handling Practices

In this study, 51.6% of the dairy farmers sampled used plastic containers and 12.1% used aluminium containers while 36.3% used both plastic and aluminium containers to handle milk (Table 5). On the other hand, half of the MBCs used plastic containers, while the other half used aluminium cans in handling milk (Table 4). Milk was bulked in aluminium containers by 41% of the dairy farmers while 59% of the farmers bulked their milk in plastic containers. All farmers in this study cleaned milk handling containers; 81.3% washed containers with warm water, 15.6% washed containers with cold water, 0.4% used both cold and warm water and only 2.7% washed the containers with hot water. Most of the milk handling containers (94.9%) were washed with soap while only 5.1% were not washed (Table 5).

All the bulking centres used cold water and a detergent for cleaning milk handling containers (Table 4). The farmers did not disinfect the equipment after washing, while for the MBCs, only 16.7% disinfected the milk handling containers (Table 4). Most (98%) of the farmer's milk handling containers were dried after washing and before handling milk while 1.6% were not dried after cleaning and before handling milk (Table 5). Most of the farmers (87.1%) sundried the containers, 10.2% dried them using a towel and 0.8% used both the towel and sun-drying. Most of the MBCs (83.33%) used towels for drying the containers after washing (Table 4).

Milk was sieved into storage containers by most of the farmers (96.5%); only 3.5% did not filter milk into containers. The dairy farmers (73.8%) who milked twice a day stored the milk using a cold water bath, 1.2% stored it by refrigeration, 3.9% stored milk by placing the container in a cool place, 5.1% boiled and placed the milk container in a cool place, while

Table 4: Hygiene practices of milk bulking centres

Hygiene Practice	Category	Frequency	Percentage (%)
Source of water	Borehole	6	100
Treatment of water	Water guard	1	16.7
	Chlorine	2	33.3
	No treatment	3	50.0
Quality test	Density, and sourness	2	33.3
	Density, sourness and Organoleptic test	2	33.3
	Density sourness, Temperature and Fat	1	16.7
	Density, sourness and Rezasurin	1	16.7
Milk containers	Aluminium cans	3	50.0
	Aluminium and Plastic containers	3	50.0
Cleaning containers	Cold water	6	100
	Detergent	6	100
	Disinfection	1	16.7
	No Disinfection	5	83.3
Container Drying	Towel	5	83.33
	Sun-drying	1	16.67
Storage temperature	Below 5°C	4	66.7
	Do not store	2	33.3

Note: Most bulking centres use non potable water for equipment cleaning.

14.5% boiled and placed the milk container in a cold water bath. Most MBCs (66.7%) cooled milk below 5°C (Table 4). The majority of the farmers (91.8%) delivered milk to bulking centres within 2 hours after milking, 7.0% within 4 hours, and 1.2% within 6 hours after milking (Table 5).

Association of personnel hygiene training with milk hygiene practices

Among the hygiene practices, availability of beddings, use of cleaning agent in washing hands and sieving milk were significantly ($p < 0.05$) associated with personnel hygiene

training (Table 6).

Microbial contamination

The mean milk microbial contamination of bulked milk samples from farmers is presented in Table 7. The TVC in bulked milk samples was $7.40 \pm 0.18 \log_{10}$ cfu/ml and was similar to $7.21 \pm 0.33 \log_{10}$ cfu/ml found in water samples but significantly higher ($p < 0.05$) than the ones in swab samples ($5.27 \pm 0.07 \log_{10}$ cfu/ml). Similar to TVC, bulked milk samples had significantly higher CC, yeasts and moulds and LAB than water and swab samples (Table 8).

Table 5: Milking equipment and milk handling practices by dairy farmers

Hygiene Practice	Category	Frequency	Percentage (%)
Milk handling containers	Aluminum	31	12.1
	Plastic	132	51.6
	Both plastic and Aluminium	93	36.3
Bulking containers	Aluminum	105	41
	Plastic	151	59
Type of water to clean containers	Warm	208	81.3
	Cold	40	15.6
	Hot	7	2.7
	Both warm and cold	1	0.4
Detergent usage in cleaning containers	Yes	243	94.9
	No	13	5.1
Equipment Drying	Yes	251	98.0
	No	4	1.6
	Sun drying	223	87.1
	Using towel	26	10.2
	Both towel and sun	2	0.8
Personnel hygiene training	Yes	214	83.6
	No	42	16.4
Sieve Milk	Yes	247	96.5
	No	9	3.5
Storage of evening milk	Water bath	189	73.8
	Refrigeration	3	1.2
	Cool place	10	3.9
	Boil and cool place	13	5.1
	Boil and Water bath	37	14.5
	Do not store	4	1.6

Hygiene Practice	Category	Frequency	Percentage (%)
Time taken to deliver milk to bulking centre	Within 2hours	235	91.8
	Within 4 hours	18	7.0
	Within 6 hours	3	1.2

Table 6: Association of Personnel hygiene training and hygiene practices

Parameter	Category	Personnel hygiene training		X ²	p-value
		Yes	No		
Availability of beddings	Yes	154	23	4.869	0.027
	No	60	19		
Use of cleaning agent to wash hands	Yes	209	36	12.191	0.001
	No	5	6		

Table 7: Means \pm stderr comparison of TVC, CC, Yeast and Moulds, and LAB of samples from dairy farmers

Sample Type	N	Log ₁₀ (cfu /ml)			
		TVC	CC	Yeast & Moulds	LAB
Milk	60	7.40 \pm 0.18 ^a	6.06 \pm 0.36 ^a	5.33 \pm 0.38 ^a	5.73 \pm 0.36 ^a
Swab	48	5.27 \pm 0.07 ^b	4.22 \pm 0.28 ^b	2.46 \pm 0.31 ^b	2.60 \pm 0.30 ^c
Water	60	7.21 \pm 0.33 ^a	5.23 \pm 0.50 ^{ab}	1.73 \pm 0.43 ^c	1.62 \pm 0.41 ^d

Correlation coefficient between Log₁₀ TVC and Log₁₀ CC = 0.447 p < 0.01

Means with the same letter superscript in a column are not significantly different at p < 0.05.

Table 8: Means \pm Standard Error comparison of TVC, CC, Yeast and Moulds, and LAB for actors

Sample	Actor	Log ₁₀ (cfu/ml)			
		TVC	CC	Yeast & Moulds	LAB
Milk	MBG Centre	7.57 \pm 0.45 ^b	5.34 \pm 0.89 ^b	5.43 \pm 0.94 ^b	6.95 \pm 0.88 ^a
	Households	7.36 \pm 0.20 ^a	6.21 \pm 0.40 ^a	5.30 \pm 0.42 ^a	5.48 \pm 0.39 ^b
Water	MBG Centre	6.19 \pm 0.79 ^b	4.36 \pm 1.21 ^b	1.48 \pm 1.06 ^b	0.00 \pm 0.97 ^b
	Households	7.41 \pm 0.35 ^a	5.56 \pm 0.54 ^a	1.77 \pm 0.47 ^a	1.94 \pm 0.43 ^a
Swab/Rinse	MBG Centre	5.23 \pm 0.12 ^b	4.34 \pm 0.48 ^b	2.48 \pm 0.59 ^b	2.19 \pm 0.52 ^b
	Households	5.34 \pm 0.06 ^a	4.53 \pm 0.25 ^a	3.21 \pm 0.31 ^a	3.20 \pm 0.28 ^a

Means with the same letter superscript for a sample type are not significantly different at p < 0.05

Discussion

The hygiene practices in the dairy industry in Lilongwe were determined and found to predispose the raw cow milk to microbial contamination. The environment in which the milk-producing animal is kept especially the sleeping place is very important in hygienic milk production (Oumer *et al.*, 2017). Most

dairy farmers provided a separate sleeping area from the feeding area and provided beddings and a higher proportion of them had concrete floors, which were expected to lower microbial contamination (Gashaw and Gebrehiwot, 2018; Oumer *et al.*, 2017). However, the frequency of changing beddings and cleaning of the dairy barns could affect microbial counts. Moreover, the use of earth floors for sleeping increases

the chances of contamination of the udder as the animals lie on that has soil which is a natural niche for microorganisms. The soiling of the sleeping places by feeds, urine and faecal matter for those animals that had the same sleeping and feeding stalls could highly contaminate the milk. Previous studies have shown that the hygienic status of the stall where milk animals sleep is very important since it predisposes the animals' udders to dirt (Saran, 1995). It has been reported that beddings' microbial counts and type are correlated with the microbial counts and types on the animals' teat ends (Zdanowicz *et al.*, 2004). Apart from milk contamination, studies have also shown that some bedding materials like sand and wood products contribute to infections of the udder like *mastitis* (Munoz *et al.*, 2006). Therefore, partitioning the feeding and sleeping places and the provision of easy-to-clean concrete floors and bedding materials may not necessarily reduce microbial contamination if hygienic practices in the stall are not met.

It was found that all the farmers washed their hands with most of them using soap and washing and drying the udder using a separate towel when more than one cow was milked, practices that could reduce 'soils' on the udder and contribute to clean milk production. The animals' udder cleanliness score has been found to correlate with milk microbial quality (Ellis *et al.*, 2007). However, the majority of the farmers and MBCs in this study were not treating their water, which was found to be contaminated with high microbial loads, hence even if proper practices were effected, the water could still contaminate the hands, the udder and the milk handling equipment and subsequently the milk (Fuquay *et al.*, 2011). After cleaning, disinfection is necessary to minimize microbial contamination by 99.9% (Schmidt, 2012). However, all the dairy farmers were not disinfecting the udder before and after milking. This could enhance milk contamination in the udder even before milking. Furthermore, the strength of the detergent/soap used to clean the udder and the cleanliness of the towel used to dry the udder influence the cleanliness of the udder and hence the chances of milk

contamination. In this study, the actors were not aware about the effective concentrations of the detergent/soap and the cleanliness of the towel used for drying the udder. This may have contributed to high microbial loads in the milk samples.

Moreover, other factors contributing to high microbial loads include the bulking of evening and morning milk, lack of cold chain facilities and the time taken to deliver the milk (Wanjala *et al.*, 2018). Most farmers bulked evening milk with morning milk but they stored the evening milk in a cold water bath which could not attain refrigeration temperatures sufficient to reduce the microbial growth rate. The evening milk therefore could have high microbial numbers and thus contaminate the morning milk, despite the swift delivery of the milk within 2 hrs of morning milking.

The results showed that plastic bucket containers were mostly used to handle milk and not the recommended aluminium cans. Moreover, all the actors cleaned the milk handling containers using soap and sun-dried them. However, none of the actors disinfected the equipment after washing. Plastic containers are difficult to thoroughly clean as compared to the aluminium containers and thus more microbes will remain on plastic containers. Resultantly, the microbes form biofilms which are persistent sources of contamination to milk (Orwa *et al.*, 2017). Previous studies have also shown that the milk microbial quality along the value chain is affected by contamination of the containers handling the milk (Wafula *et al.*, 2016; Welearegay *et al.*, 2012). In this study therefore, the milk handling containers could have contributed to the microbial loads recorded.

The provision of beddings, use of cleaning agents in washing hands and sieving milk were associated with personnel hygiene training. The actors who had received training on personnel hygiene recorded hygienic practices indicating that they practiced what they had learnt.

The high microbial load in raw milk, water, and milk-handling container surface swabs is a general indication of low levels of

milk handling hygienic practices (Mhone *et al*, 2011). Some of the key hygiene practices that influence milk quality include udder cleanliness, personnel hand washing, milk handling container type and cleaning and disinfection hence they remain areas of intervention with respect to milk hygiene (Bonfoh *et al*, 2006). The presence of coliforms, yeasts and moulds in milk at the production level confirms possible contamination with faecal material and it is an indicator of poor sanitary conditions in the production and handling of milk (Welearegay *et al*, 2012). Earlier studies showed that coliforms are correlated to milk handling container hygiene, whereby high counts were associated with low temperatures of the cleaning solution and high alkalinity of the detergent used for washing (Elmoslemany *et al*, 2009). The presence of high LAB in milk indicates poor hygienic practices since LAB are associated with milk and could anchor in poorly cleaned containers and multiply when the containers are filled with milk and if the correct storage temperature is not applied.

Conclusion

The exterior of the animals' udder, the milk handling equipment and the personnel handling milk are the major risks in milk microbial contamination in Malawi. Therefore, it is very critical that the milk handling container surfaces are properly cleaned and disinfected. Failures in the sanitation practices at the production level results to high contamination levels that no other subsequent mitigation measure can curb the losses along the value chain. More emphasis should be given to the use of aluminium cans for milking and bulking. The milking personnel hygiene and the hygiene of the animal's udder should also be emphasized so that the initial microbial contamination remains very low. If sanitation practices are improved such as the use of recommended milk containers, potable water and disinfectants at recommended concentrations, then supplemented with the milk cooling system in the value chain, milk losses can be minimized, and the dairy actors can benefit from clean milk. Moreover, training

on hygiene practices given to farmers needs to be reviewed and up-scaled in order to produce clean milk.

Abbreviations

ANOVA	Analysis of variance
CHSU	Community health science unit
GLM	General linear model
LAB	Lactic acid bacteria
MBCs	Milk bulking centres
MBGs	Milk bulking groups
SSOP	Sanitation standard operating procedure
TCC	Total coliform counts
TVC	Total Viable Counts
Y/M	Yeasts and moulds

Declarations

Availability of data and material

Please contact author for data requests.

Competing Interest

The authors declare that they have no competing interests

Funding

The authors acknowledge the support given by United States Agency for international Development as part of Feed the Future initiative, under the CGIAR Fund award number BFS-G-11-00002 and predecessor fund the Food Security and Crisis Mitigation II grant, award number EEM G-00-04-00013.

Authors' contributions

This research work was part of Richard Banda Thesis research for the award of MSc. Food Science degree of Egerton University and supervised by Joseph Wafula Matofari, John Masani Nduko, and Richard Banda was in charge of field samples collection and laboratory samples analysis under the directorship of the two supervisors. The supervisors were also

involved in the designing of the experiment, data analysis, interpretation of the results and development of this manuscript. All authors read and approved the final manuscript

Acknowledgement

We also thank Community Health Science Unit (CHSU) of the National Public Health (Microbiology) Reference Laboratory staff in Lilongwe: Dr. Watipaso Kasambara, Innocent Chibwe, and Abel Phiri. Special gratitude goes to Mr. F.D. Banda, Ellina, Precious and Stephano (late) for the assistance in questionnaire administration. We appreciate Dr. Olivier Kashongwe (PhD) and Mr. Nobert Wafula for the statistical guidance.

References

Bonfoh, B., Roth, C., Traoré, A. N., Fané, A., Simbé, C. F., Alfaroukh, I. O., et al. (2006). Effect of washing and disinfecting containers on the microbiological quality of fresh milk sold in Bamako (Mali). *Food Control*, 17, 153e161.

CAC. (2009). *Food hygiene: Basic texts fourth edition*. Joint FAO/WHO Food Standards Programme. Rome, Italy.

Ellis KA, Innocent GT, Mihm M, Cripps P, Mclean WG, Howard CV, and Grove-White D 2007: Dairy cow cleanliness and milk quality on organic and conventional farms in the UK. *J Dairy Res* 74: 302-310

Elmoslemany AM, Keefe GP, Dohoo IR, Dingwell RT. (2009) Microbiological quality of bulk tank raw milk in Prince Edward island Dairy herds. *J. Dairy Sci.* 92: 4239-4248.

Evans, J. A., Russell, S. L., James, C., & Corry, J. E. L. (2004). Microbial contamination of food refrigeration equipment. *Journal of Food Engineering*, 62, 225–232. [https://doi.org/10.1016/S0260-8774\(03\)00235-8](https://doi.org/10.1016/S0260-8774(03)00235-8)

FAOSTAT. (2014). Retrieved from www.fao.org/faostat/en/#data/QL

Fuquay J.W., Fox P.F., McSweeney P.L. (2011). *Encyclopedia of dairy sciences*. Academic Press, UK.

Gashaw, A., Gebrehiwot, E. (2018). Study on milk hygiene, quality control in the market chain in Jimma. *J Adv Dairy Res*, 2018, 6:3.

Gibson, H., Taylor, J. H., Hall, K. E., & Holah, J. T. (1999). Effectiveness of cleaning techniques used in the food industry in terms of the removal of bacterial biofilms. *Journal of Applied Microbiology*, 87(1), 41–48. <https://doi.org/10.1046/j.1365-2672.1999.00790.x>

Goode, K. R., Asteriadou, K., Robbins, P. T., & Fryer, P. J. (2013). Fouling and cleaning studies in the food and beverage industry classified by cleaning type. *Comprehensive Reviews in Food Science and Food Safety*, 12(2), 121–143. Retrieved from <https://doi.org/10.1111/1541-4337.12000>

Hasting, T. (2012). The Hygienic Design of Food Processing Plant. In J. G. Brennan & A. S. Grandison (Eds.), *Food Processing Handbook*, Second Edition. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany. <https://doi.org/10.1002/9783527634361.ch17>

Imani Development Consultants. (2004). *Review of the Dairy Industry in Malawi*. Development; Rates. Retrieved from <https://www.consultantsplas.com/resources/document/featured/DairyIndustryReviews.pdf>

Kashongwe, O. B., Bebe, B. O., Matofari, J. W., & Huelsebusch, C. G. (2017). Associations between milking practices, somatic cell counts and milk postharvest losses in smallholder dairy and pastoral camel herds in Kenya. *International Journal of Veterinary Science and Medicine*, 5(1), 57–64. <https://doi.org/10.1016/j.ijvsm.2017.01.001>

Kundhlande, G., Franzel, S., Simpson, B., & Gausi, E. (2015). Farmer-to-farmer extension approaches in Malawi: A Survey of Organizations using the approach ICRAF Working Paper No. 183. Nairobi. World Agroforestry Centre., (2016), 42. <https://doi.org/http://dx.doi.org/10.5716/WPI4384.PDF>

Munoz, M. A., C. Ahlstrom, B. J. Rauch, and R. N. Zadoks. 2006. Fecal shedding of *Klebsiella pneumoniae* by dairy cows. *J. Dairy Sci.* 89:3425–3430

Mhone, T. A., Matope, G., & Saidi, P. T. (2011). Aerobic bacterial, coliform, *Escherichia coli* and *Staphylococcus aureus* counts of raw and processed

- milk from selected smallholder dairy farms of Zimbabwe. *Int. J. Food Microbiol.*, 151, 223e228.
- Munoz, M.A., Ahlstrom, C., Rauch B. J., Zadoks, R. N. (2006). Fecal shedding of *Klebsiella pneumoniae* by Dairy cows. *J. Dairy Sci.*, 89: 3425 - 3430.
- National Statistical Office. (2012). Intergrated Household Survey (IHS 3) Report. Minister of Economic Planning and Development, Malawi Governmet.
- Orwa, J. D., Matofari, J. W., & Muliuro, P. S. (2017). Handling practices and microbial contamination sources of raw milk in rural and peri urban small holder farms in Nakuru County, Kenya. *Int. J. Livest. Prod.*, 8(1), 5–11. <https://doi.org/10.5897/IJLP2016.0318>
- Oumer, E., Tsegaye, S., Damtew, A., Feleke, A. (2017). Hygienic practices and bacteriological quality of cow raw milk from selected smallholder dairy farms of Mersa town, North Wollo, Ethiopia. *Eur. J. Exp. Biol.*, 7(4):22
- Purnell, G., & James, C. (2012). Advances in food surface pasteurisation by thermal methods. In *Microbial Decontamination in the Food Industry* (pp. 241–273). Elsevier. <https://doi.org/10.1533/9780857095756.2.241>
- Revoredo-Giha, C., Leat, P., & Jumbe, C. (2013). Developing the Dairy Processing Sector in Malawi, 9202. Retrieved from http://www.sruc.ac.uk/info/120257/food_marketing/802/assessing_contribution_of_dairy_sector_to_economic_growth_and_food_security_in_malawi
- Saka, A. R., Rao, P. S. C., & Sakala, W. D. (2003). Evaluating soil physical and chemical characteristics for describing nutrient leaching in agricultural soils. *Malawi Journal of Agricultural Sciences*.
- Saran, A. (1995): Disinfection in the dairy parlour. *Rev. sci. tech. Off. int. Epiz.* 14, 207-224.
- Schmidt, R. (2012). Basic Elements of Equipment Cleaning and Sanitizing in Food Processing and Handling Operations. Institute of Food and Agricultural Sciences. University of Florida, Gainesville.
- Sindani, G.W. (2012). *The Dairy Industry in Malawi: A Description of the Milk Bulking Groups in Malawi*. North Carolina State University.
- Walearegay, H., Yilma, Z., Tekle-Giorgis, Y. (2012). Hygienic practices and microbiological quality of raw milk produced under farm size in Hawassa, Southern Ethiopia. *Agricultural Research and Reiews*, 1: 132-142.
- Wanjala W.N., Nduko J.M., Mwendu M.C. (2018). Coliforms contamination and hygienic status of milk chain in emerging economies. *Journal of Food Quality and Hazards Control*. 5: 3-10.
- Wiggins, S. (2016). *Dairying in Malawi; The promise and challenges of dairy development*. Retrieved from http://www.sruc.ac.uk/info/120257/food_marketing/802/assessing_contribution_of_dairy_sector_to_economic_growth_and_food_security_in_malawi
- Zdanowicz, M., J. A. Shelford, C. B. Tucker, D. M. Weary, and M.A. G. von Keyserlingk. 2004. Bacterial populations on teat ends of dairy cows housed in free stalls and bedded with either sand or sawdust. *J. Dairy Sci.* 87:1694–1701.

FIRST FINDINGS ON THE PREVALENCE AND INTENSITY OF GASTROINTESTINAL PARASITES IN CAMEROONIAN ZEBUS RAISED ON A LARGE-SCALE DAIRY FARM

Sandrine Dicko Patouma¹, Arouna Njayou Ngapanya¹, Christelle Domngang Noche¹, Alain Kouam Simo¹, Philippe Vignoles² and Félicité Flore Djuikwo Teukeng^{1*}

¹Faculty of Health Sciences, Université des Montagnes, BP 208 Bangangté, Cameroon.

²Laboratory of Parasitology, Faculty of Pharmacy, 87025 Limoges Cedex, France.

Summary

Parasitological investigations on gastrointestinal helminths and *coccidia* in zebu cattle were conducted in April and July on a large-scale dairy ranch to determine the prevalence and intensity of these infections relative to the sampling dates. Using the McMaster method, parasite eggs or oocysts were counted in a total of 400 stool samples. Paramphistome eggs were the most frequent in stools followed by strongyle-type eggs and those of *Fasciola gigantica* in decreasing order. The eggs of *Moniezia*, *Trichuris* and *coccidian* oocysts were only found in some animals, regardless of the sampling date. The prevalences of *Paramphistomes* and *F. gigantica* were significantly higher in April than in July, while the differences between percentages of the other four parasite categories were not significant. Co-infections of cattle by two or three parasites were observed in 23.3-24.5% of seasonally infected zebus and the association of paramphistome + strongyle-type eggs was the most frequent. The number of paramphistome eggs per gram of stool was significantly higher in April. In the other five categories of parasites, egg or oocyst counts recorded in April and July did not show a significant difference. The range of parasite species present in the zebus from this large-scale dairy farm was similar to that previously reported by other authors on gastrointestinal parasites in traditional breeding farms in the same region. The variations in the prevalence and intensity of these infections might be due to the age of the cattle studied and the altitude of the pastures on which these ruminants were grazing.

Key words: Cameroon, coproscopy, *Fasciola gigantica*, *Moniezia*, *Paramphistomes*, strongyle, *Trichuris*, zebu.

Résumé

Premiers résultats sur la prévalence et l'intensité des parasites gastro-intestinaux chez les zébus camerounais élevés dans une ferme laitière à grande échelle. Des investigations parasitologiques ont été réalisées en avril et en juillet sur les helminthes et les coccidies gastro-intestinaux présents chez les zébus d'un important élevage laitier camerounais situé en altitude pour déterminer la prévalence et l'intensité de ces infections par rapport à la date d'échantillonnage. Les œufs de parasites ou les oocystes ont été décomptés dans 400 échantillons de selles en utilisant la méthode de McMaster. Les œufs de *Paramphistomes* étaient les plus fréquents dans les selles et étaient suivis par les œufs de strongles et ceux de *Fasciola gigantica* par ordre décroissant. Les œufs de *Moniezia*, ceux de *Trichuris* et les oocystes coccidiens n'ont été trouvés que chez quelques ruminants, quelle que soit la date d'échantillonnage. Les prévalences des *Paramphistomes* et de *F. gigantica* étaient significativement plus élevées en avril qu'en juillet, alors que les différences entre les prévalences des quatre autres types de parasites n'étaient pas significatives. Des co-infestations du bétail par deux ou trois parasites ont été observées chez 23,3 % ou 24,5 % des zébus selon la saison et l'association des œufs de type paramphistome et de strongles était la plus fréquente. Le nombre d'œufs de paramphistome par gramme de selles était significativement plus élevé en avril. Dans les cinq autres catégories de parasites, les charges en œufs ou en oocystes enregistrées en avril et en juillet n'ont pas montré de différence significative. Par rapport aux données rapportées par d'autres auteurs sur les parasites gastro-intestinaux dans les élevages traditionnels de la même région, la gamme des espèces présentes chez les zébus de cette ferme laitière à grande échelle était identique. Les variations de la

*Corresponding author email: ffnouboue@yahoo.fr

prévalence et de l'intensité de ces infections peuvent être dues à l'âge des bovins étudiés et à l'altitude des pâturages sur lesquels ces ruminants paissent.

Mots clés : Cameroun, coccidie, coproscopie, *Fasciola gigantica*, *Moniezia*, paramphistome, strongle, *Trichuris*, zébu.

Introduction

Gastrointestinal parasitic infections are common in humans and domestic animals in sub-Saharan African countries (Goldsmith and Heyneman, 1989). They constitute a major barrier to livestock production because of the direct and indirect losses they cause (Bush *et al.*, 2001). Cattle, sheep and goats of all ages are affected by numerous internal parasites, which decrease ruminant production and may cause their death (Lefèvre *et al.*, 2010). Among them, protozoa and helminths are the most important parasites. Some of these are zoonotic and therefore constitute a threat to human health (Ekong *et al.*, 2012; Macpherson and Craig, 2013). Many papers have already described the composition and characteristics of helminth species found in human faeces and ruminant stools in most countries of sub-Saharan Africa (Wymann *et al.*, 2007; Hotez and Kamath, 2009).

In Cameroon, several papers have already been published on the gastrointestinal parasites in the cattle of this country. Since the 1980s, Ndamukong *et al.* (1986, 1989) reported that gastrointestinal nematodes and cestodes were responsible for high mortality in sheep and goats. These observations were later confirmed by Awa and Njoya (1997) and Awa and Achukwi (2010). According to these last authors, up to 75% of the deaths observed in sheep and goats in northern Cameroon were due to helminths, particularly haemonchosis and monieziosis. The situation appeared to be different in traditional cattle herds from the North Region, as indicated by Chollet *et al.* (1994), who reported the high incidence of *coccidia*, *Strongyloides* and *Toxocara* in zebu calves 0-12 months of age, while *Trichuris*, *Moniezia*, *Fasciola* and *Paramphistomes* were rarely found. More conflicting results were reported in two other papers. In the Littoral

Region, *Fasciola* spp. and/or *Dicrocoelium dendriticum* were found in 81.3% of cattle slaughtered in Douala, while the stools of these ruminants were mainly infected with *Trichostrongylus* and *Haemonchus* (Ntonifor and Ndaleh, 2012). In contrast, in the North-west Region, Ntonifor *et al.* (2013) noted the predominance of six strongyle species and *Eimeria* spp. in 277 cattle, while *Entamoeba*, *Fasciola* and *Moniezia* were rarer.

These contradictory results for Cameroonian cattle can partly be explained by the climatic conditions, as this country is subjected to an equatorial climate in the south and a tropical climate (with two variants) in the north (Dubresson *et al.*, 1994; Ben Yahmed *et al.*, 2006). However, another explanation, based on differences in cattle management, cannot be ruled out. In fact, most cattle herds in Cameroon are generally reared by smallholder farmers in an extensive way (traditional system) and are mainly composed of a few heads, with ≤ 20 cattle per herd (Dieudonné, 2009). Since natural grasslands remain the only major source of food for livestock in most areas, overgrazing in some areas and/or poor management of forage in other areas (Deffo *et al.*, 2011) might be responsible for these differences in the composition of gastrointestinal parasites and species richness. In order to verify the validity of this hypothesis, further parasitological investigations were carried out on a large-scale dairy farm (Ndawara ranch) in north-western Cameroon to answer the following questions: what were the prevalence and intensity of gastrointestinal helminth and *coccidian* infections in cattle reared on this farm? Were these results comparable to those reported by other authors on traditional farms in the same area? Parasitological surveys were therefore conducted in April and July 2015 to collect stool specimens from ten zebu herds scattered across the various pastures of the ranch. The

values noted during these investigations were compared in a second step to those reported by Ntonifor *et al.* (2013) in traditional farms in the same region.

Materials and Methods

Farms studied

The Ndawara ranch (7,400 ha) is located in the Belo district, Boyo department (North-west region, Cameroon). The presence of crystallophyllian soils gives an acid pH. Its altitude is about 2000 m. The climate is characterized by a long rainy season from May to October, with average annual rainfall between 1500 and 2000 mm. The dry season runs from November to April, with an average monthly temperature of about 21.5°C in June. The region is a typical mountainous area. Its hills and valleys are covered with grass which is the main natural resource for domestic ruminants (Ntonifor *et al.*, 2013).

Several species of ruminants are reared on about 2000 ha of pastures. Of these, cattle are the main part of livestock and their number is estimated at 20,000 Bororo, Gondali or mixed zebus bred in an extensive way. These ruminants are distributed into thirty herds spread over different pastures. Ten zebu herds with a total of 1,127 animals were randomly chosen for these investigations. The management of the zebus varied from one herd to another, depending on the conformation of pastures and the experience of each cowherd.

Experimental protocol

Stool samples were taken from a total of 200 zebus in April 2015 (at the end of the dry season) and 200 in July (during the rainy season). For each sampling period, 20 cattle were randomly selected from each herd regardless of age or sex. Table I shows the characteristics of the 400 animals. Stool samples were taken directly from the rectum of each zebu or just after defecation. They were then immersed in 10% formalin, placed in boxes identified and transported to the Veterinary Laboratory at the Université des Montagnes.

The stools were analyzed using the

McMaster egg count technique (Hendrix and Robinson, 2011). Briefly, thirty grams of each sample were weighed and crushed before being mixed with 60 ml of tap water. The mixture was sieved under tap water to give a volume of 500 ml which was left for 30 min to settle. This sediment was then placed in a test tube and again left for another 30 min for further sedimentation. After removal of the liquid phase, the sediment was homogenized for 2 min and added to 4 ml of saturated zinc sulphate (ZnSO₄) solution. One millilitre of the latter mixture was transferred with a pipette onto a McMaster slide, and parasite eggs and/or *coccidian* oocysts were finally counted under a light microscope at 10x and 40x magnification. Three successive counts were performed on a total of three ml of saturated solution for each stool sample and the individual values scored for each count were averaged to obtain a mean value for one ml of solution and each category of parasites.

Five categories of eggs were considered in the stools of these zebus: i) eggs of *Fasciola gigantica*, ii) those laid by *Paramphistomes*, iii) those laid by the different gastrointestinal strongyles, iv) those laid by *Trichuris* spp. and v) those of *Moniezia* spp. *Coccidian* oocysts were also counted, regardless of the parasite species. As the aim of this study was to determine the prevalence and intensity of infections caused by the main parasite groups, no faecal culture was made to identify the different species of strongyles. In the same way, the identification of *Paramphistomes*, *Trichuris*, *Moniezia* and *Eimeria* species was not carried out. This strategy enabled a comparison of the results with those obtained by Ntonifor *et al.* (2013) during their parasitological investigations on traditional breeding farms in the same region.

Parameters studied

The prevalence of each helminth infection was calculated using the ratio of the number of infected zebus to that of ruminants studied for each sampling period. A similar method was used to determine the prevalence of *coccidian* infections. The prevalence comparison for each sampling

period was performed using a χ^2 test or a Fisher's exact test (when values were too low). The frequency of zebus simultaneously harbouring double or triple parasite infections was the second parameter and was determined for each type of co-infection using the ratio: number of co-infected zebus/total number of infected ruminants. The third parameter was the intensity of each parasite infection and was evaluated by the number of helminth eggs or that of *coccidian* oocysts per gram of stool (epg and opg, respectively). Individual values noted for this last parameter were averaged and standard deviations were calculated based on each sampling date. The normality of egg and oocyst burdens in stools was analyzed using Shapiro-Wilk normality test (Shapiro and Wilk, 1965). As the distribution of these values was not normal, the Scheirer-Ray-Hare test (non-parametric two-way ANOVA) was used to establish levels of significance. All these analyses were performed using the R 3.3.0 software (R Core Team, 2016).

The influence of zebu sex on the prevalence and intensity of parasitic infections was also analyzed. The age factor was not considered in this study, as it was evident that older cattle were the most infected.

Results

Prevalence of parasite infections

Table 2 gives parasite prevalences for both sampling dates. The most frequent helminths were *Paramphistomes* and their prevalence was significantly higher ($\chi^2 = 30.44$, $p < 0.001$) in April than in July. Strongyle-type eggs were also frequently noted in coproscopic examinations (32% in April and 36% in July), but the difference between these values was not significant. *Fasciola gigantica* was a less frequent parasite, with a significantly higher prevalence of infection ($\chi^2 = 4.26$, $p < 0.05$) in April than in July. The other three categories of parasites (*coccidian* oocysts, *Moniezia* spp. and *Trichuris* spp.) were less common in the stool samples collected during both months and no significant differences were noted between these prevalences. Excluding parasite species,

the number of infected zebus was significantly higher ($\chi^2 = 13.96$, $p < 0.001$) in April than in July.

A comparison of these prevalence values with respect to the zebu sex was also made. The prevalence of *Paramphistomes* in April was significantly higher ($\chi^2 = 4.29$, $p < 0.05$) in females than in males. On the other hand, no significant differences were noted between the values recorded in July. The prevalence values found for the other five categories of parasites in April and July showed no significant differences in the sex of zebus.

Zebu co-infections

The overall frequency of double and triple infections in zebus (Table 3) was 24.5% in April and 23.3% in July. No significant differences were found between these frequencies. The most common co-infections were those with two categories of parasites. Among these, the association of paramphistome + strongyle-type eggs was observed in 12.5% of zebus infected in April and 18.3% in July. The simultaneous presence of *Paramphistomes* and *F. gigantica* was noted only in April, with a frequency of 5.8%. The other four types of double infections were found in several animals and their corresponding frequencies were less than 3%. A total of four animals with a triple infection were noted in April. These zebus harboured *Paramphistomes* + strongyles, but with *F. gigantica* in three cases and *Trichuris* spp. in the latter case.

Intensity of zebu infections

Table 4 shows the parasite loads counted in the various stool samples. In the case of *Paramphistomes*, the mean number of eggs ranged from 23.1 to 24.0 epg in April and decreased significantly ($H = 47.24$, $p < 0.001$) to 5.2-6.1 epg in July. Strongyle-type eggs ranged from a mean of 7.1 to 12.1 epg but this variation between the two months was not significant. A similar finding was also noted for *F. gigantica* eggs which ranged from a mean of 0.7 to 2.9 epg. In the other three categories of parasites, the mean loads in infected snails were often less than 1.0 epg or 2.5 opg, and no significant difference was noted between the

Table 1: Age and sex of the 400 zebus studied in the Ndawara ranch (northwestern Cameroon) in April and July 2015.

Parameters	April	July
Sex of zebus		
Males	69	52
Females	131	148
Age of zebus		
1-2 years	55	51
3-4 years	71	88
5-6 years	45	42
> 6 years	29	19

Table 2: Prevalence of natural infections with different parasite categories in Cameroonian zebus in relation to the sampling date in 2015. A total of 200 cattle were investigated in April and 200 in July.

Parasite eggs or oocysts	April		July	
	Numbers of males/females	Overall prevalence (%)	Numbers of males/females	Overall prevalence (%)
<i>Fasciola gigantica</i>	5/13	9.0	2/5	3.5
<i>Paramphistomes</i>	38/82	60.0	23/41	32.0
Strongyle-type	17/47	32.0	23/49	36.0
<i>Trichuris</i> spp.	0/1	0.5	0/1	0.5
<i>Moniezia</i> spp.	2/0	1.0	2/1	1.5
<i>Coccidia</i>	0/1	0.5	4/7	3.5
All categories	47/112	79.5	38/86	62.0

Table 3: Frequency of Cameroonian zebus showing a double or a triple parasitic infection in relation to the sampling date in 2015.

Parasite eggs or oocysts	Number of co-infected zebus (frequency in %)	
	April	July
Total number of infected or co-infected zebus	159	120
Double infections		
<i>Paramphistomes</i> + strongyle-type	20 (12.5)	22 (18.3)
<i>Paramphistomes</i> + <i>Fasciola gigantica</i>	9 (5.8)	0
<i>Paramphistomes</i> + <i>Moniezia</i>	2 (1.2)	1 (0.8)
<i>Paramphistomes</i> + <i>coccidia</i>	1 (0.6)	3 (2.5)
Strongyle-type + <i>F. gigantica</i>	3 (1.8)	1 (0.8)
Strongyle-type + <i>coccidia</i>	0	1 (0.8)
Triple infections		
<i>Paramphistomes</i> + strongyles + <i>F. gigantica</i>	3 (1.8)	0
<i>Paramphistomes</i> + strongyles + <i>Trichuris</i>	1 (0.6)	0
Total number of co-infected zebus	39 (24.5)	28 (23.3)

Table 4: Intensity of natural infections with different parasite categories in 279 infected zebus in relation to the sampling date in 2015.

Parasite eggs or oocysts	Number of helminth eggs or coccidian oocysts per g of stools: mean value ± SD			
	April		July	
	Males	Females	Males	Females
<i>Fasciola gigantica</i>	2.3 ± 7.4	2.9 ± 8.3	0.7 ± 3.1	1.0 ± 4.3
<i>Paramphistomes</i>	23.1 ± 20.5	24.0 ± 35.3	6.1 ± 12.3	5.2 ± 10.6
Strongyle-type	7.1 ± 10.6	12.1 ± 18.1	9.7 ± 12.2	11.5 ± 19.3
<i>Trichuris</i> spp.	0	0.2 ± 2.1	0	0.2 ± 1.5
<i>Moniezia</i> spp.	1.0 ± 6.0	0	1.2 ± 6.6	0.2 ± 2.2
<i>Coccidia</i>	0	0.2 ± 2.0	2.1 ± 6.5	0.5 ± 3.1

values recorded during the two sampling dates, regardless of the parasite species.

In the six categories of parasites, the sex factor had no significant influence on the number of eggs during the two sampling dates.

Discussion

In the Ndawara ranch, the prevalence of paramphistomosis in zebus was 60% in stool samples collected in July (compared to 32% in April). The intensity of infections showed the same quantitative variation. These values are difficult to compare with other data published in Cameroon, as only Chollet *et al.* (1994) reported rare paramphistome infections in the zebu calves they studied. This is probably due to the age of the calves (0-12 months) examined by these authors. Surveys carried out in Nigeria revealed a prevalence of 56% (Bunza *et al.*, 2008) and 15.3% (Adepope *et al.*, 2014) in slaughtered cattle. In other neighbouring countries, the prevalence of paramphistome infections in cattle was 31.3% (Kanyari *et al.*, 2010) in Kenya and 37% (Nzalawahe *et al.*, 2014) or 62.8% (Nzalawahe *et al.*, 2015) in Tanzania, for example. As stool specimens were only taken in two periods over a single year, it is difficult to identify the factor that explains the significant difference in the prevalence between the two sampling periods. We therefore suggest that the most reliable hypothesis would be to relate this result to the season as previously shown in reports of other parasitological

investigations in temperate regions (Díaz *et al.*, 2007a, b; Titi *et al.*, 2010, 2014; Gonzalez-Warleta *et al.*, 2013) and subtropical or tropical countries (Rolfe *et al.*, 1991; Hassan *et al.*, 2005; Eslami *et al.*, 2011; Khan *et al.*, 2012). According to Hakalahti *et al.* (2006), rainfall had a direct effect on the dynamics of parasite populations and, consequently, on transmission of the disease.

The prevalence of *F. gigantica* infections in zebus was 9% in April, while it was only 3.5% in July. This range of values is consistent with the prevalence (6.1%) reported by Ntonifor *et al.* 2013, in stool samples from Cameroonian cattle, but is clearly lower than that (55.6%) noted by Ntonifor and Ndaleh (2012) in slaughtered cattle at Douala (these cattle came from different farms in the country). The significant increase in prevalence in April confirms the report by Keyyu *et al.* (2005) in Tanzania and can be easily explained by the infection of these animals during the rainy season of the previous year. This result confirms the fact that climatic conditions have a direct influence on the characteristics of *F. gigantica* infection in the definitive host in a given region (Spithill *et al.*, 1999; Mas-Coma *et al.*, 2009). Contrary to prevalence, the number of eggs in the stools did not show a significant increase in April and two perhaps complementary explanations may be proposed for this finding: i) egg production by an adult liver fluke in cattle was clearly lower than that of a paramphistome (Djuikwo Teukeng, personal observation), and ii) the

production of liver fluke eggs would be lower in zebu (*Bos taurus indicus*) than in European cattle (*B. t. taurus*), similar to that observed in buffaloes by Prasitirat *et al.* (1996).

Contrary to paramphistome and liver fluke infections, the prevalence of strongyle-type eggs in zebu stools varied in the same range (32%-36%), regardless of the sampling date, while their numbers were relatively stable (from 7.1 to 12.1 epg, Table 4). Although species belonging to this group of *strongyles* were not identified in this study, these results confirmed the predominance of this group compared to other categories of parasites in the prevalence and intensity of infection (with the exception of paramphistome values in April). Similar observations have been reported by Chollet *et al.* (1994) and Ntonifor *et al.* (2013) for Cameroonian cattle, by Adepipe *et al.* (2014) in Nigeria and by Kanyari *et al.* (2010) in Kenya. The absence of significant differences between prevalence values or egg loads observed in April and July indicates that the date of sampling and, by extension, the season had no influence on zebu cattle infection with this group of strongyle species.

The low values obtained for the other three categories of parasites were more difficult to comment upon. Those noted for *Moniezia* spp. agreed with data reported by Chollet *et al.* (1994) or Ntonifor *et al.* (2013) in Cameroonian cattle. The low prevalence of *Trichuris* spp. also confirmed the report by Chollet *et al.* (1994) in calves, but disagreed with that of Ntonifor *et al.* (2013) because the latter authors reported a prevalence of 18.4% in the sample of cattle studied. Prevalence values reported by Chollet *et al.* in 1994 (77.4%) and Ntonifor *et al.* in 2013 (20.9%) for *coccidia* in Cameroonian cattle, were significantly higher than those noted in this study (0.5% and 3.5%). We suggest that these differences between prevalence values for *Trichuris* or *coccidia* might be related to the age of the animals (calves or older) and climatic conditions. The associations between parasitic infections in zebu stools mainly concerned *Paramphistomes* + strongyle-type eggs (Table 3), followed by *Paramphistomes* + *F. gigantica* in decreasing order. This might be due to the

altitude of pastures on which the different zebu herds were grazing in the Ndawara ranch, as demonstrated by Nzalawahe *et al.* (2014) in Tanzania.

Conclusion

The range of parasite species observed in these zebu stools was identical to those reported by Ntonifor *et al.* (2013) in Cameroonian cattle bred in traditional breeding farms in the same region. The variations in the prevalence and intensity of these infections might be due to the age of the cattle studied and the altitude of pastures on which the animals were grazing.

Acknowledgements

The authors gratefully thank the Director of the Ndawara Ranch for his authorizations, Mrs Diallo and Mrs. Shepherds for their help in stool collection. This work was funded by the Université des Montagnes, Cameroon (BUR-CPR-2014/001).

References

- Adepipe OD, Uwalaka EC, Akinseye VO, Adediran OA, Cadmus SIB, 2014. Gastrointestinal helminths in slaughtered cattle in Ibadan, south-western Nigeria. *Journal of Veterinary Medicine*, 2014: 6 pp.
- Awa DN, Njoya A, 1997. Monitoring and control of helminthosis in local sheep in Northern Cameroon. In: Proceedings of the Fifth Annual Conference of the Cameroon Bioscience Association, Yaoundé, Cameroon, 17-19 December 1997, 15 pp.
- Awa DN, Achukwi MD, 2010. Review of livestock pathology in the central African region: epidemiological considerations and control strategies. In: Seiny-Boukar L, Boumard D (éds.), Actes du Colloque « Savanes africaines en développement : innover pour durer », Garoua, 20-23 avril 2009. N'Djamena: Prasac and Montpellier: Cirad, 13 pp.
- Ben Yahmed D, Houstin N, Seignobos C, Abwa D, 2006. Atlas du Cameroun. Paris: Editions Jeune Afrique.

- Bunza MDA, Ahmad A, Fana SA, 2008. Prevalence of paramphistomiasis in ruminants slaughtered at Sokoto Central Abattoir, Sokoto. *Nigerian Journal of Basic and Applied Sciences*, 16: 287–292.
- Bush AO, Fernández JC, Esch GW, Seed JR, 2001. Parasitism: the diversity and ecology of animal parasites. 566 pages. New York: Cambridge University Press.
- Chollet JY, Martrenchar A, Bouchet D, Njoya A, 1994. Epidémiologie des parasitoses digestives des jeunes bovins dans le Nord-Cameroun. *Revue d'Élevage et de Médecine Vétérinaire des Pays Tropicaux*, 47: 365–374.
- Deffo V, Pamo ET, Tchotsoua M, Lieugomg M, Arene CJ, Nwagbo EC, 2011. Determination of the critical period for cattle farming in Cameroon. *International Journal of Livestock Production*, 5: 59–68.
- Díaz P, Pedreira J, Sánchez-Andrade R, Suárez JL, Arias MS, Francisco I, Fernandez G, Díez-Baños P, Morrondo P, Paz-Silva A, 2007a. Risk periods of infection by *Calicophoron daubneyi* (Digenea: Paramphistomata) in cattle from oceanic climate areas. *Parasitology Research*, 101: 339–342.
- Díaz P, Paz-Silva A, Sánchez-Andrade R, Suárez JL, Pedreira J, Arias M, Díez-Baños P, Morrondo P, 2007b. Assessment of climatic and orographic conditions on the infection by *Calicophoron daubneyi* and *Dicrocoelium dendriticum* in grazing beef cattle (NW Spain). *Veterinary Parasitology*, 149: 285–289.
- Dieudonné BPH, 2009. Improving the management of dairy production systems in Cameroon. 232 pages. Doctorate Thesis, University of Pretoria, South Africa.
- Dubresson A, Marchal JY, Raison JP, 1994. *Les Afriques au sud du Sahara*. Paris: Editions Belin-Reclus.
- Ekong PS, Juryit R, Dika NM, Nguku P, Musenero M, 2012. Prevalence and risk factors for zoonotic helminth infection among humans and animals - Jos, Nigeria, 2005-2009. *Pan African Medical Journal*, 12: 6.
- Eslami A, Halajian A, Bokai ES, 2011. A survey of the bovine amphistomiasis in Mazenderan Department, north of Iran. *Iranian Journal of Veterinary Research*, 12: 52–55.
- Goldsmith R, Heyneman D, 1989. *Tropical Medicine and parasitology*. East Norwalk: Prentice-Hall International Editions.
- Gonzalez-Warleta M, Lladosa S, Castro-Hermida JA, Martinez-Ibeas AM, Conesa D, Muñoz F, López-Quilez A, Manga-González M, Mezo M, 2013. Bovine paramphistomosis in Galicia (Spain): prevalence, intensity, etiology and geospatial distribution of the infection. *Veterinary Parasitology*, 191: 252–263.
- Hakalahti T, Karvonen A, Valtonen ET, 2006. Climate warming and disease risks in temperate regions: *Argulus coregoni* and *Diplostomum spathaceum* as case studies. *Journal of Helminthology*, 80: 93–98.
- Hassan SS, Kaur K, Joshi K, Juya PD, 2005. Epidemiology of paramphistomosis in domestic ruminants districts of Punjab and other adjoining areas. *Journal of Veterinary Parasitology*, 3: 43–46.
- Hendrix CM, Robinson E, 2011. *Diagnostic parasitology for veterinary technicians*. 4th edit. Saint Louis: Elsevier.
- Hotez PJ, Kamath A, 2009. Neglected tropical diseases in Sub-Saharan Africa: review of their prevalence, distribution, and disease burden. *PLoS Neglected Tropical Diseases*, 3: e412.
- Kanyari PWN, Kagira JM, Jumanne RI, Mhoma RI, 2010. Prevalence of endoparasites in cattle within urban and peri-urban areas of Lake Victoria Basin, Kenya with special reference to zoonotic potential. *Scientia Parasitologica*, 11: 171–178.
- Kaufmann J, Pfister K, 1990. The seasonal epidemiology of gastrointestinal nematodes in N'Dama cattle in the Gambia. *Veterinary Parasitology*, 37: 45–54.
- Keyyu JD, Monrad J, Kysvgaard NC, Kassuku AA, 2005. Epidemiology of *Fasciola gigantica* and amphistomes in cattle on traditional, small-scale dairy and large-scale dairy farms in the southern highlands of Tanzania. *Tropical Animal Health and Production*, 37: 303–314.
- Khan UJ, Maqbool A, 2012. Prevalence of paramphistomosis in relation to meteorological factors. *Pakistan Journal of Zoology*, 44: 823–828.
- Lefèvre PC, Blancou J, Chermette R, Uilenberg G,

2010. Infectious and parasitic diseases of livestock. Paris: Lavoisier.
- Macpherson CNL, Craig PS, 2013. Parasitic helminths and zoonoses in Africa. London: Springer Science & Business Media.
- Mas-Coma S, Valero MA, Bargues MD, 2009. Climate change effects on trematodiasis, with emphasis on zoonotic fascioliasis and schistosomiasis. *Veterinary Parasitology*, 163: 264–280.
- Ndamukong KJN, Sewell MMH, Asanji MF, 1986. Parasitic gastro-enteritis in sheep and goats in Bamenda, N.W. Province of Cameroon. *Revue Scientifique et Technique, Série Sciences et Techniques*, 2: 101–116.
- Ndamukong KJN, Sewell MMH, Asanji MF, 1989. Disease and mortality in small ruminants in the North West Province of Cameroon. *Tropical Animal Health and Production*, 21: 191–196.
- Ntonifor HN, Ndaleh WN, 2012. Prevalence of liver fluke infections and other gastrointestinal tract parasites in slaughtered cattle in Douala, Cameroon. *Bulletin of Animal Health and Production in Africa*, 60: 438–444.
- Ntonifor HN, Shei SJ, Ndaleh NW, Mbunkur GN, 2013. Epidemiological studies of gastrointestinal parasitic infections in ruminants in Jakiri, Bui Division, North-west Region of Cameroon. *Journal of Veterinary Medicine and Animal Health*, 5: 344–352.
- Nzalawahe J, Kassuku AA, Stothard JR, Coles GC, Eisler MC, 2014. Trematode infections in cattle in Arumeru District, Tanzania are associated with irrigation. *Parasites and Vectors*, 7: 107.
- Nzalawahe J, Kassuku AA, Stothard JR, Coles GC, Eisler MC, 2015. Associations between trematode infections in cattle and freshwater snails in highland and lowland areas of Iringa Rural district, Tanzania. *Parasitology*, 142: 1–10.
- Prasitirat P, Thammasart S, Chompoochan T, Nithiuthai S, Taira S, 1996. The dynamics of antibody types and faecal egg output in cattle and buffalo following infection with 500 and 1000 *Fasciola gigantica* metacercariae. *The Thai Journal of Veterinary Medicine*, 26: 85–89.
- R Core Team, 2016. R: a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing. Available at: <https://www.R-project.org> (accessed on 27 May 2016).
- Rolfé PF, Boray JC, Nichols P, Collins GH, 1991. Epidemiology of paramphistomoses in cattle. *International Journal for Parasitology*, 21: 813–819.
- Shapiro SS, Wilk MB, 1965. An analysis of variance test for normality (complete samples). *Biometrika*, 52: 591–611.
- Spithill TW, Smooker PM, Copeman DB, 1999. *Fasciola gigantica*: epidemiology, control, immunology and molecular biology. In: Dalton JP(ed.), *Fasciolosis*. Oxon: CABI Publishing, 465–525.
- Titi A, Mekroud A, Sedraoui C, Vignoles P, Rondelaud D, 2010. Prevalence and intensity of Paramphistomum daubneyi infections in cattle from northeastern Algeria. *Journal of Helminthology*, 84: 177–181.
- Titi A, Mekroud A, Chibat MH, Boucheihkoukh M, Zein-Eddine R, Djuikwo-Teukeng FF, Rondelaud D, Dreyfuss G, 2014. Ruminant paramphistomosis in cattle from northeastern Algeria: prevalence, parasitic burdens and species identification. *Parasite*, 21: 50.
- Wymann MN, Bonfoh B, Traore K, Tembely S, Zinsstag J, 2007. Species diversity and acquisition of gastrointestinal parasites in calves aged 0-13 months in periurban livestock production in Mali. *Veterinary Parasitology*, 143: 67–73.

STRATIFIED LIVESTOCK PRODUCTION ADDS VALUE TO PASTORAL CATTLE: EVIDENCE FROM THE DRYLANDS OF KENYA

Bulle Hallo Dabasso^{1*}, Oliver Vivian Wasonga¹, Patrick Irungu² and Brigitte Kaufmann³

¹Department of Land Resource Management and Agricultural Technology, Faculty of Agriculture, University of Nairobi, P.O. Box 29053-00625, Nairobi.

²Department of Agricultural Economics, Faculty of Agriculture, University of Nairobi, P.O. Box 29053-00625, Nairobi.

³German Institute for Tropical and Sub-Tropical Agriculture, Faculty of Organic Agricultural Sciences, University of Kassel, Steinstrasse 19, 37213 Witzenhausen, Germany.

Abstract

In Africa's pastoral production systems, the body condition of livestock declines during the dry season when grazing resources become scarce, resulting in lean animals that fail to meet terminal market requirements and therefore fetch low prices. In Kenya, stratified livestock production (SLP) systems in which cattle are purchased from pastoral areas and fattened in other areas where the conditions are more favourable for their growth, are increasingly being adopted. This study evaluated the existing SLP systems practised by ranchers, traders, and agro-pastoralists as options for improving the body condition and market value of cattle produced in the arid and semi-arid pastoral areas. Data on the live weights of cattle at the time of purchase and sale, the costs of purchase and fattening, and the selling prices were collected for the period of January 2010 to June 2016. The results showed that the cattle fattened by the ranchers, traders and agro-pastoralists had average daily weight gains (\pm SD per animal) of 0.24 ± 0.07 kg ($n = 601$), 0.39 ± 0.13 kg ($n = 240$), 0.24 ± 0.08 kg ($n = 140$), respectively. In addition, the average net revenues (\pm SD per animal) (in USD) for the ranchers, traders and agro-pastoralists were 61.7 ± 34.2 , 81.3 ± 44.0 , and 55.9 ± 36.6 , respectively. The results imply that SLP is effective in improving body condition and market value of lean cattle from pastoral areas. The findings are expected to inform the development of pastoral cattle value chain in Kenya and other areas with a similar condition.

Keywords: Livestock fattening, Weight gain, Net revenue, Pastoral production, Marketing

*Corresponding author email: bulledabasso@yahoo.com

Introduction

Pastoral livestock production defines the social and cultural identity of pastoralists (Dioli, 2018) and is also an important source of food and income. It also contributes 10-40% of the agricultural gross domestic product for several African countries including Algeria, Mali, Chad, Sudan, Namibia, Ethiopia, Somalia and Kenya (African Union, 2010). The social and economic values of pastoralism are realized despite the production system being predominately based in drylands where biomass production is highly variable, both within and across seasons (Nori, 2006; Egeru *et al.*, 2014). During the dry season, both the quantity and quality of available pasture are low, and therefore the body condition of livestock declines (Kanuya *et al.*, 2006; Nyamukanza *et al.*, 2009). This implies that the livestock fall short in meeting market requirements and thus fetch low prices (Fatchamps and Gavian, 1997; Barrett *et al.*, 2003; Ayele *et al.*, 2006).

Improving the body condition and the market value of livestock that become lean in the course of the dry season is important in enhancing the socio-economic contribution of pastoral livestock production. However, a viable option for improving the body condition of the livestock produced in pastoral production systems hardly exists. The most common option is supplementary feeding of livestock (Bekele and Abera, 2008; Oddoye *et al.*, 2008), which is costly, labour intensive (Aklilu and Wekesa, 2001), and also has the possibility of compromising food security when livestock feeds are cultivated alongside with crops (FAO, 2009; Erb *et al.*, 2012).

This study evaluated SLP systems as options for improving the body condition and market value of cattle produced in pastoral areas. Stratified livestock production (SLP) has been practised widely in Africa and elsewhere. For example, in Niger and Central Mali, herders often sell weak animals at the beginning of the dry season to agriculturalists who fatten them for markets (Amano, 1995). In Botswana, farmers in semi-arid areas purchase beef cattle from pastoral areas and feed them

on sorghum residues supplemented with maize bran (Farrington *et al.*, 1989), while large scale feed-lot industries fatten cattle in ranches before selling them to the Botswana Meat Commission (Malope *et al.*, 2007).

In Kenya, SLP was started in early 1960, when the government initiated the screening of cattle produced in the pastoral areas, followed by fattening, before slaughtering them at the Kenya Meat Commission, as a strategy to produce quality beef that meets the standards of international markets (Raikes, 1981). However, the government failed to maintain the livestock marketing infrastructure including holding grounds, quarantine centres, and stock routes, which led to the discontinuation of the SLP in 1982 (AU-IBAR and NEPDP, 2006). In spite of poor marketing infrastructure, the SLP has been adopted over the last two decades (Mahmoud, 2006; Farmer and Mbwika, 2012). The adoption is driven by terminal market demands for well-finished cattle (Dabasso *et al.*, 2018) and also possibly relate to the liberalisation of the Kenyan beef market in the mid-1980s (Nyariki, 2008).

Nonetheless, there are production and marketing challenges that could hinder the performance of the SLP in adding value to cattle produced in the pastoral areas of Kenya. For instance, some ranches located in the coastal region of Kenya, are experiencing leadership struggles, illegal mining activities and lack of sufficient water (Njogu and Dietz, 2006) as well as challenges of land subdivision, mismanagement and limited market outlets (Aklilu *et al.*, 2002), which could compromise the sustainable operation of livestock fattening programs in those ranches. Moreover, the stock routes that connect the pastoral areas to the ranches are dilapidated (AU-IBAR and NEPDP, 2006). However, there also emerging opportunities which could make the SLP add value to cattle produced in the pastoral areas. These opportunities include the growing market demand for meat (Alarcon *et al.*, 2017) and the increasing use of mobile money transfer and phone communication systems that can enhance market access and reduce transaction costs (Rutten and Mwangi, 2012).

Given the background of the constraints and opportunities, there is a need to evaluate the SLP as an option for adding value to cattle produced in the pastoral areas of Kenya. The information is useful in improving the pastoral cattle value chains in Kenya in particular and in other areas in general.

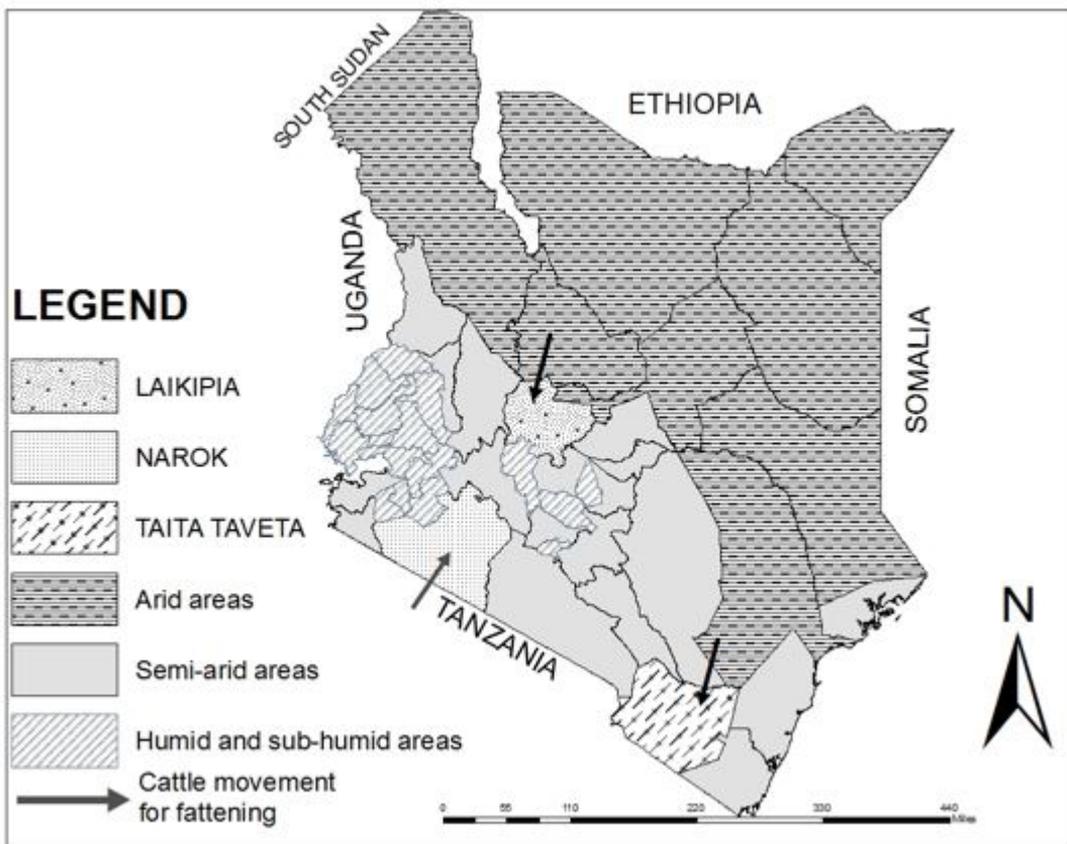
Materials and Methods

Description of the study areas

The study was conducted in Taita Taveta, Laikipia and Narok Counties, located in the semi-arid region of Kenya (Figure 1). Environmental conditions of these counties, including rainfall, relative humidity and temperature are presented in Table 1. Rainfall for these areas has a bimodal distribution, with

the short-rains season occurring in March to May and the long-rains season in October to December (Figure 2). The type of vegetation ranged from wooded savannah to open grassland in which perennial grasses including *Pennisetum mezianum*, *Digitaria* sp., *Themeda triandra*, *Cenchrus ciliaris*, *Chloris roxburghiana* and *Enteropogon* sp. are the dominant species (field notes).

The main economic activity in the areas is cattle ranching, initially started with the aim of producing breeding stock. Nonetheless, a number of ranches are now used for fattening lean cattle that were purchased from the pastoral areas (Mahmoud, 2006). This helps in controlling bush encroachment and boosts market outlet for pastoralists during periods of drought (Bell and Pramer, 2012).

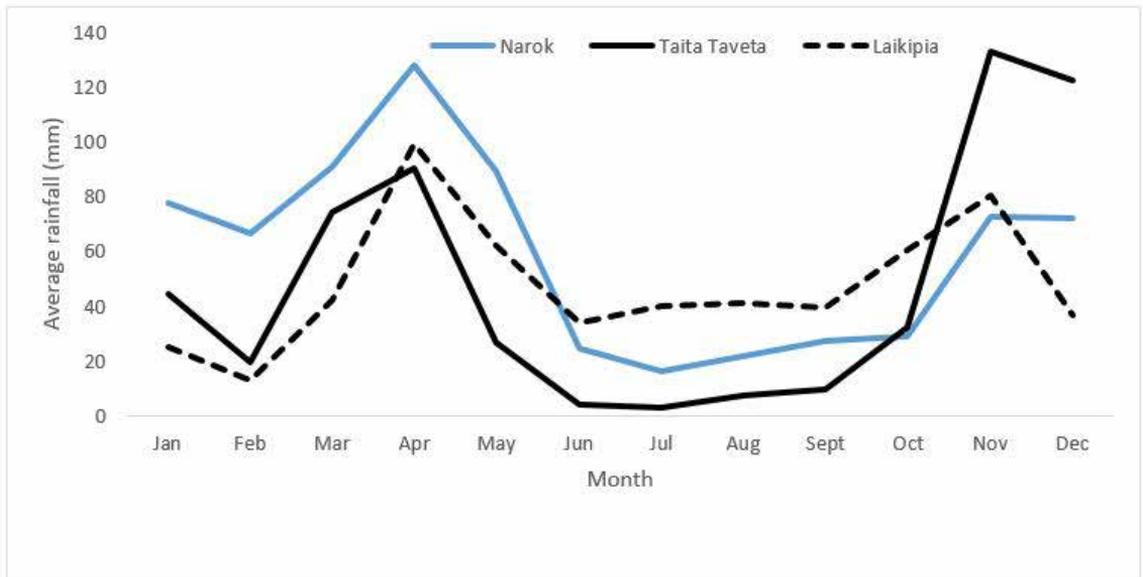


Source: Generated using ArcGIS 10.2, ESRI 2011

Figure 1: Location of the study areas

Table 1: The geographical location and environmental conditions of the study areas

Study area	Geographical location		Environmental condition			Reference
	Latitude	longitude	Temperature (°C)	Average relative humidity (%)	Annual rainfall (mm)	
Narok	0°50'-1°50' S	35°28'-36°25' E	10 - 20	61.0	463-1642	GoK, 2013b
Taita Taveta	0°46' - 4°10' S	37°36'-30°14' E	18.2 - 25	74.0	341-1,200	GoK, 2013c
Laikipia	0°18''S - 051'N	36°11''-37°24' E	16 - 26	60.0	400-750	GoK, 2013a



Source: Authors analysis of rainfall data obtained from the Kenya Meteorological Department in Nairobi, Kenya

Figure 2: Monthly average rainfall from 1980 to 2016 for Taita Taveta, Laikipia and Narok Counties of Kenya

Sampling procedure

Five ranchers, two traders, and nine agro-pastoralists were purposively selected. The selection criterion was that they purchased cattle from the pastoral areas of Kenya for fattening at the start of the data collection period (July 2015) or had complete records of costs and revenues for the cattle that were bought earlier.

The number of cattle bought by the ranchers, traders and agro-pastoralists were 601, 240 and 140, respectively. All the cattle were individually identified using their brand numbers or ear tags and their costs, revenues and weight gains calculated. The cattle were

purchased and fattened between January 2010 and June 2016.

Source of cattle, their characteristics and general management

The ranchers and traders sourced the cattle from the northern pastoral areas of Kenya while the agro-pastoralists sourced them from the southern part of the country. The cattle were of different classes, breeds, and ages and included steers, bulls, cows and heifers of the Borana breed, the Small East African zebu or their crosses, aged 3-4 years. Both the ranchers and traders regularly vaccinated the cattle against foot-and-mouth disease, anthrax,

lumpy-skin disease, and contagious bovine pleuropneumonia while the agro-pastoralists did occasional vaccination whenever there was an expected outbreak of disease. All the practitioners watered the cattle on daily basis during the wet season and once in two days during the dry season. The cattle were given ad-libitum access to salts of locally available brands, either in mixtures or separately. The ranchers and traders provided anthelmintics every three months and sprayed the cattle with acaricides on weekly basis while the agro-pastoralists gave anthelmintics every four months and sprayed acaricides on fortnightly basis.

The ranchers had their own ranches while the traders had to lease grazing resources from ranchers. The agro-pastoralists fattened the cattle in smallholder farms and supplemented them with crop residues (wheat/sorghum straws, maize stovers, and legume haulms). The cattle fattened by the ranchers and traders were sold to abattoirs and slaughterhouses in Nairobi and other major towns in the country while those by the agro-pastoralists were sold in local markets.

Data collection

Individual animal data were either collected using a data sheet that was prepared and handed over to the selected fattening entrepreneurs at the beginning of the study or acquired from their existing records. The data included the weight of the cattle at the time of purchase and at the time of sale, buying and selling prices, market levies (county fees, sale brokerage charges), costs of transportation, charges for veterinary permits, overhead costs (travel expenditures, administrative costs), herding labour (salaries, bonuses, food and medicine), feed & water charges, costs of deworming, spraying/dipping, veterinary drugs, salts/minerals, vaccination, repairs, and wages for night guards. The ranchers and traders used weighing scales to measure the weights of the cattle while the agro-pastoralists estimated the weights from the heart-girth measurements using a weighing tape (made by Dalton Supplies Ltd., England) with the help of

trained field assistants. The tape has about 95% level of accuracy in estimating live weights of East African zebu cattle from the heart-girth measurements (Goe *et al.*, 2001; Lesosky *et al.*, 2013).

Data analysis

Cattle weight gains, costs of purchase and fattening, financial losses, and the net revenues were calculated using the Microsoft Excel Spreadsheet (Version, 2013). The weight gains were calculated using equation 1 (Dunn *et al.*, 2010).

$$DWGi = \frac{FWi - IW_i}{Di} \quad (1)$$

Where; DWGi = Daily weight gain for the *i*th animal.

FWi = Final weight for the *i*th animal at the time of sale.

IWi = Initial weight for the *i*th animal at the time of purchase.

Di = Fattening period (in days) for the *i*th animal.

The purchase costs were calculated using equation 2 as described by Blanco *et al.* (2011).

$$PUi = \sum(BPi + MLi + TCi + OVi) \quad (2)$$

Where; PUi = Total purchase cost for the *i*th animal.

BPi = Buying price for the *i*th animal.

MLi = Market levy for the *i*th animal.

TCi = Transport cost for the *i*th animal.

OVi = Overhead cost for the *i*th animal.

The fattening costs were calculated using equation 3 (Ramsey *et al.*, 2005).

$$FCi = \sum(HLi + FWi + DWi + SPi + VDi + STi + VCi + OTi) \quad (3)$$

Where; FCi = Total fattening costs for the *i*th animal.

HLi = Herding labour for the *i*th animal.

FWi = Cost of feeds and water for the *i*th animal.

DWi = Cost of deworming for the *i*th animal.

SPi = Cost of spraying/dipping for the *i*th animal.

VDi = Cost of veterinary drugs for the *i*th animal.

VCi = Cost of vaccination for the *i*th animal.

OTi = Other costs for the *i*th animal (repairs, wages for night guards).

Where the costs were applicable to an entire herd, costs related to individual cattle were determined by dividing the overall costs by the number of cattle in the herd. To facilitate this, cattle deaths and transfers at the end of every month were recorded.

Financial losses relating to individual cattle were calculated by dividing the total purchasing and fattening costs incurred for the dead or lost cattle divided by the total number of cattle purchased for fattening as suggested by Ott *et al.* (1999) (equation 4).

$$LS = \frac{\sum_n^i PU + \sum_n^i FC}{n} \quad (4)$$

Where; LS = Losses per animal.

$\sum_n^i PU$ = Summation of purchase costs (buying prices, market levies, transportation, and overhead costs) incurred for the dead or lost animals.

$\sum_n^i FC$ = Summation of fattening costs (herding labour, costs of feeds and water, deworming, spraying/dipping, veterinary drugs, vaccination, repairs, night guards) incurred for the *n*th number of the dead animals.

n = Total number of cattle purchased for fattening

The net revenues were determined by subtracting the total costs of purchase and fattening from the selling prices of cattle (Belasco *et al.* 2009). The cattle were sold on-farm and no marketing costs were incurred.

In each of the SLP system, the cattle were grouped depending on location, the year of purchase, the duration of fattening and the individual practitioner involved in their management. The duration of fattening was categorized as short, medium or long depending on whether it was below, within or

above the general average for the SLP system in consideration. One-way analysis of variance (ANOVA) (IBM Corp. SPSS, 2011) was used to test whether the weight gains, costs incurred and the revenues obtained, varied with location, the year of purchase, the duration of fattening, and with individual practitioners. The values were considered significant at $P < 0.05$.

Results

Cattle weight gains, costs, selling prices and net revenues under the SLP by ranchers

The cattle ($n = 601$) fattened by the ranchers had an average initial weight of 232.1 ± 24 kg at the time of purchase and after 376.9 ± 139.5 days, attained an average weight of 315.5 ± 33.1 kg, which translated into per animal overall weight gain (OWG) and daily weight gain (DWG) of 83.4 ± 19.2 kg and 0.24 ± 0.07 kg, respectively. The average purchase cost per animal was USD 286.1 ± 70 , which encompassed the animal buying price (95.6%), the costs of transport (3.4%), overheads (0.5%), market levy (0.4%), and veterinary permit (0.1%). The fattening cost per head of cattle was USD 47.6 ± 28.1 , which included the costs of herding (37.1%), feeds and water (2.4%), deworming (11.1%), spraying/dipping (12.2%), veterinary drugs (3.7%), salts/minerals (12.7%), vaccination (6.6%) and others including repairs, wages for night guards (14.2%). The ranchers sold the animals at an average price of USD 406.77 ± 67.96 and obtained net revenue of USD 61.7 ± 34.2 per head of cattle.

The weight gains, costs, selling prices and revenues varied with location, the year of purchase, the duration of fattening and with individual practitioners (Table 2). The cattle fattened in Laikipia County had 29.6% higher OWG and 33.3% higher DWG compared to those fattened in Taita Taveta County.

Table 2: Cattle weight gains (average \pm SD kg per animal), costs, selling prices and net revenues (USD) (average \pm SD per animal) under the SLP by ranchers and their variations with location, the year of purchase, duration of fattening and with individual ranchers

Source of variation	Overall weight gain	Daily weight gain	Cost of Purchase	Cost of fattening	Selling price	Net revenue
Location	71.8 \pm 17.7 ^b	0.21 \pm 0.04 ^b	313.5 \pm 37.8 ^a	54.5 \pm 5.4 ^b	376.7 \pm 71.4 ^b	60.3 \pm 27.5 ^b
Taita Taveta (n = 273)						
Laikipia (n = 328)	93.1 \pm 14.6 ^a	0.28 \pm 0.08 ^a	261.4 \pm 81.3 ^b	66.8 \pm 24.7 ^a	431.7 \pm 53.4 ^a	80.2 \pm 30.4 ^a
Year of purchase	93.2 \pm 14.9 ^a	0.16 \pm 0.02 ^c	155.9 \pm 0.0 ^c	36.8 \pm 7.7 ^b	363.6 \pm 16.3 ^b	87.4 \pm 10.5 ^a
2010 (n= 122)						
2012 (n=302)	87.5 \pm 14.8 ^b	0.27 \pm 0.08 ^a	304.7 \pm 28.2 ^b	66.2 \pm 27.9 ^a	420.2 \pm 76.3 ^a	68.7 \pm 18.4 ^b
2014 (n= 177)	69.7 \pm 18.3 ^c	0.24 \pm 0.05 ^b	340.8 \pm 9.4 ^a	23.2 \pm 6.0 ^c	413.6 \pm 62.8 ^a	65.1 \pm 60.6 ^b
Period of fattening (days) Short (< 237) (n = 53)	78.7 \pm 15.2 ^b	0.39 \pm 0.07 ^a	263.4 \pm 0 ^b	24.8 \pm 1.4 ^a	310.4 \pm 10.0 ^c	37.5 \pm 12.7 ^b
Average (238-516) (n = 469)	81.2 \pm 19.0 ^b	0.24 \pm 0.05 ^b	309.3 \pm 53.0 ^a	51.2 \pm 30.5 ^a	423.9 \pm 65.5 ^a	48.8 \pm 14.1 ^b
Long (>516) (n = 79)	99.9 \pm 13.7 ^a	0.15 \pm 0.01 ^c	155.9 \pm 0.0 ^b	41.6 \pm 4.7 ^b	369.8 \pm 17.3 ^b	68.3 \pm 12.9 ^a
Rancher	75.8 \pm 15.9 ^b	0.35 \pm 0.08 ^a	263.4 \pm 0.0 ^c	26.9 \pm 2.6 ^c	308.9 \pm 10.0 ^e	13.5 \pm 11.1 ^d
R1 (n = 96)						
R2 (n = 100)	64.0 \pm 7.9 ^c	0.23 \pm 0.03 ^c	332.5 \pm 0.0 ^b	28.3 \pm 1.2 ^c	381.8 \pm 24.4 ^c	5.0 \pm 24.5 ^e
R3 (n = 122)	93.2 \pm 14.9 ^a	0.16 \pm 0.02 ^d	155.8 \pm 0.0 ^d	36.8 \pm 7.6 ^b	363.7 \pm 16.3 ^d	167.3 \pm 10.4 ^a
R4 (n = 77)	77.0 \pm 24.5 ^b	0.24 \pm 0.07 ^b	351.5 \pm 0.0 ^a	16.5 \pm 1.5 ^d	454.8 \pm 72.9 ^b	74.1 \pm 70.6 ^b
R5 (n= 206)	93.0 \pm 14.4 ^a	0.23 \pm 0.03 ^c	323.9 \pm 0.0 ^b	84.5 \pm 9.2 ^a	472.1 \pm 0.0 ^a	44.6 \pm 11.4 ^c

Source: Authors' analysis of the field data. Average values in the same row with different superscripts are significantly different at $P < 0.05$, $n =$ the number of cattle

Cattle weight gains, costs, selling prices and net revenues under the SLP by traders

The cattle ($n = 240$) fattened by the traders had an average initial weight of 308.9 \pm 20 kg and attained weight of 402.2 \pm 24.6 kg at the time of sale which was after 256.8 \pm 80.2 days. The average OWG and DWG per animal were 93.1 \pm 11.3 kg and 0.39 \pm 0.13 kg, respectively. The average cost of purchase, cost of fattening, selling price and net revenue per animal were USD 452.0 \pm 26.0, USD 46.3 \pm 11.9, 593.6 \pm 35.6 and USD 81.3 \pm 44.0, respectively. The costs and revenues were

influenced by the duration of fattening and also differed across the traders (Table 3). The cost of purchase included the cattle buying price (96.0%), costs of transport (2.9%), market levy (0.2%), veterinary permit (0.2%) and overheads (0.7%), while the cost of fattening included the costs of herding (36.4%), feeds and water (35.6%), deworming (4.5%), spaying or dipping (7.1%), veterinary drugs (0.9%), salts (6.2%), vaccination (3.3%) and others (repairs, wages for night guards)(6.0%).

Table 3: Cattle weight gains (average \pm SD kg per animal), costs, selling prices and net revenues (USD) (average \pm SD per cattle) under the SCP by traders and their variations with the duration of fattening and with individual traders

Source of variation	Overall weight gain	Daily weight gain	Cost of Purchase	Cost of fattening	Selling price	Net revenue
Duration of cattle fattening (days) Short (< 177) (n = 56)	89.2 \pm 11.7 ^a	0.58 \pm 0.07 ^a	465.02 \pm 0.0 ^a	29.9 \pm 0.0 ^c	613.2 \pm 48.3 ^a	109.4 \pm 48.3 ^a
Average (177.1-337.5) (n = 126)	94.2 \pm 12.2 ^a	0.37 \pm 0.06 ^b	456.4 \pm 22.1 ^b	47.7 \pm 6.7 ^b	583.5 \pm 32.1 ^c	64.9 \pm 38.3 ^a
Long (> 337) (n = 49)	94.6 \pm 6.6 ^a	0.27 \pm 0.02 ^c	425.6 \pm 31.8 ^c	51.3 \pm 3.0 ^a	596.9 \pm 0 ^b	91.1 \pm 32.3 ^a
Trader T1 (n = 116)	98.2 \pm 9.3 ^a	0.30 \pm 0.04 ^b	438.9 \pm 31.8 ^b	56.9 \pm 4.8 ^a	596.9 \pm 0 ^a	83.5 \pm 31.1 ^a
T2 (n = 115)	87.9 \pm 10.8 ^b	0.49 \pm 0.10 ^a	465.0 \pm 0.0 ^a	35.6 \pm 5.5 ^b	590.2 \pm 50.4 ^a	79.1 \pm 53.9 ^a

Source: Authors' analysis of the field data. Average values in the same row with different superscripts are significantly different at $P < 0.05$, n = the number of cattle

Cattle weight gains, costs, selling prices and net revenues under the SLP by agro-pastoralists

The cattle (n = 140) fattened by the agro-pastoralists had an average initial weight of 225.3 \pm 28.8 kg, and after 183.0 \pm 49.8 days, they attained a final weight of 268.7 \pm 33.3 kg, which translated into OWG of 43.4 \pm 18 kg and DWG of 0.24 \pm 0.08 kg per animal. The average purchase and fattening costs per animal were USD 173.5 \pm 44.1 and USD 43.4 \pm 18 respectively. The cost of purchase comprises of the animal buying price (95.7%), costs of transport (2.9%), market levy (0.6%),

veterinary permit (0.1%) and overheads (0.7%) while that of fattening included costs of herding (42.5%), feeds and water (26.4%), deworming (5.5%), spraying (7.5%), veterinary drugs (3.8%), salts (10.5%), vaccination (2.4%) and others (1.3%). The agro-pastoralists sold the fattened herd at an average price of USD 245.3 \pm 54.0 and obtained net revenue of USD 55.9 \pm 36.6 per animal. Weight gains, costs, and revenues differed with the location, the duration of fattening and with individual agro-pastoralists (Table 4).

Table 4: Cattle weight gains (average \pm SD kg per animal), costs, selling prices and net revenues (USD) (average \pm SD per animal) under the SLP by agro-pastoralists and their variations with the location, the duration of fattening and with individual agro-pastoralists

Source of variation	Overall weight gain	Daily weight gain	Cost of Purchase	Cost of fattening	Selling price	Net revenue
Location Laikipia (n = 50)	48.8 \pm 16.7 ^a	0.25 \pm 0.04 ^a	197.6 \pm 41.1 ^a	16.3 \pm 9.2 ^a	261.5 \pm 52.4	47.5 \pm 33.4 ^b
Narok (n = 90)	40.4 \pm 18.3 ^b	0.23 \pm 0.09 ^a	160.1 \pm 40.0 ^b	13.0 \pm 3.5 ^b	236.4 \pm 53.1	60.5 \pm 37.6 ^a
Period of fattening (days) short (< 133) (n = 17)	23.5 \pm 6.7 ^c	0.19 \pm 0.05 ^a	211.7 \pm 33.2 ^a	9.9 \pm 1.2 ^c	268.6 \pm 35.6 ^a	37.5 \pm 12.8 ^b
Average (133-232) (n = 100)	42.1 \pm 16.3 ^b	0.24 \pm 0.09 ^a	160.5 \pm 35.2 ^b	12.1 \pm 3.4 ^b	230.2 \pm 49.3 ^b	57.2 \pm 38.6 ^a

Source of variation	Overall weight gain	Daily weight gain	Cost of Purchase	Cost of fattening	Selling price	Net revenue
Long (> 232) (n = 23)	63.9 ± 10.0 ^a	0.23 ± 0.04 ^a	202.1 ± 56.4 ^a	26.4 ± 3.8 ^a	294.1 ± 50.9 ^a	63.7 ± 35.6 ^a
Agro-pastoralist A1 (n = 10)	22.2 ± 4.1 ^f	0.18 ± 0.03 ^c	206.2 ± 32.3 ^{ab}	10.7 ± 0.1 ^e	277.5 ± 35.4 ^a	44.7 ± 5.9 ^c
A2 (n = 14)	51.4 ± 4.1 ^b	0.24 ± 0.06 ^b	119.9 ± 33.4 ^e	16.1 ± 3.6 ^c	205.4 ± 62.3 ^c	63.7 ± 49.1 ^{bc}
A3 (n = 10)	53.5 ± 10.1 ^b	0.29 ± 0.05 ^a	157.8 ± 33.9 ^d	11.0 ± 0.3 ^{de}	243.7 ± 95.9 ^b	74.8 ± 65.1 ^{bc}
A4 (n = 7)	54.0 ± 13.8 ^b	0.33 ± 0.09 ^a	133.5 ± 20.5 ^{de}	19.5 ± 1.6 ^b	204.4 ± 19.3 ^c	51.4 ± 8.7 ^c
A5 (n = 15)	53.7 ± 8.4 ^b	0.25 ± 0.04 ^b	135.8 ± 12.8 ^d	16.2 ± 0.3 ^c	251.3 ± 20.4 ^b	99.3 ± 28.9 ^a
A6 (n = 34)	28.8 ± 17.8 ^e	0.19 ± 0.12 ^c	180.1 ± 31.2 ^c	10.4 ± 0.3 ^e	234.9 ± 41.4 ^b	44.5 ± 14.6 ^c
A7 (n = 17)	68.5 ± 4.9 ^a	0.24 ± 0.02 ^b	228.7 ± 37.2 ^a	28.7 ± 0.4 ^a	309.6 ± 38.6 ^a	52.3 ± 4.9 ^c
A8 (n = 17)	37.2 ± 12.4 ^d	0.24 ± 0.04 ^b	203.8 ± 31.1 ^b	12.0 ± 2.9 ^d	230.1 ± 35.3 ^b	14.3 ± 15.1 ^d
A9 (n = 16)	40.2 ± 7.3 ^c	0.25 ± 0.04 ^b	157.9 ± 14.4 ^d	7.7 ± 0.4 ^f	243.5 ± 44.2 ^b	77.8 ± 33.3 ^b

Source: Authors' analysis of the field data. Average values in the same row with different superscripts are significantly different at $P < 0.05$, $n =$ the number of cattle

Discussion

The purpose of this study was to evaluate whether SLP systems add value to pastoral cattle that become lean during the periods of pasture shortage in drylands. To achieve this, the study assessed cattle weight gains, costs incurred and net revenues under the SLP systems practised by ranchers, traders and agro-pastoralists in Kenya. The analysis of the weight gains provided an understanding of animal productivity and the findings were consistent with those of Asizua *et al.* (2009), who evaluated weight gain for Ankole cattle and their crosses with Borana and Friesian that were fattened on natural pastures in the semi-arid region of Uganda. The net revenues recorded in the current study depict that fattening of lean cattle from pastoral areas is a profitable undertaking. Other studies (Little *et al.* 2014; Malole *et al.*, 2014) have also reported profitability of fattening pastoral cattle through SLP systems. The study by Little *et al.* (2014) also showed that fattening of drought-stricken cattle, that were purchased from herders in southern Ethiopia during the drought of 2011 was profitable, while that by Malole *et al.* (2014) analysed the costs and revenues for indigenous beef cattle fattening systems in north west Tanzania and reported that the business is profitable, although the level of profitability

varied with the production system. In the current study, the SLP by traders in which access to grazing resources was through lease arrangements had the highest net revenue per head of cattle and therefore the most profitable. This may be attributable to the practice of pasture leasing which guaranteed access to pasture, especially during dry periods. The importance of pasture leasing for livestock production in Kenya especially during the dry season has been highlighted by Lengoiboni *et al.* (2011) who recommended that the government should recognize and formalize pasture lease agreements to enhance pastoralists' access to dry season grazing resources.

The cattle weight gains and net revenues were found to vary with the location, the duration of fattening, and with individual practitioners. This is attributable to the spatial-temporal variability of grazing resources coupled with possible differences in the management practices among the practitioners. Additionally, there is a high genotypic diversity among the East African zebu cattle (Rege *et al.*, 2001) which might have also contributed to the observed differences in the weight gains and net revenues. A study that provides a comparison of the profits earned by the practitioners under the SLP systems and under non-SLP in which herds are reared for breeding, is essential to guide the practitioners to choose

the most economically viable production system. Although a few studies (Muhuyi, 1997; Nyariki, 1990) had earlier established revenues achievable in a production system in which breeding herds of cattle were reared in Laikipia areas of Kenya, data collected concurrently with that for the SLP systems would provide an accurate comparison of the profitability between the two production systems.

This study has shown that the expenses on transportation took a larger proportion of the total purchase cost in all the SLP systems. This reflects weak market access in the pastoral livestock value chain as observed in other studies (Aklilu, 2008; Onono *et al.*, 2015). The current study further found that in the three forms of SLP, a significant proportion of the total fattening cost was spent on grazing resources (feeds and water). This observation matched with the findings by Okoruwa *et al.* (2005) who showed that expenses on feeds formed a significant percentage of the total variable cost incurred by the cattle-fattening farms located in the Ibadan region of Nigeria.

Conclusion

The findings in this study reveal that SLP is effective in improving the body condition and market value of lean cattle from the arid and semi-arid pastoral areas of Kenya. However, the animal weight gains and the profitability depend on the location, the duration of fattening, and the management practices. The study recommends marketing of lean cattle produced in the pastoral areas of Kenya through a SLP system which is particularly relevant in the face of changing land use and tenure arrangement that limit herd mobility for accessing dry season grazing resources.

Acknowledgments

This study was funded under the collaborative research project, "Reduction of Post-Harvest Losses and Value Addition in East African Food Value Chains" (RELOAD), supported through the Global Food Security Project (GlobeE) by the German Federal

Ministry of Education and Research (BMBF) in cooperation with Germany Federal Ministry for Economic Cooperation and Development (BMZ). Mr. Gitonga Thurairira of the Kenya Agricultural and Livestock Research Organization assisted in statistical analyses and Mr. Peter Mwangi of the African Drylands Institute for Sustainability helped in mapping the study areas.

References

- African Union. 2010. Policy framework for pastoralism in Africa: Securing, protecting and improving the lives, livelihoods and rights of pastoralist communities, <http://www.achpr.org/files/instruments/policy-framework>, accessed 20 April 2017
- Aklilu, Y., Irungu, P., Reda, A. 2002. An audit of the livestock marketing status in Kenya, Ethiopia and Sudan, (Organization of African Unity/Interafrican Bureau for Animal Resources, Nairobi, Kenya), Volume I
- Aklilu, Y., Wekesa, M. 2002. Drought, Livestock and Livelihoods: Lessons from the 1999-2001 Emergency Response in the Pastoral Sector in Kenya. HPN Network Paper 40. London: Overseas Development Institute
- Aklilu, Y. 2008. Livestock Marketing in Kenya and Ethiopia : A Review of Policies and Practice. Feinstein International Centre, Addis Ababa. http://www.fao.org/fileadmin/user_upload/drought/docs/Livestock%20Marketing%20in%20Kenya%20and%20Ethiopia%20-%20Aklilu%202008.pdf. Accessed 20 November 2017
- Alarcon, P., Fevre, E., E.M., Murungi, M.K., Muinde, P., Akoko, J., Dominguez-Salas, P., Kiambi, S., Ahmed, S., Hasler, B., Rushton, J. 2017. Mapping of beef, sheep and goat food systems in Nairobi □ A framework for policy making and the identification of structural vulnerabilities and deficiencies, *Agricultural Systems*, 152, 1 □ 7
- Amano, K. 1995. Dynamics of herd structures and herding strategies in West Africa: a study of market integration and ecological adaptation, *Journal of the International African Institute*, 65 (3), 351–94
- Asizua, D., Mpairwe, D., Kabi, F., Mutetikka,

- D., & Madsen, J. 2009. Growth and slaughter characteristics of Ankole cattle and its Boran and Friesian crossbreeds. *S.Afr. J. Anim. Sci.* 39, 81-85.
- AU-IBAR and NEPDP African Union Inter-African Bureau for Animal Resources and North Eastern Pastoral Development Program. 2006. Kenya livestock sector study: an analysis of pastoralist livestock products market value chains and potential external markets for live animals and meat, http://pdf.usaid.gov/pdf_docs/pnady825.pdf, accessed 20 April 2017
- Ayele, G., Jabbar, M., Teklewold, H., Mulugeta, E., Kebede, G. 2006. Seasonal and inter-market differences in prices of small ruminants in Ethiopia, *Journal of Food Products Marketing*, 12 (4), 59–77
- Barrett, C.B., Chabari, F., Bailey, D., Little, P.D., Coppock, D.L. 2003. Livestock pricing in the northern Kenyan rangelands, *Journal of African Economies*, 12 (2), 127–55
- Bekele, G., Abera, T. 2008. Livelihoods-based drought response in Ethiopia: Impact assessment of livestock feed supplementation (Pastoralist Livelihoods Initiative Program), United State Agency for International Development, <https://www.alnap.org/system/files/content/resource/files/main/bekele-and-tsehay-pia-aug-082.pdf> (Accessed 25 September 2018)
- Belasco, E.J., Taylor, M.R., Goodwin, B.K., Schroeder, T.C. 2009. Probabilistic models of yield, price, and revenue risks for fed cattle production, *Journal of Agricultural and Applied Economics*, 41 (1), 91–105
- Bell, C. and Prammer J. 2012. Linking livestock markets to wildlife conservation. In: C. Eder, D. Kyd-Rebenburg, J. Prammer (eds), *Global growing casebook insights into African Agriculture*, (Global Growing Campaign), 154–177, http://global-growing.org/sites/default/files/GGC_Lewa.pdf, accessed 16 November 2017
- Blanco, M., Joy, M., Ripoll, G., Sauerwein, H., Casasús, I. 2011. Grazing Lucerne as fattening management for young bulls: technical and economic performance and diet authentication, *Animal*, 5 (1), 113–22
- Dabasso, B., Wasonga, O., Irungu, P., Kaufmann, B. 2018. Stratified cattle production in pastoral areas of Kenya: Existing forms, driving factors and management practices, *Applied Animal Husbandry and Rural Development* Vol. 11, Issue 1, pp 22-33
- Dioli, M. 2018. Nomad aesthetic: cattle modifications among the northern Turkana of northwest Kenya, *Pastoralism: Research, Policy and Practice*, 8 (6)
- Dunn, B.H., Smart, A.J., Gates, R.N., Johnson, P.S., Beutler, M.K., Diersen, M.A., Janssen, L.L. 2010. Long-term production and profitability from grazing cattle in the Northern Mixed Grass Prairie, *Rangeland Ecology & Management*, 63 (2), 233–42
- Egeru, A., Wasonga O., Kyagulanyi, J., Mwanjalolo, M., MacOpiyo, L., Mburu, J. 2014. Spatial-temporal dynamics of forage and land cover changes in Karamoja sub-region, Uganda, *Pastoralism: Research, Policy and Practice*, 4 (6)
- Erb, K., Mayer, A., Kastner, T., Sallet, K., Haberl, H. 2012. The impact of industrial grain fed livestock production on food security: an extended literature review commissioned by Compassion in World Farming, The Tubney Charitable Trust and World Society for the Protection of Animals, UK. https://www.ciwf.org.uk/includes/documents/cm_docs/2012/t/the_impact_of_industrial_grain_fed_livestock_production_on_food_security_2012.pdf (Accessed 26 June 2018)
- ESRI. 2011. ArcGIS Desktop: Release 10. Redlands. Environmental Systems Research Institute, New York
- FAO. 2009. The state of food and agriculture 2009 Livestock in the balance, Rome, <http://www.fao.org/docrep/012/i0680e/i0680e.pdf> (Accessed 26 June 2019)
- Farrington, T., Marsh, D.K.V., Mayers, L., 1988. Winter fattening of beef cattle: From research station trials to implementation by farmers, *Agricultural Systems* 29, 165-178.
- Farmer, E. and Mbwika, J. 2012. End market analysis of Kenyan livestock & meat, a desk study, microREPORT# 184, https://www.microlinks.org/sites/microlinks/files/resource/files/Kenya_Livestock_End_Market_Study.pdf, (Accessed 20 April 2017)
- Fatchamps, M. and Gavian, S. 1997. The determinants

of livestock prices in Niger, *Journal of African Economies*, 6(2), 255–295

Goe, M.R., Alldrege, J.R., Light, D. 2001. Use of heart girth to predict body weight of working oxen in the Ethiopian highlands, *Livestock Production Science*, 69, 187–195

GoK-Government of Kenya. 2013a. First edition of Laikipia County integrated development plan for 2013-2017, (Unpublished report), <https://laikipia.go.ke/assets/file/Laikipia-CIDP-2013-2017-compressed.pdf> (Accessed 12 September 2018)

GoK-Government of Kenya. 2013b. First edition of Narok County integrated development plan for 2013-2017, (Unpublished report), www.narok.go.ke/component/j2store/downloads/getfreefile?pfile_id=990 (Accessed 12 September 2018)

GoK-Government of Kenya. 2013c. First edition of Taita Taveta County integrated development plan for 2013-2017, (Unpublished report), <https://taitataveta.go.ke/sites/default/files/Taita%20Taveta%20CIDP%20as%20at%20th%20August%202014.pdf> (Accessed 12 September 2018)

IBM Corp. SPSS. 2011. IBM SPSS Statistics for Windows (Version 20.0). IBM Corp, Armonk, New York

Kanuya N.L., Matiko M.K., Nkya R., Bittegeko S.B.P., Mgasa M.N., Reksen, O., Ropstad E. 2006. Seasonal changes in nutritional status and reproductive performance of zebu cows kept under a traditional agro-pastoral system in Tanzania, *Tropical Animal Health and Production*, 38, 511–519

Lengoiboni, M., van der Mole, P., Bregt, A.K., 2011. Pastoralism within the cadastral system: Seasonal interactions and access agreements between pastoralists and non-pastoralists in northern Kenya, *Journal of Arid Environments*, 75, 477–486

Lesosky, M., Dumas, S., Conradie, I., Handel, I., Jennings, A., Thumbi, S., Toye, P., Bronsvort, B. 2013. A live weight-heart girth relationship for accurate dosing of East African shorthorn zebu cattle, *Tropical Animal Health and Production*, 45, 311–316

Little, P.D., Dejene, D., Tiki, W. 2014. How pastoralists perceive and respond to market opportunities: the case of the Horn of Africa, *Food Policy*, 49, 389–97

Mahmoud, H.A. 2006. Innovations in pastoral livestock marketing: the emergence and the role of Somali cattle traders-cum-ranchers in Kenya. In: G. McPeak, P.D. Little (eds), *Pastoral livestock marketing: research and policy challenges*, (Intermediate Technology Publications Limited, Rugby, Warwickshire), 129–144

Malole, J.L., Kadigi, R.J., Sangeda, A.Z. 2014. Costs and Benefits of Beef Cattle Fattening Schemes in Some Selected Areas of North West Tanzania, *Iranian Journal of Applied Animal Science*, 4 (4), 699–706

Malope, P., Tsopito, C.M., Aganga, A.A., Madibela, O.R. 2007. Profitability of dry season beef feed-lotting in grain deficit countries: the case of Botswana, *Livestock Research for Rural Development*, 19 (6)

Muhuyi, W.B. 1997. A comparison of the productivity of Kenya Sahiwal cattle and their crossbreds in large dairy-dual purpose and beef Production systems, (unpublished PhD thesis, University of Nairobi)

Njogu, J., Dietz, T. 2006. Land use and tenure: entitlement rights for community-based wildlife and forest conservation in Taita Taveta, Kenya, (Institute for Development Studies, University of Nairobi, Kenya), Working Paper No. 542

Nori, M. 2006. Mobile livelihoods, patchy resources & shifting rights: approaching pastoral territories, (Draft paper for the International Land Coalition), http://www.agter.asso.fr/IMG/pdf/08_ILC_mobile_livelihoods_patchy_resources.pdf (Accessed 7 September 2018)

Nyamukanza, C.C., Scogings, P.F., Kunene, N.W. 2009. Forage-cattle relationships in a communally managed semi-arid savanna in northern Zululand, South Africa, *African Journal of Range & Forage Science*, 25, 131–140

Nyariki, D.M. 1990. Economic analysis of beef ranching: the case of Laikipia District, Kenya, (MSc thesis, University of Nairobi)

Nyariki, D.M. 2008. Price response of herd off-take under market liberalization in a developing cattle sector: panel analysis applied to Kenya's ranching, *Environment and Development Economics*, 14, 263–280

Oddoye, E.O.K., Takarma, J.F., Owusu, A. F., Johnson, V.

2008. Supplementation of grazing cattle: The use of a diet containing graded levels of dried cashew pulp. *Bulletin of Animal Health and Production in Africa* 56, 136-142

Okoruwa, V.O., Obadaki, F.O., Ibrahim, G. 2005. Profitability of Beef Cattle Fattening in the Cosmopolitan City of Ibadan, Oyo State, *Moor Journal of Agricultural Research*, 6 (1 & 2), 45–51

Onono, J.O., Amimo, J.O., Rushton, J. 2015. Constraints and efficiency of cattle marketing in semiarid pastoral system in Kenya, *Tropical Animal Health and Production*, 47 (4), 691–97

Ott, S.L., Wells, S.J., Wagner, B.A. 1999. Herd-level economic losses associated with Johne's diseases on US dairy operations, *Preventive Veterinary Medicine*, 40, 179–192

Raikes, P.L. 1981. *Livestock Development and Policy in East Africa*, (publication from the Centre of Development Research, Scandinavian Institute of African Studies, Uppsala), <http://www.diva-portal.org/smash/get/diva2:274873/FULLTEXT01.pdf> (Accessed 5 March 2018)

Ramsey, R., Doye, D., Ward, C., McGrann, J., Falconer, L., Bevers, S. 2005. Factors affecting beef cow-herd costs, production and profits, *Journal of Agricultural and Applied Economics*, 37, 91–99

Rege J.E.O., Kahi, A.K., Okomo-Adhiambo, M., Mwacharo, J., Hanotte, O. 2001. Zebu cattle of Kenya: uses, performance, farmer preferences, measures of genetic diversity and options for improved use, (Animal Genetic Resource Research I; International Livestock Research Institute, Nairobi, Kenya)

Rutten, M., Mwangi, M. 2012. Mobile cash for nomadic livestock keepers: The impact of the mobile phone money innovation (M-Pesa) on Maasai pastoralists in Kenya. In: J. Gewald, A. Leliveld, I. Pesa (eds), *Transforming Innovations in Africa: Explorative Studies on Appropriate in Africa Societies*, Leiden, Netherlands: Brill

PLANNING FOR RATIONAL USE OF ANTIBIOTICS IN TREATMENT OF BOVINE MASTITIS IN RWANDA

*Biryomumaisho S¹, Musanayire V², I Kiiza D³ and Kanyandekwe C²

¹Department of Veterinary Pharmacy, Clinical and Comparative Medicine, Makerere University, P.O. Box 7062, Kampala, Uganda

²Rwanda Agriculture Board, P.O. Box 5106, Kigali, Rwanda

³Heifer Project Rwanda, P.O. Box 4785, Kigali, Rwanda

*Formerly at Rwanda Agriculture Board, P.O. Box 5106, Kigali, Rwanda

Abstract

Subclinical *mastitis* and antimicrobial resistance are a challenge to the dairy industry in many countries particularly in the developing world. A study was conducted between January and February 2015 in five milk shade districts of Bugesera, Nyagatare (Eastern), Gicumbi (Northern), Kamonyi and Nyanza (Southern) provinces in Rwanda to assess the occurrence of subclinical *mastitis* among smallholder cattle farmers in order to identify the causative bacteria and their antimicrobial susceptibility. Of the 1,188 milk samples screened using the California *mastitis* test (CMT), 820 (70.6%) tested negative while weak and moderate somatic cell counts (SCCs) occurred in approximately equal proportions at 13% and 12% respectively. Only 42 (3.5%) of the samples were strongly positive. Chi-square tests for homogeneity of SCCs within groups viz parity, quarter which was milked and breed of cow were not significant factors for occurrence of subclinical *mastitis* ($p > 0.05$). Among milk samples that were CMT-positive, 496 samples were successfully cultured. There was no bacterial growth in 283 (57 %) milk samples. *Bacilli* were the most isolated bacterial species ($n=94$, 19%) while streptococci were the least isolated bacteria ($n=10$, 3%). The bacteria were most sensitive to *Gentamycin* (100%) followed by *Neomycin* and *Cotrimoxazole* each at 89% but most resistant to *Amoxicillin* (93%), *Streptomycin* (68%), *Ampicillin* (61%) and *Tetracycline* (61%); there was moderate resistance to *Bacitracin*. This study showed that subclinical *mastitis* is still a constraint to milk producers in Rwanda. Since most *mastitis* cases are likely to be treated before the release of culture and sensitivity laboratory tests, the results from this study showed that single or combination antibiotics comprising *Bacitracin*, *Penicillin*, *Streptomycin*, *Ampicillin* and *Tetracycline* may be used as first-line treatment options while *Gentamycin*, *Neomycin* and *Cotrimoxazole* can be used as second-line treatment options in case of failure with first line antibiotics in the treatment of *mastitis*.

Key words: Rwanda, subclinical *mastitis*, risk factors, antibiotics

Introduction

Mastitis is one of the most important diseases of dairy cattle worldwide and is manifested by a wide range of clinical and subclinical conditions. While clinical *mastitis* is easily recognized by farmers and hence milk from affected quarters excluded from sale or home consumption, subclinical *mastitis* is more difficult to detect because of its insidious nature. Subclinical *mastitis* can lead to reduced milk production (Hortet and Seegers, 1998), decreased milk quality for dairy purposes and remains one of the leading causes of losses due to rejected milk at collection centres (Persson and Olofsson, 2011). The frequency and occurrence of *mastitis* varies with geographical differences, environmental conditions and management practices in herds. The pathogens that cause *mastitis* are broadly classified as contagious and environmental. Contagious pathogens adapt to the mammary gland environment and can easily spread from one cow to another while environmental pathogens are opportunistic infections of the mammary gland and can potentially be transferred from the contaminated environment to the mammary gland of a cow during milking (Rysanek *et al.*, 2009).

Milk production in Rwanda has been on the increase in the last two decades from 50,000 metric tons in year 2000 (PSTA, 2014) to 706,030 metric tons in 2014 (NISR, 2015) making a per capita consumption of 59 litres, up from 37.3 litres in 2010. Testing for quality, however, has not expanded proportionately with increased production. Expansion of the milk market and subsequent improved returns from the sale of milk and milk products depends on enhanced milk quality. Subclinical *mastitis* is one of the leading causes of reduced milk quality and is caused mainly by bacteria (Zadoks *et al.*, 2011). The causes of *mastitis* from a contaminated environment include *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, *Streptococcus uberis* and *Streptococcus bovis* (Sharma *et al.*, 2011). *Bacilli* are also common causes of

mastitis in dairy cattle. The coliforms mainly *Escherichia coli*, *Klebsiella* and *Pseudomonas* are also causative organisms of *mastitis*. Other causes may be mycoplasma, yeast, algae, toxins, and physical trauma especially teat wounds and fistulae. In rare instances, some viruses that include bovine herpesvirus 1, bovine herpesvirus 4 and Foot and mouth disease virus have been isolated from milk from cows with subclinical *mastitis* (Wellnberg *et al.*, 2011). These viruses in addition to bovine leukaemia virus may all play an indirect role in *mastitis* development because of their immunosuppressive properties.

Approximately 25-30% of cows with chronic cases of subclinical *mastitis* may exhibit clinical symptoms that require antibiotic treatments and withholding of milk from sale, thus causing losses to the farmer (Barlow *et al.*, 2009). In Rwanda a considerable volume of milk brought to processing plants is rejected due to poor quality basing on adulteration of milk with water when tested with a lactometer and the alcohol test for the level of fermentation and presence of lactose-fermenting bacteria in subclinical *mastitis* states. Cows with subclinical *mastitis* maintain a reservoir of infection within the herd and increase the exposure of healthy cows to contagious pathogens. In Rwandan dairy cattle, limited studies have reported subclinical *mastitis* prevalence of up to 52% (Iraguha *et al.*, 2015). Generally, the prevalence of infected cows varies from 5%–75%, and quarters from 2%–40% (Merck's Veterinary Manual, 1998].

There are various methods in use for detection of subclinical *mastitis* in cattle: deviations in milk yield (Shoshani *et al.*, 2000); electrical conductivity of fore milk (Kasikci *et al.*, 2012); microorganism load, infrared thermography and udder skin surface temperature (Polat *et al.*, 2010). The other methods are the use of total and differential somatic cell counts in milk (Thurmond (1990) and indirect somatic cell counts in milk using CMT (Middleton *et al.*, 2004). None of these methods, however, is sufficient to inform the veterinarian or farmer on the course of treatment to administer and follow-up for the successful therapy of particular cases.

Moreover, it depends on whether the results are based on data collected from individual infected quarters, composite milk from the four quarters of an individual cow or bulk milk.

Antimicrobial compounds are widely used to treat and prevent clinical and sub-*mastitis*. The treatment regimens comprise of intramammary antibiotic infusions and when in systemic reactions caused by toxemia / bacteraemia and characterised by raised body temperature, homologous or synergistic antibiotics to intramammary infusions are injected. The common intramammary antibiotics registered and authorised for use in Rwanda are in four main groups. These are β -lactam antibiotics (*Penicillin*, *amoxicillin* and *Ampicillin*); aminoglycosides (*Gentamycin*, *Neomycin* and *Streptomycin*); *Tetracyclines* and sulphonamides (*Cotrimoxazole*). A variety of supportive treatments such as frequent milk removal, oxytocin injections, intraruminal or intravenous fluids, steroidal and non-steroidal anti-inflammatory drugs can be used (Shim *et al.*, 2004). However, these treatments are usually administered without evidence of their efficacy and this is one of the causes of treatment failure hence the need for antibiotic selection based on pathogen isolation and in vitro susceptibility testing. In addition to costs incurred, the overuse and in some instances the misuse of antibiotics may result in emergence and entrance of resistant bacteria species in the human food chain (White and McDermott, 2001). The other methods with great potential in the treatment and control of *mastitis* include use of bacteriophages (Chibani-Chennoufi, *et al.*, 2004); vaccination (Bannerman and Wall, 2005; Cullor *et al.*, 2013); plant-derived natural products with antimicrobial action (Domadia *et al.*, 2007) and recombinant cytokines (Alluwaimi, 2004). The use of animal derived immunomodulators naturally produced by mammals such as lactoferrin from saliva, tears and milk also has great potential (Hafez *et al.*, 2013).

The importance of *mastitis* stretches beyond individual farmers and milk processors to public health because of the extensive use of antibiotics in its treatment. The worries

about antimicrobial resistance, milk quality and antibiotic residues in milk are a matter of concern to consumers of milk and its products and the whole society. This study, therefore, was undertaken to understand the extent of subclinical *mastitis* in Rwandan dairy cattle, identify the causative pathogens and their antimicrobial susceptibility patterns.

Materials and Methods

Study area

During the 2015 epidemiosurveillance of livestock diseases, milk samples were collected from five milkshed districts of Rwanda namely Kamonyi and Nyanza (Southern Province), Bugesera and Nyagatare (Eastern Province) and Gicumbi (Northern Province) (Figure 1). Selection of the districts was based on their importance in milk production in the country.

Collection and CMT of milk samples

A total of 1,188 individual teat milk samples were obtained from 297 lactating cows on 66 farms and screened for subclinical *mastitis* using commercial BOVIVET CMT reagent. On each farm, the teats of selected cows were washed with clean warm water, dried and disinfected with 70% ethanol. A small sample of milk, approximately 5 ml from each quarter was milked directly onto each of the four wells of a plastic CMT paddle. An equal amount of CMT reagent was added and the mixture rotated clockwise and anticlockwise for 30 seconds. Samples from each quarter were recorded as negative if there was no colour change on the milk-CMT mixture (less than 250,000 somatic cells). A score of + was assigned if the sample was weakly positive with thickening of the mixture but not forming a gel (250,001-800,000 SCC). The thickening could in some cases disappear when rotation was maintained for a few more seconds. A score of ++ (800,001-1,500,000 SCC) was assigned when immediate thickening of the mixture, with a slight gel formation were noted. When the mixture was further swirled, it moved toward the centre of the cup, exposing the bottom of the outer edge. A score of +++

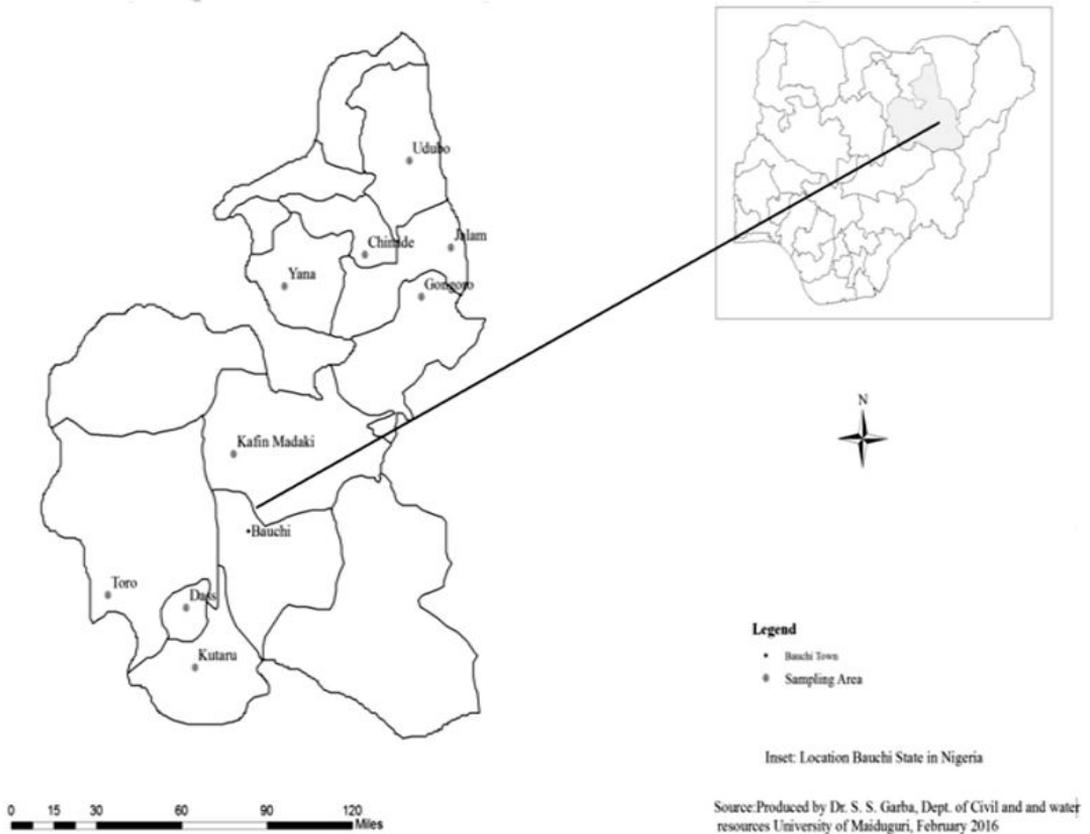


Figure 1: Map of Rwanda showing the sampled milkshed districts

(> 1,500,001 SCC) was assigned when a gel was formed and the surface of the mixture became elevated (like a fried egg). The central peak remained projected even after the CMT paddle rotation was stopped. For each tested teat, 2ml of milk samples were milked into sterile test tubes, kept at 4°C in a cool box and submitted to the laboratory for bacteriological and antibiotic sensitivity analyses.

Risk factors for occurrence of subclinical mastitis

The breed of cows that were sampled were classified as Ankole or Improved if they were crossed or pure bred. The parity of each sampled cow was noted. Individual quarters which were sampled were also noted.

Sample processing

The isolation of pathogens was done on blood agar plates and classified according to

the Bergey's Manual of Systematic Bacteriology comprising of the Gram staining technique, microscopic observation and determining the biochemical profiles. The catalase test using hydrogen peroxide was used to distinguish the two groups of Gram positive cocci viz. Staphylococcus and Streptococcus species. Rabbit plasma solution was used to confirm the presence of Staphylococcus aureus against other coagulase negative Staphylococcus species. The confirmation of Gram negative species was done using 4% potassium hydroxide.

Antibiotic sensitivity tests

McFarland 0.5 was taken as the reference to measure the turbidity of bacterial suspensions. The pathogen isolates, Staphylococcus aureus and other coagulase negative Staphylococci, Streptococci, coliforms and bacillus species were spread on Mueller Hinton Agar plates and exposed to available

commercial antibiotic disks preloaded with *Penicillin G* (10µg), *Bacitracin* (10µg), *Neomycin* (30µg), *Cotrimoxazole* (25µg), *Streptomycin* (10µg), *Ampicillin* (10µg), *Gentamycin* (10µg) and *Tetracycline* (30µg). The plates were incubated overnight at 37°C. Antimicrobial effectiveness was determined using a graduated caliper by measuring the zone of inhibition and the results were recorded in millimeters. Inhibition zones were interpreted as sensitive, intermediate and resistant as indicated in Table 1.

Statistical analysis

Descriptive statistics for somatic cell counts (SCCs) among different quarters were summarised in pie charts and presented as percentages; the bacteria that were isolated from milk samples were also presented as percentages in a pie chart. The antibiotic sensitivity test results were presented in bar graphs against bacterial growth inhibition zones (mm). Somatic cell counts were tested for equal distribution / independence within groups (breed, parity and quarter which was milked); Chi square statistics were estimated and p values below 0.05 were significant and implied that SCCs were not equally distributed by the respective risk factor.

Risk factors for occurrence of subclinical mastitis

Of the risk factors, none was

significantly associated with the occurrence of subclinical mastitis when the Chi-square test for homogeneity of SCCs was compared within breed, quarter which was milked or parity of the cows ($p > 0.05$) (Table 2). Milk samples that were negative for subclinical mastitis comprised 70.6%; proportionally, the quarters that contained low positive (Post +) and moderately high SCCs (Post ++), (250,001-1,500,000) occurred in equal proportion at 13% and 12% respectively; 3.5% of all the samples contained more than 1,500,000 somatic cells in the milk.

Milk culture, bacteria species identification and their antibiotic susceptibility

Agar plate culture results of milk samples are shown in Figure 2. Overall, among 496 individual teat milk samples that were successfully cultured, there was no bacterial growth in 283 (57 %) milk samples. Coliforms were isolated from 20 (4%) samples. *Bacilli* were isolated from 94 (19%) samples. *Staphylococcus aureus* from 10 (2%) and other *Staphylococci* from 74 (15%) of the samples. The least isolated individual bacteria were streptococci 15 (3.0%). Antibiotic sensitivity tests

Figure 3 shows sensitivity results of bacteria to nine commonly used antibiotics in the treatment of mastitis in Rwanda as either single or combination therapies. Of the 496 milk samples that were cultured, 283 (57%)

Table 1: Key for interpretation inhibition zones

Antibiotic	Inhibition zone diameter (mm)		
	Resistant	Intermediate	Susceptible
<i>Penicillin G</i> (PG)	≤ 11	12-21	≥ 22
<i>Bacitracin</i>	≤ 8	9-12	≥ 13
<i>Gentamycin</i>	≤ 9	10-12	≥ 13
<i>Streptomycin</i>	≤ 11	12-14	≥ 15
<i>Ampicillin</i>	≤ 11	12-13	≥ 14
<i>Tetracycline</i>	≤ 14	15-18	≥ 19
<i>Amoxicillin</i>	≤ 13	14-16	≥ 17
<i>Neomycin</i>	≤ 12	13-16	≥ 17
<i>Cotrimoxazole</i>	≤ 10	11-15	≥ 16

Table 2: Risk factors for occurrence of subclinical mastitis

	SCC scores	<250,000	250,001-800,000	800,001-1,500,000	>1,500,001	Total	%
Breed	Ankole	70	7	14	5	96	8.1
	Improved	769	153	133	37	1,092	91.9
	Subtotal	839	160	147	41	1,188	100
Breed Chi square = 4.27, df=3, p= 0.23							
Quarter	Fore	422	76	71	25	594	50
	Hind	417	84	76	17	594	50
	Subtotal	839	160	147	42	1,188	100
Quarter Chi square = 2.12, df=1 87, p= 0.54							
Parity	1-3	436	78	71	23	608	51.2
	4-7	403	82	76	19	580	48.8
	Subtotal	839	160	147	42	1,188	100
Parity Chi square = 1.29, df=3, p= 0.73							
Percentage distribution of SCCs		70.6	13.5	12.4	3.5	100	

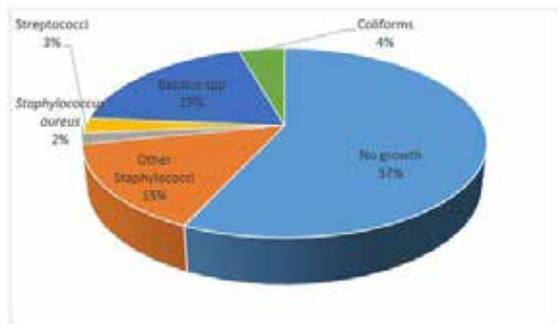
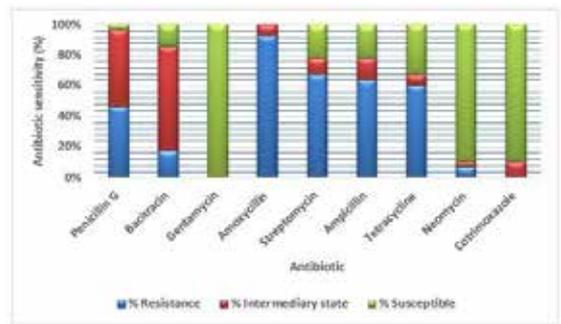


Figure 2: Species of bacteria isolated from milk samples



PG = Penicillin G, BAC= Bacitracin, GTM = Gentamycin, STR=Streptomycin, AMP=Ampicillin, TET=Tetracycline, AMO=Amoxycillin, NEO=Neomycin, COT=Cotrimoxazole

Figure 3: Antibiotic sensitivity test results from cultured milk samples

showed no bacterial growth. Of the remaining 213 samples, bacterial growth and subsequent sensitivity testing were performed on 28 representative colonies. The highest sensitivity of 100% was observed with *Gentamycin* and in the second position (89%) of the cultured bacteria were sensitive to *Cotrimoxazole* and *Neomycin*. The highest resistance was observed for *Amoxycillin* (93%), *Streptomycin* (68%), *Ampicillin* (64%) and *Tetracycline* (61%). Moderate sensitivity / resistance was observed in *Penicillin* (50%) and *Bacitracin* (67%).

Discussion

Assessment of subclinical mastitis in dairy cattle was carried out in five milksheds districts of Rwanda namely Bugesera and Nyagatare (Eastern Province); Kamonyi and Nyanza (Southern Province) and Gicumbi (Northern Province). Overall, the finding that 70.6% of the sampled quarters had SCC less than 250,000 per millilitre of milk indicates that most of the milk produced by the cattle farming community in Rwanda is clean. Conversely, 29.4% of all the four quarters were affected by

one of the three grades of subclinical *mastitis* viz weakly positive (Pos +, 13%), moderately positive (Pos ++, 12%) and strongly positive (Pos +++, 3.5%). The high percentage of clean milk may be due to increased effectiveness of the veterinary extension services together with the message of increased milking hygiene at household level by the Rwanda Agriculture Board. Generally, awareness messages about hygienic milk production are being taken seriously by the farmers. These figures are lower than those obtained by Iraguha *et al.*, (2015) and Mpatswenumugabo *et al.*, (2017) who obtained 52% and 50.4% respectively. Mwabonimana *et al.*, (2015) found an even higher prevalence of sub-clinical *mastitis* in 30 milking cows in Nyabihu and Musanze districts of Northern Rwanda. Notably, sub-clinical *mastitis* survey results vary for various reasons that may include the season when sampling was done and the herd management systems among others. Generally, the prevalence of *mastitis*-infected cows varies from 5%–75% (Merck's Veterinary Manual, (1998).

The risk factors investigated for occurrence of *mastitis* included breed, quarter and parity where the cows were reared. When SCCs was tested for distribution homogeneity of the Chi square values, none of these factors were associated with the occurrence of subclinical *mastitis*. These results confirm that other factors either individually or in combination contribute to the development of *mastitis*. Several studies have consistently showed that cow, udder, milker and environmental hygiene constitute the main factors (Mahantesh *et al.*, 2014). Others are milk yield whereby high yielding cows are at more risk whether or not pre- and post-milking dips are used.

The bacteria isolated in the present study were streptococci (3%), coliforms (4%), *Bacilli* (19%) and Staphylococci (17%). Of the staphylococci, 2% were *S. aureus* and the rest were other Staphylococcus species (15%). These findings indicated that causes of subclinical *mastitis* are mainly contaminative as coliforms and other staphylococci (not *S. aureus*) are categorised as sources from the environment rather than from other

quarters (Radostatis *et al.*, 2000). The common coliforms include *Escherichia coli*, *Klebsiella* and *Enterobacter aerogenes* among others. The results from other studies in Rwanda give conflicting findings: for instance, Kamana *et al.*, (2014) isolated coliforms, *Staphylococcus aureus*, *Salmonella* and *Listeria monocytogenes* from milk samples along the milk chain. Conversely, Iraguha *et al.*, (2015) in a limited study found more coliforms (87%) in milk samples. This implies that there are varied causes of subclinical *mastitis* in different areas that should be constantly updated in more robust studies.

The antibiotics the bacteria were sensitive / resistant to as shown in Figure 3 can be classified broadly into four major groups: β -lactam group (*Penicillin*, *amoxicillin* and *Ampicillin*); Aminoglycosides (*Gentamycin*, *Neomycin* and *Streptomycin*); *Tetracyclines* (*Tetracycline*) and Sulphonamides (*Cotrimoxazole*). The isolated bacteria were most sensitive to aminoglycosides *Gentamycin* and *Neomycin* at 100% and 89% respectively. The bacteria were more resistant to *Amoxicillin*, *Streptomycin*, *Ampicillin* and *Tetracycline*. Moderate resistance was observed with *Bacitracin* and *Penicillin*. Basing on antibiotic groups, bacteria were most sensitive to aminoglycosides but more resistant to β -lactam antibiotics. These results are similar to those of Lollai *et al.*, (2016) who found *Tetracycline* and *Streptomycin* resistant bacteria in milk drawn from small ruminants at 64 and 1024 MIC $\mu\text{g ml}^{-1}$. In a study of milk hygiene in western Uganda, Sajjakambwe *et al.*, (2017) found that the bacterial isolates were mainly resistant to *Penicillin* and *Tetracycline*; a pattern similar to that observed in this study.

In clinical veterinary practice, cows that are reported with sub-and clinical *mastitis* are mainly treated with antibiotics. However, many veterinarians record varying degrees of success and in many cases do not access suitably equipped laboratories to conduct culture and antibiotic sensitivity tests on the isolated bacteria in milk samples. In our experience, the process of conducting culture and sensitivity tests on bacteria isolated from milk samples takes up to 36 hours. Farmers and

veterinarians alike cannot wait for 36 hours before commencing treatment of *mastitis*. Thus knowledge of antibiotics that are likely to give better treatment results becomes useful. The results from this study have identified antibiotics to which bacteria isolated from milk were sensitive or resistant. In case of treatment failure with the common antibiotics on the market (first line intramammary antibiotic infusions) we have identified effective second line antibiotics viz *Gentamycin*, *Neomycin* and *Cotrimoxazole* that can be used in Rwanda.

Conclusions

Based on the results from this study, it can be concluded that subclinical *mastitis* is still a constraint to production of clean and hygienic milk in Rwanda and contaminative bacteria are the major causes. Our clinical veterinary practice shows that once *mastitis* is detected and milk samples are taken for culture and sensitivity tests of the causative microbes, laboratory results are expected within 36 hours. This calls therefore, for the rational use of intramammary infusion antibiotics. In this study, we identified suitable first line antibiotics to which bacteria have developed some degree of resistance (*Bacitracin*, *Penicillin*, *Amoxicillin*, *Streptomycin*, *Ampicillin* and *Tetracycline*). These can be used to initially treat cows as laboratory results are being awaited. The second line antibiotics were those which registered high levels of sensitivity (*Gentamycin*, *Neomycin* and *Cotrimoxazole*) and can suitably be administered when first line antibiotics have not been effective in the treatment of subclinical and clinical *mastitis*.

Acknowledgements

This study was funded by Rwanda Agriculture Board (RAB) through the Veterinary Services Unit, Section of Microbiology of the National Veterinary Laboratory at Rubirizi, Kigali. The authors would like to thank the livestock keepers, District and Sector Veterinary officers for their assistance.

Conflict of interest

The authors wish to state there is no conflict of interest regarding publication of this paper.

References

- Alluwaimi AM, 2004. The cytokines of bovine mammary gland: prospects for diagnosis and therapy. *Research in Veterinary Science*; 77 (3): 211–222.
- Bannerman DD and Wall RJ, 2005. A novel strategy for the prevention of *Staphylococcus aureus*-induced *mastitis* in dairy cows. *Information Systems for Biotechnology (isb) news report*; 1–4.
- Barlow J, White L, Zadoks RN and Schukken YH, 2009. A mathematical model demonstrating indirect and overall effects of lactation therapy targeting subclinical *mastitis* in dairy herds. *Preventive Veterinary Medicine*; 90:31–42.
- Chibani-Chennoufi S, Dillmann ML, Marvin-Guy L, Rami-Shojaei S and Brussow H, 2004. *Lactobacillus plantarum* bacteriophage LP65: new member of the SPOI-like genus of the family Myoviridae. *Journal of Bacteriology*; 186: 7069–7083.
- Cullor J, Fenwick B and Smith B, 2013. Active immunization with *E. coli* J5 and its protective effects. *Immunobiology and Immunopharmacology of Bacterial Endotoxins*; 18: 265.
- Domadia P, Swarup S, Bhunia A, Sivaraman J, Dasgupta D, 2007. Inhibition of bacterial cell division protein FtsZ by cinnamaldehyde. *Biochemical Pharmacology*; 74: 831–840.
- Dulin AM, Paape MJ and Nickerson SC, 1988. Comparison of phagocytosis and chemiluminescence by blood and mammary gland neutrophils from multiparous and nulliparous cows. *The American Journal of Veterinary Research*; 49 (2) 172–177.
- Hafez SM, Ismael AB, Mahmoud MB and Elaraby AA, 2013. Development of new strategy for non-antibiotic therapy: bovine lactoferrin has a potent antimicrobial and immunomodulator effects. *Advances in Infectious Diseases*; 3: 185–192.

- Hortet P and Seegers H, 1998. Calculated milk production losses associated with elevated somatic cell counts in dairy cows: review and critical discussion. *Veterinary Research*; 29 (6): 497-510.
- Iraguha B, Hamudikuwanda, H and Mushonga B, 2015. Bovine *mastitis* prevalence and associated risk factors in dairy cows in Nyagatare District, Rwanda. *Journal of South African Veterinary Association*; 86 (1): 1228. doi: 10.4102/jsava.v86i1.1228.
- Kamana O, Ceuppens S, Jacxsens L, Kimonyo A and Uyttendaele M, 2014. Microbiological quality and safety assessment of the Rwandan milk and dairy chain. *Journal of Food Protection*; 77 (2): 299-307.
- Kasikci G, Cetin O, Bingol EB and Gunduz MC, 2012. Relationship between electrical conductivity, somatic cell count, California *mastitis* test and some quality parameters in the diagnosis of subclinical *mastitis* in dairy cows. *Turkish Journal of Veterinary and Animal Sciences*; 36 (1): 49-55.
- Lollai SA, Ziccheddu M, Duprè I and Piras D, 2016. Characterization of resistance to *Tetracyclines* and aminoglycosides of sheep *mastitis* pathogens: study of the effect of gene content on resistance. *Applied Microbiology*; 121 (4): 941-951.
- Mahantesh M, Kurjogi M and Basappa KB, 2014. Epidemiology of bovine *mastitis* in cows of Dharwad District. <http://dx.doi.org/10.1155/2014/968076>.
- Merck's Veterinary Manual. 8th Ed, 1009
- Middleton JR, Hardin D, Steevens B, Randle R and Tyler JW. 2004. Use of somatic cell counts and California *mastitis* test results from individual quarter milk samples to detect subclinical intramammary infection in dairy cattle from a herd with a high bulk tank somatic cell count. *Journal of American Veterinary Medical Association*; 224: 419-423.
- Mpatswenumugabo JP, Bebora LC, Gitao GC, Mobegi V, Iraguha B, Kamana O and Shumbusho B, 2017. Prevalence of Subclinical *Mastitis* and Distribution of Pathogens in Dairy Farms of Rubavu and Nyabihu Districts, Rwanda. <https://doi.org/10.1155/2017/8456713>.
- Mwabonimana MF, Rekeraho J, Ntiringanya M and Bimenyimana F, 2015. Cost benefit analysis of bovine *mastitis* in Nyabihu and Musanze districts, Rwanda. *International Journal of Novel Research in Life Sciences*; 2 (4): 21-31.
- National Institute of Statistics of Rwanda, 2015. Rwanda Statistical Yearbook of November 2015.
- Persson Y and Olofsson I, 2011. Direct and indirect measurement of somatic cell count as indicator of intramammary infection in dairy goats. *Acta Veterinaria Scandinavica*; 53 (1): 15.
- Polat B, Colak A, Cengiz M, Yanmaz LE, Oral H, Bastan A, Kaya S and Hayirly A, 2010. Sensitivity and specificity of infrared thermography in detection of subclinical *mastitis* in dairy cows. *Journal of Dairy Science*; 93: 3525–3532.
- Radostatis OM, Gay CC, Blood DC and Hinchcliff KW, 2000. *Veterinary Medicine, Diseases caused by bacteria I*; 9th Edition: 701-747.
- Rysanek D, Zouharova M and Babak V, 2009. Monitoring major *mastitis* pathogens at the population level based on examination of bulk tank milk samples. *Journal of Dairy Research*; 76(1):117–123.
- Sharma N, Singh NK and Bhadwal MS, 2011. Relationship of Somatic Cell Count and *Mastitis*: An Overview. *Asian-Australian Journal of Animal Science*; 24 (3): 429 – 438.
- Shim EH, Shanks RD and Morin DE, 2004. Milk loss and treatment costs associated with two treatment protocols for clinical *mastitis* in dairy cows. *Journal of Dairy Science*; 87 (8): 2702-2708.
- Shoshani E, Leitner G, Hanochi B, Saran A, Shpigel N and Berman A, 2015. Mammary infection with *Staphylococcus aureus* in cows: progress from inoculation to chronic infection and its detection. *Journal of Dairy Research*; 67: 155.
- Ssajakambwe P, Bahizi G, Setumba C, Kisaka SM, Vudriko P, Atuheire C, Kabasa JD and Kaneene JB, 2017. Milk Hygiene in Rural Southwestern Uganda: Prevalence of *Mastitis* and Antimicrobial Resistance Profiles of Bacterial Contaminants of Milk and Milk Products. *Veterinary Medicine International* doi: 10.1155/2017/8710758.
- Strategic plan for Transformation of Agriculture (PSTA III), 2014. Results Framework; 43-45.

Thurmond MC, 1990. A method to estimate the somatic cell count of milk from a mastitic quarter using composite somatic cell count. *Canadian Journal of Veterinary Research*; 54:190-194.

Wellnberg GJ, Wim van der Poel HM and Oirschot TT, 2002. Viral infections and bovine *mastitis*. *Veterinary Microbiology*; 88 (1): 27-45.

White DG, McDermott PF, 2001. Emergence and transfer of antibiotic resistance. *Journal of Dairy Science*; 84: (E. Suppl) E151–E155.

Zadoks RN, Middleton JR, McDougall S, Katholm J and Schukken YH, 2011. Molecular epidemiology of *mastitis* pathogens of dairy cattle and comparative relevance to humans. *Journal of Mammary Gland Biology and Neoplasia*; 16 (4): 357-72.

PREVALENCE OF *HAEMONCHUS CONTORTUS* IN KALAHARI RED GOATS REARED IN THE SOUTH-WEST NIGERIA

*Okwelum N¹, Akinkuotu O A², Shittu O O¹ and Oluwatosin B O¹

¹Institute of Food Security, Environmental Resources and Agricultural Research, Federal University of Agriculture, Abeokuta, Nigeria.

²College of Veterinary Medicine, Federal University of Agriculture, Abeokuta, Nigeria.

Abstract

This study was carried out in Federal University of Agriculture Abeokuta, Nigeria. The research was aimed at determining the prevalence of *Haemonchus contortus* in the Kalahari Red Goat herd reared in the hot humid tropics through faecal egg count techniques. One hundred and ten faecal samples were collected from 110 goats of mixed sex from the Kalahari Red goat herd. Parasitological methods were used for worm isolation, identification and faecal egg load determination. Data generated were analysed using the SAS Statistical package. The results obtained revealed the prevalence rate of *Haemonchus contortus* as 31.82% in the goat population. The mean egg count was 728.57 ± 177.07 per gram of faeces (EPG) with a range of 100 – 4900 EPG as an overall analysis. The mean egg count amongst the female goats was 783 ± 203.42 with a range of 100-4900 EPG which was the same with the overall range while the mean egg count in the male goats was 400 ± 184.40 with a range of 100 – 900 EPG. Thus, the females had a higher EPG than the males. The prevalence rate of 31.82% is of economic importance and should be of concern in the Kalahari Red goat herd health management operations. The strict control of parasites both on pastures and in the animal host has to be adopted. Continuous general hygiene, boosting of dietary nutrients and the use of conventional dewormers alternated with forages with proven antihelthic properties are ameliorative steps.

Key words: *Haemonchus contortus*, prevalence, Kalahari Red goats, hot humid tropics.

PRÉVALENCE DES *HAEMONCHUS CONTORTUS* CHEZ LA CHÈVRE ROUGE KALAHARI ÉLEVÉS DANS LE SUD-OUEST DU NIGÉRIA

Résumé

Cette étude a été réalisée à l'université fédérale de l'Agriculture Abeokuta, Nigéria. La recherche visait à déterminer la prévalence des *Haemonchus contortus* dans le troupeau de chèvre rouge Kalahari élevé dans les zones tropicales humides chaudes grâce à des techniques de comptage faecal egg. Cent dix échantillons de matières fécales ont été prélevés de 110 chèvres de sexe mixte du troupeau de chèvre rouge du Kalahari. À l'aide des méthodes parasitologiques pour l'isolement de ver, identification et oeuf fécale chargent détermination. Données obtenues ont été analysées à l'aide du logiciel statistique SAS. Les résultats obtenus ont révélé le taux de prévalence de *Haemonchus contortus* 31,82 % dans la population caprine. Le nombre d'oeufs moyen était de 728.57 ± 177.07 par gramme de fèces (EPG) avec une gamme de 100 – 4900 EPG comme une analyse globale. Le nombre d'oeufs moyen parmi les chèvres femelles était de 783 ± 203.42 avec une gamme de 100-4900 EPG qui était la même chose avec la fourchette globale tandis que le nombre d'oeufs moyen dans les chèvres mâles était $400 \pm 184,40$ avec une gamme de 100 à 900 EPG. Ainsi, les femelles avaient un EPG plus élevé que les mâles. Le taux de prévalence de 31,82 % est d'une importance économique et devrait être source de préoccupation dans les opérations de gestion de la santé de troupeau chèvre. Un contrôle strict du parasite aussi bien sur les pâturages et l'animal hôte doit être adopté. Hygiène générale continue, une amplification des nutriments alimentaires et l'utilisation de vermifuges classiques alternée avec des fourrages avec éprouvée antihelthic propriétés vont améliorateur.

Mots clés : *Haemonchus contortus*, prévalence, chèvres Kalahari rouge, chaud des tropiques humides.

*Corresponding author email: drjhephzi@yahoo.com

Introduction

Africa has a goat population of 174 million which represents 31% of the world total. Nigeria's goat population is 34.5 million according to Onakpa *et al.*, 2010. Goat is the second most import livestock in Nigeria due to its high population. The Kalahari Red goats have their origin from South Africa and was reported to have been developed mainly as a meat goat (Kotze *et al.*, 2004; Simela and Merkel, 2008). The breed has been well adapted to the arid and semi-arid savannah with good foraging and excellent mothering abilities thus are regarded as "minimum care/maximum profit" breed (Kotze, 2004). They are also known to be hardy with good economic traits such as fecundity, good mothering ability huge body mass and resistant to diseases and parasites and the need to be inoculated is far less than other breeds (Stonehaven, 2011). The genetic diversity study of the Kalahari Red goat population was reported to be high as revealed by the mtDNA analysis (Bemji *et al.*, 2014). The KR goat was imported into Nigeria in 2011 by the Federal University of Agriculture, Abeokuta, Nigeria.

Endo-parasitism has been identified by Chiezey *et al.*, 2008 as a complex disease hindering efficient and profitable livestock production especially in goats worldwide. Amongst several endo-parasites of goats, *Haemonchus contortus* is the most important and has been reported to be the cause of most outbreaks of acute and subacute parasitic gastroenteritis of goats according to Fakae *et al.*, 2004.

It is important to evaluate the parasitic infestation levels as an indicator of health since gastrointestinal parasitism has been noted to be a serious cause of loss in productivity and death in goats (Githiori *et al.*, 2006). According to Akhtar *et al.* (2000), high the prevalence of endo-parasites is primarily due to high temperatures in the hot-humid south western Nigeria which facilitates parasite multiplication and transmission.

Mahusoon *et al.* (2004) and Nwosu *et al.* (2007) noted that helminth infestations in goats have negative impacts on productivity

and health which is manifested through morbidity, mortality, treatment and control of the diseases.

This study was carried-out to identify and determine the prevalence of *Haemonchus contortus* infestation in the Kalahari Red goats reared in the Institute of Food Security, Environmental Resources and Agricultural Research (IFSERAR), Federal University of Agriculture, Abeokuta (FUNAAB), Nigeria which is situated in the hot humid tropics.

Materials and Methods

Study site:

The study was carried out in IFSERAR, FUNAAB, Nigeria. One hundred and ten faecal samples were collected from Kalahari Red goats raised in the IFSERAR Farm in the month of August 2013 for the identification of the prevalent endo-parasites in the goat herd. The faecal samples were analysed in the parasitology laboratory in the College of Veterinary Medicine, FUNAAB Abeokuta, Nigeria.

Gastrointestinal data collection:

About 5g of fresh faecal sample was collected from each goat under aseptic conditions. The samples were labelled, then transferred to the Parasitology Laboratory of the College of Veterinary Medicine, Federal University of Agriculture Abeokuta (FUNAAB) and kept in a refrigerator at 4o C until further examinations were carried out. Both the McMaster egg count and floatation methods as described by Urquhart *et al.* (1996) were performed to screen the samples for quantitative and qualitative analysis, respectively.

Floatation method was conducted as follows:

Water and salt (KOH) solution was prepared as floatation medium, and poured into the faecal sample in a beaker. It was filtered and the faecal debris discarded. The filtrate was then poured into a test-tube, filled to the brim and covered with the cover slip. The filtrate was allowed to stay for 30 minutes. The cover slip was then removed, placed on a

clean slide and viewed under the microscope at a magnification of $\times 40$.

Quantitative analysis (McMaster Egg Count Technique):

About 3g of faecal sample was added to 42ml of water and mixed thoroughly. It was filtered into a test-tube to about 15ml. The solution in the test-tube was then centrifuged in a bench centrifuge for 45 minutes at 1500 revolutions per minute (RPM) and the supernatant was discarded. The sediment was mixed thoroughly, after which there was an addition of salt to the sediment. The two chambers of McMaster slide were filled with the sediment and viewed under the microscope. The number of eggs was counted for a single chamber and multiplied by 100 to give the total egg load.

Statistical Analysis:

The data generated was analysed using the SAS Statistical Package in a General Linear Model (GLM) and significant means were separated using Duncan's Multiple Range Test of the same package at ($p < 0.05$) level of significance. Significantly different means were further expressed in descriptive statistics to show their trend graphically.

Results and Discussion

The prevalence rate of *Haemonchus contortus* was 31.82%. Out of the 110 faecal samples examined, 35 were found to be positive with *Haemonchus contortus* infection. The prevalence rate is illustrated graphically in Figure 1. The mean egg count was 728.57 ± 177.07 per gram of faeces (EPG) with a range of 100 – 4900 EPG as an overall analysis.

The mean egg count amongst the female goats was 783 ± 203.42 which was in the same range as the overall mean count while the mean egg count in the male goats was 400 ± 184.40 within the range of 100 – 900 EPG. This is illustrated in Figure 2. The higher prevalence rate recorded in the female animals than in the males in this study is in agreement with the reports of Okwelum *et al.*

(2012) who reported a higher prevalence rate of gastrointestinal parasite infestation in female animals than in males and also Onyenwe *et al.* (2005) who reported a higher prevalence rate of *Haemonchus contortus* infestation in female than in males amongst the Red Sokoto breed of goat.

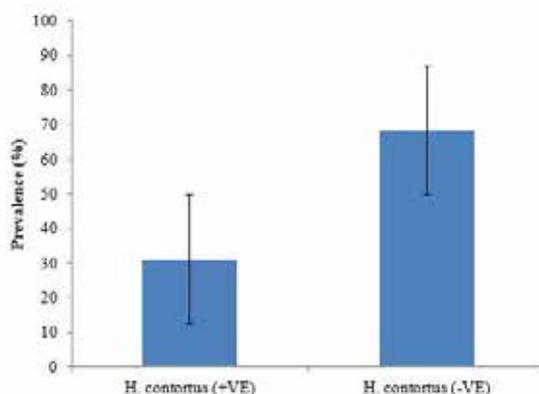


Figure 1: Prevalence of *Haemonchus contortus* in Kalahari Red Goats

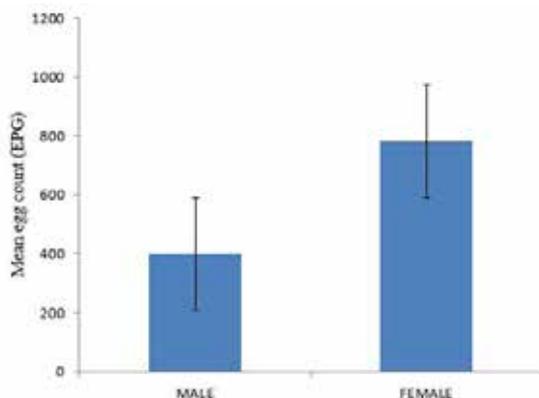


Figure 2: *Haemonchus contortus* load amongst sexes in Kalahari Red goats

Different methods are used for the diagnosis of haemonchosis and these vary in their sensitivities and cost. A conventional method of identification is the microscopic examination of faecal materials (Mahdi and Ali, 2004; Hamed *et al.*, 2005; Yatswako *et al.*, 2007; Ayinmode and Fagbemi, 2010). In some studies, it was determined that the sensitivity of the ELISA was higher than those faecal examination methods (El-Shazly *et al.*, 2002; Yilmaz *et al.*, 2008). El-Shazly *et al.* (2002) also stated that faecal examination technique

showed the lowest sensitivity when compared to ELISA and the polymerase chain reaction (PCR) for diagnosis of endoparasites. Hence the sensitivity of the methods of diagnosis in this study is low and so this prevalence rate recorded should be worrisome.

Conclusion

The prevalence rate of 31.82% recorded is of economic importance and so adequate control measures need to be put in place to ensure minimum worm loads both on the pastures and in the animals. Resistant does should be selected for breeding as resilience is genetic and this will give rise to healthier breeding flocks from resistant progenies. Allowing animals to feed on browse plant paddocks, the feeding of hay and high quality diets will help to control the worm infestation. Regular deworming programmes with conventional antihelmithics and alternative medicinal forages of proven antihelthic value need to be adopted. The periodic use of anaemia detection kits such as FAMACHA will also be very useful in the diagnosis of worm infestations and thus assist in early detection and prompt useful treatment. FAMACHA is a calibrated card used by Veterinarians to detect anaemia in goats from the colour or pigmentation of the ocular membranes.

The strict control of parasites both on pastures and in the animal host has to be adopted. Continuous general hygiene, boosting of dietary nutrients and the use of conventional dewormers alternated with forages with proven antihelthic properties are ameliorative steps in similar tick infested areas.

References

Akhtar, M. S., Iqbal, Z., Khan, M. N. and Muhammad, L. (2000). Anthelmintic activity of medicinal plants with particular reference to their use in animals in Indo-Pakistan subcontinent-A Review. *Small Ruminant Res.* 38: 99-107.

Ayinmode, A. B., Fagbemi, B. O. (2010). Prevalence of *Cr yptosporidium* infection in cattle from southern

Nigeria. *Veterinarski. Archiv.* 80(6): 723-731.

Bemji MN, Awotunde, EO, Olowofeso O, James I J, Oduguwa BO, Okwelum N and Osinowo OA. (2014). Maintenance of mtDNA diversity in Kalahari Red goat of South Africa imported to Nigeria. *Animal Genetic Resources.* 55: 39-46.

Chiezey, N. O., Aganubi, O. J. and Oyedipe, E. O. (2008). The reproductive performance of sheep carrying natural infection of gastro-intestinal nematodes. *Asia Journal of Animal and Veterinary Advances* 3(4): 236 – 243.

El-Shazly AM, Gabr A, Mahmoud MS, Aziz, S. S., Saleh, W.A. (2002). The use of Ziehl-Neelsen stain, enzyme-linked immunosorbent assay and nested Polymerase Chain Reaction in diagnosis of cryptosporidiosis in immunocompetent,- compromised patients. *J. Egypt Soc . Parasitol.* 32: 155-166.

Fakae, B. B., Musongong, G.A., Chiejina, S. N., Behnke, J. M., Ngongeh, L.A. and Wakelin, D. (2004). Variability in resistance of the Nigeria West African Dwarf goats to abbreviated escalating trickle and challenge infections with *Haemonchus contortus*. *Vet. Parsitol.* 122: 51-65.

Githiori, J., Anthanasiadou, S. and Thamsborg, S. M. (2006). Use of plants in novel approaches for control of gastrointestinal helminths in livestock with emphasis on small ruminants. *Vet. Parasitol.* 139: 308-320.

Hamedi, Y., Safa, O. and Haidari, M. (2005). *Cryptosporidium* infection in diarrhoeic children in southeastern Iran. *Pediatr. Infect. Dis . J.* 24: 86-88.

Kotze, A., Swart, H., Grobber, J. P. and Nemaangani, A. (2004). A genetic profile of the Kalahari Red goat breed from Southern Africa. *South Afr. J. Anim. Sci,* 34(1): 10–12.

Mahdi, N. K. and Ali, N. H. (2004). Cryptosporidiosis and other intestinal parasitic infections in patients with chronic diarrhoea. *Saudi Med. J.* 25: 1204-1207.

Mahusoon, M. M., Pepera, A. N. F., Pepera, E. R. K. and Pepera, K. A. (2004). Effect of Molybdenum supplementation on circulating mineral levels, nematode infection and body weight in goats as related to season. *Trop. Agr. Res.* 16: 128-136.

Nwosu, C. O., Madu, P. P. and Richards, W. S. (2007). Prevalence and seasonal changes in the population of gastrointestinal nematodes of small ruminants in the semi-arid zone of North-eastern Nigeria. *Vet. Parasitol.* 144: 118-124.

Okwelum, N., Iposu, S. O., Andabai, G. H., Sanwo, K., Smith, O. F., Takeet, O. V. A., Oduguwa, B. O., Amole, T. A., Rahman, S. A., Olugbogi, E. I., and Famakinde, S. A. (2012). Prevalence of Gastrointestinal Parasites in Nigerian Muturu Cattle in Ipokia Local Government Area of Ogun State, Nigeria. *Nigerian Journal of Science* 46: 51-55.

Onakpa, M. M., Onifade, K. and Umar, M. A. (2010). Effects of Diminazene Aceturate and Ivermectin administration on semen and serum parameters of Red Sokoto bucks. *Adva. Sci. Res.* 1(2): 61-65.

Onyenwe, I. W., Onwe, C., Onyeabor, A. and Onunkwo, J. I. (2005). Abattoir-Based Study of the Susceptibility of Two Naturally Infected Breeds of Goat to *Haemonchus contortus* in Nsukka Area of Enugu State, Nigeria. *Animal Res. Intern.* 2(2): 342 – 345.

Simela, L. and Merkel, R. (2008). The contribution of chevon from Africa to global meat production. *Meat Sci.*, 80(1): 101–109.

Stonehaven, S. (2011). About the Kalahari Red (available at http://www.stonehavenstud.com.au/kalahari_red.htm). Last modified 13 May 2011, Retrieved 07 February 2014.

Urquhart, G. M., Armour, J., Duncan, J. L., Dunn, A. M. and Jennings, F. W. (1996). *Helminthology*. *Vet. Paras.* Blackwell Publishing Chapter. 1, pp 12-17.

Yatswako, S., Faleke, O. O., Gulumbe, M. L., Daneji, A. I. (2007). Cryptosporidium oocysts and Balantidium coli cysts in pigs reared semi-intensively in Zuru, Nigeria. *Pak. J. Biol. Sci.* 10: 3435–3439.

Yilmaz, H., Zeynep, T. and Mautalip, C. (2008). Investigation of cryptosporidiosis by enzyme-linked immunosorbent assay and microscopy in children with diarrhoea. *Saudi Med. J.* 29(4): 526-529.

Director of Publication

Prof. Ahmed Elsawalhy

Editor in Chief

Dr. Simplicie Nouala

Editors

Dr. Edward Musiwa Nengomasha
Prof. James Wabacha
Dr. Mohamed Batu Duramany Seisay
Dr. N'Guetta Austin Bosso

Reviewers

Prof. Abdu Ayuba Paul
Prof. Abdullahi Alhaji Magaji
Dr. Adama Sow
Prof. Adel Abdel Azeem Mahmood Fayed
Dr. Amadou Traore
Prof. Ayayi Justin Ayih-Akakpo
Prof. Bassirou Bonfoh
Dr. Benedicta O. Mbu Oben
Prof. Benjamin Obukowho Emikpe
Dr. Bockline Omedo Bebe
Dr. Cyprien F. Biaou
Prof. Etienne Pamo Tedonkeng
Dr. Gilbert Komlan AKODA
Dr. Henri Kabore
Dr. Jacques Somda
Dr. James Okwee-Acai
Dr. Jean Marcel Mandeng
Dr. Jean Claude Fotsa
Prof. John David Kabasa
Prof. John Osita Arinze Okoye
Dr. Joseph Simbaya
Dr. Komlan AKODA
Dr. Langelihle Simela
Prof. Malek Zrelli
Dr. Norber Mbahin
Prof. Osama Rajab Mohamed Elwaer
Dr. Patrick Irungu
Dr. Samuel Wakhusama
Dr. Sarah Ossiya
Prof. Serge Niangoran Bakou
Dr. Tadele Tolosa Fulasa
Prof. Tarnagda Zekiba
Prof. Timothy Uzochukwu Obi
Dr. Unesu Ushewokunze-Obatolu
Dr. William Olaho Mukani

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
March 2018

Aims and scope

The Bulletin of Animal Health and Production in Africa (BAHPA) of the African Union Inter-African Bureau for Animal Resources (AU-IBAR) is a scientific journal which publishes articles on research relevant to animal health and production including wildlife and fisheries contributing to the human wellbeing, food security, poverty alleviation and sustainable development in Africa. The bulletin disseminates technical recommendations on animal health and production to stakeholders, including policy makers, researchers and scientists in member states. The Bulletin is the African voice on animal resources issues specific to Africa.

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

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

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

Language

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

Manuscripts Submission

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

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

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

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

Types of contribution

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

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

Review Articles: should cover subjects falling within the scope of the bulletin, which are of active current interest. Papers need not contain original work or ideas. They will be reviewed for completeness, accuracy, style and suitability of content by referees familiar with the subject and the Editor-in-Chief.

Editorial: articles are short articles describing news about the bulletin or the opinion of the editor-in-chief, the publisher or a guest editor of a thematic series.

Letters to the Editor: the bulletin welcomes letters to the editor. The purpose of Letters to the Editor is to provide a forum for positive and constructive views on articles and matters published in the bulletin. Letters to the Editor must not exceed 300 words. Letters to the editors include technical reports from countries or projects.

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

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

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

News and announcements: BAHPA is pleased to publish information on animal health and production activities/meetings. Please send the following information to the Editor: Date of the event, title, organization offering the event, location and contact information.

Submission Guidelines

Full papers of original research

All manuscripts submitted to BAHPA should include the following features:

1. On cover page of the manuscript, the following should be clearly written/inserted: the corresponding author, name of the institution, title of the manuscript, names of the authors, the addresses of the authors and the e-mail address of the corresponding author. The corresponding author should ensure that all the other authors consent to their names being included. The consent should be sent directly by co-authors to the editor via email.
2. Each original article should be divided into Abstract and Keywords, Introduction, Materials and Methods, Results, Discussion, conclusion, Acknowledgments and References. A textbox containing a public brief on the study for the benefit of policy makers should also be provided. This textbox will not be included in the published article but will be compiled and published in a separate edition at the end of the year.
3. Title, which should be concise, preferably not more than 15 words long, followed by the author(s) name(s) and institution(s) to which work should be attributed and address for correspondence, if different.
4. The Abstract should not be longer than 300 words giving a synopsis of the work and should contain the objectives, briefs description of materials and methods, highlights of significant results, conclusions and recommendations. Up to six keywords should be provided..
5. The Introduction should contain the problem statement, the hypothesis and the objective of the work and cite recent important work undertaken by others.
6. Materials and Methods should describe materials, methods, apparatus, experimental procedure and statistical methods (experimental design, data collection and data analysis) in sufficient detail to allow other authors to reproduce the results. This part may have subheadings. The experimental methods and treatments applied shall conform to the most recent guidelines on the animal's treatment and care. For manuscripts that report complex statistics, the Editor recommends statistical consultation (or at least expertise); a biostatistician may review such manuscripts during the review process. Cite only textbooks and published article references to support your choices of tests. Indicate any statistics software used.
7. Results should be presented clearly and concisely, in a non-

repetitive way. Subheadings may be accepted.

8. Discussion of significance should be focused on in the interpretation of results. Subheadings are not accepted in this section.
9. Acknowledgements. Where necessary acknowledgements of grants and technical assistance should be included under this heading. Please also include any potential conflict of interests if appropriate. Suppliers of materials should be named and their location (town, state/county, country) included.
10. State the conclusions, and any implications that may be drawn from the study.

Short Communications: Manuscripts should contain original data and be limited to 1500 words. The number of tables and figures are limited to two. A limited number of references should be included. Headings are not allowed in short communications.

Sequence of Preparation

1. The data files must be PC/Windows-compatible. The text should be prepared using standard software (Microsoft Word) format; do not use automated or manual hyphenation. Please do not include footnotes.
2. Use Times New Roman 12 point font for all text except for tables and figures where Times New Roman 10 font should be used.
3. Use 1 inch margins on top, bottom, left and right margins,
4. Every line on the text should be numbered.
5. Use double line spacing for body of text. For Abstract, Figures, Tables and References use single line spacing.
6. Place page numbers in the lower right hand corner of your manuscript.
7. Run "the spell check" and "grammar check" on the entire file before submission using either the UK English or French standard.
8. Avoid using abbreviations for the names of concepts. Use ordinary words for variable names – not code names or other abbreviations. Use the same name for a variable throughout your text, tables, figures and appendices. Names of organizations and research instruments may be abbreviated, but give the full name (with abbreviation in brackets) the first time you mention one of these.
9. References should take the following form: In the text, a reference identified by means of an author's name should be followed by the date of the reference in parentheses. When there are more than two authors, only the first author's name should be mentioned, followed by ' *et al.*'. In the event that an author cited has had two or more works published during the same year, the reference, both in the text and in the reference list, should be identified by a lower case letter like 'a' and 'b' after the date to distinguish the works. Examples: Abayomi (2000), Agindotan *et al.*, (2003), (Kelebeni, 1983), (Usman and Smith, 1992), (Chege, 1998; Chukwura, 1987a,b; Tijani, 1995, 1993), (Kumasi *et al.*, 2001)

The use of reference managing software is encouraged

The authors should be cited in a chronological order by year and then by a or b; in the reference list they should be listed alphabetically.

Please ensure that references in the text exactly match those in the manuscript's reference list. Check each reference in the text to see that you have the complete citation in the reference section of the paper in the desired style. In the references section, references are listed in alphabetical order.

Examples of References

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

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

Illustrations

Please send the figures as separate files and do not import them into the text file. Put all tables, figures, diagrams and artwork on separate pages. Each figure, table, and bibliographic entry must have a reference in the text. References to tables and figures in the text should be by number and not to "table below" or "figure below". The Editor will place them in the appropriate place in the text of article during the final edit. Tables and figures should be numbered consecutively. Please submit the data for figures in black and white.

Abbreviations, Symbols and Nomenclature

All specifications must be stated according to the S.I. system. Concentrations of chemical solutions are to be given in mol/l. All other concentrations should be given in % (volume or weight). Any abbreviations of chemical, biological, medical or other terms should only be employed when it is certain that they are internationally known. The full name must be stated in brackets when the abbreviation is first used. Names of micro-organisms and zoological names should be italicized in the manuscript.

Ethical guidelines

BAHPA adheres to the below ethical guidelines for publication and research. Experimentation will only be published if such research has been conducted in full accordance with ethical principles. Manuscripts containing experimentations must be accompanied by a statement that the experiments were undertaken with the understanding and written consent of each subject and according to the above mentioned principles. Editors reserve the right to reject papers if there are doubts as to whether appropriate procedures have been used.

1. When experimental animals are used the methods section must clearly indicate that adequate measures were taken to minimize pain or discomfort.
2. All studies using animal subjects should include an explicit statement in the Material and Methods section identifying the review and ethics committee approval for each study, if applicable. Editors reserve the right to reject papers if there is doubt as to whether appropriate procedures have been used.

Revising your article

When you submit a revised version of your article in response to the referees' comments, you must accompany it with a detailed list of the changes made (ignoring typographical errors, but mentioning additional paragraphs, changes to figures, etc) suitable for transmission to the referee. Where changes have been made in response to the referees' remarks it is important to mention this and indicate where they can be found. You may also wish to send in a second copy of your article with the changes marked or underlined.

You should go through the referees' comments and for each comment mention whether you followed their suggestion or whether you disagree and wish to respond to the comment. If a referee has misunderstood a point, it is not necessarily their fault and may have been caused by ambiguity or lack of clarity in your article which needs to be corrected. Some authors copy out each of the referees' comments in turn and include their response immediately after. In other cases responses can be made referring back to the reports. Finally, please make sure that you send your revised article to us and not simply the original version again. This is a common mistake, especially when authors send in their work electronically. Electronic revised articles should contain all text and graphics files needed to generate the revised version, and not just those files that have changed.

By observing these guidelines you will be assisting the referees, who give up their time to review manuscripts. If you prepare your article carefully, this can save valuable time during the publication process.

Appeal of Decision

Authors who wish to appeal the decision on their submitted paper may do so by e-mailing the editorial office with a detailed explanation for why they find reasons to appeal the decision within 14 days.

Proofs

One set of proofs will be sent to the author to be checked for printer's errors and should be returned within three days.

Offprints

25 offprints of each article will be supplied free of charge. Additional offprints may be ordered and paid for at the proof stage. Each extra offprint costs US \$5.00.

Subscriptions

The annual subscription fee, including postage (surface mail) and handling is USD 100.00. Air mail charges are available upon request.

Back volumes

Back issues are also obtainable upon request at similar charges.

Desktop Publisher
Fahim Franz Kremeier

