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CLINICAL MASTITIS IN RED SOKOTO GOATS: PREVALENCE, PRESENTATION, ASSOCIATED FUNGAL AGENTS AND THEIR ANTIMYCOTIC SUSCEPTIBILITY PATTERNS IN A SEMI-ARID REGION OF NIGERIA

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Abstract

This study was designed to determine the prevalence of clinical mastitis in Red Sokoto goats (RSG), the fungal agents associated with the disease and their antimycotic susceptibility patterns. Lactating RSG were examined for clinical mastitis by collecting milk samples for mycotic examination and antimycotic susceptibility patterns. Of a total of 127 lactating RSG, 38 (29.9 %) had clinical mastitis. Out of this, 22 (57.9 %) were unilateral, while 16 (42.1 %) were bilateral. Six (15.8 %) RSG had teat injuries, whereas 32 (84.2 %) had no injuries. Based on age, 15 (39.5 %) were ≥3 - <4 years, 12 (31.6%) were ≥2 - <3, while 3 (7.9 %) were ≥four years. Twenty-eight (73.7 %) of the mastitic RSG were from an intensive management system, while 10 (26.3%) were from extensive management system. Twelve (31.6%) of the RSG with clinical mastitis were in their 3rd parity, 10 (26.3 %) in their 2nd parity while 8 (21.1 %) each were in the 1st and 4th parities. The onset of mastitis in most goats was after the second kidding 21 (55.3%). Thirteen fungal isolates made up of 8 species were recovered. *Aspergillus niger* was the most common fungus with four (30.7%) isolates. This was followed by *Aspergillus fumigatus* and *Fusarium chlamydosporium* with 2 (15.4%) isolates each. *Aspergillus lentullus*, *Aspergillus nidulans*, *Aspergillus terreus*, *Candida albicans* and *Histoplasma capsulatum* each had 1 (7.7%) isolate. The fungi were more susceptible to *Amphotericin B*, *Fluconazole*, and *Nystatin*. The study showed a higher prevalence of clinical mastitis and fungal agents with more moulds than yeasts isolated.

Keywords: Clinical mastitis; Red Sokoto Goat; Mycotic agents; Nigeria.

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Introduction

Mastitis is an inflammatory condition of the mammary gland, characterized by changes in the physical characteristics of the mammaric parenchyma or milk (Nazifi *et al*., 2011). It may be infectious or non-infectious in origin (Bradley, 2002). The condition is established to be the most acute disease in the dairy industry irrespective of animal species, due to its devastating economic effects on farmers (Radostits *et al*., 2000). Mastitis accounts for about 38% of the total direct loss of common production diseases (Kossaibati and Esslemont, 1997). It occurs whenever the animals are bred, fed, and managed to increase milk supply (Sharma, 2015). The higher the level of milk production, the higher the chances of mastitis, unless there are stringent control measures. The disease may occur either clinically or subclinically, with the former characterized by changes in milk colour, the presence of blood tinges or clots, and a large number of leukocytes in the milk (Ameh *et al*., 1993). There is often swelling, heat, pain, fibrosis, and indurations in the mammary gland (Ameh *et al*., 1993; Ameh and Tari, 2000). These signs of clinical mastitis can be detected by visual observation of the udder and the milk samples. However, in subclinical mastitis, no visible inflammation is seen, but there is a reduction in milk synthesis and changes in the microbial content of the milk secreted (Menzies and Ramanoon, 2001; McDougall *et al*., 2002; Contreras *et al*., 2007).

Studies on the prevalence of mycotic mastitis are few because most studies concentrate on the predisposing factors and antibiotic susceptibility patterns of the disease. In a recent survey, Danmallam and Pimenov (2019) reported an overall prevalence of 40.4 % for caprine mastitis in three regions of Nigeria, suggesting a substantially high prevalence of the condition in the country. The reported prevalence of subclinical mastitis is higher than that of the clinical form (Ferdous *et al*., 2018; Danmallam and Pimenov, 2019). In subclinical mastitis, the prevalence ranged from 12.7 % to 38.7 % (Kostelič *et al*., 2009; Gebrewahid *et al*., 2012; Pirzada *et al*., 2016; Tambuwal and Jibrin, 2017; Ferdous *et al*., 2018; Danmallam and Pimenov, 2019) while that of clinical mastitis ranged from 8.0 % to 11.7 % (Ferdous *et al*., 2018; Danmallam and Pimenov, 2019). Most of these studies centered on the preponderance of bacteriological agents as the primary causes, although samples collected from studies in clinically mastitic goats suggested that other agents apart from bacteria were responsible for mastitis (Ameh *et al*.,1993; Ameh and Tari, 2000).

Fungi are widespread and known to cause thrush, disseminated candidosis, cryptococcosis, and mastitis in man and animals (Malinowski and Kłossowska, 2002; Asfour *et al*., 2009). Studies on mastitis in cows suggested that mycotic mastitis accounts for 2–13 % of all cases (Lagneau *et al*., 1996; Krukowski *et al*., 2000; Krukowski *et al*., 2006). The incidence of mastitis due to fungi is usually low in dairy herds but occasionally it may occur in high proportions, mainly when there is trauma to the teats that can establish fungal infections in does (Gonzalez *et al*., 2001; Jand *et al*., 2003; dos-Santos and Marin, 2005; Spanamberg *et al*., 2009). The treatment of does with refractory mastitis using antimycotic drugs has been successful after several unsuccessful therapies with antibiotics (Sudhakara *et al*., 2018), signifying the involvement of fungi and other agents in caprine mastitis.

In Nigeria, Sahel goats, West African Dwarf goats (WAD), and Red Sokoto goats (RSG) are the three main breeds of goat, although RSG is the most widely distributed breed (Blench, 1999). The RSG is highly sought due to its superior skin and Moroccan leather (Shittu *et al*., 2008). However, available results suggest that mastitis is a significant health problem in this breed of goats with adverse impacts on its welfare and productivity (Shittu *et al*., 2008; Tambuwal and Jibrin, 2017). This study was designed to ascertain the prevalence of clinical mastitis in RSG, the fungal agents associated with the disease, and their antimycotic susceptibility patterns. Information obtained from this study will support preventive measures to reduce the incidence of the disease through effective therapeutic management.

Materials and Methods

Study location and animals

The study was conducted in households with backyard goat farms, while laboratory analysis was carried out at the Central Laboratory of the City campus, Usmanu Danfodiyo University, Sokoto, Nigeria. Sokoto lies between 5° and 6° East and between 13 and 14° North with an average annual temperature of 40°C and a yearly rainfall of 300mm – 1200 mm. The sample population was lactating RSGs from backyard flocks in Sokoto metropolis. The study was approved by the Faculty of Veterinary Medicine Animal Research Ethics Committee (FAREC), Usmanu Danfodiyo University, Sokoto, Nigeria.

Study design

A cross-sectional study of goats in backyard goat farms was carried out in Sokoto metropolis. During each visit, lactating RSGs were separated and clinically examined for mastitis. Milk samples were collected from those diagnosed with clinical mastitis and taken to the Laboratory for mycological examination and antimycotic susceptibility testing.

Sample size

The sample size was calculated using the formula described by Thrusfield (2018);

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

Where n = sample size,
P_{exp} = expected prevalence,
d = desired absolute precision.

Using a prevalence of 8.0 % obtained by Danmallam and Pimenov (2019), at the desired absolute precision of 0.05;

$$n = \frac{(1.96)2\ 0.08\ (0.92)}{(0.05)2}$$

n = 113.1
However, 127 RSGs were examined.

Clinical examination

Each half of the udder down to the teat were physically examined for features of clinical mastitis by palpation to detect visible inflammation and injury of the udder, presence of indurations, nodules, masses, hardness, reaction to pain, hotness of the udder, and fibrosis. The age of the RSGs were determined using their dentition, as described by Umar *et al.* (2018). Information on the management system, parity, and onset of the condition were obtained.

Samples collection

The RSGs diagnosed clinically mastitic were restrained, and their teats were disinfected with 70 % ethanol. The udders were then expressed to collect 10 ml of milk samples aseptically into Bijou bottles. This was done by collecting milk mid-stream to avoid sampling of contaminated milk. The sample bottles were adequately sealed, appropriately labeled, and transported on ice packs to the Laboratory for analysis.

Mycological analysis of milk samples

One milliliter of each milk sample was directly inoculated into Oxoid Sabouraud's dextrose agar (SDA) and Brain-heart infusion agar (BHIA) each containing Gentamicin at 50 mg/L. The inoculated SDA plates were incubated in the darkroom at ambient room temperature for 7 - 15 days, while the BHIA plates were incubated at 37°C for 2 - 3 days. The colonial, morphological and microscopic identifications were carried out on the cultured samples on both media according to the methods of Kidd *et al.* (2016). The microscopic identification was made at a magnification of ×40 by staining the fungal colonies on glass slides with lactophenol cotton-blue using thermal fixation as described by Kidd *et al.* (2016).

All isolates were sub-cultured on SDA plates containing gentamicin and incubated at ambient room temperature for 3 - 7 days. After that, individual colonies were picked and stored on SDA slants in sterile sample bottles until ready for use, as described by Mbuk *et al.* (2016).

Antimycotic susceptibility tests

Antimycotic susceptibility tests were carried out on all the isolates using commercial antimycotic discs. The antimycotic drugs employed were: *Amphotericin B* (10µg), *Fluconazole* (25µg), *Nystatin* (100 IU), Terbinafine (1µg), and Voriconazole (1µg). The isolates tested were inoculated into Sabouraud's Dextrose Broth (SDB) with Gentamycin, and the inoculum was standardized to 0.5 McFarland and incubated for 6 hours. A sterile cotton wool swab was soaked into the inoculum. The swab was used to streak the entire surface of Mueller Hilton Agar (MHA) before placing antimycotic discs 15 mm apart as previously described (Shalini *et al*., 2015; Mohamed *et al*., 2019). The plates were incubated at 35°C for fungi and read after 3 - 5 days. The zones of inhibition were measured in millimeters (mm) and compared against a reference standard, which contained measurement ranges and their equivalent qualitative categories of antimycotic susceptibility (Sevtap, 2007; Jean *et al*., 2017).

Data analysis

Data obtained were analysed using descriptive statistics and presented in Tables.

Results

The prevalence of clinical mastitis is presented in Table 1. Out of a total of 127 lactating RSGs examined, 38 were clinically mastitic, representing a prevalence of 29.9 %. The presentation of clinical mastitis among RSG is shown in Table 2. Based on laterality, 16 (42.1 %) RSGs had bilateral mastitis, with 1 of them being supernumerary, 22 (57.9 %) had unilateral mastitis (13 right, 9 left). A total of 6 (15.8 %) RSGs with clinical mastitis had teat injuries, while 32 (84.2%) had no injuries. The majority of the RSGs with clinical mastitis were aged ≥3 - <4 years 15 (39.5 %), followed by age group ≥2 - <3 12 (31.6 %), while age group ≥4 years had only 3 (7.9 %) goats with clinical mastitis. Further details are in Table 2. Twenty-eight (73.7 %) mastitic RSGs were from an intensive management system, while 10 (26.3 %) were from an extensive management system. Based on parity, 12 (31.6 %) mastitic RSGs were in their 3rd parity, 10 (26.3 %) were in their 2nd, while 8 (21.1 %) mastitic RSGs were each of the 1st and 4th parity groups. The onset of mastitis was after the second kidding in 21 (55.3 %) RSGs, while it began after the first and third kidding in 11 (28.9 %) and 6 (15.8 %) RSGs, respectively.

A total of 13 isolates of fungal pathogens were recovered from 11 mastitic RSGs, indicating a prevalence of 28.95 % of fungal agents in the RSGs with clinical mastitis but a prevalence of 8.7 % of fungi in the 127 goats examined (Table 3). The 8 fungal species isolated comprised of four (30.7 %) *Aspergillus niger*; 2 (15.4 %) *Aspergillus fumigatus* and 2 (15.4 %). *Fusarium chlamydosporium* Others were 1 (7.7 %) *Aspergillus lentullus*, 1 (7.7%) *Aspergillus nidulans*, 1 (7.7%) *Aspergillus terreus*, 1 (7.7 %) *Candida albicans* and one (7.7 %) *Histoplasma capsulatum*.

The antimycotic susceptibility patterns of the fungi isolated from the RSGs is presented in Table 4. The *Aspergillus niger* isolates were sensitive to *Amphotericin B*, *Fluconazole*, and *Nystatin* but resistant to Terbinafine and Voriconazole. The *Aspergillus fumigatus* isolates were susceptible to *Amphotericin B* and *Nystatin*, but resistant to *Fluconazole*, Terbinafine, and Voriconazole. However, the *Fusarium chlamydosporium* were susceptible to *Amphotericin B* and *Fluconazole*, but resistant to *Nystatin*, Terbinafine, and Voriconazole. *Aspergillus lentullus*, *Aspergillus terreus*, and *Candida albicans* were resistant to all the anti-fungal drugs tested. The *Aspergillus nidulans* isolate was susceptible to *Amphotericin*

Table 1: Prevalence of clinical mastitis among red Sokoto goats in backyard farms in a semi-arid region of Nigeria in Sokoto, Nigeria

Number examined	Number Clinical Mastitic	Percentage (%)
127	38	29.9

Table 2: Presentation of clinical mastitis in Red Sokoto Goats in backyard farms in a semi-arid region of Nigeria (n = 38)

	Frequency	Percentage (%)
Laterality		
Bilateral	16	42.1
Unilateral: right 13(59.1%), left 9(40.9%)	22	57.9
Teat injury		
Present	6	15.8
Absent	32	84.2
Age		
≥1 - <2 year	8	21.1
≥2 - <3 years	12	31.6
≥3 - <4 years	15	39.5
≥ 4 years	3	7.9
Management System		
Intensive	28	73.7
Extensive	10	26.3
Parity		
First	8	21.1
Second	10	26.3
Third	12	31.6
Fourth	8	21.1
Onset		
After first kidding	11	28.9
After second kidding	21	55.3
After third kidding	6	15.8

Table 3: Prevalence of fungi species isolated from clinically mastitic Red Sokoto Goats in backyard farms in a semi-arid region of Nigeria

Number of goats examined	**Number clinically mastitic**	**Prevalence of isolates in examined goats**	**Prevalence of isolates in clinically mastitic goats**
127	38	11/127 (8.7 %)	11/38 (28.9 %)
Fungal species		**Number of isolates**	**Percentage of isolate**
Aspergillus niger		4	30.7
Aspergillus fumigatus		2	15.4
Fusarium chlamydosporium		2	15.4
Aspergillus lentullus		1	7.7
Aspergillus terreus		1	7.7
Candida albican		1	7.7
Aspergillus nidulans		1	7.7
Histoplasma capsulatum		1	7.7
Total		**13**	**100 %**

Table 4: Antimycotic susceptibility pattern of fungi isolated from Red Sokoto Does with clinical mastitis

Fungal species	Percentage susceptibility to antimycotic				
	AMP	FLU	NYA	TER	VOR
Aspergillus niger	75%	100%	75%	0%	0%
Aspergillus fumigatus	100%	0%	100%	0%	0%
Fusarium chlamydosporium	50%	50%	0%	0%	0%
Aspergillus lentullus	0%	0%	0%	0%	0%
Aspergillus terreus	0%	0%	0%	0%	0%
Candida albican	0%	0%	0%	0%	0%
Aspergillus nidulans	100%	100%	100%	100%	0%
Histoplasma capsulatum	100%	100%	100%	0%	0%

AMP - Amphotericin B (10 µg), FLU – Fluconazole (25 µg), NYS – Nystatin (100 IU), TER – Terbinafine (1 µg), VOR -Voriconazole (1 µg)

B, *Fluconazol*e, *Nystatin*, and Terbinafine, but resistant to Voriconazole. The *Histoplasma capsulatum* isolate was sensitive to *Amphotericin B*, *Fluconazol*e, and *Nystatin* but resistant to Terbinafine and Voriconazole.

Discussion

A prevalence of 29.9 % for clinical mastitis in Red Sokoto Goats was observed in this study. This is similar to the 31.7% prevalence reported in Tanzania (Karimuribo *et al*., 2006), but higher than earlier reports of clinical mastitis of goats in Nigeria, where prevalence rates ranging from 5.7 % to 17.0 % were reported (Ameh *et al*., 1993; Ameh and Tari, 2000; Danmallam *et al*., 2018; Danmallam and Pimenov, 2019). It was also higher than the reports of Muhana (2014) in Iraq, Megersa *et al*. (2010) in Ethiopia, and Ferdous *et al*. (2018) in Bangladesh, where 3.5 %, 4.3 %, and 11.7 % prevalence were reported, respectively. The high prevalence of clinical mastitis in the present study may be associated with increased susceptibility of Red Sokoto goats (RSG) to mastitis (Shittu *et al*., 2008). RSG has high milk yield that may be incompletely stripped during lactation due to low physical obstruction in the teat canal. This predisposes their gland cisterns to pathogens that compromise the immune system leading to infection of the udder. Previous studies have shown that mastitis in goats occurs due to invasion of the mammary glands by pathogenic agents (Alawa *et al*. 2000). The results of the present study showed that the prevalence of unilateral mastitis in RSGs was higher than that of bilateral mastitis. This agrees with previous reports in Nigeria (Ameh *et al*. 1993; Ameh and Tari, 2000) but differs from the description of Ferdous *et al*. (2018) in Bangladesh. The variation may be attributed to the differences in geographical location, breed, and milking practices in each area. These are known factors that determine mastitis in goats (East *et al*., 1987; Menzies and Ramanoon, 2001; Zenebe *et al*., 2014). Right half mastitis was more prevalent than the left half mastitis, similar to previous reports (Ameh *et al*. 1993; Ameh and Tari, 2000; Ferdous *et al*., 2018; Abubakar *et al*., 2020). This is believed to be caused by the tendency of the goat to lie down on its right side from bulk feed in the rumen (Shittu *et al*., 2008). This predisposes teats on the affected side to contamination with pathogenic agents from the ground capable of causing intramammary infections.

The majority of the clinically mastitic goats in this study had no teat injury, consistent with the reports of Megersa *et al*. (2010) and Ferdous *et al*. (2018). In other studies, a significant number of teat injuries in lactating goats were reported during clinical (Ameh *et al*., 1993; Ameh and Tari, 2000; Danmallam and Pimenov, 2019) and subclinical (Zamin *et al*., 2010) mastitis. The limited number of teat injuries in this study may have been caused by the early detection of a high number of clinical mastitis. Belina *et al*. (2016) observed

that late detection of mastitis could lead to blind quarters due to teat injury. In the present study, RSG aged ≥3 - <4 years had the highest frequency of clinical mastitis. This is consistent with previous reports of goats with mastitis (Ameh and Tari, 2000; Pilau *et al*. 2011; Ferdous *et al*. 2018). Mastitis in goats lingers for a long time, with most acute cases becoming chronic (Bergonier *et al*., 2003), hence may not be detected early. This may account for the high frequency in goats ≥3 - <4 years. Also, an increase in the age of goats is believed to lead to an increase in milk yield and susceptibility to mastitis (Zeng *et al*., 1999).

Clinical mastitis was more in goats under intensive management than those on extensive management, similar to the report of Megersa *et al*. (2010). Poor management in intensive goat farming enhances the risk of developing mastitis based on the pollution of the house by micro-organisms from animal discharges. In Nigeria, most goats reared under the intensive system are raised on floors with beddings heavily contaminated with urine and feces that boost microbial growth (Lawal-Adebowale, 2012). These beddings play a crucial role in the transmission of infection from animal to animal within the intensive system. This may have influenced the high frequency of mastitis in this management system.

The frequency of clinical mastitis increased as parity advanced up to the third pregnancy. This was comparable to the reports of Moroni *et al*. (2005) and Megersa *et al*. (2010). It is believed that intramammary infections that cause mastitis occur as the parity of animals increases due to lingering diseases from past lactations that were poorly or incompletely milked (Moroni *et al*., 2005). RSG are seldomly milked, making it difficult to diagnose early mastitis without swelling of the udder (Alawa *et al*., 2000). Also, the majority of the mastitic goats in the present study were diagnosed after the second kidding. This reinforces the observation that mastitis in RSGs is not detected early, probably due to incomplete milking during previous lactation, leading to chronic mastitis that manifests clinically as enlargement of the mammary gland (Addo *et al*., 1980; Alawa *et al*., 2000).

This study detected a prevalence of 28.9 % of fungal agents in RSGs with clinical mastitis but a prevalence of 8.7 % of fungi in lactating RSGs. This is higher than the 1.74 % reported by Muhana (2014) in Iraq but lower than the 14.2 % reported by Ilhan *et al*. (2016) in Turkey. It is also lower than the 12.1 % and 35.6 % prevalence reported by Costa *et al*. (1993) and Zhou *et al*. (2013) in dairy cows. These differences may be attributed to breed and species variation coupled with poor environmental hygiene of the farms we examined due to contaminated litter. Prior to this study, there were no documented reports on the prevalence of mycotic mastitis in RSGs nor any other caprine species in Nigeria. It is possible that previous studies on caprine mastitis were associated with fungal agents, but the preponderance of bacteria is often highlighted. The majority of the fungal isolates obtained in this study were moulds similar to an earlier report in goats (Mishra *et al*., 1996; Muhana, 2014) and sheep (Las-Heras *et al*., 2000) but contrary to reports by Costa *et al*. (1993) and Delavenne *et al*. (2011), where more yeasts were isolated. This may be associated with the management practices instituted on the farms sampled, which tend to promote the growth of moulds over yeasts. Isolation of yeast, an opportunistic organism, has been associated with dairy animals during milking (dos-Santos and Marin, 2005). More than 50% of the fungi isolated in the present study were *Aspergillus* spp, with *Aspergillus niger* accounting for the highest frequency. This is consistent with the report of Muhana (2014) in Iraq, but different from the reports of Ilhan *et al*. (2016) in Turkey, who reported *Candida spp* as the most frequent fungi in goat milk. The variation may be caused by the difference in the design of the study. Our study was based on clinical mastitis, while Ilhan *et al*. (2016) isolated *Candida spp* from subclinical mastitis.

Aspergillus and *Candida spp* are normal flora found on the udder of clinically healthy goats (Ilhan *et al*., 2016). *Histoplasma capsulatum*, known as a human and animal dermatophyte (Kidd *et al*., 2016), was also found in this

present study, suggesting a potential zoonotic challenge to consumers of goat milk in the study area. The fungal agents isolated in this study are widespread in the environment, especially in unhygienic environments. Most antimycotic agents used in treating animals with mastitis are centered on pathogenic yeasts with no clear evidence of consistency in their efficacy (Krukowski *et al*., 2006). However, in this study, most of the fungal isolates were susceptible to *Amphotericin B*, *Fluconazole*, and *Nystatin*, while none of the fungi were susceptible to Voriconazole. This makes the trio of antimycotic agents the drugs of choice in treating fungal infections in mastitic RSGs in the study area. None of the five antimycotic agents (*Amphotericin B*, *Fluconazole*, *Nystatin*, Terbinafine, and Voriconazole) was effective against the pathogenic yeast *Candida albicans*. This is contrary to the observation of Mbuk *et al*. (2016) in which *Amphotericin B* was reported to be the most effective agent against Candida species in lactating cows. The difference may be attributed to the fact that the disc used in this study was specific for C. albicans, unlike that for Candida species used by Mbuk *et al*. (2016). It is, therefore, essential to determine the antimycotic susceptibility pattern of *Candida albicans* in mastitic RSGs using other anti-fungal agents.

Conclusion

This study detected a prevalence of 29.9 % for clinical mastitis in RSGs, with majority occurring under intensive management and from the third birth. A prevalence of 8.7 % for fungi was established with more moulds than yeasts isolated and *Aspergillus* species was the predominant fungal isolate. The fungi were more susceptible to *Amphotericin B*, *Fluconazole*, and *Nystatin* than other antimycotic agents used.

Conflict of Interest

The authors do not have any conflict of interest regarding the publication of this work.

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ASSESSMENT OF WATER AND FEED AS SOURCES OF MYCOTIC INFECTION IN POULTRY FARMS IN THREE LOCAL GOVERNMENT AREAS IN IBADAN, OYO STATE.

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Abstract

The majority of farmers in Ibadan and across the south-western region of Nigeria get feed materials from feed mills and other sources. The conditions of storage of the feed ingredients are usually without proper ventilation and the span of storage is not known to most of the farmers. Water and feed are usually served to poultry without any investigation on the presence or load of fungal agents in them. This study was carried out to assess the presence of fungi in the feed and water served to poultry in the study area. A total of 42 water samples and 43 feed samples were collected at different specific points across 7 farms in 3 local government areas in Ibadan, Oyo State of Nigeria. Each of the samples was inoculated and cultured both at room temperature and at 37^{0}C and studied for fungal growth. The fungal species isolated from the water samples and their respective frequency of occurrence were *Aspergillus fumigatus* (7.14%), *Aspergillus terreus* (1.19%), *Aspergillus niger* (7.14%), *Aspergillus* flavus (1.19%), *Penicillium spp*. (8.33%), *Mucor spp* (3.57%). and *Fusarium spp*. (2.83%). From the feed samples, *Aspergillus fumigatus* (27.90%), *Aspergillus niger* (6.98%), *Aspergillus* flavus (19.77%), *Penicillium spp*. (16.27%), Rhizopus arrhizus (9.30%) and *Mucor spp*. (5.81%) were isolated. These findings showed that the water and feed samples contained fungal agents that could be sources of infection to poultry as well as posing a threat to public health. The need for effective prevention and control of the spread of these pathogens through feed and water in poultry is important towards achieving disease free production as well as the production of wholesome poultry and poultry products.

Keywords: Fungi, poultry, feed, water, Ibadan, Nigeria

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Introduction

Food is very important for the survival of mankind. There cannot be life without food. But the importance and availability of food are as important as its safety. Although, the majority of people give little thought to the safety of the food that they eat (Habib *et al*., 2015). The feed of animals plays a very key role in the eventual availability of milk, meat, and egg for human consumption. It is not only animals that will be affected if the quality of animal feed is reduced, but it will also affect humans, the final consumers of the animal products. (Stefi *et al*.,2016).

Water, as the most abundant and essential commodity to man, is also very essential to animals including poultry (Auwal and Taura, 2013). A greater percentage of the world population, especially the people of the developing world do not have access to safe drinking water. Nigeria for instance, in coastal West Africa, has abundant water bodies; but the water is still not naturally safe (Auwal and Taura, 2013). This in turns affects the availability of safe water for animals' consumption, including poultry. Poultry feed may be carriers for a wide variety of microbes, including the pathogenic fungi (Webster and Weber, 2007). Fungi are generally adapted to the low moisture contents of the poultry feeds and they actively grow in many substrates especially stored grains and seeds (Webster and Weber, 2007). They usually manifest in the caking of feed and production of mycotoxins (Islam *et al*., 2014). The advancement of the poultry industry in many areas of the world is constrained by a number of factors of which a major one is the outbreak of diseases (Islam *et al*., 2014). The diseases affecting the industry are responsible for about 30% mortality of chickens every year of which, the major causes are microorganisms (such as bacteria, fungi, viruses), mineral and vitamin deficiencies, and parasites. (Islam *et al*., 2014)

Animal feeds are easily contaminated with colonies of fungi. These fungi use up the nutrients in the feed for their metabolism and growth and subsequently produce secondary metabolites that are harmful to the animal system. The various metabolites they produce cause diverse metabolic disturbances that usually result in poor productivity (Webster and Weber, 2007). Handling contaminated feeds poses a health challenge to the workers as they are exposed to the fungi and mycotoxins (Viegas *et al*., 2016).

Human and animal infections caused by fungi are becoming more and more important in recent times, and the contamination of feed and water especially are associated with changes in taste and odor (DEFRA, 2011). It is also associated with many health challenges such as asthma and various respiratory problems. (Auwal and Taura, 2013). This investigation was carried out to assess the presence of fungi in the feed and water served to poultry in 7 poultry farms located in 3 Local government areas in Ibadan, Oyo State, Nigeria.

Materials and Methods

A total of 42 water samples and 43 feed samples were collected from Ido, Lagelu and Ibadan-North Local government areas of Oyo State. The water samples were aseptically collected at three different points on the farm: the tap closest to the water source (either borehole or well), point of storage within the well and from the drinkers (either the nipple line or open drinker0. Feed samples were collected at three different spots on the farms: the feed mills on the farms or from the main feed stores for those without feed mills, the stacks of feed within the pens and directly from the feeders.

Fungal Analysis

10mls of each water sample was collected into a plastic test tube, covered and centrifuged at 2000 rpm for 10 minutes. The supernatant was decanted to leave about 0.5 ml in the test tube. From this, 2 wire loopfuls were taken for inoculation.

One gram of each feed sample was dissolved in sterile distilled water to make up a volume of 10 mls. It was then left to rest for 30 minutes and subsequently vortexed. Two

loopfuls of the supernatant were taken for inoculation. For each sample, two plates were inoculated on Sabouraud's Dextrose Agar impregnated with chloramphenicol (50 mg/ml) and gentamycin (50mg/ml) (Yousefi, *et al*., 2013). The plates were incubated at 37^{0}C and 25^{0}C. The plates were observed every other day for growth and read between 7-10 days. The identification of the fungi was done using their colonial morphology and microscopic characteristics.

Results

From a total of 84 plates inoculated with water samples, 24 (28.57%) were positive for fungal growth. The culture, isolation and identification of the organisms recovered from the water samples revealed that *Penicillium spp*. grew on 7 plates (8.33%), *Aspergillus fumigatus* and A. niger grew on 6 plates (7.14%) each, *Mucor spp*. on 3 plates (3.57%) while *Fusarium* spp. and *Aspergillus terreus* were recovered on 2 (2.38%) and 1 (1.14%) plates respectively as shown in table 1.

Out of the 86 plates that were inoculated with feed samples, 56 (65.12%) were positive for fungi. Table 2 shows the variety of fungi that were isolated from the feeds within the study area. In all, 7 fungal species representing 4 genera were recovered from the feed samples. These included *Aspergillus*, Penicillium, Mucor, and *Fusarium*.

Aspergillus fumigatus had the highest occurrence in the feed samples and it was recovered on 24 (27.9%) of the plates inoculated. *Aspergillus* flavus had a frequency of 17 (19.77%), while *Penicillium spp*. was recovered on 14 (16.27%) plates. Rhizopus arrhizus was grown on 8 (9.3%) plates. *Aspergillus niger* was recovered on 6 (6.98%) plates. *Mucor spp*. had the least frequency of 5 (5.81%) plates.

ORGANISM	WATER SAMPLES		TOTAL	PERCENTAGE GROWTH
	AT ROOM TEMP.	AT 37^{0}C		
Aspergillus fumigatus	3	3	6	7.14
Aspergillus terreus	0	1	1	1.19
Aspergillus niger	5	1	6	7.14
Aspergillus flavus	0	1	1	1.19
Pennicillium spp.	7	0	7	8.33
Mucor spp.	1	2	3	3.57
Fusarium spp	2	0	2	2.38

Table 2: Fungi isolated from water samples at room temperature and at 37^{0}C

ORGANISM	WATER SAMPLES		TOTAL	PERCENTAGE GROWTH
	AT ROOM TEMP.	AT 37^{0}C		
Aspergillus fumigatus	12	12	24	27.90
Aspergillus niger	1	5	6	6.98
Aspergillus flavus	7	10	17	19.77
Pennicillium spp.	9	5	14	16.27
Rhizopus arrhizus	1	7	8	9.30
Mucor spp.	2	3	5	5.81

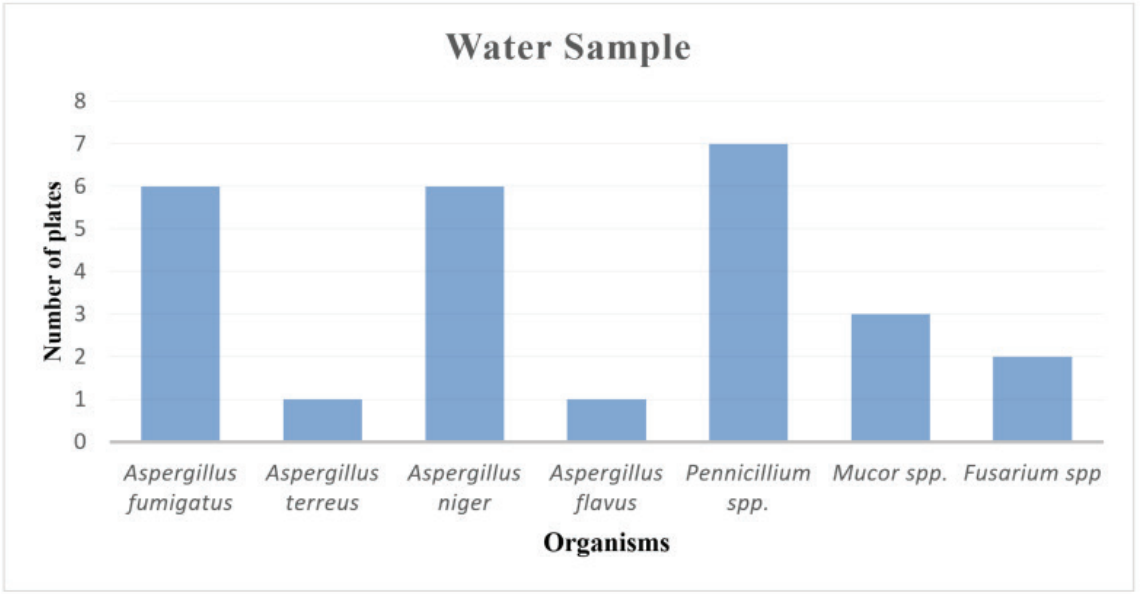

Figure 1: Graphical representation of the fungi isolated from the water samples

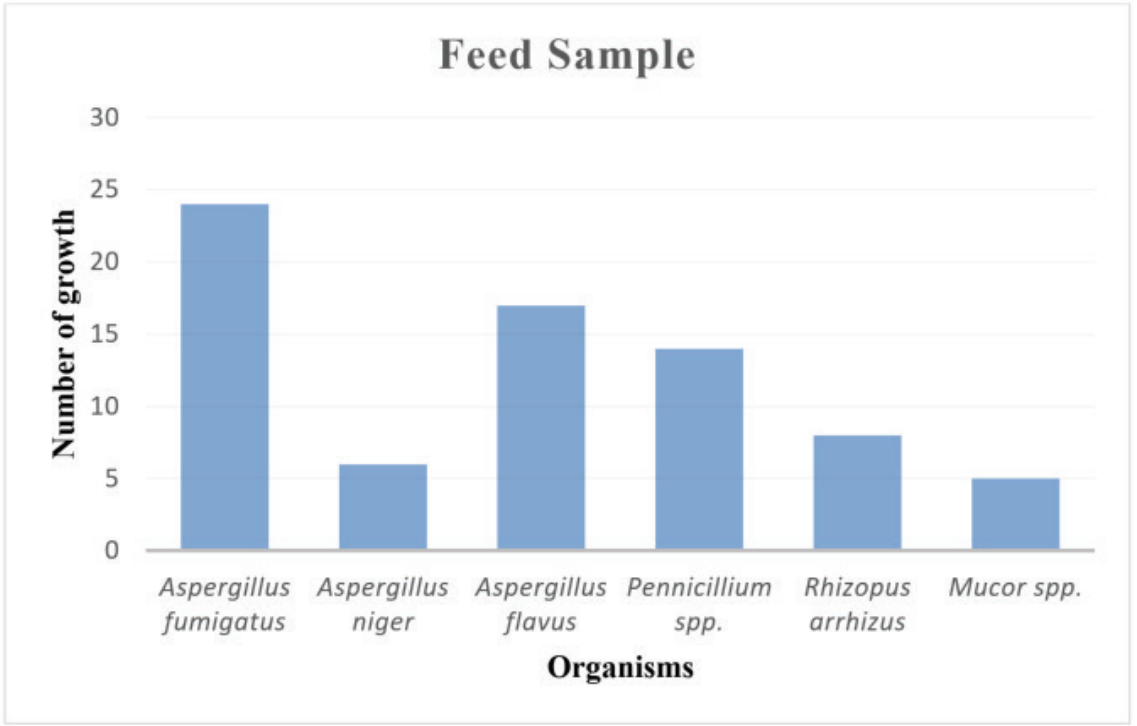

Figure 2: Graphical representation of fungi isolated from feed samples

Discussions

The abundance of *Penicillium spp*. in the water samples is clearly supported by the results of studies conducted by Auwal and Taura (2012) who reported that *Penicillium spp* are bundantly distributed in water and they have the ability to survive various water treatments. Thus, they can be found in various water networks. Heating of the water seems to be the only method that inhibits the viability of Penicillium hyphae and spores. The implication of *Penicillium spp*. and other fungal agents in water has been well associated with asthma, allergy, and other serious respiratory problems from several studies worldwide (Schwab and Straus, 2004 and Cooley, *et al*,.1998).

One of the most frequently isolated genera from these results is *Aspergillus*. This was consistent with the findings of Auwal and Taura (2012), Gunhild *et al*., 2006 and Arvanitidou *et al*., 1999; 2000). Fungi of the genus *Aspergillus* are well-known to produce aflatoxins (B1, B2, G1, and G2). These mycotoxins are known to be the most toxic, hepatocarcinogenic natural products (Bennett and Klich, 2013).

Fusarium was also recovered in the studies by Okpako *et al*., 2009 in Calabar, Nigeria. *Fusarium* species have been identified as agents of superficial infection such as keratitis, oncomycosis, cutaneous infection, and infections of burns and wounds. But the agent has also been isolated in deep-seated and disseminated infections in immunocompromised patients; especially with neutropenic patients (Guarro and Gene, 1995).

In this study, fungal contamination was found to be present in high proportions in the feed samples (64.3%). This finding is similar to the results of studies carried out earlier in Nigeria by Uwaezuoke and Ogbulie (2008), Obi and Ozugbo (2007), Osho *et al* (2007) and Aliyu *et al*. (2016). This is further supported by the findings from other regions of the world such as in Brazil (Olivera *et al*., 2006; Simas *et al*., 2007) and Pakistan (Saleemi *et al*., 2010),

Shareef (2009) reported the main contaminating genus to be from the *Aspergillus* spp. with a percentage frequency of about 91% and Aliyu (2016) also reported that the main contaminating fungi in poultry feed were from the genus *Aspergillus*, with specific emphasis on A. fumigatus and A. flavus, the well-known aflatoxigenic species. Aliyu later identified *Aspergillus* and Penicillium as the typical fungal genera usually contaminating poultry feeds.

The fungal agent with the highest occurring frequency in the feed samples collected in this study was *Aspergillus fumigatus*, followed by A. flavus, while *Mucor spp* had the least frequency. The organisms isolated also supported the results previously recorded by Krnjaja *et al*., 2008 who identified Mucor, *Aspergillus*, Penicillium, and *Fusarium* species with the highest frequency recorded in Mucor (76%) and least in *Fusarium* (15.6%) species. Arne *et al*., (2011) reported that the dominant genera of fungi isolated from poultry feeds are *Aspergillus*, *Fusarium*, Mucor, and Penicillium. *Aspergillus fumigatus* still remains a major pathogen that affects the respiratory tracts of birds. It produces an acute infection in young birds with high morbidity and mortality in flocks. In adults, the disease is more chronic. It results in severe respiratory distress, granuloma air sacculitis, and pneumonia (Arne *et al*., 2011).

Aspergillus flavus still remains renowned for the elaboration of its toxic metabolites, aflatoxins. Although there are about 18 types of aflatoxins that have been identified, only 4 are found in feeds. They are aflatoxin B1, B2, G1 and G2 (Yin, *et al*., 2015). Aflatoxin B1 is considered most toxic and is listed as a group I human carcinogen by the International Agency for Research on Cancer (Yunus *et al*., 2011)

Penicillium species were also observed in the poultry feeds in Telangana, India (Rajender, *et al*, 2017). Organisms of the genus Penicillium are known to be of varying degrees of mycotoxigenicity. Research work carried out by Girisham and Reddy (2016) revealed 8 different mycotoxins with the Ochratoxin A (OTA) as the predominating mycotoxin.

The other fungi that were isolated but with a relatively low frequency are Rhizopus arrhizus, *Aspergillus niger*, and *Mucor spp*. All these organisms have significant influences on the health of the birds. There could also be an

outbreak if the organisms are consumed in an infective dose (Webster and Weber, 2007).

The presence of fungi and aflatoxins in the feed ingredients that are served to livestock can greatly influence the quality of the finished feed. This will subsequently affect the health of the animals and humans. It is, therefore, critical to determine the presence of pathogenic agents and other potential sources of contamination in the feed production process. (Donna *et al*, 2017).

The methods of transport and storage of finished feeds by retailers and farmers are potential factors encouraging mycotic growth. When the feeds are transported from mills to the farms or within farms, they are usually compacted and reduced to smaller particles. This further increases the chances of fungal growth. These in addition to the high humid environment coupled with the long periods of storage help the multiplication of fungal organisms and further contamination. (Donna *et al*, 2017).

Conclusions

Fungal contamination of animal feeds with the consequent mycotoxin production is one of the major threats to human and animal health (Mariana *et al*., 2014). The high fungal recovery in the studies on the water and feed samples of poultry indicates a potential hazard to both animals and humans. The high occurrence of fungal species along the food chain is of great public health concern. (Aliyu, *et al*., 2016)

Species of fungi that are pathogenic, toxigenic and allergenic were isolated from the samples of feed and water in the study area. Thus they are present in the feed and water served in the poultry farms. These may likely be aerosolized into the air, thereby causing significant infection in immunocompromized poultry workers. (Hageskal, *et al*., 2009).

Furthermore, since the species isolated are also pathogenic to humans, exposure of humans to these mycotic agents in sufficient doses could cause disease in healthy individuals. A regular mycotoxicological analysis should therefore be carried out to ascertain the safety of poultry water and feed.

Feeds and feedstuffs are excellent media for the growth of fungi; a very high standard of hygiene is necessary to prevent feed contamination. One of the best ways to control feed contamination and mycotoxin problems is to investigate potentially toxigenic fungal populations in feeds (Krnjaja, *et al*, 2008). Milled feeds should not be stored for long periods of time. Also, a high standard of hygiene should be maintained to prevent feed contamination and subsequent mycotoxin production.

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MICROMORPHOLOGY OF THE URINARY BLADDER OF THE AFRICAN STRAW-COLOURED FRUIT BATS (EIDOLON HELVUM) FROM EASTERN NIGERIA

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Abstract

The microanatomy of the urinary bladder of the African straw-coloured fruit bats (Eidolon halvum) from Eastern Nigeria was investigated as the importance of bats in viral and bacterial disease transmission is growing. Whilst the microanatomy of the urinary bladder of most bat species has been reported there is dearth of information from available literature on that of the species under study. The urinary bladders were collected from animal samples sacrificed for reproductive anatomy baseline studies from a previous study. They were fixed in 10% neutral buffered formal saline and subsequently processed for routine histology. The urothelium was of stratified transitional epithelium and the lamina propia/sub*mucosa* contained smooth muscle cells, collagen fibres and blood vessels. PAS positive eosinophlic anucleated spherical to rectangular blocked-shaped structures were seen in the lumen in close association with the epithelium. These structures may be neutral mucin secretory bodies with a protective role in the wild against the bats' highly acidic urine. The *tunica muscularis* layer contained smooth muscle cells with various orientations from circular to oblique and longitudinal which can be related to the need to withstand urine pressure as the organ gets full. This study will contribute to the growing body of knowledge on bat biology for more investigative studies and aid pathologists in disease diagnosis.

Keywords: Nigeria, bat, urothelium, mucin, neutral, histology.

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Introduction

Generally, the urinary bladder is an oval shaped muscular distensible organ that holds urine under low pressure, which can be emptied by means of the urethra (Gopal, 2015). In mammals, it is located in the pelvis just above and behind the pubic bone when empty but can reach the abdomen when full. Urine is made in the kidney and goes into the urinary bladder by means of two tubules called ureters. The nature and volume of urine is related to feeding habits and water conservation mechanisms for environmental adaptation (Gopal, 2013). Bats are the only flying mammals and they have a wide range of feeding and roosting habits, social behaviour and body system biology (Kerth, 2003). The African straw-coloured fruit bat, Eidolon helvum is commonly found in moist and dry tropical rain forests (Hayman *et al*., 2012).

In recent years, bats are known to have many ecologically and economically important roles which include: sources of animal protein in prey and predator relationship models, arthropod natural control, seed dispersal, pollination, materials and nutrient distribution and recycling (Boyles *et al*., 2011;). Most importantly bats have been implicated in numerous infectious diseases agents such as Ebola virus, Marburg virus, Leptospirosis, Staphylococcus and histoplasmosis and are hence a source of big concern in zoonoses and public health (Plowright *et al*., 2015; Allocati *et al*., 2016; Olatimehin *et al*., 2018). Recent reports on bat population fluctuation mappings have associated increased levels of environmental stress, including rapid deforestation with increased bat contact with man, hence sporadic epidemics of associated viral infections such as rabies and Ebola viruses (Afelt *et al*. 2018a; Afelt *et al*. 2018b). The zoonotic importance has led to increased interest in bats, especially the urinary bladder where urine, one of the suspected routes through which bats indirectly transmit infectious pathogens, is stored (Mann *et al*., 2015; Carol *et al*., 2018) This current study relied on the histomorphology of the urinary bladder of Eidolon helvum in understanding the cellular structure of this organ and its associated secretions. The aim was to fill the knowledge gap in the available literature, provide baseline information for more investigative studies and help pathologists in characterizing diseases for enhanced management.

Materials and Methods

Thirty-seven adult male African straw-coloured fruit bats were used in this study. The identification of the bats was by the description given by Rosevear (1965) and Defrees and Wilson (1988). Male bats were captured between January 2017 and September 2018 using mist nets at nightfall near their roosts in the Obiagu community of Enugu State of Nigeria. Upon capture, the bats were transported in plastic cages with tree branches hanging on the ceiling of the cages mimicking a roost, to the laboratory unit of the Department of Veterinary Anatomy University of Nigeria Nsukka.

Adult male bats were identified by the presence of a bright orange collar (Rosevear 1965) and fusion of the epiphyseal plates in the finger bone joint of the fourth metacarpal-phalangeal bones (Kunz and Anthony, 1982; Anthony, 1988). All bats were humanely handled and cared for in compliance with the guidelines authorised by the Institutional Animal Care and Use Committee of the University of Nigeria, Nsukka (FVM-UNN-IACUC-2019-0710), The bats were weighed and euthanized by deep anaesthesia with a mixture of xylazine (2.2mg/kg; Xylamax, Bimeda Canada) and ketamine hydrochloride (13mg/kg; Rotexmedica, Trittau, Germany) administered intramuscularly within 6 hours of capture. The urinary bladders were harvested, fixed in 10% neutral buffered formalin and processed for routine histology through dehydration in graded concentrations of ethanol, clearing in xylene and embedding in paraffin wax. The sections 5µm thick were stained with haematoxylin and eosin; Mason trichrome; Periodic acid Schiff (PAS); Alcian Blue (AB); and combined AB/PAS (Bancroft and Stevens, 1990). They were viewed and photomicrographs were taken with a Motican

2001 camera (Motican UK) attached to an Olympus microscope.

Results

At a lower magnification, the walls of the urinary bladder showed four layers; the *mucosa* with a prominent urothelium, tunica sub*mucosa*; *tunica muscularis* and the *tunica adventitia* (Fig.1). The *mucosa* was thrown into longitudinal *mucosa*l folds lined by stratified epithelium. The superficial epithelial cells were dome shaped cuboidal cells to few squamous cells. Whilst the number of layers varied from 3 to 6, the deep cells were mostly cuboidal. The *mucosa* lacked lamina muscularis *mucosa*e, thus the lamina propria blended with the sub*mucosa* to become the lamina propria-sub*mucosa* and formed the cores of the *mucosa*l folds (Fig. 2). This layer contained abundant blood vessels, some smooth muscle fibres and slightly dense irregular collagen fibres (Figs. 3 & 4). Some eosinophilic spherical to rectangular block-shaped structures were observed in the lumen in close association with the epithelium and the lamina propria (Fig. 4). These structures were PAS positive (Figs. 5 & 6). Combined AB/PAS staining showed that these spherical bodies were only PAS positive (Fig. 7). The muscle layer contained abundant smooth muscle cells and its general architecture was thick comprising inner and outer longitudinal muscle fibres. Circularly and obliquely oriented muscle fibres were seen sandwiched between the longitudinal muscle fibres (Figs. 8 & 9).

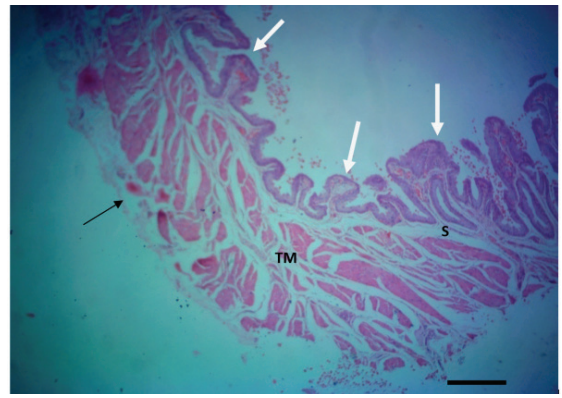

Fig. 1: Transverse section of urinary bladder of *Eidolon helvum* showing *mucosa*l folds (white arrows), thin sub*mucosa*(s), *tunica muscularis* (TM), and *tunica adventitia*(black arrow). H&E (Scale bar = 4µm).

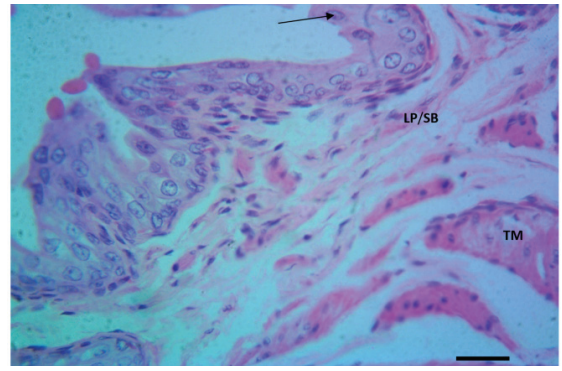

Fig. 2: Section of the urinary bladder of *Eidolon helvum* lined by transitional epithelium (black arrow). Note the lamina propria/sub*mucosa* (LP/SB) containing fibrous tissue and blood vessel .Observe the *tunica muscularis* (TM) containing smooth muscles in longitudinal and oblique orientation. H&E (Scale bar = 40µm).

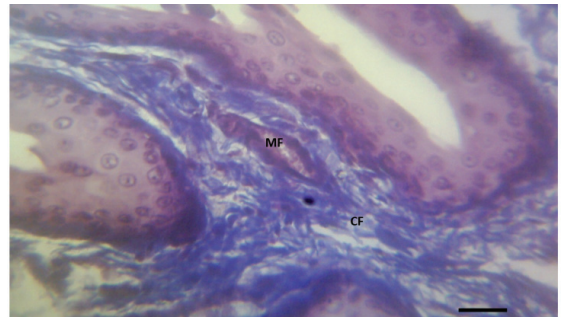

Fig. 3: Section of the urinary bladder of *Eidolon helvum* showing lamina propria/sub*mucosa* (LP/SM) collagen fibers that are slightly dense and some muscle fibers (MF). Mason Trichrome (Scale bar = 40µm).

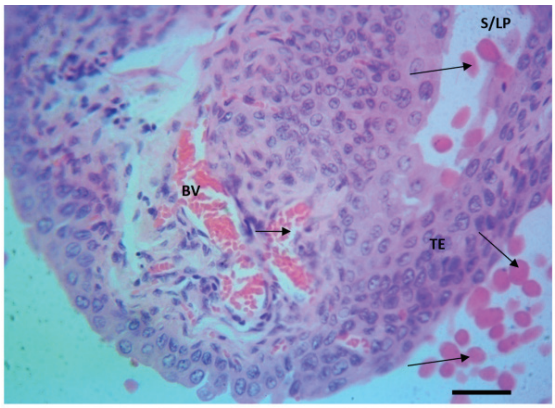

Fig.4: The urinary bladder of *Eidolon helvum* showing the *mucosa* lined by the transitional epithelium (TE) and abundant blood vessels (BV) in the lamina propria-sub*mucosa*. Note the eosinophlic spherical to rectangular block- shaped structures (black arrows). H & E (Scale bar = 40µm).

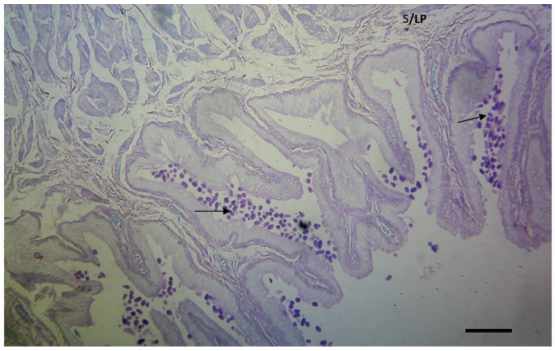

Fig. 5: Section of urinary bladder of *Eidolon helvum* showing PAS positive reaction by the eosinophlic spherical to rectangular block- shaped structures (black arrows). PAS (Scale bar = 10µm).

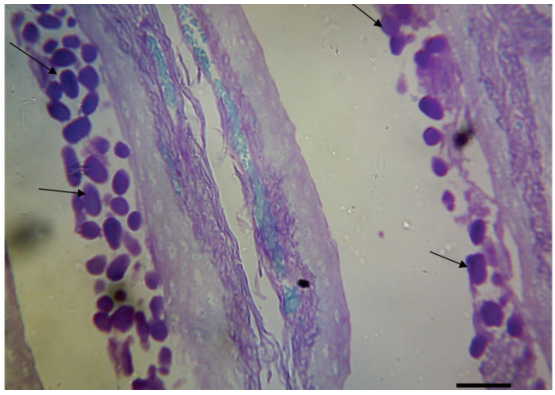

Fig. 6: Section of the urinary bladder of *Eidolon helvum* showing PAS positive reaction by the eosinophilic spherical to rectangular block- shaped structures (black arrows) at higher magnification. PAS. (Scale bar = 40µm).

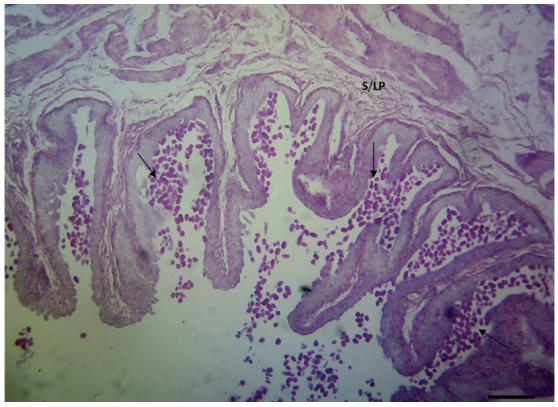

Fig.7: Section of the urinary bladder of *Eidolon helvum* showing only PAS positive reaction by the eosinophilic spherical to rectangular block- shaped structures (black arrows). AB/PAS (Scale bar = 10µm).

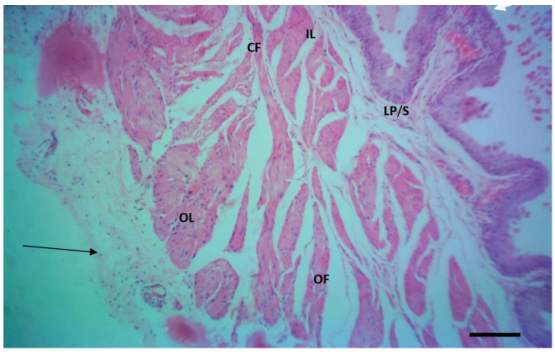

Fig. 8: Transverse section of the urinary bladder of *Eidolon helvum* showing a tunica *mucosa*l fold (white arrow), lamina propria/sub*mucosa*(LP/S). Note that the *tunica muscularis* contained thick smooth muscles ,comprising inner and outer longitudinal fibers(IL & OL). Circular fiber (CF) and obliquely oriented fibers (OF) sandwiched between the longitudinal fibers. Observe the loose fibrous tissues of the *tunica adventitia* (black arrow). H&E (Scale bar = 10µm).

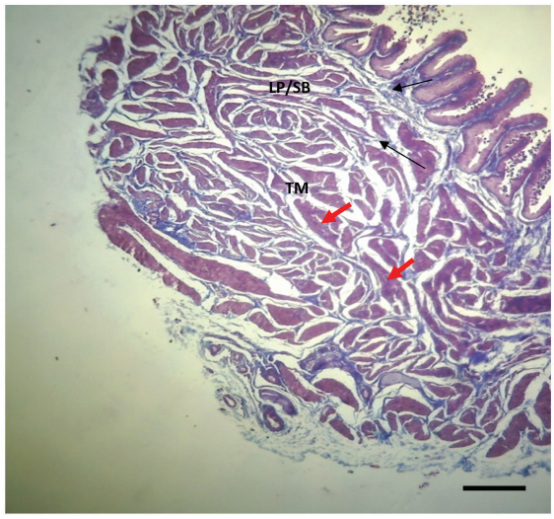

Fig. 9: Section of the urinary bladder of *Eidolon helvum* showing thick abundant smooth muscles (red arrows), in the tunica mucularis (TM). Note the presence of Collagen fibers (black arrows) embedded in the lamina propria/sub*mucosa* (LP/SB) /, . Mason Trichrome (Scale bar = 4µm).

Discussion

The histology of the urinary bladder of the African straw-coloured fruit bat was investigated in this study. The presence of the four tunics as shown in this study indicates conformity to the general plan of tubular organs comprising of the *mucosa*, sub*mucosa*, muscularis, and the adventitia. The urothelium under study consisted of stratified transitional epithelium. This epithelium has been associated with protective functions from toxic urine, signalling system, and capacity for distensibility to accommodate more urine (Acharya *et al*., 2004; Birder and Andersson, 2013; Winder *et al*., 2014 Fry and Vahabi, 2016). Stratified epithelium has also been reported in the human urinary bladder with indented nuclear shape (Jost *et al*., 1989). Whilst no distinct muscularis *mucosa* layer was seen in this study, its observation with well developed intercellular junctions and glycogen granules have been reported in humans (Dixon and Gosling, 1983). But the presence of smooth muscles cells in the lamina propria-sub*mucosa* as seen in this study has been described as the muscularis *mucosa*e of the human urinary bladder, whilst questioning the existence of a sub*mucosa*l layer in this organ (Karl-Erik and McCloskey, 2014; Fry and Vahabi, 2016). Lack of a distinct muscularis *mucosa*e layer reported in this study brings close contact between the lamina propria and the sub*mucosa*, hence the designation lamina propria/sub*mucosa* with slightly dense irregular collagen presence may have protective and communication enhancing functions for the urothelial signalling system (Birder and Andersson, 2013). This proximity enhances vascular communication between the urothelium, deeper structures in the sub*mucosa* and the *tunica muscularis* with supplies of nutrients and signalling molecules. The PAS positive eosinophlic spherical to rectangular blocked-shaped structures in the lumen in close association with the epithelium and lamina propria may be neutral mucin secretory bodies with a protective role against bats' highly acidic urine (Hales, 2014), but further studies are needed to clearly elucidate on their structure and function as mucous glands have been reported in suburethral regions of some Indian bats (Gopal, 2015).

In the urinary bladder under study, the well developed *tunica muscularis* maybe the detrusor muscle layer, presented a circularly to obliquely oriented fibers. This structural orientation has been related to the capacity to hold urine despite pressure from different directions even though not well developed relative to other mammals, as also seen in the uterus. It also helps the muscle layer to exert adequate force during the expulsion of urine (Fry *et al*., 2004). The mammalian urinary bladder is mostly extra-peritoneal in the pelvic cavity, hence the absence of a serosal coat as observed in this study. The presence of *tunica adventitia* has also been reported in the Indian bat Rousettus lschenaulti urinary bladder (Gopal, 2015).

Conclusion

The urinary bladder of the African straw-coloured fruit bat from this study is well adapted for life in the wild. This was deduced from the presence of a well developed protective urothelium and neutral mucin

secretory bodies against acidic urine content. The suburothelial structures of the lamina propria-sub*mucosa* most likely support and protect the urothelium. The *tunica muscularis* coat serving as the major support for holding and releasing urine, from this investigation is relatively well developed and oriented to adequately provide this function in this species of bats. This study hence will fill the knowledge gap in the available literature, provide baseline information for more investigative studies and help pathologists in characterizing diseases for enhanced management.

Acknowledgements

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FACTORS INFLUENCING PASTORAL HOUSEHOLD LIVESTOCK-DEPENDENT INCOMES IN SELECTED AREAS OF TURKANA AND WEST POKOT COUNTIES OF KENYA.

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Abstract

This study was designed to determine factors influencing pastoral household livestock-dependent incomes in selected areas of Turkana and West Pokot counties in Kenya, and to find out if the choice of veterinary delivery systems is one of the factors. The main goal of the study was to shed more light on the strategies to adopt in order to increase pastoral household livestock-dependent incomes in the two counties. The purpose of this paper, is therefore, to provide baseline data to facilitate the tracking of progress made towards the improvement of pastoral household livestock-dependent incomes in the two counties since the devolution of government functions, following the adoption of a new national Constitution that was promulgated in March 2013. The study analyzed the social and economic factors that influence pastoral household livestock-dependent incomes in the selected areas. The hypothesis tested was that animal health service delivery in the arid and semi-arid lands (ASALs) is not adequate to improve pastoral incomes and livelihoods

Field surveys for the collection of information were conducted using questionnaires, while socio-economic data were used to draw up a 'check list' for a number of variables to determine factors affecting household income in the two counties. Descriptive and regression analyses were carried out for data collected from a total of 160 respondents (80 from Turkana county and 80 from West Pokot county), which included household income and expenditure, herd size, cost of delivery of veterinary services, household size, age of household head, level of education of household age, gender of household age, availability of credit to herders, availability and acceptability/satisfaction of veterinary services, and service demand. Semi-structured interviews were used to elicit specific information regarding the monthly animal health expenditure for a period of one year for the households involved and the mode of service delivery was obtained by identifying the most used animal health delivery systems in each area. The delivery systems considered included veterinarians (public or private), self-treatment using modern medicines, self-treatment using traditional medicines, or the use of community based animal health workers (CAHWs).

The results indicated that in Turkana county, the level of education, acceptability/satisfaction with available services and distance to the nearest veterinary clinic showed positive and significant ($p \leq 0.05$) influences on pastoral household livestock derived incomes. Household size showed a positive and marginally significant ($p \leq 0.10$) influence whereas accessibility showed a negative and marginal ($p \leq 0.10$) influence on pastoral household livestock incomes. The other variables (age of household head, cost of service delivery, and mode of service delivery) had positive but insignificant effects on household incomes, except for satisfaction with the available services, which had a negative but insignificant influence on household income.

In west Pokot county, the level of education of the household head also showed a positive and significant ($p \leq 0.05$) influence on pastoral household livestock incomes. The cost of service delivery showed a positive but marginal ($p \leq 0.10$) influence on pastoral household livestock incomes. Household size, age of household head, and mode of service delivery had positive but significant effects on household incomes whereas accessibility, availability, and acceptability of services had negative but insignificant influences on

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household incomes.

From the results, it was concluded that the delivery of animal health services in the target areas at the time of the study, was neither effective nor sustainable and thus, it was not able to positively influence pastoral incomes and livelihoods. It is recommended that the government of Kenya, in collaboration with stakeholders in the livestock sub-sector takes measures to streamline research and extension on socio-economic factors that affect livestock production and marketing and improve the delivery of animal health services in the two counties in order to significantly impact on pastoral household livestock derived incomes and livelihoods. It is further recommended that the Government and other stakeholders should explore an alternative animal health care delivery model for the Arid and Semi-Arid Lands (ASALs) other than CAHWs that is effective and acceptable within the current policy and legal frameworks.

Key words: pastoral household livestock incomes, extension services, service demand, animal health care service providers, access to credit.

Introduction

It has been observed that since the 1980s, the funding for the livestock sub-sector, and the agricultural sector in general, has been declining in Kenya. Whereas the total agricultural sector used to receive 10% of the total government budget in the 1960s, the funding level decreased to 7.5% in the 1980s and to a dismal 3% in the 1990s. During the financial year 2005/2006, the entire agricultural sector received about 5-7% of the total budgetary allocation. Indeed, since the financial year 2002/2003, the total agricultural sector budgetary allocation has been about 3% of the total government budget, with the livestock budget accounting for only 1% of this proportion, which is equivalent to about 0.25% of the national Gross Domestic Product (GDP) (Kenya fiscal year budgets 2002/2003; 2003/2004; 2004/2005; 2005/2006). This is as opposed to the contribution of the livestock sub-sector to the national GDP that is currently estimated at about 10%. In the financial year 2008/2009, the agricultural sector received 4.5% of the national budget against 7% in 2007/2008. In the financial year 2008/2009, the livestock sub-sector received Kenya shillings (KSh) 4.56 billion (0.6% of the national budget) against a requirement of KSh 10 billion (1.3%) of the budget (Ministry of Livestock Development, 2009). This included KSh 800 million for control of Rift Valley Fever (RVF), KSh. 420 million, for the control of Pestes des Petits Ruminants (PPR) and KSh. 700 million for drought response. The budget for emergency interventions was KSh 557 million. As a result of inadequate funding for disease control, the vaccination coverage for diseases including foot and mouth (FMD), and Contagious Bovine Pleural Pneumonia (CBPP) in the Arid and Semi-Arid Lands (ASALs) of Kenya has been extremely low and irregular resulting in the current status of frequent outbreaks and spread of these diseases.

This study was therefore designed to determine factors influencing pastoral household livestock-dependent incomes in selected areas of Turkana and West Pokot counties in Kenya, and to find out if the choice of veterinary delivery systems is one of the factors, the main goal of the study being to shed more light on the strategies to adopt in order to increase pastoral household livestock-dependent incomes in the two counties. The study analyzed the social and economic factors that influence pastoral household livestock-dependent incomes in selected areas of Turkana and West Pokot districts of Kenya. The hypothesis tested was that animal health service delivery in the arid and semi-arid lands (ASALs) is not adequate to improve pastoral incomes and livelihoods

The purpose of this paper, is therefore, to provide baseline data to facilitate the tracking of progress made towards the improvement of pastoral household livestock-dependent incomes in the two counties since the devolution of government functions, following the adoption of a new national Constitution that was promulgated in March 2013.

Materials and Methods

Study area

The study was conducted in 2010 in West Pokot and Turkana counties in the Rift Valley region of Kenya. Following the promulgation of a new national constitution in August 2010 and the subsequent adoption of a devolved system of government from March 2013, the Rift Valley Province was dissolved and apportioned into devolved Counties. As a result, West Pokot and Turkana districts became Counties. Most Government functions were devolved and the delivery of animal health services became the responsibility of County governments. West Pokot and Turkana Counties which border Uganda, South Sudan and Ethiopia, are occupied by pastoral, agro-pastoral and sedentary Pokot and Turkana communities. The area has a harsh climate with unreliable rainfall that renders the areas very low in arable agricultural potential. They are only suitable for extensive rearing of indigenous livestock. Livestock production is the main economic activity in the two areas and accounts for 93.2% of most household incomes and 95% of most households' employment.

Sources and collection of data

Primary (raw) data were collected through questionnaire interviews, focus group discussions (FGDs) and other participatory methods. A questionnaire for the herders was developed, pre-tested on selected households in the study areas and adjusted as appropriate. In each of the two areas, a total of 80 households were selected. Data were collected on household size and characteristics, number of livestock owned, sources of income, access to markets and roads, access to water, access to veterinary and extension services, and affordability of the services. Identification and training of enumerators from the local communities was carried out before the actual fieldwork was undertaken. The rapporteurs were trained to ensure that they did not deviate from the required protocol, thereby reducing bias in the sample data collected.

Selection of study units and sampling

A stratified random sampling procedure was used to collect the socio-economic data. (Mugenda and Mugenda,1999). A list of all divisions in each of the counties was obtained from the provincial administration. Then two divisions in each county that had the highest cattle population were purposively selected to form the primary sampling units (PSUs). Selection of these PSUs was followed by the selection of households and finally the determination of the individuals within the households to be included in the study. A list of households in each selected division was obtained from which a sample frame was drawn. Random sampling was done using random numbers generated using a computer that enabled a deliberate unbiased sampling process, so that each sampling unit in a group had an equal probability of being selected (Levy and Lameshow, 1996). A sample size of 40 households per division was considered making a total of 80 households per county, a sufficiently large and acceptable sample size for social studies (Freund and Benjamin, 2006).

Data collection

Each household was visited individually and data collected on selected variables namely, size of house hold, the age of the herder, level of education of the household head, gender of the household head, satisfaction with veterinary services available, distance to the nearest veterinary clinic or animal health service deliverer, actions taken whenever ones animals got sick, availability and accessibility of veterinary services, availability of extension services, cost of veterinary services, herd size, availability of credit to herders, household income and expenditure, service demand and response time.

Data analysis and analytical models

Both descriptive and regression analysis was done, with descriptive statics being analyzed for all the selected variables.

Descriptive analysis

Data collected was analyzed mainly for descriptive statistics. Descriptive statistics were used to provide summaries about the sample measures that included means, ranges, mode and variation (Sternsten, 1996). Descriptive statistics were used to analyse household incomes and expenditure, herd sizes, costs of delivery of animal health services, household sizes, ages of household heads, levels of education of household heads, gender of household heads, monthly incomes, availability of credit, acceptability of veterinary services and service demand.

Regression Analysis

Model specification

Regression models were constructed for continuous dependent variables. Linear regression models were used to analyze both quantitative and qualitative responses. Other regression formulations involving the choice of animal health service delivery as a binary choice dependent variable were tried and these were the logit, Probit and linear probability model (LPM) regressions. These approaches are used to estimate models involving dichotomous response variables. This was done because the model that fits the data set better could not be determined a priori. Unfortunately, none of the binary choice models fitted the available data. Thus ordinary least squares (OLS) models were found to fit some of the data better. However, the OLS regression results depicted the presence of heteroscedasticity with respect to herd size. Herd size was therefore used to weight the data so as to obtain weighted least squares (WLS) model (Madalla, 2001). As a result, the R2, t and F values increased significantly.

The following general equation represents the base model used for the analysis.

$$Y_i = \alpha + \beta X_i + \mu_i \quad i = 1,2,3,\ldots,N$$

Where Yi is the continuous dependent variable for household i, α is the intercept term Xi is the explanatory variable for household I, β is the parameter, and μi is the error term, μi~ (0,σ2) of the unknown effects on the dependent variable. The specific expanded OLS model including more explanatory variables for estimation can then be written as in Equation (1):

$$Y_i = \alpha + \beta_1 X_{1i} + \beta_2 X_{2i} + \ldots + \beta_n X_{ni} + \mu_i \quad)$$

Where:

Y_i = Income from livestock for HH_i
α = Constant
X_{1i} = Cost of delivery for HH_i
X_{2i} = Acceptability by HH_i
X_{3i} = Herd size for HH_i
X_{4i} = Accessibility to services by HH_i
X_{5i} = Availability of services to HH_i
X_{6i} = Education of the herder HHi
X_{7i} = Extension services to HH_i
X_{8i} = Availability of credit to HH_i
X_{9i} = Gender of HH_i
X_{10i} = Household size HH_i
X_{11i} = Income level HH_i
X_{12i} = Age of herder HH_i
X_{13i} = Service demand HH_i
X_{14i} = Mode of animal health service delivery for HH_i
X_{15i} =response time
HH stands for Household

The degree of responsiveness of pastoral household livestock-dependent incomes to changes in the factors included in the model were quantified and the policy implications interpreted. It was hoped that the study would hed more light into strategies to adopt to increase pastoral household livestock-dependent incomes, one of the strategies being improving the quality of veterinary service delivery.

Results and Discussion

Descriptive statistics

The results of the variables tested are as follows:

Age of the herder: According to the survey, the majority of the respondent herders' ages

in both counties were in the age class of >46 years. There were more elderly herders (average age of the household age was greater than 46 years of age) than younger ones. Contrary to the findings of this study, it was expected that the average age of the herder was changing with more young people taking full charge of households and providing herd management. While Shiferaw and Holden (1998) underscored the positive correlation between age and perception of problems in a farming system, Bellon and Taylor (1993) argued that older persons are less likely to engage in productive farming practices.

Household size: The results indicated that by the time of the study, the average household size was 9.89 in West Pokot county whereas that of Turkana county was 7 adult equivalents. Since the number of people living in a household is a determinant of the household's availability for labor, it was expected that the bigger the size of the household, the higher the productivity and hence the household income. The results concurred with the findings of Henry (1990) who reported that the human population in the arid and semi-arid lands (ASALs) particularly the more drought prone arid areas has various patterns of concentration.

Level of education of household head: Of the herders interviewed in both counties, the majority were illiterate. Households headed by educated individuals are less likely to be poor compared to those of uneducated heads. This is because educated heads of households have higher income earning potential and more alternative income earning opportunities, and are therefore better able to improve the quality of their respective households' welfare (Krishna *et al*., 2004; Mango, *et al*, 2004). Muyanga (2004) pointed out that education provides an opportunity for pastoral households to diversify their livelihood portfolios especially through employment as a source of wages and remittances.

Gender of household age: All (100%) of the 160 households sampled in the two counties were male headed. Because of cultural and religious norms, women are deprived of property ownership rights and given lower status in all of the pastoral communities. They are also denied participation in traditional leadership and control of key assets and are given marginal benefits from divorce and inheritances of common properties (Marinda and Heidhues, 2004).

Satisfaction with the available veterinary services: Veterinary services in the two counties were provided by Veterinarians, Animal Health Auxillaries, Livestock Officers and Community Animal Health workers (Lewa, Mbithi, Nyariki, Muchina and Wabacha; 2020). The results from the study indicate that relatively more herders in Turkana county were satisfied with the animal health care services available (mainly CAHWS) than those in west Pokot county. Herders in West Pokot county may have been more exposed and more aware of quality veterinary services and would not accept services by CAHWs, since by the time of the study, in West Pokot county, there was a private veterinarian who was distributing quality veterinary drugs through Animal Health Auxiliaries (AHAs) to herders.

Table 1 shows the findings on satisfaction with veterinary services available in West Pokot and Turkana counties.

Table 1: Satisfaction with veterinary services available

Response	County			
	West Pokot District		Turkana District	
	Number of Households	Percentage of households	Number of households	Percentage of households
Very satisfied	6	7.5	38	47.5
Just satisfied	29	36.3	32	40
Not at all satisfied	45	56.3	10	12.5
Total	**80**	**100**	**80**	**100**

Distance to nearest Veterinary Clinic or Animal Health Service Deliverer: The majority of herders in Turkana county covered shorter distances (<5 Km) to the nearest animal health service deliverer. On the contrary, in West Pokot county, the majority of the herders covered longer distances (6-10 KM) to the nearest animal health service deliverer. This difference in distances may have been attributed to the presence of a private veterinary practice in West Pokot county (in Kapenguria division) and herders may have preferred walking longer distances to access quality veterinary drugs from the veterinarian whereas in Turkana county, herders walked shorter distances to CAHWs who lived amongst them in the communities. Table 2 shows the average distances covered to the nearest veterinary clinic or to the nearest AHSD.

Table 2: Average distances covered by herders to the nearest veterinary clinic and animal health service in west Pokot and Turkana counties

	West Pokot District		Turkana District	
	Number of Households	Percentage of households	Number of households	Percentage of households
<5	13	16.3	62.0	77.5
6-10	41	51.3	0.0	0.0
11-15	18	22.5	2.0	2.5
>15	8	10.0	16.0	20.0
Total	**80**	**100**	**80**	**100**

Response time: In both study areas, animal health service providers, took on average, longer than expected to respond to a herder's call leaving the herders with no other option but to treat their own animals.

Table 3 shows average time taken by the animal health service providers to respond to herders' calls in West Pokot and Turkana counties.

Action taken by herders whenever their animals were sick: The results of the study indicated that the majority of herders in west Pokot County treated their own animals whereas the majority of herders in Turkana county called an animal health service provider. In West Pokot County, herders accessed quality drugs from the private veterinarian located in Kapenguria, whereas in Turkana county, most herders sought veterinary services from Community Animal Health Workers (CAHWs) who lived with them in the communities and hence the difference.

Table 4 shows actions taken by interviewees whenever their animals were sick

Table 3: Average time taken by the animal health service deliverer to respond to a herders call in West Pokot and Turkana counties.

Time	County			
	West Pokot District		Turkana District	
	Number of Households	Percentage of households	Number of households	Percentage of households
Hours	33	41.3	24	30
Days	27	33.8	23	28.8
Week	9	11.3	11	13
>Week	11	13.8	22	28.2
Total	**80**	**100**	**80**	**100**

Table 4: Actions taken by interviewees whenever their animals were sick

Action taken	County			
	West Pokot District		Turkana District	
	Number of Households	Percentage of households	Number of households	Percentage of households
Treated Own Animals	47	58.8	33	41.25
Called an AHSD	29	36.3	42	52.8
Slaughtered	4	5	5	6.25
Total	**80**	**100**	**80**	**100**

Extension services: Only 18/80 (22.5%) of the herders interviewed in West Pokot county accessed extension services, and none of the herders interviewed in Turkana county accessed the services. From a market economic perspective, customers of animal health services have limited knowledge and information on the available treatment/ preventive options (Leonard 1993; 2000). This information asymmetry may result in adverse selection of a veterinary service.

Table 5 shows a summary of explanatory variables affecting household income in West Pokot and Turkana counties.

Table 5: Summary of explanatory variables affecting household income in West Pokot and Turkana counties

Variable	Unit definition	Average recorded	
		West Pokot	Turkana
Herd size	TLUs	39.34	90.3
Household size	Adult equivalents	9.89	7
Age of household head	Scale	>46	>46
	1-4: The larger the older	(mode = 4)	(mode = 4)
Level of education of household head	Scale	Illiterate	Illiterate
	1-4: The larger the higher	(mode = 1)	(mode = 1)

Variable	Unit definition	Average recorded	
Acceptability of service (satisfaction with service)	Scale	Not satisfied by available service	Very satisfied with service
	1-3		
	The lower the better	(mode = 3)	(mode=1)
Accessibility to service	Scale	6-10 KM	6-10 KM
(Distance to the nearest veterinary service	1-4	(mode = 2)	(mode = 2)
	The lower the fewer the kilometers		
Availability of veterinary services (Hours taken by AHSD to respond to herder's call)	Scale	Hours	Hours
	1-4		(mode = 1)
	The higher the more	(mode = 1)	
Availability of extension services	Binary	80 herders do not access extension services	80 do not herders access extension services
	1 for yes		
	2 for No		
Service demand (action taken when animals are sick)	Scale	5	
	1-6	Self-treatment	

TLUS:Total Livestock Units

Results of regression analysis

Two models involving multiple regression analysis were applied using the base Equation (1). These were the ordinary least squares (OLS) and the weighted least squares (WLS). The exact regression procedures involved in the estimations of the models are as shown below:

1. *OLS:* This involved the direct application of the base equation, where all the classical linear regression assumptions on the error term were assumed to hold.
2. *WLS:* When OLS regression analysis was done, the factors were examined at a 5% significance level in both counties. In West Pokot, none of the factors was significant at this level, but in Turkana county, only two factors were significant, namely acceptability/satisfaction with available veterinary services (Community Animal Health Workers), and the response time (time taken by an animal health service provider to respond to a herder's call). When the level of significance was raised to 10%, only response time became marginally significant in West Pokot county, whereas the mode of service delivery became marginally significant in Turkana county.

Because of the low R2 (Coefficient of determination) in the OLS and the evidence of heteroscedasticity, brought about by herd size, a form of weighting was applied to the data before running an OLS regression to obtain WLS parameters. An OLS was then run to

obtain WLS and the factors were examined again at 5% and 10% levels of significance. The level of education of the household heads in both counties was significant at 5%. In Turkana county, also significant at 5% were acceptability of available animal health services and distance to the veterinary clinic. When the significance level was raised to 10%, in Turkana county, accessibility, response time and household size became marginally significant, whereas in West Pokot county, the cost of service delivery per month became marginally significant. Tables 6 and 7 report the OLS and the WLS results for Turkana county.

Table 6: Factors influencing pastoral household livestock incomes: Ordinary Least Squares (OLS) Regression Coefficientsa for Turkana county using household incomes as the regressand.

Factor	β	t	Sig.
Level of education of household head	.024	.192	.848
Age of household head	.129	1.236	.221
Cost of service delivery per month	-.147	-1.449	.152
Accessibility	-.039	-.392	.696
Satisfaction	.042	.419	.676
Acceptability	.287	2.209**	.031
Distance to vet clinic	.123	.960	.340
Mode of delivery	.201	1.895*	.062
Response time	.318	2.696**	.009
Herd size (TLUs)	.162	1.535	.129

[a]Dependent variable: Monthly income level of herder (month)
***Significant at 5%; *Significant at 10%; R2 = 0.376; Adj R2 = 0.276;*
*F = 3.731***

Table 7: Factors influencing pastoral household livestock incomes: Weighted Least Squares (WLS) Regression Coefficientsa,b for Turkana county using household income as the regressand and herd size as the weighting variable.

	β	T	Sig.
Level of education of household head	.334	3.816**	.000
Age of household head	.099	.900	.371
Cost of service delivery per month	.143	1.613	.112
Accessibility	-.159	-1.837*	.071
Satisfaction	-.110	-1.272	.208
Acceptability	.184	2.015**	.048
Distance to vet clinic	.431	3.620**	.001
Mode of delivery	.133	1.194	.237
Response time	.171	1.903*	.061
Household size	.181	1.689*	.096

[a]Dependent variable: Monthly income level of herder (month)
[b]Weighted by herd size (TLUs)
***Significant at 5%; *Significant at 10%; R2 = 0.543; Adj R2 = 0.475;*
F = 7.963**

Table 8: Factors influencing pastoral household livestock incomes: Ordinary Least Squares (OLS) Regression Coefficients a for West Pokot county Using household income as the regressand

	β	t	Sig.
mode of delivery	.117	.991	.325
Household size	-.029	-.246	.807
Level of education of household head	.173	1.511	.135
Age of household head	.104	.879	.382
Cost of service delivery per month	.119	1.039	.303
Availability of extension services	-.139	-1.214	.229
Accessibility to service (Distance to the nearest road/ markets)	-.184	-1.592	.116
Acceptability of service (satisfaction with service)	-.196	-1.686*	.096
Herd size (TLUs)	-.006	-.047	.962

[a]*Dependent variable: Monthly income level of herder (month)*
**Significant at 10)%; R2=0.167; Adj R2=0.060; F=1.557*

Table 9: Factors influencing pastoral household livestock incomes: WLS Regression coefficientsa for west Pokot countyusing household incomes as the regressand and herd size as the weighting variable.

	β	t	Sig.
Household size	.184	1.540	.129
Level of education of household head	.283	2.431**	.018
Age of household head	.167	1.446	.153
Cost of service delivery per month	.210	1.742*	.087
Accessibility to service (Distance to the nearest road/ markets)	-.173	-1.428	.158
Availability of extension services	-.055	-.469	.641
Acceptability of service (satisfaction with service)	-.124	-1.072	.288
mode of delivery	.041	.352	.726

[a]*Dependent Variable: Monthly Income level of herder (month)*
[b]*Weighted Least Squares Regression - Weighted by Herd size (TLUs)**Significant at 5%; *Significant at 10)%; R2=0.246; Adj R2=0.147; F=2.492**

Hypothesis Testing

Focusing on the WLS model which explains more than the OLS model by virtue of its R2 value and F statistics, the results indicated that in Turkana county, levels of education of the household heads, acceptability, and distance to the nearest veterinary clinic showed positive and significant ($p \leq 0.05$) influences on pastoral household livestock incomes. Household size showed positive and marginal significance ($p \leq 0.10$) influence whereas accessibility showed negative and marginal ($p \leq 0.10$) influence on pastoral household livestock income. The rest of the variables including the age of household head, cost of service delivery, and mode of service delivery had positive but insignificant effects on household income, except for satisfaction with available services, which had a negative but insignificant influence on household income. In West Pokot county, the level of education of the household head also showed positive and significant ($p \leq 0.05$) influence on pastoral household livestock incomes. The cost of service delivery showed positive but marginal ($p \leq 0.10$) influence on pastoral household livestock income. Household size, age of

household head, and mode of service delivery had positive but significant effects on household incomes whereas accessibility, availability and acceptability of services had negative but insignificant influences on household incomes.

The significant variables are described below: Education level of the household head: In both counties, this factor was significant at a 5% level of significance. The education level of the household head influenced the monthly income of a household positively. The result supported the set hypothesis, which was positive. This may have been due to the reason that learned household heads made more informed decisions with regards to the choice of animal health service deliverers. The results were consistent with the findings of Peter *et al*., (2006) which showed that that with increased diversification into desirable assets and livelihoods (including education) households can remain active in the pastoral economy.

Household size: Household size was marginally significant in Turkana county (at a 10% level) and was not at all significant in West Pokot County. In Turkana county, it had a positive coefficient, indicating that larger households had more income. Larger pastoral families are expected to be secure in terms of labor provision, and therefore can afford to maintain larger herds resulting in bigger incomes, compared to smaller households (Dahl and Hjort, 1979).It has been argued that the availability of child herding labor influences diversification of pastoral livelihoods since households with a larger family labor force would be more willing to devote labor to another income generating activity in addition to pastoralism (Farah, Nyariki, Ngugi, and Musimba, 2003).

Acceptability of available services: In Turkana county, this factor was significant at a 5% level, whereas in West Pokot county, it was not at all significant. In Turkana county, CAHWs were more accepted than in West Pokot county, since they were the only services available in most of the study area and this led to more utilization of the services which impacted positively on household livelihoods. In West Pokot county, herders did not utilize a lot of services offered by CAHWs, and most of them could also not afford quality services provided by the only private veterinarian available. A study by Riviere-Cinnamond and Eregae (2003), demonstrated that community's acceptance of CAHWs led to higher production and income. This finding is in agreement with findings by Van *et al*., (2004) who reported that acceptability is affected by the attitude of the herders towards the animal health service provider and their evaluation of the costs of interventions.

Distance to the nearest veterinary clinic: In Turkana county, distance to the nearest veterinary clinic was significant (5% level of significance) whereas in West Pokot county, this factor was not at all significant. The reason for this observation was because in Turkana county, CAHWs lived in the community and were easily accessible. Their utilization led to positive impact on livelihoods. Availability of animal health service providers is often affected by the physical distance between him/her and the herder (Woods 2000). This in turn affects productivity and household income. Leonard (2004), also reports that the most prominent transaction in animal health service provision particularly in developing countries is distance. In Uganda, Koma (2000), and Woods (2000) found that greater distance to animal health providers noticeably reduced demand for their services.

Accessibility of animal health care: As hypothesized, accessibility to animal health care was expected to increase household incomes through increase in productivity (Heffernan, 2001; Dawit, 2003). Accessibility in Turkana county was marginally significant (At a 10% level of significance) whereas the factor was not at all significant in West Pokot county. However, in Turkana county, accessibility to animal health care influenced household incomes negatively. This could be explained by the fact that the herders could not differentiate between self-treatments and alternative animal health services that they could have opted for.

Response time: In Turkana county, the response time was marginally significant (at a 5% level of significance). Response time had a positive coefficient indicating that herders were most likely to use animal health services which were offered on time, therefore increasing productivity and income as well. This finding was consistent with the findings of Kathiravan et. al., (2009), who reported that the herder whose dependency on livestock for livelihood (as is the case in our study area) loses faith in animal health service providers who are not easily available. Increase in response time decreased the utilization of the services

Other Parameters Tested

Other parameters tested included the following:

(i) Cost of service delivery

The cost of delivery was marginally significant in West Pokot county, but not at all significant in Turkana county. As hypothesized, the cost of animal health service delivery was expected to lower household income through decrease in productivity, since high cost of delivery was expected to reduce demand. In the contrast, the cost of service delivery influenced household incomes positively. This finding is in agreement with the results of previous study done by Ahuja *et al*., (2002), which indicated that price is not an important determinant of the decision to use veterinary services.

(ii) Affordability of animal healthcare services

The affordability parameter was not tested for significance since it did not vary amongst the interviewees in both counties. This parameter evaluated the minimum necessary level of preventive and curative animal health care in each county.

(iii) Access to credit

At the time of the study, none of the interviewees had access to formal financial arrangements, and as a result, this parameter was not included in the model for analysis since it was not a variable.

Conclusion

After weighing, amongst other factors affecting household incomes in Turkana county, it was concluded that the level of education of household head, accessibility to and acceptability of animal health services, distance to veterinary services, time taken by the animal health service deliverer to respond to a herder's call and household size significantly influenced household incomes. The effects of cost of animal health services delivery, satisfaction with the animal health services offered and the mode of animal health service were so weak as to lack significance in the sample chosen.

In as far as the cost of animal health services delivery was concerned, the clients were observed to seek for quality of care dictated by their particular conditions and gave only secondary consideration to the price. This study further concluded that the delivery of animal health services in the target counties is neither effective nor sustainable and thus, in its current state, shall not improve pastoral incomes and livelihoods.

Recommendations

The study recommends the following:

- *Research:* It was observed that some key socio-economic factors, which also influence livestock production such as level of education of herders, cost of service delivery, accessibility of the services and acceptability of animal health services, have not been given sufficient attention in the past. It is recommended that the government of Kenya in collaboration with stakeholders in the livestock sub-sector also takes measures to streamline research and extension on socio-economic factors that affect livestock production and marketing.
- It is also recommended that the county governments should increase investments in the livestock sector, through prioritization of livestock in the development planning processes in the County governments;
- Animal Health Service Delivery should

be improved in the two Counties in order for it to significantly impact on pastoral incomes and livelihoods. This could be achieved through exploration of an alternative animal health care delivery model for the Arid and Semi-Arid Lands (ASALs) other than CAHWs that is effective and acceptable within the current policy and legal frameworks.

- Other recommended interventions that need to be put in place to increase the efficiency of the delivery of animal health services in ASALs include recruitment of sufficient veterinarians and animal health technicians specifically for the ASALs to increase accessibility to professional/ government animal health services, and also reduce distances to veterinary clinics, services and other inputs into the livestock value chain; Prioritization of livestock in the development planning process at the county governments; focus on enhancing level of education of pastoralists; an integrated approach to livestock development at the county governments; development of county policies that enhance economic viability of pastoralism; extension of the county extension programs to pastoral areas; and promotion of alternative livelihoods to pastoralists

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MOLECULAR CHARACTERIZATION AND BOTTLENECK ANALYSIS OF RABBIT BREEDS IN NIGERIA USING MICROSATELLITE MARKERS

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Abstract

The domestic rabbit (*Oryctolagus cunniculus*), continues to lag behind in terms of genetically inclined research necessary for its characterization in Nigeria, hence this study, which investigated the genetic characterization and bottleneck analysis of rabbit populations in Nigeria using microsatellite markers.

A total of one hundred rabbit blood samples were randomly collected across rabbit breeds, (New Zealand White (25), Californian White (25), New Zealand Red (25) and the Chinchilla (25) from different farms across Nigeria through saphenous venupuncture. Genomic DNA was extracted using the Norgen DNA extraction kit and then amplified for microsatellite markers.

The Bottleneck analysis sought to determine recent bottleneck (heterozygosity excess) in rabbit populations. The analysis revealed the heterozygosity excess values obtained were non-significant ($P > 0.05$) warranting the rejection of the null hypothesis that suggested a genetic bottleneck in the rabbit populations. These results were consistent with the normal L-shaped distribution of allele frequency revealed by the mode-shift indicator test in all populations indicative of no recent bottleneck in the rabbit populations.

Furthermore, a principal coordinate analysis defined two genetically distinct populations of the four breeds with the Chinchilla, New Zealand Red and Californian White as cluster one and the New Zealand White rabbit populations as cluster two with considerable variation within itself.

The findings showed that there were high levels of genetic disparity among the commonest breeds of Rabbits adapted to Nigeria, which encourages further genetically inclined research to facilitate the identification and development of effective breeding strategies crucial for the preservation and conservation of this animal genetic resource in Nigeria.

Keywords: Rabbit; principal coordinate analysis; characterization; saphenous vein; genetic bottleneck; microsatellite

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Introduction

The domestic rabbit (Oryctolagus cuniculus) has a high reproductive potential, fast growth rate, early sexual maturity, short gestation period, short generation interval and the ability to re-breed shortly after kindling thus, breeding all year-round (Irlbeck, 2001).

The historical record of rabbit keeping in Nigeria is not available; despite speculations of the commencement of rabbit rearing with the advent of slave-trading and the European invasion of Africa, when most exotic agriculturally important crops and livestock were introduced (Onifade *et al*., 1999).

Rabbit production in Nigeria is subsistence oriented, non-commercial and characterized by inadequate or no evidence of genetic characterization research as compared with the other livestock genetic resources in Nigeria (Omotoso *et al*., 2019).

Furthermore, the significantly low population size of rabbits representing the various breed lines in Nigeria warranted investigation into the possibility of a recent genetic bottleneck effect on the stock.

In recent years, molecularly inclined characterizations have been carried out in other African countries particularly Northern Africa, based on reports on Ghana (Lukefahr, 2000); Egypt (Grimal *et al*., 2012 ; El-Asker *et al*., 2016) and Tunisia (Ben-Larbi *et al*., 2014), in order to facilitate documentation, improvement and conservation of rabbit genetic resources.

There is inadequate genetic research crucial for the characterization of rabbit breeds in Nigeria , which would define the structure and facilitate conservation of this genetic resource (Omotoso *et al*., 2019). A deeper understanding of the genetic characterization of the rabbit populations would motivate effective breeding methodologies necessary for moving cuniculture to a commercial level in Nigeria (Omotoso *et al*., 2019).

Microsatellites which are highly resourceful in the genetic appraisal of several species populations, was therefore employed in the genetic characterization and ascertainment of a recent bottleneck event among the four commonest breeds in Nigeriain order to provide an essential requisite for the identification, management and improvement of the micro livestock populations, thus facilitating their conservation in the country.

Materials and Methods

Blood sampling and DNA extraction

A total of one hundred rabbit blood samples were collected from four rabbit breeds (New Zealand White -25, Californian White -25, New Zealand Red-25 and the Chinchilla-25) from different farms and research institutes across Nigeria. Approximately 1ml of blood was collected aseptically into 5 ml ethylene di-amine-tetracetic acid (EDTA) tubes from each of the rabbits through the saphenous rear leg venupuncture. which was placed in an icebox and transported to the Biotechnology laboratory of the Department of Animal breeding and genetics, Federal University of Agriculture, Abeokuta, and was stored at -20°C until DNA Extraction was carried out using Norgen DNA extraction kits with strict adherence to the manufacturer's guidelines. All animal sampling and handling was done with strict adherence to the Nigerian animal welfare ethical standards.

Polymerase chain reaction and microsatellite genotyping

Seven microsatellite markers (SAT3, SAT8, SAT12, SOL 3, SOL 8, SOL 28 and SOL 30) used for this study were presented in Table 1. The polymerase chain reaction carried out for the amplification of isolated DNA was prepared in a 25.00 µL cocktail mixture which contained 1.00 µL of DNA, 2.50 µL of 10 × buffer, 1.00 µL of 25 mM dNTPs, 2.00 µL primer (1.00 µL of each forward and reverse), 0.20 µL of Taq polymerase, 2.20 µL of 25 mm/Mol Mg2+ and 16.10 µL distilled water. A denaturing temperature of 94°C (1 minute) and annealing temperatures ranging from 52°C - 60°C for the seven microsatellite primers were used.The initial extension was done at 72°C for 1 minute followed by the final extension at 72°C for 10 minutes (Table

1). The products generated were subjected to 12 % polyacrylamide gel electrophoresis on an ABI 3730 DNA Sequencer. The DNA bands on the gels were scored based on the size of the DNA ladder with Gene Scan 3.1.2 (Figure 1). The bands were designated as alleles and the data was entered into an Excel Worksheet.

Data Analyses

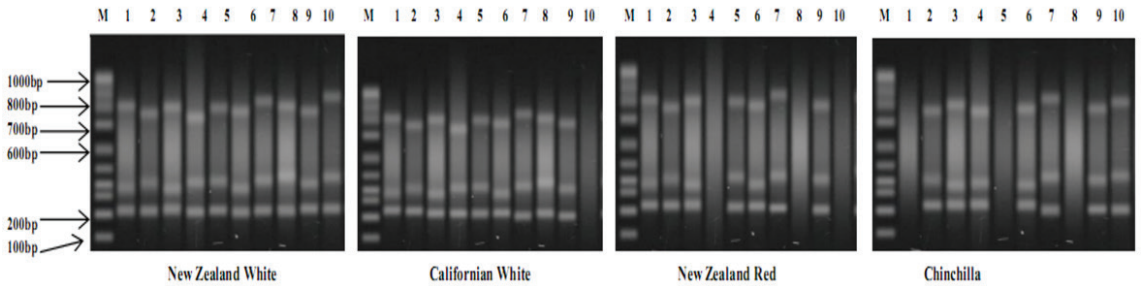


Figure 1: *Gel Electrophoretogram of rabbit breeds*

Allele frequencies, observed heterozygosity (HO) andexpected heterozygosity (HE) were estimated for seven microsatellite markers using the microsatellite analyzer (version 4.05); which is a platform independent analysis tool for large microsatellite datasets (Dieringer and Schlotterer, 2003). F-statistics was obtained using the Genepop 4.1 program (Raymond and Rousset 1995; Rousset 2008

To test for evidence of a recent genetic bottleneck, the program BOTTLENECK (Piry *et al*., 1999) was used. The program tested for the departure from mutation-drift equilibrium based on heterozygosity excess/deficiency. The Wilcoxon signed rank test was used to test for heterozygosity excess under two mutation models, infinite alleles (IAM) and the step- wise mutation model (SMM). The method of graphical representation of mode-shift indicator was also used for assessing distortion in allele frequency, which is indicative of possible bottleneck.

The allele data was plotted as Principal coordinate analysis (PCoA) using GenAlEx™ software (Peakall and Smouse, 2006).

Results and Discussion

The allelic frequencies of the seven microsatellite loci amongst the rabbit populations in Nigeria are presented (Table 1).

The total number of alleles observed across microsatellites varied between 8 (SAT3 and SOL3) and 17 (SOL28). The mean number of alleles identified in the entire rabbit population was 11.142±1.164, indicative of a high level of genetic variation among rabbit populations (Table 2).

Heterozygosity is a population genetic diversity parameter defined as the probability that two alleles taken at random from the population are different. The average heterozygosity is a robust measure of genetic variability within a population as it takes into account all levels of genetic variation rather than just classification into monomorphic or polymorphic variations.

The observed heterozygosity which is the percentage of loci heterozygous per individual, while the expected heterozygosity, otherwise known as gene diversity in this study (0.8230) was higher compared to those reported in Chinese rabbits (Zhu *et al*., 2004), European rabbit (Queney *et al*., 2000), Tunisian rabbits (Ben-Larbi *et al*., 2014) but similarities in range were observed with those reported for Egyptian rabbits (El-Aksher *et al*., 2016), European rabbits, (Surridge *et al*., 2008) and Chinese rabbits (Xin-sheng *et al*., 2008).

The observed heterozygosity obtained in this study was lesser than the values for the expected heterozygosity in most populations. This could be attributed to one or both of the following: segregation of non-amplifying (null) alleles and a scoring bias (heterozygotes scored wrongly) (Zhu *et al*., 2004). The high

level of heterozygosity recorded in this study could be attributable to the heterozygosity of the breeds from the historic mixing of strains of different populations.

The values of FIS were positive at two loci indicating a within population heterozygotes deficiency while five loci were characterized by negative FIS values indicating homozygosity deficiency and/or heterozygosity excess. The FIS estimates ranged between -0.0681 (SOL3) to 0.0788 (SAT12). The mean inbreeding coefficient of the individual relative to the sub-population FIS was -0.0201., This indicated the existence of outbreeding (heterozygosity excess) within the rabbit population, which suggested the mating of unrelated individuals at a high unprecedented rate possibly through indiscriminate negative assortative mating among the rabbit breeds in Nigeria.

The Bottleneck analysis (Piry *et al*., 1999) was used to investigate the hypothesis of a recent bottleneck. The Wilcoxon sign-rank

Table1: The allele frequencies of the seven microsatellite markers in the rabbit populations in Nigeria

Locus	Allele	Frequency	Locus	Allele	Frequency
SAT 3	146	0.1381	SOL 8	136	0.1705
	148	0.1513		138	0.2216
	150	0.1579		140	0.0966
	154	0.0263		142	0.1079
	156	0.1776		144	0.0056
	160	0.0197		146	0.1079
	162	0.0987		148	0.0398
				150	0.0852
SAT 8	122	0.1705		152	0.0795
	124	0.2216		154	0.0057
	126	0.0332		156	0.0738
	128	0.1079		158	0.0057
	130	0.0439			
	132	0.0781	SOL 28	236	0.0059
	134	0.0852		238	0.1012
	136	0.0795		240	0.0238
	138	0.0439		242	0.1191
	142	0.0636		244	0.0238
	144	0.0739		248	0.0774
				250	0.0238
SAT 12	111	0.0867		252	0.0297
	113	0.0533		254	0.0059
	115	0.0200		256	0.1012
	119	0.0667		258	0.0357
	121	0.0200		260	0.0238
	123	0.1733		264	0.1969
	127	0.2667		266	0.0057
	129	0.0733		268	0.0238

Locus	Allele	Frequency	Locus	Allele	Frequency
	131	0.0333		270	0.0059
	133	0.2667		272	0.1429
SOL 3	164	0.1154	SOL 30	152	0.0779
	166	0.1089		154	0.0389
	168	0.1731		156	0.1234
	170	0.0064		158	0.0065
	172	0.1795		160	0.0389
	174	0.1089		162	0.1623
	176	0.1474		164	0.0325
	178	0.1603		166	0.0065
SOL 30	168	0.1429			
	170	0.0065			
	172	0.1364			
	174	0.2273			

Table 2: Genetic characterization parameters across markers among four rabbit breeds in Nigeria

Marker	Allele size range (bp)	Annealing temperature (°C)	T_{NA}	H_O	H_E	A_R	A_E	F_{IS}
SAT3	146-162	60	8	0.8184	0.8511	8.50	6.72	-0.0662
SAT8	122-144	55	11	0.7418	0.7813	6.15	4.57	-0.0387
SAT12	111-133	58	10	0.7910	0.8267	7.64	5.77	0.0788
SOL3	164-178	58	8	0.7869	0.8392	7.88	6.21	-0.0681
SOL8	136-158	60	12	0.8184	0.7989	6.20	4.97	-0.0041
SOL28	236-272	52	17	0.8294	0.8551	9.91	6.90	-0.0584
SOL30	152-174	52	12	0.8103	0.8091	7.51	5.24	0.0154
MEAN	-	-	11.142	0.7995	0.8230	7.68	5.77	-0.0201
SEM	-	-	1.164	0.011	0.010	0.494	0.336	0.020

H_O = Homozygosity, H_E= Heterozygosity, T_{NA}= total number of alleles, A_R= Allelic richness, A_E = Effective number of alleles, F_{IS} = heterozygote deficiency, H_{WE}= hardy Weinberg equilibrium F_{ST} = genetic differentiation, PIC= polymorphism information content.

test under three mutations models IAM, TPM and SMM and shift mode test were used to find out a recent bottleneck (heterozygosity excess) in the four rabbit breeds. The heterozygosity excess values obtained were non-significant ($P > 0.05$) warranting the rejection of the null hypothesis that suggested a genetic bottleneck in the rabbit populations. These results were consistent with the normal L-shaped distribution of allele frequency revealed by the mode-shift indicator test in all populations indicative of no recent bottle neck in the rabbit populations (Table 3).

Table 3: Test for a recent bottleneck event among the four rabbit populations in Nigeria

Breeds	Test	IAM	TPM	SMM	Mode-shift indicator
New Zealand White	Wilcoxon	0.9804	0.4687	0.5937	Normal "L" shaped
Californian White	Wilcoxon	0.9375	0.4542	0.5977	Normal "L" shaped
New Zealand Red	Wilcoxon	0.9375	0.6875	0.5937	Normal "L" shaped
Chinchilla	Wilcoxon	1.0000	0.5687	0.8125	Normal "L" shaped

IAM: Infinite allele model, TPM: Two phase model, SMM: Stepwise mutation model

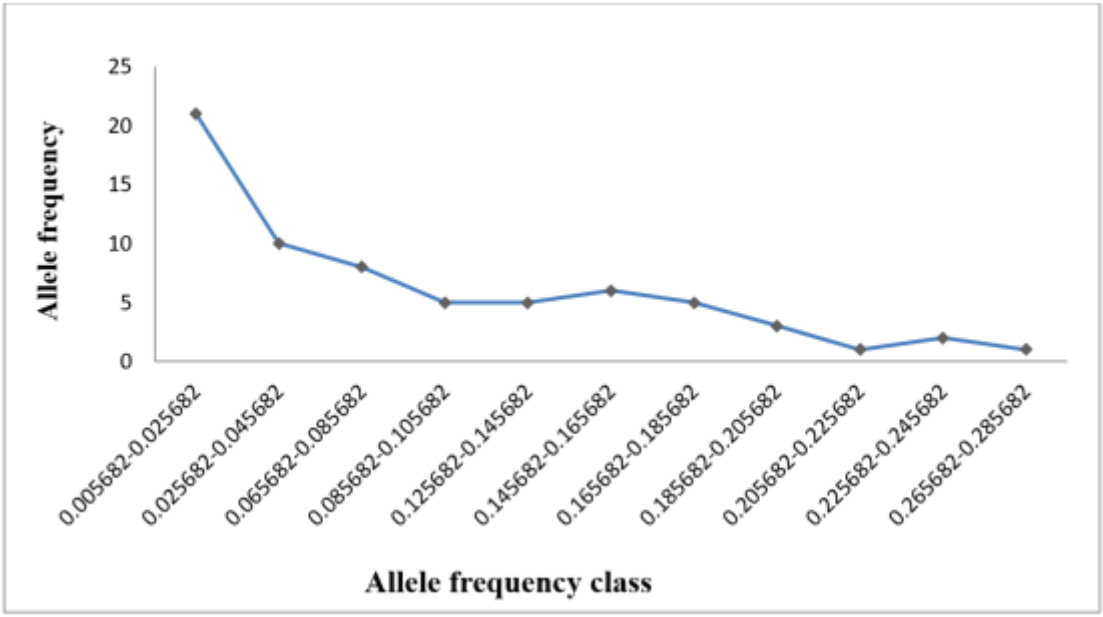
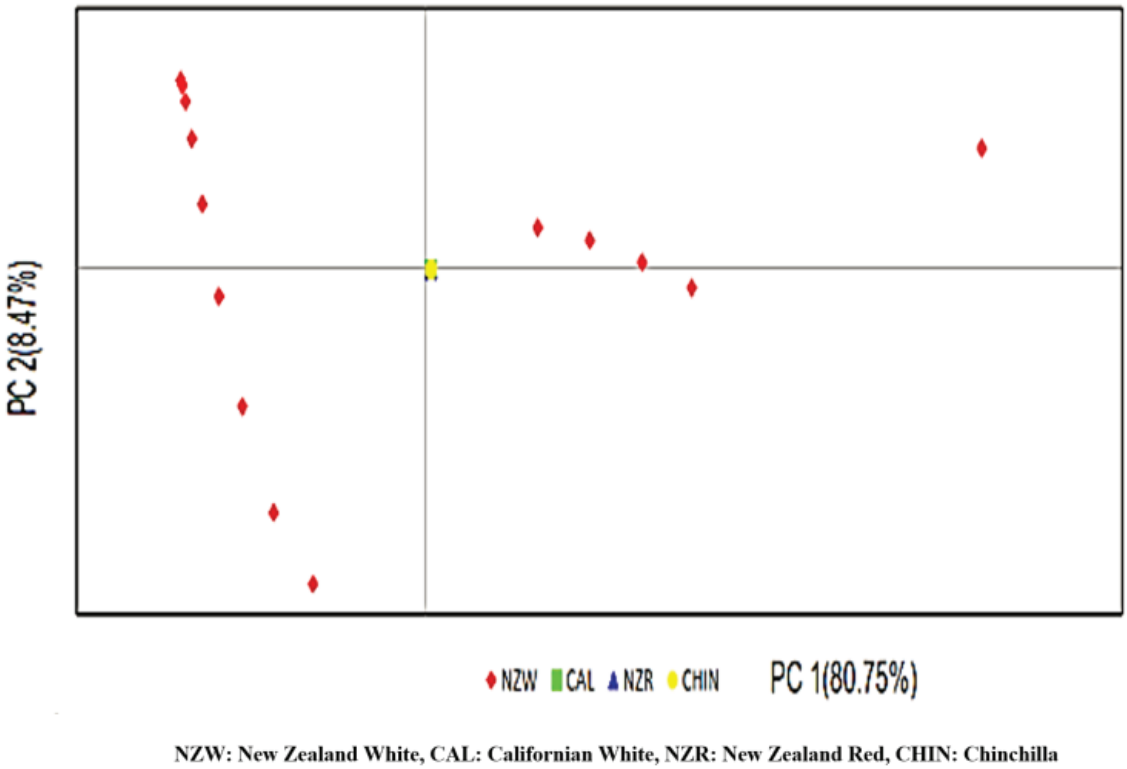


Figure 2: L-shaped mode-shift graph showing lack of recent genetic bottlenecks in the four rabbit populations in Nigeria

The Principal coordinate analysis (PCoA) revealed two genetically distinct populations with one (PC1) consisting of the Chinchilla, New Zealand Red and Californian White grouped together as a cluster on Axes, while the New Zealand White rabbit populations (PC2) were grouped as clusters with significant variation within them (Figure 3). This was indicative of a high level of homogeneity among the Californian white, Chinchilla and New Zealand Red rabbit breeds in Nigeria, while the New Zealand White expressed significant levels of genetic distinction among the rabbit populations examined and within itself.



NZW: New Zealand White, CAL: Californian White, NZR: New Zealand Red, CHIN: Chinchilla

Figure 3: Principal Coordinate Analysis based-allele frequency

Conclusion

The molecular characterization of the Nigeria rabbit population using microsatellites showed that rabbits in Nigeria represent a high genetic reservoir, which is characterized with a higher within breed variation than between breed variation as a result of the low values of genetic differentiation with regards to the inbreeding estimates. This indicates a relatively high out-breeding among the rabbit breeds.

All microsatellite markers used for the analysis are highly polymorphic and informative for genetic diversity studies, since the all loci PIC value in this study was greater than the threshold value of 0.5 (i.e. the value at which the microsatellite marker can be regarded as being informative).

The absence of a bottleneck effect or genetic drift suggests there has not been any recent reduction in the effective population size. Furthermore, it is noteworthy that geographic isolation is a crucial factor for differentiation between populations, and no clear geographic and/or reproductive isolation pattern was observed among the breeding rabbit populations in this study. Since animals from different Nigerian geographic settlements were considered as a homogenous population based on the Principal Coordinate analysis, it is therefore assumable that the influx of gene flow between breeds is attributed to the indiscriminate breeding and mating patterns practiced by Nigerian subsistence rabbit farmers with the breeds, which may explains a low identified structure between the four commonest rabbit breeds in Nigeria.

It is therefore recommended that the subsistence Nigerian rabbit farmers be sensitized on the crucial need for breed preservation, while the animal breeder organizations in Nigeria should speedily engage conservational approaches in the resuscitation of the genetic/breed dilution plaguing the *Oryctolagus cunniculus* population in Nigeria.

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HAEMATOLOGICAL AND SERUM BIOCHEMICAL ALTERATIONS ASSOCIATED WITH MICROSCOPIC KIDNEY LESIONS IN DROMEDARY CAMELS IN NORTHERN NIGERIA

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Abstract

An assessment of renal function is required in routine dromedary health investigations when kidney lesions are endemic in a population. In this study, haematological and serum biochemical alterations were evaluated in dromedary camels identified to have microscopic kidney lesions in order to determine the occurrence of renal dysfunctions related to haematopoiesis, excretion of nitrogenous waste products, electrolyte balance and the conservation of plasma proteins. Venous blood was collected from camels with or without anticoagulants during a cross-sectional survey of kidney lesions among slaughter camels at the Maiduguri abattoir in northern Nigeria. Haematological parameters were estimated using EDTA-anticoagulated blood while serum from clotted blood was used to measure the concentrations of biochemical parameters. The mean values of the parameters were compared among the groups with assortments of tubular, interstitial and glomerular lesions. The parameters were within the reference intervals. There were no significant variations among the groups and normal azotemic variables (creatinine, urea and uric acid) did not correlate with the values of packed cell volume. Therefore, the camels with kidney lesions did not have haematological and serum biochemical changes associated with such lesions, implying that abnormal indicators of renal dysfunction in the camels might be clinically rare as the animals remain apparently healthy. These findings point to the hardiness of camels in the local harsh climatic environment.

Keywords: Renal dysfunction, haematological parameters, serum biochemical parameters, kidney lesions, dromedary camels, Nigeria

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Introduction

Dromedary camels (*Camelus dromedarius*) in northern Nigeria are among the sedentary or pastoral herds (Jaji *et al.* 2017) that could have health issues requiring veterinary attention. Macroscopic and microscopic kidney lesions occurred in apparently healthy slaughter camels in Maiduguri (Hassan *et al*., 2015; Waziri *et al*., 2019). Similar lesions were previously reported in camel populations from other countries (Taha *et al*., 2007; Salem and Hassan, 2011; Rezaie *et al*., 2014; Kojouri *et al*., 2014; Saini *et al*., 2015; Tharwat *et al*., 2018a; b). Renal failure could be a challenging functional deficit faced by camels in the arid and semi-arid environments where there is poor water supply and an exaggerated need to manage body water conservation through anatomical and physiological adaptations of the kidneys (Wilson, 1984; Abdalla and Abdalla, 1979; Abdalla, 2020). Laboratory investigations of camel kidney diseases involve the use of blood samples of affected animals to search for altered parameters relevant to the diagnosis of renal dysfunctions.

Renal dysfunction caused by kidney lesions is associated with impaired haematopoiesis and altered full blood counts, because the kidneys secrete erythropoietin that regulates erythrocyte and platelet production and the bone marrow responds to the toxic effects of the uremic syndrome (Dodds and Nicholls, 1983; Zachee *et al*., 1994). Renal secretory tumours or renal tissue damage may increase or decrease erythropoietin production, respectively; with effects on erythropoietin-dependent erythropoiesis resulting in erythrocytosis (polycythemia) or anemia (Leung, 2013). Abnormalities in total and differential leukocyte counts often occur in individuals with kidney diseases (Agarwal and Light, 2011; Arai *et al*., 2018) because of inflammatory conditions associated with the disease (Tharwat *et al*., 2018a).

The kidney plays a role in maintaining the plasma concentrations of proteins and electrolytes, such that dysproteinemia and dyselectrolytemia occur in individuals with kidney lesions (Dhondup and Qian, 2017). Elevations of plasma creatinine, uric acid and urea concentrations are indications of failure of the kidney to excrete nitrogenous waste products of metabolism through glomerular filtration (Zhang *et al*., 2017). Camels with kidney lesions were previously reported to have abnormal levels of serum biochemical parameters, indicating *azotemia*, *hyperglycaemia*, *hyperproteinemia* or *hypoproteinemia*, *hypoalbuminemia*, *hyperglobulinaemia hyponatremia*, *hypochloridemia*, *hypercalcemia*, *hyperphosphatemia* and *hypermagnessemia* (Barakat *et al*., 2017; Tharwart *et al*., 2018a, b).

Dromedary camels with kidney lesions encountered at slaughter in Nigeria (Waziri *et al*., 2020) might have renal dysfunctions that could affect haematological and serum biochemical parameters influenced by the kidney. The objective of this study was to assess the haematological and serum biochemical alterations in dromedary camels with assortments of microscopic kidney lesions.

Materials and Methods

Study design

The study was an observational, cross-sectional investigation of dromedary camels (*Camelus dromedarius*) for haematological and serum biochemical alterations associated with kidney lesions that were previously described and reported (Waziri *et al*., 2020) in Maiduguri (Latitude 11°N and Longitude 13°E), Borno State, Nigeria, where dromedary camels were slaughtered for meat (Baba *et al*., 1994). The camels selected in the study were adult (> 1.0 year old) males and non-pregnant females (Waziri *et al*., 2020). During antemortem examination, the blood samples were collected from the camels for full blood counts and serum biochemistry. After histological examination of the kidneys and collection of data, the camels with kidney lesions were sorted into groups with mild tubular lesions (MLT, n=9) moderate tubular lesions (MDT, n=60), glomerular and tubular lesions (GAT, n=4), interstitial and tubular lesions (IAT, n=13), as well as glomerular, interstitial and tubular lesions (GIT, n=7) for

data analysis. Comparison and correlation of the haematological and serum biochemical parameters of the groups were done to ascertain whether the variety of lesions in the groups influenced the parameters.

Blood sample collection

Blood samples (15 ml) were collected from the jugular vein of each camel, and put into plain sample tubes (10 ml) and EDTA-containing sample tubes (5 ml). The blood samples were transported on ice packs to the laboratory. Serum was harvested from the clotted blood samples and stored for subsequent biochemical analysis.

Determination of haematological parameters

Packed cell volume (PCV), red blood cell count (RBC), haemoglobin concentration (HB), total leucocyte [TLC] and absolute differential leucocyte counts (DLC) were determined by standard procedures (Jain, 1998). The haematocrit method was used to determine the PCV. The haemoglobin concentration was determined using the cyanmethaemoglobin method, the haemocytometer method was used to determine RBC and TLC while DLC was determined using the thin blood film. The mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular haemoglobin (MCH) were calculated as previously described (Jain, 1998).

Determination of biochemical parameters

Reagents test kits (Randox laboratories Ltd., UK; Biosystems Ltd) were used to determine serum total proteins, albumin, urea, creatinine, uric acid, calcium and phosphorous concentrations. Serum potassium and sodium concentrations were determined by flame photometry, while the titrimetric method was used to determine serum chloride and bicarbonate ion concentrations. The serum globulin concentration was calculated by subtracting the value of the albumin concentration from the total protein concentration, while the albumin/globulin ratio was calculated using the albumin and globulin concentrations.

Statistical Analysis

Data were summarised as means ± standard deviations. Analysis of variance (ANOVA) followed by Dunnett's post-hoc test was used to compare means among groups and differences in means were considered significant at $p<0.05$. The relationships of PCV values with the concentrations of serum creatinine, urea and uric acid were assessed using correlation coefficients (r) with test of significance at $p = 0.05$. Computer software (GraphPad Prism 5.0) was used for statistical analysis.

Results

The haematological parameters of dromedary camels with various kidney lesions are presented in Table 1. There were no significant ($p>0.05$) variations among dromedary camels with the various lesions.

Serum biochemical parameters of dromedary camels with various kidney lesions are presented in Table 2. All biochemical parameters did not significantly ($p>0.05$) vary among the groups with the various lesions.

The relationships between packed cell volume values and serum uric acid, urea, or creatinine concentrations are presented in Fig 1. PCV did not significantly ($p>0.05$) correlate ($r=0.02 – 0.19$) with the serum uric acid, urea or creatinine concentrations.

Discussion

The results of this study showed that the kidney lesions were not associated with anemia. The nephropathic camels had erythrocyte parameters within the reference intervals reported for camels (Gupta *et al*., 1979; Mohamed and Hussein, 1999; Farooq *et al*., 2011; Waziri *et al*., 2019). Camel kidney disease did not also affect the PCV levels in previous reports (Salem and Hassan, 2011; Tharwat *et al*., 2018a). It was expected that an anemic camel would have a PCV of <19% but none of the camels had the PCV below the critical cut-off. Chronic kidney

Table 1: Haematological parameters of dromedary camels with kidney lesions* grouped into mild tubular lesion (MLT), moderate tubular lesion (MDT), glomerular and tubular lesions (GAT), interstitial and tubular lesion (IAT), and glomerular, interstitial and tubular lesions (GIT).

Parameters	MLT (n=9)	MDT (n=60)	GAT (n=4)	IAT (n=13)	GIT (n=7)
Packed Cell Volume (%)	26.00±3.09	26.36±4.34	26.00±4.69	26.33±7.70	27.29±5.53
Haemoglobin concentration (g/dl)	11.44±2.73	11.57±2.74	11.73±3.57	10.56±3.46	12.10±1.85
Red Blood Cell (x106/µl)	7.31±1.12	7.17±1.42	6.90±1.29	7.10±2.53	7.35±1.25
MCV (fl)	35.93±4.12	37.22±3.89	37.73±2.41	37.66±2.74	37.26±4.46
MCHC (gm/dl)	43.62±6.59	43.55±5.60	44.40±6.51	39.84±5.93	45.11±7.18
MCH (pg)	15.85±3.76	16.28±3.03	16.80±3.24	15.00±2.53	16.71±2.71
Total Leucocyte (x103/µl)	11.77±2.01	12.46±2.71	13.08±3.48	12.32±4.70	12.61±3.26
Neutrophils (103/µl)	3.67±0.90	3.90±1.05	4.25±1.70	4.26±1.70	3.81±1.26
Lymphocytes (103/µl)	6.64±1.46	6.98±1.79	7.38±1.72	6.61±2.84	7.33±2.14
Monocytes (103/µl)	0.51±0.20	0.61±0.22	0.48±0.17	0.50±0.32	0.49±0.24
Eosinophils (103/µl)	0.96±0.20	0.98±0.27	0.98±0.13	0.97±0.39	0.97±0.35
Basophils (103/µl)	0.01±0.32	0.00±0.02	0.00±0.00	0.01±0.03	0.03±0.05
Neutrophil-lymphocyte ratio	0.57±0.18	0.58±0.17	0.56±0.11	0.65±0.11	0.54±0.22

#No significant (p>0.05) difference among groups for all parameters
**Lesions described by Waziri et al. (2020)*

Table 2: Serum biochemical parameters of dromedary camels with kidney lesions* grouped into mild tubular lesion (MLT), moderate tubular lesion (MDT), glomerular and tubular lesions (GAT), interstitial and tubular lesions (IAT), and glomerular, interstitial and tubular lesions (GIT).

Parameters	MLT (n=9)	MDT (n=60)	GAT (n=4)	IAT (n=13)	GIT (n=7)
Total Protein (g/dl)	7.87±2.97	7.69±2.30	8.36±0.88	6.23±1.37	9.49±4.77
Albumin (g/dl)	4.31±0.79	4.45±0.98	4.18±0.19	3.97±0.70	5.43±3.39
Globulin (g/dl)	3.56±2.78	3.24±2.21	4.19±1.02	2.26±0.93	4.07±4.31
Albumin/Globulin Ratio	0.29±0.43	0.33±0.59	0.11±0.04	0.25±0.27	0.57±0.85
Uric Acid (mg/l)	7.71±6.98	6.38±5.82	4.38±1.94	7.67±6.84	5.80±3.69
Urea (mmol/l)	2.88±4.63	2.64±4.85	2.85±2.01	4.75±5.47	4.63±4.01
Creatinine (µmol/l)	86.27±63.76	73.36±51.61	73.22±35.59	86.24±51.98	87.32±44.20
Chloride ion (mmol/l)	168.89±11.67	166.82±23.87	164.00±9.09	159.54±28.00	166.29±18.60
Bicarbonate ion (mmol/l)	20.89±2.67	21.62±4.29	21.25±0.96	20.85±2.23	24.00±7.39
Sodium ion (mmol/l)	138.22±6.26	137.85±6.21	133.25±3.59	137.54±6.55	136.71±7.65
Potassium ion (mmol/l)	4.10±1.78	4.37±2.22	3.15±0.91	4.09±1.80	3.84±2.32
Calcium ion (mmol/l)	2.04±0.87	1.63±0.73	1.33±0.20	1.43±0.61	1.75±0.73
Inorganic Phosphates (mmol/l)	1.33±0.22	1.45±0.34	1.44±0.05	1.55±0.39	1.32±0.13

#No significant (p>0.05) difference among groups for all parameters
**Lesions described by Waziri et al. (2020)*

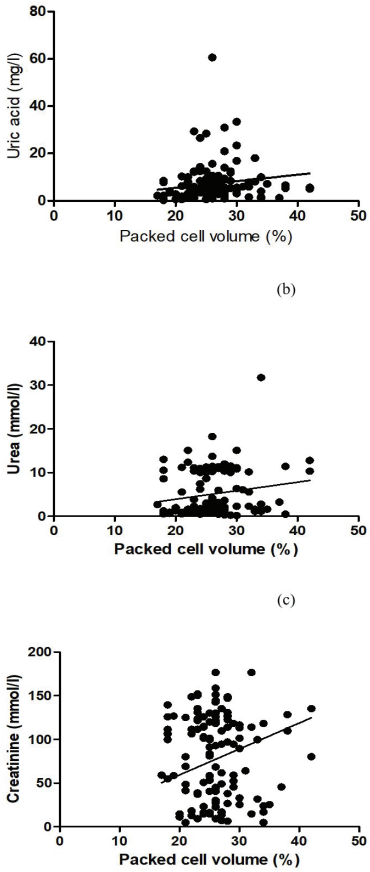

Figure 1: Relationship between packed cell volume and uric acid (a, r=0.20, p>0.05), or urea (b, r=0.18, p>0.05) or creatinine (c, r=0.02, p>0.05)

disease may cause non-regenerative anemia (normocytic and normochromic) because of the suppression of erythropoiesis due to suboptimal erythropoietin production from the endocrine peritubular fibroblasts in the cortex of the kidney (Leung, 2013). It seemed that the kidney damage in the camels did not destroy or undermine the function of the erythropoietin-producing cells. Even when the lesions reduced renal erythropoietin production, it was possible that extrarenal augmentation of erythropoietin secretion could take place to stabilize erythrocyte parameters. Moreover, inflammatory nephritides could have a negative influence on the erythrogram through the circulating pro-inflammatory cytokines that impair erythropoiesis and shorten erythrocyte lifespans (Morceau *et al*., 2009; Libregts *et al*., 2011). In this report, erythrocyte parameters were from camels with kidney lesions that were predominantly tubular nephrosis rather than chronic nephritides and pro-inflammatory cytokines might not have affected the erythrocyte mass.

The TLC and absolute DLC of the camels with various kidney lesions did not vary among the groups and their values were within the reported reference intervals for camels (Gupta *et al*., 1979; Mohamed and Hussein, 1999; Farooq *et al*., 2011; Waziri *et al*., 2019). Camels with kidney lesions may have normal leucocyte counts (Salem and Hassan, 2011) or leukocytosis with neutrophilia (Tharwat *et al*., 2018a), and uremia occurs, sometimes, without affecting the leucocyte count (Sarabandi *et al*., 2015). Elevated TLC could predict the odds of chronic kidney disease (Fan *et al*., 2017) with increased granulocyte (neutrophil and eosinophil) and monocyte counts and decreased lymphocyte count (Agarwal and Light, 2011), but low TLC and DLC tend to be associated with progression of the disease (Arai *et al*., 2018). Neutrophilia and lymphopenia are predictors of increased mortality of individuals with chronic kidney disease (Reddan *et al*., 2003). Pro-inflammatory cytokines induce the elevation of leucocyte counts but a switch to anti-inflammatory cytokine secretion in the course of the disease may lead to a decline in leucocyte counts. Camel nephritides with renal recruitment of leucocytes trigger the mobilization of leucocytes into the circulation from the bone marrow (Tharwat *et al*., 2018a) and reduction in leucocyte counts may follow with time because of leucocyte and myeloid apoptosis and bone marrow exhaustion (Arai *et al*., 2018). However, when the kidney lesions are not associated with remarkable systemic conditions, alterations in leucocyte counts may not be expected as was the case in this study.

The serum biochemical parameters of the camels were not significantly altered, implying that disruption in glomerular and tubular functions could not be associated with the kidney lesions. Serum urea, creatinine and uric acid concentrations were the indicators of glomerular failure (Vaidya and Bonventre,

2006; Vaidya *et al*., 2008; Zhang *et al*., 2017) that remained within normal values (Mohamed and Hussein, 1999) in the nephropathic camels studied. Camels with kidney lesions were reported to have increased serum creatinine and/or urea levels (Salem and Hassan, 2011; Barakat *et al*., 2017; Tharwat *et al*., 2018a, b). Kidney disease in camels caused decreased serum total protein concentrations (Barakat *et al*., 2017) and albumin (Tharwat *et al*., 2018a), perhaps, as a result of albuminuria (Gansevoort and de Jong, 2009). In other cases, it caused increased serum protein and globulin concentrations (Tharwat *et al*., 2018a, b) due to immunoglobulin production stimulated by antigens and immune-mediated conditions. Dyselectrolytemia was not indicated in the nephropathic camels studied although it was expected in renal tubular abnormalities (Dhondup and Qian, 2017). Electrolyte balance was stable in the affected camels because of the renal functional reserve capacity. However, in pyelonephritis of camels, there were previous reports of decreased serum sodium and chloride concentrations (Tharwat *et al*., 2018a) and increased serum calcium, inorganic phosphate and magnesium concentrations (Tharwat *et al*., 2018b).

There was no correlation between PCV values and serum creatinine, urea or uric acid concentrations. The need for this analysis arose because of the wide variability of PCV values in camels (Waziri *et al*., 2019), and the possibility of the transition from normal values to abnormal ones occurring within the reference interval, with alterations of azotemic variables. Earlier reports showed that azotemic variables could increase in proportion with the degree of anemia in individuals having chronic kidney disease (Callen and Limari, 1950; Eschbach, 1989). This study clarified that the status of the variables for assessment of renal function did not affect PCV values.

Conclusion

The clinical implication of this study was the capacity of camels to avert the pathophysiological (haematological and serum biochemical) consequences of kidney lesions reported by Waziri *et al*. (2020). Therefore, the ability of the camels to tolerate kidney damage could be another indication of the hardiness of camels in the harsh climatic environment of northern Nigeria. In practice, serious clinical conditions associated with renal dysfunctions could be rare in the local camels in spite of the kidney lesions encountered at postmortem examinations.

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Conflict of Interest

The authors declare no conflict of interest with respect to the research and publication.

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NUTRITIONAL EFFECTS OF DIETARY INCLUSION OF BOILED RUBBER SEED MEAL AS PARTIAL REPLACEMENT FOR SOYABEAN MEAL ON GROWTH PERFORMANCE OF SPRAGUE-DAWLEY RATS AS A MODEL FOR PIGS

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Abstract

The study generated data on the effects of graded levels of boiled rubber seed meal (BRSM) on the growth performance and economy of gain of rats. Twenty four Sprague-Dawley rats (12 males and 12 females) were randomly selected and allocated to 4 dietary treatments containing varying amounts of BRSM (0, 50, 100 and 150 g RSM kg^{-1} diet) in a complete randomized design such that there were 6 rats (3 males and 3 females) with one rat per replicate. Feed and water were provided ad libitum for 4 weeks. The addition of the graded levels of the BRSM to rat diets significantly ($P< 0.05$) influenced feed intake ($r = -0.67$), final body weight ($r = -0.70$) and body weight gain ($r = -0.85$). However, the inclusion of BRSM in diets had no significant ($P> 0.05$) effects on the efficiency of feed utilisation ($r = 0.19$) and water consumption ($r = -0.08$). In addition, there were no health-related problems or mortalities attributable to the level of BRSM in the diet. The examination of several internal body organs at the termination of the 4-week study revealed no macroscopic deviation from the normal in terms of gross tissue changes. Also, dietary BRSM had no significant effect on the relative organ weights of the experimental animals. The cost per gram of feed declined as more BRSM was included to replace soya bean meal. The diet that contained the highest amount of BRSM was cheaper. Furthermore, feed cost per gram live weight gain was lowest for rats on the 150 g kg^{-1} BRSM diet and highest for the 50 g kg^{-1} diet. Inclusion of 150 g BRSM kg^{-1} diet might be beneficial in terms of cost effectiveness.

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Introduction

In developing countries, intensive animal production, particularly the monogastrics, is hindered by the dependence on the importation of feed raw materials resulting in the high cost of feed, which is a sequel to the competition between man and animals for these same feed ingredients. Consequently, there is the need for alternative feedstuffs which must be ingredients with less competition from other secondary industrial users and producers, and are readily available in commercial quantities and at affordable prices (Adesehinwa *et al*., 1998). One of such cheap alternative feedstuffs for all classes of animals is the rubber (*Hevea brasiliensis*) seed cake. Rubber seeds are under-utilized in most rubber producing countries, because they are left to rot on rubber plantations.

The use of rubber seed meal as a source of protein in animal feeding systems has been limited due to the presence of toxic cyanogenic glycosides and other biochemically active compounds (Eka *et al*., 2010; Daulay *et al*., 2014; Sharma *et al*., 2014; Udo *et al*., 2016; Ulodo *et al*., Farr *et al*., 2019a). There is a wide variety of methods of processing rubber seeds to reduce their content of biochemically active compounds and hence their toxicity. These methods comprise of different combinations of drying, soaking, boiling and fermentation of whole seeds. All of these reduce the total hydrogen cyanide and other biochemically active contents of the seeds. In a recent study, Farr *et al*. (2019a) evaluated four simple methods of processing (soaking in water, sun-drying, boiling in water, and roasting) in terms of chemical compositions and energy values of the resultant rubber seed meals. The results indicated that the boiling of rubber seeds in water for 30 minutes followed by sun-drying significantly reduced the contents of the hydrogen cyanide and the other biochemical active compounds of the resultant rubber seed meal compared with the other processing methods.

The boiled rubber seed meal (BRSM), previously identified to be the best type of rubber seed meal based on a chemical evaluation study (Farr *et al*., 2019a), rat growth trial (Farr *et al*., 2017) and a subsequent reproductive trial (Farr *et al*., 2019b), was used in the this study which aimed to determe the effects of the incremental replacement of dietary soya bean meal (one of the most important protein sources for the feed industry), with boiled rubber seed meal on the growth performance, physiological parameters and economy of gain using laboratory rats as a model for pigs. The high cost associated with pig experimentation, coupled with the long delay in growth responses, limits their use in the routine evaluation of feedstuffs. The laboratory rat has proved a valuable model for investigations into basic processes of nutrition (Okafor and Anyanwu, 2006; Lewis *et al*., 2006; Donkoh *et al*., 2012, Chavlon-Demersay *et al*., 2017; Farr *et al*., 2017) and tests with laboratory rats have been used in the development of methods designed to replace the use of live animals, such as growing pigs.

Materials and Methods

Processing Method

The rubber seeds used in the study were obtained from the Rubber Plantations Section of the Department of Crop and Soil Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana. The seeds were de-hulled and partially sun-dried for 24 hours at an ambient temperature of about 30°C. The partially dried, de-hulled rubber seeds were placed in a 20 litre aluminum bowl containing water and subjected to heating at a temperature of about 100°C for 30 minutes with a rubber seed to water ratio of 1:3. After heating for about 30 minutes, the water was decanted and the boiled seeds sun-dried for 3 days at ambient temperatures ranging from 30°C to 35°C. The rubber seeds were turned over periodically and collected overnight to protect the seeds from being moistened by dew. The dried boiled seeds were ground using a hammer mill and stored in polythene sacs.

Dietary Treatments

Four experimental diets were formulated: a control diet containing no rubber seed meal and three other treatment diets in which the processed RSM (boiled rubber seed meal, BRSM) was incorporated at 50, 100 and 150 g kg^{-1} to replace soyabean meal (Table 1). The experimental diets were formulated to be isocaloric but not iso-nitrogenous.

Table 1: Composition of experimental rat diets

	Level of dried rubber seed meal, g kg^{-1}			
	0	**50**	**100**	**150**
Ingredients, g kg^{-1}				
Maize	550	550	550	550
Fishmeal	120	120	120	120
Soyabean meal	160	110	60	10
Rubber seed meal	0	50	100	150
Wheat bran	150	150	150	150
Oyster shell	5	5	5	5
Dicalcium phosphate	5	5	5	5
NaCl	5	5	5	5
Vitamin/trace mineral premixa	5	5	5	5
Chemical analysis, g kg^{-1} DM				
Crude protein	216.8	204.1	191.4	179.2
Crude fibre	37.83	39.36	40.88	42.41
Ether extract	39.35	47.35	54.53	63.35
Calcium	8.75	8.74	8.66	8.71
Phosphorus	8.34	8.23	8.11	8.00
ME (MJ kg^{-1}) b	12.78	12.75	12.71	12.68

[a]Vitamin/mineral premix specified to provide the following kg^{-1} diet: vitamin A 10,000 IU; D 2000 IU; K 3 mg; riboflavin 2.5 mg; niacin 12.5 mg; cobalamin 0.05 mg; pantothenic acid 5 mg; choline 175 mg; folic acid 0.5 mg; zinc 25 mg; iron 0.5 mg; copper 50 mg; cobalt 625mg; iodine 0.5 mg; selenium 0.3 mg; chlorine 1.6 g; sodium 1.3 g; magnesium 2mg; sulphur 0.4 g; potassium 3.0 g.

[b]Calculated from data of NRC (1998) and the estimated metabolizable energy value of BRSM (9).

Experimental Animals and their Management

Twenty four (12 males and 12 females) Sprague Dawley growing rats (mean body weight of 73 g) were kept individually in raised stainless steel cages with intact floors in a room with a 12 h light/dark cycle. The rats were randomly allocated to the four experimental diets such that there were 6 rats (3 males and 3 females) per diet. Animals in the treatments were balanced for sex and weight. The rats were dewormed using Ivermectin before the start of the trial. Each rat had access to its respective diet for a 28-day period. Water was available ad libitum.

Parameters Measured

Growth Parameters

Rat growth performance was assessed by measuring: initial body weight, feed intake, body weight gain, feed conversion efficiency (feed: gain), mortality, cost per g of feed, and feed cost per g weight gain.

Physiological Parameters

After the experimental period, the possible effects of BRSM on the weights of some body organs were assessed. Two rats (1 male and 1 female) from each treatment were euthanized following anesthesia by chloroform

inhalation. The abdomen of each rat was opened by an incision along the mid-ventral line and the skin and musculature folded back to expose the internal organs. The heart, liver, kidney, spleen, lung and intestines were excised, weighed immediately and expressed as g g^{-1} live weight to ensure uniformity in comparison. The heart, liver, kidney, spleen, lung and intestines were examined to determine whether the diets had resulted in any gross pathological changes.

Statistical Analysis

The dietary treatment effects for all the variables measured were statistically analysed. The computations were performed using the general linear models procedure of SAS (2003). The data were subjected to regression analysis to show the effects of including BRSM in diets on performance. Differences between the means were determined by the use of Duncan's multiple range test (Steel *et al*., 1997) and considered significant if $P<0.05$.

Results and Discussion

The detailed analytical data for the boiled rubber seed meal (BRSM), in comparison with that of soyabean meal (SBM), are presented in Table 2.

BRSM contained more fibre, ether extract and calcium but had less crude protein, ash, phosphorus, potassium and magnesium compared to values reported by AEC (1987) for SBM. The calculated metabolizable energy content of BRSM (12.56 MJ kg^{-1}) was higher compared to the value of 9.75 MJ kg^{-1} reported for SBM. This may have been due to the differences in the ether extract (fat) content of BRSM and SBM.

The general performance of the experimental population is presented in Table 3. Average feed consumption per rat for the 4 week period ranged from 262.8 g to 353.7 g. Feed intake was significantly ($P<0.05$) affected by the inclusion of BRSM in the diet. The feed consumption of rats fed on the control diet was not different ($P>0.05$) from that of rats given the diet containing 50g kg^{-1} diet. However, rats fed on the diets containing higher concentrations of BRSM (100g and 150g BRSM kg^{-1} diets) registered significantly ($P<0.05$) lower feed intakes. The following correlation between the level of BRSM in the diet and feed intake was found: Y (feed intake) = 348.4 - 0.42X ($r = -0.67$; $P< 0.05$) where X is the level of BRSM in the diet

There was little difference in the average rat weights at the start of the feeding

Table 2: Chemical composition of BRSM

Item	BRSM[a]	SBM[b]
Dry matter (%)	88.5	90.0
Crude protein (%)	18.4	46.0
Crude fibre (%)	8.25	5.0
Ether extract (%)	17.5	1.50
Ash (%)	1.25	6.00
Calcium (%)	0.29	0.25
Phosphorus (%)	0.42	0.65
Potassium (%)	1.58	2.0
Magnesium (%)	0.25	0.27
Hydrocyanic acid (mg/100 g DM)	4.6	-
Metabolizable energy (MJ/kg-1)	12.56	9.75

[a]*Adapted from Farr et al. (2019a)*
[b]*Adapted from AEC (1987)*

trial for rats fed on diets containing 0, 50, 100 and 150 g BRSM kg^{-1} in place of soya bean meal. However, the body weight gains of rats fed on the diet containing no BRSM were different from those given different amounts of BRSM. Rats on the BRSM-containing diets registered lower weight gains. Regression of body weight gain against the level of BRSM in the diet yielded the following equation: Y (weight gain) = 78.77 – 0.0996X (r = -0.85); P<0.01).

The efficiency with which feed was converted to gain (feed: gain ratios) did not show any consistent trend, although animals fed on the control diet (devoid of BRSM) were slightly more efficient in converting feed to weight gain, followed by those on the 150 g kg^{-1} diet. Regression of feed conversion ratio against the level of BRSM yielded the linear regression equation: Y (feed:gain) = 4.644 + 0.0005X (r = 0.19; P>0.05). Even though there were no significant (P>0.05) differences in the ability to convert feed to weight gain, the lower growth performance rate may have been due to the lower protein and higher fibre contents of the BRSM-containing diets as well as the reported presence of cyanogenic glycosides which affect the growth performance of animals. Protein is the major macronutrient in animals which provides essential and non-essential amino acids for protein synthesis and energy for maintenance and growth (Kim *et al*., 2002). Animals need protein to grow and most importantly, to develop muscle tissue (muscles contain chiefly protein and water). Protein is made up of amino acids, the 'building blocks' of protein, linked together in chains (Wu, 2010).

Table 3: Effects of varying amounts of BRSM on growth performance, organ weights, and economy of gain of rats

	Level of BRSM (g kg^{-1} diet)				SEM	r values and level of significance
Parameter	**0**	**50**	**100**	**150**		
Growth performance						
Feed intake, g	353.7[a]	341.0[ab]	262.8[c]	309.5[b]	11.5	-0.67
Initial body weight, g	71.7[a]	76.8[a]	68.2[a]	73.5[a]	8.95	-0.12
Final body weight, g	152.2[a]	150.8[a]	131.3[c]	141.0[b]	12.5	-0.70
Body weight gain, g	80.5[a]	74.0[b]	63.2[c]	67.5[c]	7.93	-0.85
Feed: gain	4.49[a]	4.89[a]	4.71[a]	4.63[a]	0.46	0.19
Water intake, ml	470.7[a]	483.8[a]	446.2[a]	488.5[a]	12.1	-0.08
Mortality, %	0	0	0	0	-	-
Organ weights, g g^{-1}LBW						
liver	0.05[a]	0.05[a]	0.05[a]	0.05[a]	0.007	0.0
heart	0.004[a]	0.005[a]	0.004[a]	0.004[a]	0.001	-0.26
kidney	0.012[a]	0.008[a]	0.009[a]	0.010[a]	0.002	-0.38
lung	0.011[a]	0.010[a]	0.009[a]	0.007[a]	0.002	-0.29
spleen	0.006[a]	0.007[a]	0.008[a]	0.006[a]	0.0025	-0.13
intestines (empty)	0.055[a]	0.048[a]	0.050[a]	0.057[a]	0.009	-0.25
Economy of gain						
Cost/g feed, GH¢	0.020	0.0193	0.0184	0.0175	-	
Feed cost per						
g weight gain, GH¢	0.0898	0.0944	0.8667	0.8103		

*SEM – Standard error of the mean; NS – non-significant (P≥0.05); *P≤0.05*
abcMeans within a row with different superscripts are significantly different
US$ 1 equivalent to GH¢5.0 (Ghana cedis)

High dietary fibre has been reported to negatively affect the digestibility of proteins and energy (Fernandez and Jorgensen, 1986; Chebeauti *et al*., 1991; Noblet *et al*., 1993). The boiled rubber seed meal used in this study was reported by Farr *et al* (2019a) to contain 4.6 mg hydrogen cyanide/100 g DM compared to 60.95 mg/100 g DM for the raw (unprocessed) rubber seed meal.

Cyanogenic glycosides on hydrolysis yield toxic hydrocyanic acid (HCN). The cyanide ions inhibit several enzyme systems, depress growth through interference with certain essential amino acids and the utilization of associated nutrients (Kumar, 1992; Ukpebor *et al*., 2007; Uluodo *et al*., 2018). Ravindran (1983) found impaired performance traits in pigs fed more than 10% rubber seed meal. This effect was, however, attributed to a deficiency of lysine and methionine rather than to cyanogenic glycosides present in the meal.

Even though there was a tendency towards decline in feed intake by rats with the increasing levels of BRSM in the diet, this was not correlated with the amount of water consumed. The amount of water drunk by rats during the course of the trial was not correlated with the level of BRSM in the diet: Y (water intake) = 465.93 – 0.048X ($r = -0.082$; $P>0.05$). This observation is in contrast with studies that have previously shown that the absolute water intake of animals is positively correlated with feed intake (Harris and van Horn, 2003; Donkoh *et al*., 2012)

There were no indications of ill-health among the rats on the various dietary treatments and no mortalities were recorded during the study. The relative organ weights of the experimental animals are presented in Table 3. Organ weights are widely accepted in the evaluation of test article-associated toxicities (Black, 2002; Bucci, 2002; Wooley, 2003). The structure and size of organs such as the liver, heart and gastro-intestinal tract are often indications of the physiological state of the body. Organ weight changes are often associated with treatment-related effects. Alterations in liver weight may suggest treatment-related changes including hepato-cellular hypertrophy (for example, enzyme induction or peroxisome proliferation) (Greaves, 2000; Amacher *et al*., 2006; Juberg *et al*., 2006). Elevated heart weight may be evidence of myocardial hypertrophy that is often macroscopically and microscopically difficult to recognize (Thiedemann, 1991; Greaves, 2000). Changes in kidney weight may reflect renal toxicity, tubular hypertrophy or chronic progressive nephropathy (Greaves, 2000). In this study, the liver, heart, kidney, lungs, spleen and intestinal weights for rats fed on diets containing graded levels of BRSM were not significantly ($P>0.05$) different from those fed the control diet devoid of BRSM. At the termination of the 4-week study, no gross organ (liver, heart, kidney, lungs, spleen and intestines) changes were observed at necroscopy in all the experimental rats.

The cost per g of feed and the feed cost per g weight gain are presented in Table 2. The cost per gram of feed declined as more BRSM was included to replace soyabean meal. The diets that contained higher amounts of BRSM were cheaper, that is, GH¢ 0.020, GH¢ 0.0193, GH¢ 0.0184 and GH¢ 0.0175, respectively. This was solely attributed to the price disparities between BRSM and soya bean meal. Although the rubber seeds were obtained free of charge, the boiled rubber seed meal was assigned a value of GH¢ 1.25 per kg, being the cost of picking the seeds, transportation and processing. The prevailing cost per kg for soyabean meal, which the boiled rubber seed meal replaced in the experimental diets, was GH¢ 3.00. Consequently, the cost per g of the control diet (devoid of BRSM) was higher than the various boiled rubber seed meal-containing diets. Feed cost per gram live weight gain was lowest for rats on the 150 g kg^{-1} diet and highest for the 50 g kg^{-1} diet. The higher cost of feed conversion registered by rats on the 50 g kg^{-1} diet may be attributed to the poorer efficiency of feed utilization.

Based on the results of this study, boiled rubber seed meal could be harnessed as a supplement for formulating pig and poultry feeds. The 150 g kg^{-1} diet inclusion rate of BRSM might be beneficial in terms of cost effectiveness and that the seasonal variations

in the prices of conventional feedstuffs such as maize and soyabean meal would make the use of processed rubber seed meal in animal diets more attractive.

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PROSPECTS FOR IMPROVING PRODUCTION PERFORMANCE OF INDIGENOUS GOAT BREEDS IN NORTHERN ZAMBIA

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Abstract

Indigenous goat breeds in Zambia though acclaimed to be adapted, disease resistant and thus resilient are constrained with a lot of challenges that has limited their productivity. This study was undertaken in Northern and Muchinga provinces of Zambia to analyze these constraints and proffer best practices to improve the production performance of indigenous goat breeds. Farmers and farmer groups were engaged by administering questionnaires and focus group discussions, respectively, using enumerators trained in participatory research methods. The identified constraints centred mostly on the environment (inadequacies of the production systems and management practices) and absence of genetic improvement. It was noted that matings were largely uncontrolled, indiscriminate and depended on the few free running male goats in the community which was a consequence of negative selection in the males through sales of heavier goats. The prospects for improving the production and productivity of indigenous goat breeds therefore lie in a twin-pronged approach addressing the environment in which the goats are raised and improving on the genetics available to the smallholder farmers. Market development along the value chain and training of farmers in husbandry skills were emphasized in the seeming transformation and commercialization of the production system to improve productivity and income from sales.

Keywords: Goat, constraints, performance, environment, genetic improvement, Zambia

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Introduction

Goats have been known to contribute to the sustenance of small and marginal farmers in tropical Africa by playing an important role in income generation, capital storage, employment generation and household nutrition (FAO, 1991). Goats have demonstrated a remarkable ability to survive extended periods of drought and they acted as life savers, especially for the resource-poor rural farmers. Tropical indigenous goat breeds possess adaptive traits, which enable them to survive and produce under extreme conditions. Odubote (1996) highlighted some of these traits to include heat tolerance, resistance to specific diseases, ability to use poor quality feeds and to survive under irregular supply of feed and water.

Goats are therefore a very important animal genetic resource and their improvement has been identified as one of the pathways to sustainably improve the livelihoods of small scale or rural farmers in Zambia due to the relatively low goat population in the smallholder farming areas. The indigenous goats of Zambia which predominate the smallholder holdings were mainly the Gwembe, Sinazongwe (Valley) and Plateau breeds and were well described in the farm breed survey report by Zulu *et al* (2003). The farm breed survey also revealed that management system among rural livestock farmers was poor leading to high mortality and lack of good bucks was found to be a common problem resulting into communal use of bucks. Ahmadu and Lovelace (2002), however, noted that the local Zambian goats have a high production potential, which were yet to be fully exploited by goat keepers in the country. The performance data in their study, revealed that the fertility rate, kidding rate and prolificacy were high and comparable, though the pre-weaning mortality were very high, averaging slightly above 45% across the three breeds. This was indicative of deficiencies in the production system and management practices such as poor and inadequate nutrition, poor disease control, losses arising from diseases, predators and accidents, among others (Odubote *et al* 1992; Odubote, 2020).

To address the above mentioned deficiencies, the government, donor agencies and the farmers have mostly focused on the genetic option of crossbreeding the indigenous goat breeds with the high performing imported African breeds such as the Bantu, Boer, Red Kalahari, Swaka-Lala; and exotic breeds which include Saanen, Anglo-Nubian and Toggenburg. Most of these efforts were not successful and sustainable due to the high input requirement, poor husbandry skills among farmers and the harsh environmental conditions. It must be noted that there were little or no efforts directed at within breed genetic improvement of the indigenous goat. There was also paucity of information in literature on the production performances and estimates of genetic parameters for traits of economic importance in the indigenous breeds of goats in Zambia. However, with the FAO (2007), Interlaken declaration of the Global Plan of Action for Animal Genetic Resources, there has been renewed efforts aimed at developing the indigenous animal genetic resources.

In view of these factors and the impact of climate change the Government of the Republic of Zambia recently secured funding to improve the production of indigenous breeds of livestock in the Northern and Muchinga provinces which were characterized by very low livestock populations and equally low numbers of households keeping livestock compared with other provinces (Odubote, 2020). The project will among other activities carry out restocking of animals in a pass on scheme and put up infrastructure facilities to enhance livestock production and productivity. The husbandry skills of the smallholder farmers as beneficiaries are therefore crucial for the attainment of the project's goals of farming diversification and improved livelihoods.

This study was therefore undertaken to analyze the goat management practices employed by smallholder farmers in the two target provinces in order to identify shortcomings in the management practices and provide recommendations on best practices in the management of indigenous livestock breeds. The study also highlighted opportunities for

community based goat genetic improvement.

Materials and Methods

Sampling

The study was carried out in five selected districts of the Northern (Kasama) and Muchinga (Isoka, Mafinga, Mpika and Nakonde) provinces of Zambia. Five livestock farmers keeping indigenous livestock breeds (randomly selected from each of two randomly selected registered livestock farmer groups) per district were sampled. Focus group discussions were held with two registered livestock farmer groups from each of the selected districts.

Design of a survey instrument

A structured questionnaire was designed for use with smallholder farmers to obtain information on the pertinent goat management practices on their farms. The information collected was used for the focus group discussion to further explore on the production systems and management practices employed in the two provinces. The focus group discussion elaborated on the challenges of smallholder goat farmers and best practices that the communities can adopt for sustainable management. The survey instrument and focus group discussion topics were pre-tested on small number of smallholder farmers within the target provinces before being rolled out in the field.

Recruitment and Training of Enumerators:

Two enumerators were recruited from each of the selected districts and the main criteria were minimum educational qualification of Certificate in animal production course and two years working experience with livestock farmers in the district. The enumerators were trained on best practices for community management of indigenous goat breeds and data collection using focus group discussions.

Data collection

Prior informed consent was obtained from the individual farmers and communities. The enumerators were supervised and monitored by the researcher in the data collection using the pre-designed and structured questionnaires and the subsequent focus group discussions. A total of 50 farmers were interviewed between September and November 2019. The information gathered formed the ground norm for 10 focus group discussions covering such areas as goat housing, labour requirement, watering points, land ownership, feeds and feeding, grazing land management, health care and disease control, reproduction and breeding strategies, manure management, marketing and record keeping.

Data analysis:

The information obtained from the focus group discussions were summarized to interrogate the inadequacies of the production and management systems and highlight the best practices to employ for improved production performance and productivity which are hereby presented.

Results and Discussions

Land ownership

It was noted in all the five districts that community land was vested in the village headmen who then allocated a piece of farmland to each of the farmers. However, the land along the streams/swamps/dambos was reported to be community owned as well as the grazing lands. This land ownership practice which was without tenure rights has been a limiting factor to long term farming and the planting of perennial crops or sown pastures. This has led to increased pressure on community land for cultivation and grazing. Women were noted to be disadvantaged therefore limiting their roles, investments and expansion profile (FAO 2012).

Water points

The farmers mentioned that they provided water in water troughs for the goats mostly during the dry season when water was scarce. This was normally done by any household member available on the day although it was mostly done by women. The farmers depended on streams and nearby

rivers although it was noted that provision of adequate and clean water for the animals was not of high priority for the farmers. It is however, important that farmers should be made aware that the availability of clean water is synonymous with life and important for many functions and processes of the body. Water harvesting techniques (Maher *et al* 2019) such as runoff harvesting, flood water harvesting and groundwater harvesting- wells, boreholes, dams should be introduced and promoted for farmers to have access to water bodies to sufficiently water their animals all year around and especially during the long dry season. The distances covered to access to water points was noted as another hindrance which will become increasingly important with declines in rainfall amount and variability as a result of negative effect of climate change. Establishment of communal water points in close proximity would be beneficial.

Labour

In both provinces, the care and management of the goats was carried out by all the household members. The goats needed close attention during the rainy season which coincides with the cropping season when they have to be closely monitored to ensure they did not stray onto fields and cause damage. The labour requirements were mainly during the day to ensure the goats were tethered. During the dry season, the goats do not require monitoring, hence labour requirements were lower. Ownership of livestock is greatly influenced by culture and small stock in many communities are left under the responsibility of women and children (Bett *et al*., 2008; FAO, 2012), who are more involved with their care and management. The targeted training of women in goat farming practices would thus lead to higher adoption rates.

Housing

The housing of goats in all the five districts have similar designs were made out of chopped trees or poles, thatched roof and the floor were raised off the ground using poles. Feeding and water troughs were generally not provided and same with bedding materials in the shelter. The goats were reported to be housed for the night and let out the following morning for grazing nearby. However, during the rainy season, the goats were housed for longer hours during the rainy season than in the dry season. During the rainy season, the goats were tethered to trees close to the farmers' houses to prevent them from straying onto crop fields.

All the goats were housed together and were not separated by age or sex. However in Mafinga, farmers stated that they usually remove the heavily pregnant female goats from the herd to prevent miscarriages through injuries during fights. Also the new mothers and their kids were separated and housed either in the family kitchen or the house to help warm the kids and avoid injuries of the young by the aggressive adults. Highly aggressive bucks were also usually separated from the herd.

It was evident that farmers were limited in their management practices since the animals graze for most of the day during the dry season. The farmers did not have direct control over feeds and feeding, watering, disease prevention and control, matings and manure collection. Confinement of animals for some period of the day and at night through housing facilities using locally available materials could be a very good entry point for interventions in developing management best practices. Adequate housing facilities could lead to decrease in losses arising from predators, thefts, accidents and opportunistic diseases. It will also reduce the energy loss in scavenging for food and water. A good housing layout limits injuries and losses due to thefts, predators, parasites and diseases; offers protection from inclement of weather- heat, cold and rain; provide comfort for the animals to be productive without stress. Odubote *et al*., (1992), observed that housing facilitates implementation of several improved management practices such as feeding, watering, health care, castration, weaning, record keeping and controlled mating in a conducive environment for both farmers and animals. The ease of cleaning of the different parts especially the floor and collection of manure helps to maintain a hygienic environment. The housing

floor design with raised and slatted floor do enhance manure collection (CCARDESA and GIZ 2017c).

Grazing management

All the respondents reported that the goats were not herded but were open grazed during the dry season. The goats feed on the following naturally occurring grasses such as natural Hyparrhenia spp., Star grass, Elephant, Guinea, Rhodes. Others include shorter grasses, such as Cynodon dactylon, Digitaria setivalva, Heteropogon contortus and Microchloa caffra.

There were no deliberate efforts aimed at maintaining or improving the quality of pasture on the grazing land through regular seeding, introduction of new species/varieties and controlling grazing. It was clear from the study that the types of grasses available in the natural communal grazing lands were limited and the quality had degraded over the years without any form of rejuvenation. It was therefore to be expected that the nutritional benefits to the animals were minimal or sub optimal to sustain the animal and this was more critical in the long dry season. The effects of sub optimal nutrition are likely to include poor body condition, poor growth and poor fertility.

CCARDESA and GIZ (2017b) had noted that there must be conscious communal efforts through community regulations to restore, improve on the quality of the pasture, promote a diversity of forages available with high yielding grasses through pasture renovation or rejuvenation and introduction of leguminous forages, controlled grazing, encourage grazing rotation and manage the carrying capacity of the grazing lands (through adjusting stocking density) at any particular time to avoid over grazing and degradation of the grassland. The above had earlier been suggested by Simbaya, (2002). The communal grazing land must be managed in such a way as to provide quality grass and forages during the long dry season. This calls for possible establishment of communal grazing land management team in each locality in partnership with the Department of Livestock Development.

The alternative to the communal grazing land above was for each farmer to have own sown pasture which will ultimately guarantee quality and diversity of pasture available for the goats especially during the long dry season when the quality of the grasses in the communal grazing land are at the lowest. The goats were reported to roam freely in the dry season after fields have been harvested.

Feeding-cultivated, collected and processed fodder

Most of the farmers in the five districts reported growing similar type of crops namely maize, cassava, millet, groundnuts, beans, pigeon peas, bambara nuts, pumpkins and assorted vegetables. These crops were grown for sale and household consumption. The crop residues especially the maize stovers, groundnuts helms, soya beans, sorghum, millet, cassava leaves, sweet potato leaves and rice husks were usually given to the goats as feed even as they were allowed to graze in the harvested fields. In the dry season, the goats were free to forage for feed around the homestead while tethered. The goats feed on leaves of banana, mango and moringa, as well as leaves and soft stems of plants such as acacia plants/trees. The main source of feed throughout the year for the goats was naturally occurring pasture.

The farmers in all the districts mentioned that they did not cultivate any crop or fodder specifically as forage for the goats, neither are multipurpose trees or shrubs grown specifically for the same purpose. The farmers in Nakonde and Mpika did not collect any naturally occurring fodder or purchase any feed for the goats. However, farmers in Isoka, Mafinga and Kasama collected naturally occurring fodder for the goats like local grass, fresh-shooting mango leaves/branches, acacia species and Moringa to feed the goats. Some farmers from Isoka, Mafinga and Kasama reported purchasing maize bran as supplementary feed for the goats. The farmers mixed maize bran with salt or sunflower cake which were fed to the goats.

Good husbandry practices dictate that an animal should be fed a balanced diet for normal functioning of the body – maintenance,

growth, pregnancy and milk production (as the case may be). This diet should contain adequate amounts of carbohydrates, proteins, minerals and vitamins in the right proportion. It was noted from the study that grasses formed the bulk of feed for the ruminants though the quantity and quality of grasses consumed could not be ascertained. Nonetheless, the animals' body condition was indicative of the limited feeding regime as well as the long distances covered by the animals from homesteads to the communal grazing lands. It is therefore necessary that farmers should plant their own pastures to guarantee adequate feeds of good quality for the animals all year round.

Hence, production of Brachiaria, and other multi-purpose fodder trees/shrubs and leguminous forages should be encouraged as being promoted by the Ministry of Fisheries and Livestock (Department of Livestock Department, 2018). The farmer must identify sources (grazing land, sown pasture or purchase) of continuous supply of fodder such as Stargrass, Napier and Brachiaria. Some other high-protein fodder shrubs, such as Calliandra, Leucaena, Gliricidia and Desmodium, can be grown around the edges of the plot. The quality of feeds such as straw, crop residues, or dry fodder can be enhanced by processing/ treatment (to form hay or silage) to improve their digestibility and therefore meet the nutritional requirements of the different classes of goats. The above becomes even more critical during the long dry season and will help the animals to be in good body condition throughout the year.

Another shortcoming observed was that crop production, crop residues and the agricultural industry by-products were not integrated into the feeding of livestock as either main or supplementary feeding as recommended by CCARDESA and GIZ (2019a). The goats require supplementary feed, such as concentrates to meet their nutritional requirements, fast growth and good health especially during periods of pregnancy and lactation (Odubote *et al* 1992). The supplements can be home made using cheap and locally sourced ingredients. Energy sources include maize, sorghum, millet and their by-products; protein sources include legume grains such as sunflower, soyabean and by-products; minerals and vitamins can also be provided using bone meals and from pasture. Concentrate feeds generally provide more digestible nutrients than roughages, which increases the digestibility of feed and generally improves animal productivity. However, access to and availability of feed and potential competition with direct human consumption, are major challenges.

Integrating agroforestry with goat production though not common in Zambia, has the potential to address smallholder farmer's challenges of lack of fodder for animals. Agroforestry technologies such as biomass transfer and fodder banks in a "cut and carry" system could be introduced. It is a fact that agroforestry is mostly promoted and practised in Eastern province unlike in the Northern and Muchinga provinces of Zambia. Agroforestry, if prominently positioned could be an excellent source of fodder from trees, shrubs and leguminous plants for goats. The main challenge is that goats being indiscriminate browsers must not be left unattended to if allowed in the fields as they are likely to cause damage to the tree barks due to their intensive browsing ability and feeding on buds, shoots and leaves of woody plants.

In summary, Simbaya (2002) had noted that the most important sources of feed for ruminants in the smallholder sector are natural pastures and crop residues and therefore suggested the following:

- There is a need to train farmers in conservation of excess herbage in the wet season which will help to improve utilization of natural pastures.
- Farmers must be encouraged to collect, dry and stack all crop residues after harvest for use late in the dry season, instead of allowing animals to graze them in situ.
- The use of fodder trees by smallholders should be encouraged.
- Farmers need to be encouraged to establish fodder gardens, so that they can cut and carry feed to their stock.
- Farmers should be encouraged to select

suitable stock and to match animal numbers to the feed resources.

Health care and disease control

The farmers in all the districts mentioned that they have not had any serious outbreaks of diseases affecting goats. However, common ailments include Diarrhoea, Anaplasmosis, Pneumonia, Pink Eye, Helminthiasis and Bloat. There were no community by-laws in all the five districts on disease control, disease management and bio-security. Though farmers were advised to spray, dip and vaccinate their animals, the farmers were not monitored to check if they did. The community did not have any measures in place for checking new goat arrivals in their areas for any disease as a bio security measure.

Globally, health care and disease control is hinged on three pronged strategies which are prevention, detection and treatment. CCARDESA and GIZ (2017e) had postulated that the above are key in improving animal health and productivity, reducing mortality and morbidity, and preventing further outbreaks. Prevention is the cheapest method of disease control. Improving farm biosecurity measures is important to protect the farm from incoming diseases as well as to help prevent and contain outbreaks of diseases and spread to other farms. This can be achieved through limiting number of people (and animals from neighbourhood) visiting the farm and making contact with the animals as well as to help prevent outbreaks of diseases to other farms. Provision of footbaths where practicable are equally important. Such measures such as routine and regular deworming, dipping, spraying and vaccination programmes must be inculcated in the smallholder farmers. Adequate feeding of animals promote good health and enable the animal to withstand a number of opportunistic infections and diseases.

The farmer must be conversant with the herd and alert to any behaviour change which may be indicative of a problem. Early detection of animal diseases and subsequent prompt notice to veterinary services for treatment are very critical. The veterinary assistants and the department of veterinary services must be notified immediately there was a sick or dead animal for quick diagnosis and prompt treatment and/or preventative measures. In addition, communal efforts through vigilance, rules and regulations aimed at preventing and combatting disease outbreaks cannot be overemphasized. The farmers are encouraged to follow general animal health management protocols.

Reproduction

Goat breeding was not controlled in all the districts except Kasama. The bucks ran freely among the flocks in the community especially during the dry season. Female goats in the herd were available for mating. The farmers borrowed bucks from neighbouring farms to mate with their does when there were no suitable bucks on the farm or noticed desirable traits in a neighbouring herd. Even with the buck sharing practice, mating still remain uncontrolled. However, in Kasama district, where controlled mating was practiced, whenever the bucks was selected, the other males were tied up to ensure they did not mate with the females in the vicinity.

Since the study revealed uncontrolled mating was the norm, it is important that the farmers should take the lead in ensuring controlled matings. Controlled mating allows for synchronization of management practices and operations on the farm. Controlled mating starts with the accurate and timely detection of oestrus and subsequent successful mating with selected males. A doe found on heat in the morning should be served in the afternoon/evening; but if detected in the evening, it should be served the following morning. Missed heat periods translate to no mating and is therefore a lost opportunity for conception and gestation which are costs to production in terms of time and resources. The same is true for unsuccessful mating. The profitability of livestock production is dependent on the female being serviced, conceiving and successful delivery of healthy young animal(s). It is therefore important that breeding animals must be properly managed and practices to control mating such as culling,

castration and correct mating ratios are observed.

Breeding strategies

In Isoka, Kasama and Nakonde, the farmers reported that the breeds they currently raise (Gwembe, Sinazongwe (Valley) and Plateau breeds) were inherited from their parents and also provided in a farmer empowerment pass-on programme. The farmers in Mpika and Mafinga also reported that they inherited the breeds they currently keep from their parents and also due to availability in the area when they decided to rear goats. Farmers in Kasama, Isoka and Mpika reported that they build up their herds by utilizing matings within the herds without resorting to new animal acquisitions. In Nakonde the farmers acquired new goats by purchasing or in some cases as gifts or dowry payments. In Mafinga, the farmers mentioned purchasing or exchanging bucks to avoid inbreeding. The farmers mostly purchased or bred from within the community (neighbouring farms). The farmers do also made arrangements with neighbouring farmers to borrow their bucks for mating.

The farmers in the five districts reported keeping only local breeds of goats. The important traits in breeding goats mentioned by farmers included: size and conformation, multiple births, short kidding interval, docility, mothering ability, disease resistance and tolerance to heat. They also requested from the seller for information on ownership of the goat in question, health of the goat, number of parturitions/parity, age of the animal and history of multiple births (twins and triplets). Pedigree was also considered important although in most cases they did not ask about it. However, there was no conscious and deliberate selection for traits in breeding males and females.

The study further revealed gaps in the supply, availability and access to improved breeding males for mating within the community. Haile *et al*., (2011) had noted the above in Ethiopia in what was regarded as negative selection for body weight since the males with higher body weight were sold off to gain more income. It was also noted that the smallholder farmer was not actively involved in the production of the breeding males but only took advantage when available. It would therefore be logical and critical that the smallholder farmers take keen interest in the selection of breeding animals and the mating system to employ in order to improve on the productivity of the flock. Controlled mating with selected bucks are essential in delivering on superior male genetics. Though the men were noted to be the main decision makers when it comes to breeding, it is important that the women who did most of the management practices be given some latitude to contribute in the decision making process.

Identification and evaluation of bucks is very crucial in the selection and management of superior males within the flocks. It is therefore essential that selection criteria and breeding objectives are established for each breed. The management of such superior males at farm, farmer group or community level, should be given adequate attention through farmer-owned and farmer-led initiatives such as community based breeding programme (CBBP) supported by the Government and development partners. Ojango *et al* (2016) was of the opinion that a community breeding programme geared towards the optimal use of available resources and incorporating gender integrated innovative technologies can be developed using indigenous breeds.

The above scenario therefore presents an opportunity for the genetic improvement of indigenous breeds of goats. The farmers in a group will determine the breeding objectives and traits of economic importance to focus on being a long term strategy. Farmers will select within each flock, bucks which typically represent the ideal characteristics of the breed and also based on progeny performances. It must also be agreed that only the selected males be allowed to mate within the group to avoid dilution of efforts. This means that all the males that are not required for breeding would be removed for sale or castrated. This will ensure that the superior performance of the selected males can be passed on to the

following generation. Concurrent with the above, is that the farmer is freed from the costs associated with keeping and maintaining the poor performing males. The income earned from disposal of the unwanted males can then be channelled towards the care and management of the productive group.

Haile *et al* (2011), and Ojango *et al* (2017a, b) charted the process for instituting a CBBP. It must be noted that genetic improvement through creation of breeding nucleus and continuous supply are very key in the design of CBBP. It is essential that superior animals within the community are continuously evaluated, selected and pooled to create an open breeding nucleus. It is from these pools that superior males are drawn. It is also important for the purpose of avoiding inbreeding to diversify the breeding nucleus using herds from distant communities. The success and predictability of any genetic improvement programme is invariably hinged on adequate recording system (CCARDESA and GIZ 2019d) and trained personnel in animal breeding and genetics which is lacking in most eastern and southern African countries, including Zambia (Zonabend *et al* 2013).

Formation of indigenous livestock breed associations will also help in maintaining desirable qualities of the various indigenous breeds by instilling rules and regulations. It will also help promote the ideals and branding of the indigenous livestock breeds and products

Manure management

The farmers from Isoka and Mpika reported that they generally do not collect goat manure because of their preference for cattle manure. Others mentioned that during the dry season, they sometimes collect the goat manure to fertilize the vegetable gardens and also mixed with maize residue as well as other materials to make compost manure for the fields. In all instances, the manure was not treated. Nevertheless, goat manure has been reported by CCARDESA and GIZ (2019c) to be an excellent source of plant nutrients (notably Nitrogen and Potassium) and therefore could reduce the need for chemical fertilizers. The fact that chemical fertilizers account for substantial cost of crop production means appropriate application of manure can help lower the costs.

The effective collection, proper storage and correct application of goat manure are very important to maintain good goat house hygiene, prevent possible spread of diseases, reduce odour and more importantly apply in garden as organic fertilizer. Housing design especially the flooring and roofing are very critical in manure management. Proper roofing and flooring can contribute to reduce runoff especially during the raining season while the slatted floor not only help with collection but also prevent leaching of nutrients. The manure management system works best with a good housing system which allows for the dung and urine excreted to be collected in an effective and efficient manner as quickly as possible and on a regular basis for storage (CCARDESA and GIZ 2019c).

However, despite the body of knowledge supporting manure application, most farmers do not subscribe to the practice mostly due to inadequate information. Information on the importance of manure application to crop fields to improve soil fertility and reduce cost of crop production must be widely disseminated to smallholder farmers.

Record keeping

The respondents while acknowledging the importance of record keeping, failed to keep records. They mentioned not being able to sustain the momentum of keeping records after being taught and hence relied on recollection of events which were mostly inaccurate. However, management practices hinged on such inaccurate records are unlikely to be effective. Farmers should be encouraged to keep accurate records of activities, events and occurrences on the farm in order to keep track of production and productivity. Record keeping is a useful tool for monitoring performance and decision making and for planning of activities such as vaccination, selection of breeding stock, culling etc. It is also important that these records should be the

basis for business decisions and management practices on the farm to financially benefit the enterprise. Livestock extension and advisory services should emphasize on record keeping. The above will also help in generating the much needed data for policy and planning (Odubote, 2019)

Closely related to the above is the fact that most of the goats were not individually identified using ear, collar tag or other forms of identification. Unique identification of individual goats is however a requisite for accurate record keeping especially for performance records and the identification of superior animals for genetic improvement (Haile *et al*., 2011).

Use and sale of goat and goat products

In the five districts, the farmers reported that the common reason for keeping goats was for sale to respond to household cash requirements. The goats were rarely used for meat consumption but to meet household needs such as purchase of maize, fertilizers and school fees and this was in agreement with the report of Namonje-Kapembwa *et al* (2016). The goats were sold or exchanged within the community and occasionally farmers travelled into town to find buyers. In few cases, traders came from outside the community to buy goats. It was noted that farmers in Mafinga and Nakonde districts sold their goats in Malawi and Tanzania thus exploiting the trans-border trade. In Nakonde, there was high demand for live goat due to the high Muslim community from Tanzania coupled with several restaurants in Nakonde and Tunduma which are trading hubs at the Zambia-Tanzania border.

It was clear from the study that livestock production in the study areas was not taken as an economic venture. There was no value addition as only live animals were traded. Farmers were thus at the mercy of the traders as the outlets or markets were equally limited and the numbers being traded were not only low but also erratic and spontaneous. This scenario could also open up opportunities for the smallholder farmers to engage in specialized markets such as the group fattening of male kids for sale, the sale of does and young bucks as breeding animals; sale and or hiring out of mature males for breeding within the community and the processing of hides and skins. The burden of maintaining and carrying extra animals not required for breeding can also be transferred to the group in exchange for returns in a pooled arrangement. Individual smallholder farmers may also not be in a position to slaughter a goat and quickly complete sale of the carcass before it becomes spoilt (thus incurring loss) given the lack of cold storage facilities within the community. However, where the market exists for live goats (Tanzania and Malawi borders), livestock production must be tailored to meet the demand while also avoiding oversupply which may lead to a glut in the market

These considerations make it imperative for the smallholder farmers to form cooperatives not only for knowledge sharing but also for pooling resources to achieve economies of scale which would translate into reduced costs of production through bargaining power, bulk purchase of input supplies such as feed supplements, drugs, vaccines and veterinary services as suggested by Namonje *et al* (2016). In addition, livestock and livestock products can be bulked and put up for sale in larger numbers to pre-arranged customers and at regular interval times. It also calls for increased investments by cooperatives to take advantage of the market opportunities which include sale of culled and fattened live animals, breeding animals, value added meat products and forages. Vigorous branding of the indigenous livestock breeds products can further help create a niche market as currently is the case with village chickens.

It is therefore important for livestock cooperative farmer groups to explore goat value addition activities rather than only selling live animals. Goat meat processing through salting, curing, sun drying, fermentation and smoking leads to improvement in taste or shelf life extension (preservation). Value addition thus increases the value of livestock as the meat products and also the by-products of the processing (blood, offal and bones) can also create business lines such blood meal, bone

meals for the livestock and other industries.

Conclusions

It was evident that goat production among the farmer groups in Northern and Muchinga provinces, though at an infant stage of development characterized by the low input and low output traditional system, has a huge potential given the proximity to market, good vegetation and abundant water bodies. Investments in the production system and interventions in the management practices through training in husbandry skills and extension services offer prospects for improved performance and productivity. Farmer groups led community based breeding programmes with support of government and research agencies offer the scope and latitude for the genetic improvement of the indigenous breeds of goats through retaining of selected males of superior performance.

Statement of Interest

There is no conflict of interest

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

The Editor in Chief
March 2020

Aims and scope

The Bulletin of Animal Health and Production in Africa (BAHPA) of the African Union Interafrican B*ureau* for Animal Resources (AU-IBAR) is a scientific journal which publishes articles on research relevant to animal health and production including wildlife and fisheries contributing to the human wellbeing, food security, poverty alleviation and sustainable development in Africa. The bulletin disseminates technical recommendations on animal health and production to stakeholders, including policy makers, researchers and scientists in member states. The Bulletin is the African voice on animal resources issues specific to Africa.

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

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- Beekeeping and honey bees
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- wildlife management
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- Food safety and food hygiene
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- Emerging and re-emerging issues in animal resources
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Letters to the Editor: the bulletin welcomes letters to the editor. The purpose of Letters to the Editor is to provide a forum for positive and constructive views on articles and matters published in the bulletin. Letters to the Editor must not exceed 300 words. Letters to the editors include technical reports from countries or projects.

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

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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.

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