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SEROPREVALENCE OF FMD IN CATTLE, SHEEP AND GOATS IN SOMALILAND

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Summary

This study was conducted in the period July 2011 to February 2012 in Berbera veterinary Quarantine in North West Somalia (Somaliland) to determine the seroprevalence of FMD in cattle, sheep and goats using PrioCHECK®-FMDV NS ELISA. A total of 1080, 840 and 2080 sera samples from cattle, sheep and goats, respectively, were examined by ELISA against 3ABC NSP of FMDV. The result revealed that, the seroprevalence of FMD in cattle was (200/1080) 18.52%. Higher ($p< 0.05$) prevalence (20%) was found in summer season. The seroprevalence was higher ($p>0.05$) in age group > 4 year (147/530) 27.74% as compared with age group 2- 4 year (51/300) 17% and the age group < 2 year (0. 8%). The overall seroprevalence of FMD in sheep and goat in the three regions in Somaliland, Burao, Borma and Hargeisa was (366/2880)12.71%. Among the districts seroprevalence of FMD was higher ($p>0.05$) in Buroa district (160/900) 17.78% as compared with Borma (143/1080) 13.24% and Hargeisa 7%. Higher (($p<0.05$) seroprevalence was found in sheep (143/840) 17.03% as compared with goats (223/2040) 12.71%. For sheep and goats higher (($p<0.05$) seroprevalence was recorded in summer season 14.56% as compared with the winter 11.2%. Higher ($p<0.05$) seroprevalence (21.43%) was recorded in sheep >3 years as compared with goats (17%), while the seroprevalence recorded for age group 6month to 1year was 6% in sheep and 2.67% in goats. In conclusion the seroprevalence of FMD in cattle, sheep and goats was found to be high in the studied districts of Somaliland. A further study to determine the serotypes is recommended. Furthermore, given that the animals entering the market chain come from the neighboring countries there is a need to enhance the inspection and certification at the border points. The movement of animals in search of pasture or trade across the national borders in the region calls upon a regionally coordinated and harmonized FMD control strategy especially for trade with Middle East countries including Egypt.

SEROPREVALENCE DE LA FIEVRE APHTEUSE CHEZ LES BOVINS, OVINS ET CAPRINS DAN LE SOMALILAND

Résumé

Cette étude a été réalisée, pendant la période de juillet 2011 - février 2012 dans la quarantaine vétérinaire de Berbera dans le Nord-Ouest de la Somalie (Somaliland), avec pour but de déterminer la séroprévalence de la fièvre aphteuse chez les bovins, les ovins et les caprins en utilisant le test PrioCHECK ® FMDV NS-ELISA. Au total, 1080, 840 et 2080 échantillons de sérum prélevés respectivement sur des bovins, ovins et caprins, ont été examinés en utilisant le test ELISA 3ABC PNS afin de détecter le virus de la fièvre aphteuse. Les résultats ont révélés, que la séroprévalence de la fièvre aphteuse chez les bovins était de18,52% (200/1080). Une prévalence plus élevée ($p <0,05$) de 20% a été observée en saison estivale. La séroprévalence était plus élevée ($p> 0,05$) chez le groupe d'âge> 4 ans (147/530, soit 27,74%) comparée au groupe de 2 à 4 ans (51/300, soit17%) et groupe au d'âge <2 ans (0,8%.). La séroprévalence globale de la fièvre aphteuse chez les ovins et caprins dans les trois régions du Somaliland (Burao, Borma et Hargeisa) - était de 12,71% (366/2880). Au niveau des districts, la séroprévalence de la fièvre aphteuse était plus élevée ($p> 0,05$) dans le district de Buroa (160/900, soit17,78%) comparé à celle

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du district de Borma (143/1080, soit13,24%) et à celle de Hargeisa 7%. Une séroprévalence élevée ((p <0,05) a été identifiée chez les ovins (143/840, soit17,03%) comparé aux caprins (223/2040, soit12,71%). Pour les ovins et les caprins (p <0,05) une séroprévalence plus élevée a été enregistrée pendant la saison estivale (14,56%) comparé à celle enregistrée en hiver (11,2%). Une séroprévalence élevée (p <0,05) (21,43%) a été enregistrée chez les ovins> 3 ans comparé aux caprins (17%), tandis que le taux de séroprévalence enregistré pour le groupe âgé de 6 mois à 1 an était de 6% chez les ovins et de 2,67% chez les caprins. En conclusion, la séroprévalence de la fièvre aphteuse chez les bovins, ovins et caprins a été jugée élevée dans les districts étudiés du Somaliland. Il est recommandé de mener une étude plus approfondie pour déterminer les sérotypes impliqués. De plus, étant donné que les animaux entrant dans la chaîne de commercialisation proviennent des pays voisins, il est nécessaire de renforcer l'inspection et la certification aux points frontaliers. Le mouvement des animaux à la recherche de pâturages ou à des fins de commerce à travers les frontières nationales dans la région exige une stratégie régionale coordonnée et harmonisée de lutte contre la fièvre aphteuse, en particulier pour le commerce avec les pays du Moyen-Orient, y compris l'Egypte.

Introduction

Foot and Mouth disease (FMD) is highly contagious viral disease of cloven-hoofed domestic and wild animals. It is widely distributed and occurs most commonly in Asia, Africa, the Middle East, and parts of South America (Kitching, 1999). In pastoral areas of Africa outbreaks of FMD are reported frequently but the disease remains largely uncontrolled using conventional methods (Thomson and Bastos, 2005). Foot and Mouth Disease Virus (FMDV) is classified within the Aphthovirus genus as a member of the Picornaviridae family and is a highly infectious disease agent that causes severe vesicular disease (Thomson, 1995). It contains a single stranded RNA molecule and has seven major serotypes: A, O, C, SAT1, SAT 2, SAT3 and ASIA 1. Infection with one serotype does not confer immunity against another (OIE Manual, 2005). Recovered animals or their products are considered most important sources of the virus and can be a means of spread.

FMD in adult sheep and goat is frequently mild or inapparent, but can cause mild mortality in young animals. Sheep have importance role in the epidemiology of FMD and there have been numerous examples in past where small ruminants have been responsible for the introduction of FMD in a previous – free country. The difficulty in making a clinical diagnosis makes the development of more rapid screening tests to assist in control programs a priority (Kitching and Hughes, 2002).

The epidemiology of FMD in Africa has been reviewed (Vosloo *et al.,* 2002). The salient features of the disease in Africa include; the presence of six FMDV serotypes including serotypes O, A, C, Southern African Territories (SAT) 1, SAT 2 and SAT 3..The disease is of high economic importance especially to countries that have an intensive animal industry. It is one of the most important economic diseases of livestock (Bronsvoort *et al.*, 2004). The economic importance of the disease is not only due to the ability of the disease to cause losses of production, but to the restrictions on the trade of animals and their products both locally and internationally (James and Rushton, 2002). FMD has a great potential for causing severe economic losses in susceptible animals (OIE, 2000) Greater losses can result from refusal of FMD free countries to import livestock and livestock products from infected regions (Kahrs, 2001).

FMD virus has 4 structural proteins (SP) (VP1,VP2,VP3 and VP4) forming the capsid. Replication of the virus during infection, results in the production of a numbers of non – structural proteins (NSP) some of which are immunogenic. Non-structural protein (NSP) 3ABC antibody is considered to be the most reliable indicator of present or past infection with foot and mouth disease virus (FMDV) in vaccinated animals. An indirect ELISA has been developed using purified His-tagged 3ABC fusion protein as antigen, for detection of the antibody response to FMDV NSP 3ABC in different animal species (Lu *et al* 2007).

Several techniques for confirmation of FMDV have been described in the OIE Manual of Diagnostic Techniques, but there is still need for considerable effort for developing rapid, accurate tests for use on a wider scale (Clavijo *et al.,* 2004).

Sero-surveillance will be improved by using the new nonstructural protein (NSP) enzyme-linked immunosorbent assays (ELISAs) now available, which offer the potential to identify animals exposed to any of the seven serotypes in a single, affordable test (De Diego *et al* 1997).

Thus, the aim of this study was to determine the seroprevalence of FMD in sheep, goats and cattle in four districts, namely Hargeisa, Buroa, Borama and Wajaale which are high risk areas for FMD, due to the presence of regular trans-boundary movement of live animals across Somalia, Ethiopia border.

Material and Methods

Study area

This study was carried out between July 2011 and February 2012 in Berbera Veterinary quarantine international North West Somalia (Somaliland).

Livestock production systems in the studied area

The Horn of Africa is endowed with a vast animal resource and it is estimated that it is the home to 119 million cattle, 209 million sheep and goats, and 14 million camels. The contribution of the livestock sectors to livelihoods, food security and national economies is enormous. Trade in livestock and livestock products is an important commerce between the Horn of Africa countries and the Middle East and also substantial intra-regional cross-border trade of live animals also occurs among the Horn of Africa countries.

The livestock production systems practiced in Somalia depends on the region, availability of labour, herd sizes and types of livestock kept. There are four main livestock production systems namely, nomadic pastoralism, agro-pastoralism, settled mixed farming and urban stall feeding. Livestock movement occurs mainly in search of grazing pastures and water and also for trade. From April to October the animals graze on both sides of the border with Ethiopia on the Highland Plateau. They move freely across the border since the communities living in this region are the same. The movements are likely to lead to exchange of virus strains endemic in the regions. During the dry season (November to March) the pastoralists move the animals northwards to the coastal areas. They then move inland at the start of the rains in April. Movement from the eastern part of the country to the western part is rare.

Livestock markets and trade

Major trade routes transect the country mainly towards the major export ports. During a single day at Burao/Yirowe, as many as 10,000 head of sheep and goats may be sold for export. Livestock sales for local slaughter and consumption contribute another 350-400 to these daily livestock sale numbers. Burao/Yirowe is the largest livestock market in Somaliland, handling 66-70% of all sheep and goats exports and 60-65% of all camel exports on their way to Berbera. The second largest market is Hargeisa, and smaller numbers of exports pass through the remaining Somaliland markets. (FSAU 2001)

Berbera veterinary quarantine is an important quarantine in Somaliland. Table 1 shows the number of livestock exports from the quarantine to Middle East region including Saudia Arabia and other gulf countries.

Animals studied

I- cattle

A total of 1080 cattle admitted to quarantine station from Wajaale town located 80 km west of Hargeisa.. wajaale town is a major hub that links Ethiopia and Somaliland. Wajaale district is 4km^2 and the species most reared in cattle mainly for subsistence purposes. Cattle exported from Gebilay region and its neighborhood are inspected and vaccinated in Wajaale district. All imports destined for the major port of Berbera from Ethiopia go through this strategic border, with a twin Ethiopian (sister) town on the other side of the border also called Wajaale.

Table 1: Sheep and Goats cattle and camels Exported from Berbera Quarantine, 2009-2011 (head per year)

Year	No. of animals		
	sheep and goats	Cattle	camels
2009	597029	6150	169
2010	1946081	24267	44334
2011	4597694	45833	57086
Total	15416	2054584	

Table 2: Seroprevalence of FMD in sheep and goats according to districts

Species / locality	Sheep			Goats			Total sheep and goats		
	Total	positive	%	Total	positive	%	Total	positive	%
Hargeisa	300	28	9.34	600	35	5.84	900	63	7
Buroa	200	55	27.50	700	105	15	900	160	17.78
Borma	340	60	17.65	740	83	11.22	1080	143	13.24
Total	**840**	**143**	**17.03**	**2040**	**223**	**10.94**	**2880**	**366**	**12.71**

P > 0.05

II- sheep and goat

A total of 840 sheep and 2040 goats that were admitted to Quarantine from the three districts Hargeisa, Buroa and Borma were enrolled for the study.

Sample collection:-

A total of 3960 blood sera samples were collected from (840 sheep, 2040 goats and 1080 cattle). A total of 10 ml of blood samples were collected from peripheral blood aseptically via jugular puncture, using vacutainer tubes with a separate needle for each sample, allowed to clot, and transferred on ice to the laboratory of the Saudi - Emirates Veterinary Quarantine at Berbera city of Somaliland. The sera were separated by centrifugation at 2000 rpm for 4 min and aspirated in eppendorf tubes using Pasteur Pipettes, identified and stored at -20°C until testing.

Serological test

The kit used was the commercial PrioCHECK®-FMDV NS (Prionics Lelystad B.V., Platinastraat 33, Netherlands) product, lot number FBT1139T. The PrioCHECK®-FMDV NS detects antibodies directed against the non-structural 3ABC protein of FMD. The ELISA detects FMDV infected animals independent of the serotype that causes the infection and independent of the fact that the animal is vaccinated or not. The ELISA can be used to test serum samples of cattle, sheep, goats and pigs (Sorensen *et. al.,* 1998). The PrioCHECK®-FMDV NS is a blocking ELISA. The test plates are coated with 3ABC specific monoclonal antibody (mAb), followed by incubation with antigen (3ABC protein). Consequently, test plates of the kit contain FMD NS antigen captured by the coated mAb).

The test was carried out as per the manufacturer's instructions. The tests were performed by dispensing the test samples to the wells of ELISA plate, Incubation overnight at 21-22^{0}C, After incubation the plate is washed and conjugate is added to all wells, incubated at 21-22^{0}C for one hour. FMD NS specific antibodies, directed against the non-structural proteins, that may be present in the test sample will bind to 3ABC protein and will block the binding of the mAb-HARPO. After incubation, the plate is washed and the Chromogen (TMB) substrate is dispensed to all wells incubates for 15 minutes at room temperature. (22 ± 3°C) the color development is stopped. Color development measured optically at a wavelength of 450 nm shows the presence of antibodies directed against FMDV. Samples give percent of inhibition IP<50% considered negative and that give IP≥50% considered positive.

Table 3: The seroprevalence of FMD in sheep and goat by age group

Species / Age	Sheep			Goat		
	Total examined	positive	%	Total examined	positive	%
6 month – 1year	200	12	6	450	12	2.67
> 1-3 year	280	26	9.29	650	48	7.38
> 3year	360	105	21.43	940	163	17
Total	**840**	**143**	**17.03**	**2040**	**223**	**10.94**

P > 0.05

Table 4: The seroprevalence of foot and mouth in cattle by age group

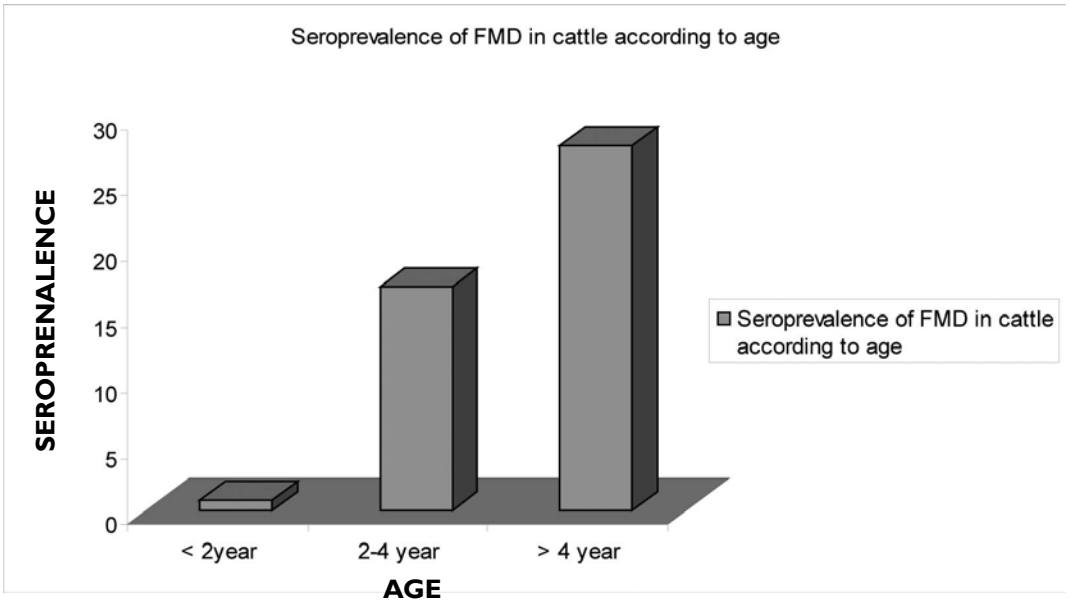
Age	Total	Positive	%
< 2year	250	2	0.8
2-4 year	300	51	17
> 4 year	530	147	27.74
Total	**1080**	**200**	**18.52**

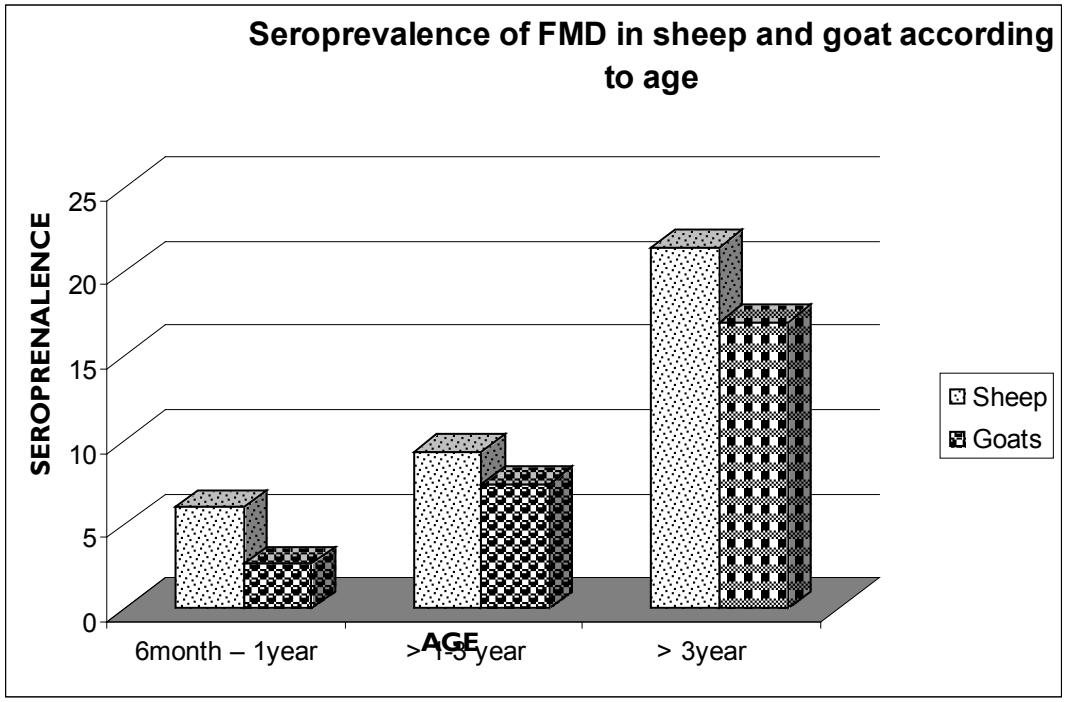
P > 0.05

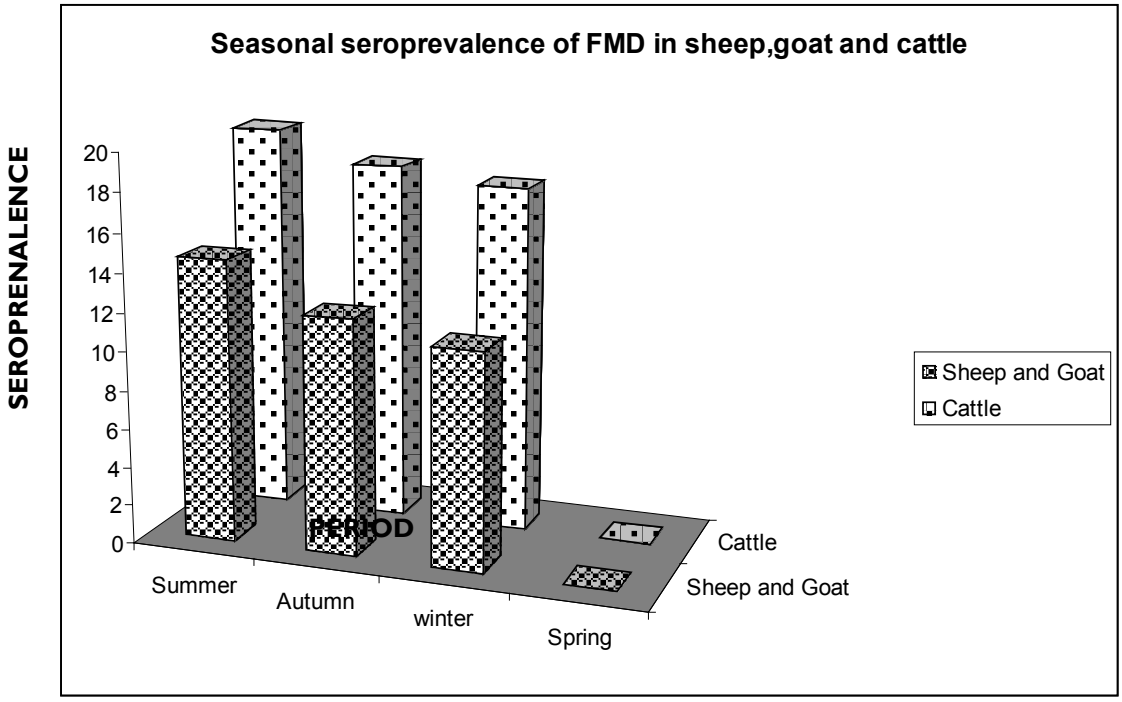
Table 5: Seroprevalence of FMD in sheep, goat and cattle according to season

Species / Season	Sheep and Goat			Cattle		
	Total	Positive	%	Total	Positive	%
Summer	900	131	14.56	270	54	20
Autumn	1440	175	12.16	270	50	18.5
winter	540	60	11.2	540	96	17.8
Spring	0	0	0	0	0	0
Total	**2880**	**366**	**12.71**	**1080**	**200**	**18.52**

P > 0.05




Seroprevalence of FMD in sheep and goat according to age
SEROPRENALENCE
25
20
15
10
5
0
6month – 1year
>1-3 year
> 3year
AGE
Sheep
Goats


Seasonal seroprevalence of FMD in sheep,goat and cattle
SEROPRENALENCE
20
18
16
14
12
10
8
6
4
2
0
Summer
Autumn
winter
Spring
PERIOD
Cattle
Sheep and Goat
Sheep and Goat
Cattle

Results

The overall seroprevalence of FMD in sheep and goat in the 3 regions in Somaliland was 12.71% (Table 2). Among the different districts the seroprevalence of FMD was higher in Buroa district 17.78% as compared with Borma 13.24% and Hargeisa 7%. According to species higher (($p< 0.05$) seroprevalence was found in sheep 17.03% followed by goats 10.94%. The overall seroprevalence of FMD in cattle from Wajaale town was 18.52%.

Table 3 Fig 2 shows the higher seroprevalence recorded in >3 year age group 21.43% in sheep and 17% in goat, and the lowest seroprevalence found in age group 6 month to 1year age group 6% in sheep and 2.67% in goat also the higher seroprevalence was recorded in age group > 4 year in cattle 27.74% and low seroprevalence (0.8%) recorded in age group < 2 year Table 4 and Fig 1 and in sheep and goat

Higher ($p< 0.05$) prevalence was found in summer months in cattle (20%) and sheep (14.56%) and low seroprevalence of 17.8% and 11.2% was recorded in winter season in cattle and (sheep and goats), respectively (Table 5).

Discussion

The role of sheep in maintaining of FMD virus remains uncertain, so the design of appropriate disease control measures is problematic (Balinda *et al.,* 2009). In particular, it is not proven that prophylactic vaccination program of small ruminants is essential to control the disease, owing to the cost of vaccination; small ruminants are still not routinely vaccinated unless there is an association with large number of cattle (Kitching and Hughes, 2002). The result obtained is similar to that obtained by (Abdulahi *et al.,* 2011).

Seroprevalence documented in this survey higher as compared with previous reports of 8.18% (Molla *et al.*, 2010) and 9.5% (Megersa *et al.,* 2009) in South Ethiopia (South Omo, Sidama and Gamogofa zones). On the other hand, the seropositivity findings of this study were similar to the overall seroprevalences of 21% and 26.5% reported by Shale *et al.* (2004) and Rufael *et al.* (2008), respectively. The high prevalence of the disease in case of this study could be attributable to unrestricted high herd mobility, continuous contact and intermingling of different herds at water points and communal grazing areas. These results also agree with the result obtained by Dukpa *et. al.* (2011) who made cross sectional serological surveys conducted between March and December 2009 to determine the distribution of foot-and-mouth disease and also to validate the current passive surveillance system in Bhutan. A total of 1909 sera collected from cattle, goats, sheep and pigs, from 485 herds in 106 villages, were tested using a foot-and-mouth disease non-structural protein 3ABC ELISA.

The obtained result is in agreement with the result of Ehizibolo *et al. (*2010) who reported in Nigeria significant seroprevalence of FMD (9.3%) and (15%) in sheep and goat, respectively, using virus-infection associated antigen. In 2007, Balinda *et al.*, (2009) recorded that mean prevalence estimates of antibodies towards FMDV NSP was 14% in goats and 22% in sheep in Kasese district in Uganda, while Bushenyi was still free. The difference between these two districts probably reflects different levels of FMDV challenge attributed to the variation in exposure rates which again in part may be as a result of the differing husbandry practices. Lazarus *et al* (2012) made a serological survey conducted between 2009 and 2011 in six Border States and two other states that lie on the major cattle trek routes in Nigeria. Using PRIOCHECK FMD-3ABC NS protein ELISA, they found that the seroprevalence of FMD in small ruminants (sheep and goat), was 41.66 and 21.81%, respectively.

Despite reports that the small ruminants (sheep and goat) are susceptible, and represent a risk of infection to cattle and plays an epidemiological role in FMD; analyses indicate that cattle have a greater risk of infection than sheep and goat (Balinda *et al.,* 2009). However, the high degrees of seropositivity found in small ruminant is unlikely to be connected with infections and possible viraemia in sheep and goats and these represent significant risks to naïve cattle population since these smallstock move together and often lead the cattle along the trek-route and can shed the virus for long time without showing apparent clinical signs

while infecting other animals (Arzt *et al.,* 2011). It will be important to determine the specific role of sheep and goat in the epidemiology of FMD in West and Central Africa.

The age-specific seroprevalence revealed an increasing prevalence as the age increases, in which higher seroprevalence was recorded in age group > 4 year in cattle 27.74% and low seroprevalence (0.8%) recorded in age group < 2 year Table 3 and Fig 1 and in sheep and goat Table 3 Fig 2 the higher seroprevalence recorded in >3 year age group 21.43% in sheep and 17% in goat, and the lowest seroprevalence found in age group 6month to 1 year age group 6% in sheep and 2.67% in goat. These results are agreement with the report of Chepkwony *et al (*2012), who reported that seroprevalence varied significantly between ages with the older animals showing a higher risk of infection with FMD virus compared with younger animals. Out of all animals that tested positive for at least one serotype, 1-2 year age bracket realized a prevalence of 38.2%, 2-3 year olds realized 54% while 3-4 year age bracket realized seroprevalence of 80%, and with (Gelaye *et. al.,* 2009). This may be attributable to the young cattle being herded in homestead areas and hence having less chance of exposure. In addition, in disease-prevalent districts of the study area, the cattle herds follow seasonal patterns in search of good pasture and water and the herds are usually composed of adult males and non-lactating and non-pregnant female cows and are therefore more exposed to FMD than younger age group. This is in agreement with a report of Murphy *et. al.,* (1999). Those animals aged >4 years may have acquired the infection from multiple serotypes and/or infections. Antibodies to non-structural proteins can be detected in infected cattle and for up to 1 year after infection (Mackay *et. al.,* 1998), while antibodies to the structural proteins can last 1–2 years (McCullough *et. al.,* 1992) and even longer. This indicates that higher prevalence in old cattle in this study is likely due to constant re-exposure to FMD.

Higher ($p< 0.05$) prevalence was found in summer months in cattle (20%) and sheep (14.56%) and low seroprevalence of 17.8% and 11.2% was recorded in winter season in cattle and (sheep and goats), respectively (Table 5).

In conclusion the seroprevalences of FMD were found to be high in the districts of Somaliland. Identification and characterization of the serotypes of FMD virus in the study area as well as sero-prevalence surveys in wildlife and smallstock is recommended so as to determine the role that these animals may play in the transmission of FMD. This is very important in the understanding of the disease and also for the implementation of efficient prevention and control measure. Effective control of FMD in Somalia will require imposition of animal movement restrictions, the use of multivalent vaccines to avoid the economic impact of FMD, enhanced border inspection and certification and implementation of a regionally coordinated and harmonised FMD control strategy.

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THE EFFICACY OF ALBENDAZOLE AND MOXIDECTIN IN THE CONTROL OF NEMATODE INFECTION IN DAIRY CATTLE

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Abstract

The objective of this randomized controlled field trial was to determine and compare the efficacies of two anthelmintics, moxidectin and albendazole on gastrointestinal nematodes (GIN) in smallholder dairy cattle in Kenya in June to August 2010. On the first visit, faecal samples were collected from the rectum of 419 cattle that were above three months of age on 128 smallholder dairy farms. Faecal egg counts (FECs) for GIN eggs were conducted using the modified McMaster method, and larval cultures were done on pooled samples for each farm to determine the GIN genera encountered. The cattle were allocated to three treatments groups (albendazole, moxidectin, and placebo groups), using a blocked random allocation method. A second faecal sampling and FEC was done on the recruited cattle two weeks post-treatment, with laboratory staff again blinded to each sample's group status. Statistical analyses were conducted to determine the efficacies of the two anthelmintics mentioned relative to the placebo group. The prevalence of GIN infections in the study population was 13.8%, in large part due to 75% of the cattle being managed using zero-grazing. Haemonchus, Trichostrongylus and Oesophagostomum were found on 28%, 20% and 15% of the 128 farms, respectively. The newer moxidectin had significantly better efficacy (95.8%) than albendazole (74.9%) for treating GINs in smallholder dairy cattle in Kenya.

Key Words: *Albendazole, Moxidectin, small holder dairy cattle, Nakuru District, Mukurweini District.*

L'EFFICACITE DE L'ALBENDAZOLE ET DE LA MOXIDECTINE DANS LE CONTROLE DE L'INFECTION AUX NEMATODES CHEZ LES BOVINS LAITIERS

Résumé

L'objectif de cet essai contrôlé randomisé sur site était de déterminer et de comparer les niveaux d'efficacité de deux anthelminthiques, la moxidectine et l'albendazole, sur les nématodes gastro-intestinaux (NGI) chez les bovins laitiers des petites exploitations au Kenya, de juin à août 2010. À la première visite, sur 128 petites fermes laitières, des échantillons fécaux ont été prélevés dans le rectum de 419 bovins âgés de plus de trois mois. Une numération des œufs fécaux (FEC), pour les œufs GIN, a été effectuée selon la méthode modifiée de McMaster, et des cultures larvaires ont été réalisées sur des échantillons groupés pour chaque exploitation, afin de déterminer les genres GIN identifiés. Les animaux ont été répartis en trois groupes de traitement (albendazole, moxidectine, et groupe sous placebo) en utilisant une méthode de répartition aléatoire. Un deuxième prélèvement fécal et une FEC ont été effectués sur des bovins sélectionnés deux semaines après le traitement, le personnel de laboratoire étant laissé aveugle quant à l'état du groupe de chaque échantillon. Des analyses statistiques ont été réalisées pour déterminer les niveaux d'efficacité des deux anthelminthiques susmentionnés par rapport au groupe sous placebo. La prévalence des infections GIN chez la population étudiée était de 13,8%, en grande partie dû au fait que 75% les bovins étaient gérés en stabulation. Les nématodes Haemonchus, Trichostrongylus et Oesophagostomum ont été trouvés respectivement dans 28%, 20% et 15% des 128 fermes laitières. La nouvelle moxidectine avait une

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efficacité significativement meilleure (95,8%) que celle de l'albendazole (74,9%) pour le traitement de GI chez les bovins laitiers des petites exploitations laitières au Kenya.

Mots-clés : *Albendazole, Moxidectine, bovins laitiers des petits fermiers, District de Nakuru, District de Mukurweini.*

Introduction

The use of anthelmintics in cattle has reduced the impacts of gastrointestinal nematodes (GIN) on productivity. However, their frequent use and under-dosing have contributed to the emergence of nematode populations resistant to the drugs available in the market (Michel, 1985). Therefore, randomized controlled field trials to determine their efficacy are needed to assist veterinarians and farmers in decisions on which anthelmintic products should be used.

Albendazole, a benzimidazole derivative, is an anthelmintic drug that has been widely used around the world against nematodes for decades, with the specific advantage of being effective against round worms, tapeworms and flukes both in cattle and small ruminants (Guitian *et al.* 1999). According to Maingi *et al.,* (1998), the most commonly used anthelmintics in Kenya are benzimidazoles and levamisoles. Studies carried out in Nyahururu District of Kenya confirmed the presence of resistance to albendazole and levamisole in sheep (Maingi *et al.,* 2001). It is thus possible that resistance to albendazole in cattle could have also developed. Studies done in Argentina using benzimidazoles indicated faecal egg count (FEC) reductions of 68% in cattle compared with the expected 95% that would demonstrate good anthelmintic efficacy (Suarez *et al.,* 2006). Moxidectin (pour-on) is a relatively new anthelmintic to African cattle farmers with a very wide safety margin (Kahn & Line, 2010). A study carried out in Mexico by Maritonera-Diez *et al.,* (2005) showed the efficacy of moxidectin pour- on to be 100% at day 28, dropping to 33% at day 60. The current study was meant to determine the efficacy of moxidectin pour-on compared with oral albendazole on smallholder dairy farms in Kenya.

Material and Methods

Study area

The trial was carried out in Mukurweini District of Nyeri County and Nakuru District of Nakuru County in Kenya. Nyeri County is one of the five counties of Central Province and forms part of Kenya's central highlands (Ministry of Planning and National Development, 2005). Dairy farming is an important enterprise in Mukurweini District, with the farmers practicing zero-grazing methods, where pastures are cut and carried to the cattle (Ministry of Livestock Development, 2008).

Nakuru County is one of the 14 Counties of the Rift Valley Province (The Constitution of Kenya, 2010), and lies within the Great Rift Valley. Dairy farmers in the area practice both zero-grazing, and semi-zero-grazing, where the cattle are housed but allowed to graze at certain times (Ministry of Livestock Development, 2008).

Study design

A total of 419 head of cattle on 128 smallholder farms (64 from each district) were enrolled to the field trial. In Nakuru, a simple random selection was employed at the farm level using a sampling frame of the dairy farms provided by the District Livestock Production Officer. For logistical reasons, a purposive sampling method was used in Mukurweini, as the research was conducted alongside another project comparing smallholder dairy farms with and without biogas digesters (Dohoo *et al.,* 2012a; Dohoo *et al.,* 2012b). In Mukurweini district, all cattle that were above three months of age on the selected farms were sampled for the study. In Nakuru district, some farms were larger, and therefore on farms that had large herds of cattle, the animals were systematically randomly selected, such that no more than 10 animals were sampled per farm.

The study was carried out between 16th June 2010 and 30th August 2010. On the first visit (day 0), faecal samples (minimum of 5 g) from each selected animal were collected from the rectum. They were examined for nematode eggs at the parasitology laboratory at the Faculty of Veterinary Medicine, University of Nairobi Kabete. Faecal egg counts (FEC) of GIN eggs were conducted using the Modified McMaster technique (Wood *et al.,* 1995). Animal owners and laboratory staff were blinded to the treatment groups of the animals.

Sampled cattle were randomly allocated into one of the three groups. This was done by a random block allocation method, with the first treatment being picked randomly by selecting numbers from a hat without replacement until all numbers were selected.

- Group 1 – control group, only oral and pour-on placebos
- Group 2 – moxidectin 0.5% pour-on at 0.5 mg/kg body weight (licensed dose) poured along the dorsal midline of the animal, and an oral placebo.
- Group 3 – albendazole drench at 10 mg/kg body weight (licensed dose) and a pour-on placebo.

Because treatment with albendazole comes with a warning not to use on cows/heifers pregnant less than 45 days or on milking cows (milk withdrawal), milk cows and bred heifers were only allocated to groups 1 or 2. A second faecal sampling and FEC was done on the recruited cattle two weeks post-treatment, with laboratory staff again blinded to each sample's group status.

Data handling and Statistical Analysis

The anthelmintic efficacy (%) post-treatment for each of the anthelmintic medicines was estimated according to Wood *et al.,* (1995): % Efficacy = ((Mean FEC Control – Mean FEC Treatment) / (Mean FEC Control)) * 100. The formula uses the post-treatment FECs assuming that the pre-treatment FECs are the same due to the random allocation of animals in the groups. Arithmetic mean was used as opposed to geometric mean, as the geometric means tend to overestimate drug efficacies, and thus, are unable to pick up small levels of anthelmintic resistance (Waruiru *et al.,* 2003). The 95% confidence intervals were calculated around the efficacy percentages.

Results

For the two visits, the sample numbers, and mean FECs of the 3 treatment groups are presented in Table 1. On the first visit, the prevalence of GIN infections in the study population was 13.8%, in large part due to 75% of the cattle being managed using zero-grazing. There were no differences in mean FECs among groups on the first visit, as expected, demonstrating an effective random allocation process. *Haemonchus*, *Trichostrongylus* and *Oesophagostomum* were found on 28%, 20% and 15% of the 128 farms, respectively.

On the second visit, 2 of the 419 enrolled animals were not sampled and tested by FEC because they were not at the farm at the time of the visit. The mean FEC was significantly higher in the placebo and albendazole groups compared with the moxidectin group, while the albendazole-treated animals had a numerically but not statistically significantly lower FEC than the animals in the placebo group (Table 1). The moxidectin group had a significantly higher efficacy (95.8%) than the albendazole group (74.9%).

Discussion

In this randomized controlled, blinded, field trial, moxidectin pour-on had an efficacy of 95.8%, which is slightly lower than a study that was carried out in Mexico by Maritorena-Diez *et al.,* (2005), which placed the efficacy of moxidectin pour-on at 100% after 28 days. Compared with the expected 95% that would demonstrate good anthelmintic efficacy, this relatively new anthelmintic to the African marketplace appears to have very good efficacy against GINs in cattle in Kenya. However, because moxidectin does not work against trematodes, cattle at risk of trematode infection should be dewormed periodically with an anthelmintic with theoretical or known efficacy against trematodes, such as albendazole. This study did not assess the efficacy of albendazole on trematodes.

Table 1. Number of cattle allocated to the anthelmintic treatment, the mean epg count 14 days post treatment and the 95% confidence interval.

Treatment Group	Number Sampled	Mean FEC [95% CI][1]	Percentage efficacy [95% CI]
Placebo	180	13.3 [6.5, 20.1]	-
Moxidectin	179	0.56 [0, 3.2]	95.8% [93.7, 97.9]
Albendazole	60	3.3 [0, 7.9]	74.9% [69.0, 80.8]

1 FEC = Faecal egg count; CI = confidence interval

Albendazole had an efficacy below 80% in the current study, which may be partly because it has been in use for a long time as the drug of choice for dairy farmers in Kenya, who buy the anthelmintic over the counter (Wanyangu *et al.,* 1994). A study carried out in Nyandarua (Maingi *et al.,* 2001) reported resistance to benzimidazoles in sheep. However, another study carried out on goats in Kenya by Waruiru *et al.* (2003) estimated the efficacy of albendazole at 96.9% at day 21. Our efficacy percentage (74.9%) was similar to another benzimidazole study done in Argentina, which had an efficacy of 68% (Suarez *et al.,* 2006). Perhaps there is less albendazole resistance among goats in Kenya than among cattle.

Benzimidazoles are sold over the counter under many varying brand names by different manufacturers, which lead the farmers to think they are switching anthelmintics (Wanyangu *et al.,* 1994), which could have contributed to resistance over time.

Conclusions

Moxidectin had a very good GIN efficacy in cattle in Kenya, and had a higher efficacy compared with albendazole Farmers should be encouraged to use a variety of anthelmintic drugs, in rotation, including moxidectin, to ensure proper GIN control, in consultation with their local veterinary service providers.

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A STUDY ON THE EFFICIENCY OF NATURAL AND SYNTHETIC PROSTAGLANDINS FOR ESTRUS SYNCHRONIZATION IN JENNIES

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Abstract

A study was conducted to determine the efficiency of natural and synthetic prostaglandins in estrus synchronization of jennies. In the experiment, jennies were randomly assigned to Lutalyse (n=5) and Estrumate treatment (n= 7) groups. *Ovarian follicular* activity was determined ultrasonically. Serum was collected for progesterone assay. The days to estrus, days to ovulation, estrus length, size and number distribution of follicles, and size of the preovulatory follicle were recorded to compare ovarian function. The mean (±SD) number of total follicles was 12.9±3.8 for lutalyse and 13.0±0.5 follicles for estrumate treatments. The mean (±SD) days to estrus, days to ovulation, diameter of the preovulatory follicle and the rate of ovulation for lutalyse groups were 2.3±0.5days, 11.0 ±1.2days, 37.4±2.4mm and 80%, respectively. The same parameters for estrumate treatment were 3.9±0.7 days, 39.5±2.7mm, and 100%, respectively. There was a significant difference ($p<0.05$) in the mean size of the dominant follicles between the two groups; estrumate treatment resulted in the largest dominant follicles. Estrumate also produced shorter days to estrus and days to ovulation ($p<0.05$). In conclusion, both lutalyse and estrumate can be used for estrus synchronization; however, estrumate gives a relatively better response compared with lutalyse for estrus synchronization in jennies.

Key words: *estrus synchronization, estrumate, jennies, lutalyse.*

UNE ETUDE SUR L'EFFICACITE DES PROSTAGLANDINES NATURELLES ET SYNTHETIQUES POUR LA SYNCHRONISATION DE L'ESTRUS DE L'ANESSE

Résumé

Une étude a été menée dans le but de déterminer l'efficacité des prostaglandines naturelles et synthétiques dans la synchronisation des chaleurs de l'ânesse. Dans le cadre de l'étude, des ânesses ont été réparties de manière aléatoire dans des groupes de traitement par la lutalyse (n = 5) et l'estrumate (n = 7). L'activité folliculaire de l'ovaire a été déterminée par ultrasons. Du sérum a été prélevé pour un dosage de la progestérone. Les jours pré-oestrus, les jours pré-ovulatoires, la durée de l'oestrus, la taille, le nombre et la répartition des follicules, et la taille du follicule pré-ovulatoire ont été enregistrés pour comparer la fonction ovarienne. Le nombre total moyen de follicules était de 12,9 ± 3,8 pour le traitement par la lutalyse et de 13,0 ± 0.5 pour le traitement par l'estrumate. Les moyennes des jours pré-oestrus, des jours pré-ovulatoires, des diamètres du follicule pré-ovulatoire et des taux d'ovulation pour les groupes traités par la lutalyse étaient respectivement de 2,3 ± 0,5 jours, 11,0 ± 1,2 jours, 37,4 ± 2,4 mm et 80%. Les mêmes paramètres pour le traitement par l'estrumate étaient respectivement de 3,9 ± 0,7 jours, 39,5 ± 2,7 mm, et 100%. Une différence significative ($p <0,05$) a été notée au niveau de la taille moyenne des follicules dominants entre les deux groupes : le traitement par l'estrumate a engendré les plus grands follicules dominants. L'estrumate a également causé une diminution du nombre de jours pré-oestrus et pré-ovulatoires ($p <0,05$). En conclusion, la lutalyse et l'estrumate peuvent être toutes les deux utilisées pour la synchronisation oestrale, mais l'estrumate donne une réaction relativement meilleure par rapport à la lutalyse pour la synchronisation oestrale chez l'ânesse.

Mots-clés : *Synchronisation oestrale, estrumate, ânesses, lutalyse.*

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Introduction

Reproductive efficiency is known to be the product of successful estrus detection and conception rates (De la Sota *et al.,* 1998). Detection of estrus in group of randomly cycling females is time consuming, laborious and subject to human error (Hafez and Hafez, 2000). All these problems can be possibly solved by synchronization of estrus and ovulation time, so that the females would be in estrus within a predictable time (Blanchard *et al.,* 1999; Arthur *et al.,* 2001). In many parts of Africa where nearly 90% of agricultural operations depend on manual labour. Donkeys play an extremely important role in the agricultural sector (Fesseha, *et al.,* 1997; Martin-Curran and Smith, 2005). Ethiopia with an estimated 5.2 million heads has the largest donkey population in Africa and the second largest in the world after China. As often constrained by prevailing traditional management systems, modern donkey production with more options of controlling the reproductive functions and improving performance through breeding have not been properly implemented.

Prostaglandin ($PGF_{2\alpha}$) administered as a single intramuscular injection during day 5 through 17 of the estrus cycle will induce regression of the corpus luteum (CL) and subsequent return to estrus within 36 to 72 hours. However, prior to and from days 18 to 21 of the estrus cycle, the CL is refractory to $PGF_{2\alpha}$ (Arthur *et al.,* 2001). While much is known about other equines, very little is known about donkeys. The presence of species specific factors influencing synchronization of estrus and the variable time to ovulation during estrus, and prolonged duration of behavioral estrus in equines are some of the factors that make synchronization problematic (Kojima *et al.,* 2000; Bearden *et al.,* 2004; Samper *et al.,* 2006; Samper, 2008). Therefore, the objectives of this study were to determine the potential use of estrus synchronization drugs in jennies and compare the efficiency of natural and synthetic prostaglandins in estrus synchronization.

Materials and Methods

Study area

This study was conducted in Debre Zeit located at about 45km southeast of Addis Ababa at $8^0 7^0$N Latitude and 39^0E longitude at altitude of 1990m above sea level and situated in central Ethiopia. The climate is characterized by a bimodal rainfall with the short rainy season occurring from March to May preceded by a long dry season from October to February. The long rainy season occurs from June to September. The relative humidity is 52% and the annual rainfall of 866mm of which 84% falls during the long rainy. The mean maximum and minimum temperature ranges are 26^0C and 14^0C, respectively.

Study animals

A total of eighteen apparently healthy and none pregnant jennies whose ovarian follicular activities were previously determined (Sida/SAREC research project, 2007) were used in this study. The jennies were 6 - 14 years old, had a body weight of 120 - 140kg, and a body condition score of 3 to 4 (on a 0 - 5 scale). The jennies were housed in a closed barn during the night but were allowed to graze natural pasture during the day time. They were also supplemented with grass hay, and water was provided *ad libitum* daily. All the jennies had been regularly dewormed previously. However, treatment was repeated for common internal and external parasites with Invermectin (Vermic® Centrovet, Chile) at a rate of 1ml/50kg orally one week before the start of the experiment.

Study design

Trial I: synchronization with Lutalyse (natural prostaglandin)

The jennies were randomly assigned to two groups: Lutalyse treatment (n=5) and Control (n=4). The jennies were given 1ml (equivalent to 5mg/ml) intramuscular injection of Lutalyse (Dinoprost tromethamine, Lutalyse®, USA) and the same treatment was repeated on Day 14. They were scanned using ultrasound (Mindray, veterinary digital ultrasonic imaging system, Hong Kong) prior to the administration

of Lutalyse and every other day starting on day 3 post injection for a total of 126 jenny days (JD) to determine the ovarian follicular activity. The animals were allowed to come in contact with jacks daily for about 20 minutes and were observed for manifestation of estrus. The number of follicles in each ovary, the diameter of the three largest follicles in each ovary (including the dominant/preovulatory follicle), the number of days to estrus, length of estrus, and days to ovulation, were recorded and used for later comparison of ovarian functions.

Trial II: synchronization with Estrumate (synthetic prostaglandin)

The jennies were randomly assigned to two groups: Estrumate treatment group (n=7) and control groups (n=2). The jennies were given 0.5ml (equivalent to 125 µg) intramuscular injection of Estruate (Cloprostenol®, Schering-Plough Animal Health Corp, Germany). They were similarly scanned using ultrasound (Mindray, Vet Digital Ultrasonic Imaging System, Hong Kong) prior to the administration of Estrumate and every other day starting on day 3 post injection for a total of 54 JD, to determine the ovarian follicular activity. The presence of estrus was observed daily. Ovarian function was studied similarly from the number of follicles in each ovary, the diameter of the three largest follicles in each ovary, the diameter of the dominant/preovulatory follicle, days to estrus, length of estrus, days to ovulation and ovulation rate. Follicular sizes were determined using the internal electronic caliper of the ultrasound. Ovulation was confirmed after a sudden disappearance of a preovulatory follicle and ultrasonic detection of corpus luteum in the following days.

Data analysis

All data were stored in Microsoft Excel sheet and all computations and comparisons for each variable interaction were performed using SPSS for Windows (SPSS version 15.0. 2006, Chicago, USA). The data were described and results were presented as mean (±Standard Deviation). Variables were compared using student t-test, ANOVA and Chi Square. P-value was held at 0.05 to determine significance of differences.

Results

Synchronization with Lutalyse

Except one jenny in the control group, no preovulatory follicles were present at the time of lutalyse injection in all animals. A corpus luteum was detected in 3 of the 5 treated jennies during scanning on the day of lutalyse treatment. All of the jennies exhibited signs of colic, straining, distress, frequent recumbency, sweating and urination shortly (10-15minutes) after injection. These signs receded soon and all the animals returned to normal state within 60 minutes of the injection. The mean (±SD) total number of follicles in both ovaries for all jennies was 12.6± 3.9 (range 6-21); while it was 12.9±3.8 follicles for Lutalyse treatment, and 12.2±4.2 follicles for Lutalyse control groups. Table 1 presents summary of the follicular data for both the treatment and control groups.

Follicular growth pattern with respect to the total number of follicles in both ovaries in the treatment and control groups were similar after the second injection (Figure 1). The mean (±SD) days to estrus, days to ovulation, and the rate of ovulation were 2.3 ± 0.5 days, 11.0 ± 1.2days, and 80%, respectively. Only 1 jenny ovulated after the first injection of lutalyse while 4 ovulated during the second injection. The average duration of estrus was 8days (range 6-12 days).

The particular elements of behavioral manifestations of estrus in those jennies that showed estrus included lowering of the head with forward extension of the neck, jawing, backward depressing of the ears against the neck, raising the tail, frequent urination, positioning, vocalization, flehman reaction, mounting other jennies, and winking.

Synchronization with Estrumate

Jennies injected with estrumate did not show any clinical signs of side effects. The mean (±SD) total number of follicles in both ovaries for all jennies was 12.5 ±4.9 follicles (range 5-23), while it was 13.0±5 follicles for the treatment and 9.3±1.5 for the control groups. Follicular growth pattern showed a steady decrease in the total number of follicles in the treatment group (Figure 2) while it

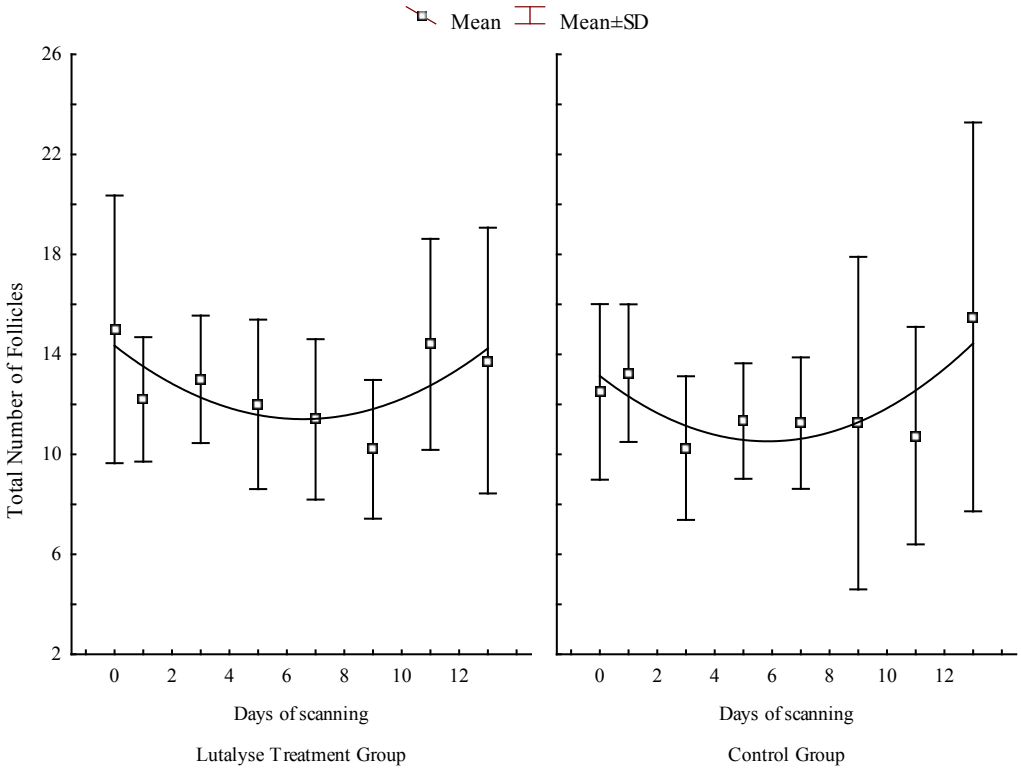

Figure 1: Follicular development pattern in the treatment and control jennies (n=9) from the days of the second treatment of Lutalyse (Day 0)

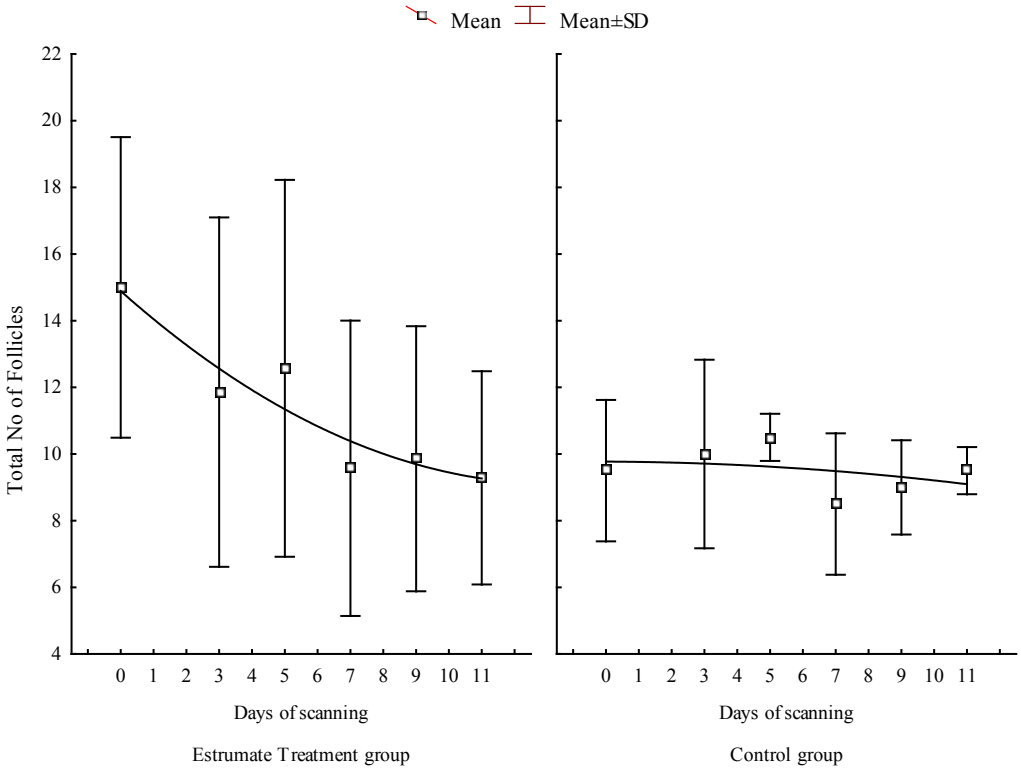

Figure 2: Follicular development pattern with respect to total number of follicles in Estrumate treated and control jennies (n=9)

remained relatively unchanged for the control group. The mean (±SD) days to estrus, days to ovulation, diameter of the preovulatory follicle and the rate of ovulation in estrumate treatment group were 3.9± 0.7 days, 9.1 ± 1.6 days and 100%, respectively. The average length of estrus was 6.3 days (range 5 – 8 days). All the basic elements of behavioral estrus were present but their manifestation was weaker in spite of apparent follicular activity evidenced by the presence of large follicles (>20mm).

There was a significant difference ($p<0.05$) in the mean diameter of the first largest follicles between the jennies of the two trials. Estrumate treated jennies had the largest follicular diameter in both ovaries (23.8±8.6mm for left and 17.2±7.9mm for right ovaries), compared with the jennies treated with Lutalyse (17.5±7.3mm for left and 17.1±7.8 for the right ovaries). However, there was no significant difference both in total number of follicles and diameter of the preovulatory follicle between the trials.

In the Trial-I, 3 out 5 jennies (60%) showed estrus signs on Day 2 post Lutalyse injection while in Trial-II, 4 out of 7 jennies (57%) showed estrus on Day 4 of Estrumate injection. The days to estrus was significantly shorter ($p<0.05$) with Lutalyse treatment while the days to ovulation was significantly shorter ($p<0.05$) with Estrumate treatment. Preovulatory follicles were relatively larger in Estrumate treated groups (39.45 ± 2.7mm) than Lutalyse treated jennies (37.35 ± 2.4mm) but the difference was not statistically significant. The maximum diameter of the preovulatory follicle (41mm) was found in jennies treated with Estrumate.

Discussion

The dose of both lutalyse and Estrumate were based of the dose rates recommended for mares (Samper, *et al.,* 2006). Thus the present study showed that dose rates recommended for mares also work for jennies. However, clinical side effects were more pronounced with lutalyse requiring further studies to determine the optimal dose rate for jennies to induce luteolysis. Estrus manifestations and follicular activities with regards to the mean number of follicles, the diameter of the preovulatory follicles and length of estrus found in both trials are closely similar to previous studies for donkeys (Henry *et al* 1991; Meira *et al* 1995; Henry *et al.,* 1998; Blanchard, *et al.,* 1999; Lemma *et al.,* 2006).

The variations in the number of ovarian follicles visible at different stages of the reproductive cycle and the maximum size of a preovulatory follicle in jennies have also been previously discussed (Meira *et al.,* 1995; Lemma *et al.,* 2006). Carluccio *et al.,* 2006; 2008) similarly reported a mean preovulatory follicular size of 39± 0.27mm in jennies following $PGF_{2\alpha}$ treatment.

The length of estrus, duration from treatment to estrus found with lutalyse treatment of this study (8 days and 2.3±0.5 days) are in close agreement to previous reports (Henry *et al.,* 1998; Carluccio *et al* 2006; 2008) but shorter than the 4.4±1.6 days reported by Blanchard *et al.,* (1999). On the other hand, the duration from treatment to ovulation was similar to the finding by the later authors. This study demonstrates that donkeys are responsive to different doses and preparations of prostaglandin administered at any time following ovulation. Different studies indicated the use of natural prostaglandin to be effective in synchronizing estrus in mares starting on day 6 post ovulation through day 18 of the cycle. After treatment, the mares were known to return to estrus in 4-5days, and ovulate 10-12 days post injection (Bearden *et al.,* 2004; Samper, 2008). However, the large dose normally recommended for mares is of concern due to the side effects. No reports so far exist on the use of estrumate in jennies for estrus synchronization but results of the current study are generally in agreement with reports for mares (Samper, 2008).

Conclusion

From the present study it is concluded that estrus can be synchronized using both natural and synthetic prostaglandins at a dose rate recommended for mares. However, synchronization with estrumate treatment produced a relatively better response than lutalyse treatment. Although days to estrus

Table 1: Size distribution of follicles in both ovaries of treatment and control jennies in Trial-I and II

Parameter of ovarian function	Lutalyse (126JD)		Estrumate (54JD)	
	Treatment	Control	Treatment	Control
Size of largest follicle on the date of treatment [mm]	15.1 (±1.1)	20.1 (±8.2)	18.8 (±5.7)	17.9 (±0.4)
Size of largest follicle in LOV [mm]	17.5 (±7.3)	19.3 (±9.2)	23.8 (±8.6)	19.7 (±5.7)
Size of largest follicle in ROV [mm]	17.1 (±7.8)	16.1 (±5.9)	17.2 (±7.9)	11.8 (±4.3)
Size of the preovulatory follicle [mm]	37.4 (±2.4)		39.5 (±2.7)	

JD= Jenny days; LOV= Left ovary; ROV= Right ovary

were shorter and estrus manifestations were overt during lutalyse treatment, follicular activity was better with estrumate treatment as evidenced by the appearance of larger follicles and higher incidence of ovulation. A further study on the optimal doses of lutalyse for jennies is recommended.

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Impact

In many African countries, the contribution of donkeys in the agricultural sector is crucial. Donkeys in most instances were considered as small horses regarding reproduction which they are not. Detection of estrus in group of randomly cycling females is time consuming and laborious. Synchronization of estrus and ovulation time solves such problem so that the females would be in estrus within a predictable time. Particularly, synchronization of estrus, little known for tropical jennies, is highly useful in the selection of jennies, and jacks with useful traits to breed in a more controlled manner and contribute to better reproductive management.

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PREVALENCE OF CRYPTOSPORIDIUM OOCYST IN CALVES GRAZING ALONG RIVER RIMA BANK IN SOKOTO, NIGERIA

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Abstract

The present study was conducted to investigate the point prevalence of Cryptosporidium oocysts infection in calves grazing along the bank of Rima River Sokoto in October 2011. The river bank is a converging zone for domestic animals reared in different quarters of the town and the surrounding settlements. A total number of 2,959 cattle were enumerated out of which 147 (4.97%) were calves. Faecal samples were collected from 100 (68.02%) calves by convenient sampling technique. Formol-Ether sedimentation and modified Ziehl-Neelsen staining techniques were used to identify the cryptosporidium oocysts in the faecal samples. Faecal consistency was also used to identify diarrhoeic and non-diarrhoeic calves. Cryptosporidium oocysts were identified in 33 (33.0%) of the calves examined. The detection rate was higher among the male calves (38.46%) than females while the Rahaji breed had the highest prevalence of 62.5%. A total of 6 (18.18%) among the positive cases were diarrhoeic. The differences in prevalence based on sex, breeds and presence of diarrhoea were not statistically significant. Calves may become sources of Cryptosporidia infection to man and other animals in the study area through unrestricted movements and interactions with the environment.

Keywords: *Calves, Cryptosporidium, Diarrhoea, Rahaji, Rima River, Sokoto.*

PREVALENCE DE L'OOCYSTE DE CRYPTOSPORIDIUM CHEZ LES VEAUX PAISSANT LE LONG DE LA RIVE DU FLEUVE RIMA A SOKOTO (NIGERIA)

Résumé

La présente étude a été réalisée dans le but d'évaluer la prévalence ponctuelle de l'infection aux oocystes de Cryptosporidium chez les veaux paissant le long de la rive du fleuve Rima à Sokoto en octobre 2011. La rive est une zone de convergence des animaux domestiques élevés dans les différents quartiers de la ville et les villages environnants. Un nombre total de 2959 bovins a été recensé, dont 147 (4,97%) veaux. Des échantillons fécaux ont été recueillis à partir de 100 (68,02%) veaux selon la technique d'échantillonnage pratique. Les techniques de sédimentation formaline –éther et de coloration Ziehl-Neelsen modifiée ont été utilisées pour identifier les oocystes de Cryptosporidium dans les échantillons fécaux. La consistance des matières fécales a également été utilisée pour identifier les veaux diarrhéiques et non diarrhéiques. Les oocystes de Cryptosporidium ont été identifiés dans 33 cas (33,0%) des veaux examinés. Le taux de détection était plus élevé chez les veaux mâles (38,46%) que chez les femelles, et la race Rahaji avait la plus forte prévalence de 62,5%. Au total, 6 (18,18%) des cas positifs étaient diarrhéiques. Les différences de prévalence fondées sur le sexe, la race et la présence de diarrhée n'étaient pas statistiquement significatives. Les veaux peuvent devenir des sources d'infection à cryptosporidia pour l'homme et d'autres animaux dans la zone d'étude à cause des mouvements illimités et des interactions avec l'environnement.

Mots-clés : *veaux, Cryptosporidium, diarrhée, Rahaji, fleuve Rima, Sokoto.*

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Introduction:

Cryptosporidiosis is an infection caused by the protozoan *Cryptosporidium* which is characterised by acute onset of gastroenteritis (Atherton *et al.,* 1995) and has been identified as the cause of many outbreaks of diarrhoea in humans and animals all over the world (Fayer *et al.,* 2000; Fayer, 2004). *Cryptosporidiosis* has been reported to affect mainly very young and immunocompromised animals. Calves and lambs of 1 to 3 weeks of age appear to be most susceptible (Ayeni *et al.,* 1985; Colville and Berryhill, 2007). *Cryptosporidium* has been found in 2.4% to 100% of calves and cattle (Castro-Hermida *et al.,* 2002), 1.45% to 59% of lamb and sheep (Nouri, M and Karami, 1991; Causape *et al.,* 2002) and 4.6% to 6.4% of kids and goats (Gorman *et al.,* 1990; Gorman Minas *et al.,* 1994). In Nigeria, Ayinmode and Fagbemi (2010), obtained 28.1% (n=96) from 7-12 months old calves and 27.3% (n=84) from calves less than 6 months in a south west state.

Like in animals, immunocompromised and young humans suffers severe and life threatening, profuse, intractable diarrhoea with *Cryptosporidium* species (Smith, 1990), particularly people living with HIV/AIDS (Radostis *et al.,* 2004). Animal to human transmission has been established (Reynoldson, 1999) and importance of water as a route of transmission has been increasingly recognised (Casemore, 1990; Anonymous, 1990). Contact with contaminated drinking and recreational waters with humans or animal faeces are major sources of waterborne zoonotic transmission. In addition, *Cryptosporidium parvum* oocysts were detected in almost all of the environmental waters tested (Smith and Rose, 1998). Cattle manure is a recognized source of *Cryptosporidium parvum* oocysts (Hiepe and Buchwalder, 1991; Pell, 1997) and unless manure management or treatment strategies are used to minimize oocysts viability or transport to water. Similarly, disposal of faeces, farmyard manure or other contaminated waste in land-based dump sites, when followed by periods of heavy rainfall can lead to Cryptosporidium oocyst contamination of water courses (OIE, 2005).

It was observed that the presence of animals along the Rima River coupled with the process of grazing and indiscriminate deposition of animal faeces along the river bank can lead to environmental and water pollution with microorganism. Therefore, the main objectives of the present study were to determine the prevalence of cryptosporidiosis in calves grazing along Rima River bank and to examine the potential risk posed to animals and man by consumption of water from the river.

Material and Method

The Rima River is located north of Sokoto metropolis and it is a major source of raw water to the Sokoto main water treatment plant that is situated at a short distance to the river bank. The river also serves as source of irrigation for vegetable farms and converging area for domestic animals from different households and surrounding settlements. The animals are collected from different households in the morning by paid herders, moved on hoof towards the river where they are grazed and watered till evening when they return to their respective homes. The team moved in on the 22nd October, 2011, enumerated the cattle herds present at that point in time, identified the calves and selected the sampled calves by convenient sampling technique based on cooperation from the herders.

Faecal samples were collected *per rectum* of calves with gloved hand using convenient sampling technique (Cameron, 1999), put in sterile bottles and transported to the Veterinary Public Health Laboratory, Usmanu Danfodiyo University, Sokoto. The faecal matter were processed by formo-ether sedimentation technique and identification of the oocysts by modified Ziehl-Neelsen (mZN) staining as described in OIE Manual (OIE , 2005) and then examined for oocysts under the microscope using × 40 object (low power) magnification (Cheesebrough, 1999). During faecal collection the sexes of the animal were determined using external genitalia, age estimation by asking for the calving date and confirmed by the dentition, breeds through their external body features and presence of diarrhoea was recorded. Detection rate(s) of *Cryptosporidium* oocysts in each sample were compared by sex, age, breeds and presence or absence of diarrhoea, using Chi-square test at 5% level of significance.

Table 1: Prevalence of *Cryptosporidium* oocysts in calves in relation to sex

Sex	Number of sample examined	Number of samples positive	Prevalence (%)
Male	26	10	38.46
Female	74	23	31.03
Total	**100**	**33**	**33.00**

P – value = 0.491, X^2 (DF), 0.474(1)

Table 2: Prevalence of *Cryptosporidium* oocysts in relation to presence or absence of diarrhoea

Diarrheic status	Total Sample	Positive	Prevalence (%)
Diarrheic	26	6	23.07
Non diarrheic	74	27	36.48
Total	**100**	**33**	**33.00**

P – value = 0.221, X^2 (DF), 1.565(1)

Table 3: Prevalence of *Cryptosporidium* oocysts in different breeds identified

Breeds	Total Sample	Positive	Prevalence (%)
Cross	42	15	35.71
Rahaji	26	10	62.50
Sokoto Gudali	24	6	25.00
White Fulani	8	2	25.00
Total	**100**	**33**	**33.00**

P – value = 0.702, X^2 (DF), 1.417(3)

Results

Out of 100 bovine faecal samples examined, 33 (33%) were positive for *Cryptosporidium* oocysts. Oocysts detection rate of 38.46% and 31.08% were obtained for male and female calves respectively (Table 1). In this study, no significant association (P>0.05) exist between the three variables analysed (sex, age, breeds and presence or absence of diarrhoea) in calves. Of the total 100 samples collected, 26 (26%) were diarrheic, out of which, 6 (23.07%) were positive for infection, while out of 74 non diarrheic samples, 27 (36.48%) were positive for *Cryptosporidium* oocysts (Table 2). Four different breeds were identified during sampling, and these include Cross, Rahaji, Sokoto Gudali and White Fulani. Infection rate was highest for Rahaji breeds (62.5%) and lowest among Sokoto Gudali and White Fulani (25%) (Table 3).

Discussion

The faecal carriage of Cryptosporidium oocysts in calves in this study is relatively high and similar to some reports from Nigeria, Africa and rest of the world (Joachim *et al.,* 2003; Faleke *et al.,* 2006; Kreausukon *et al.,* 2008; Ayinmode and Fagbemi, 2010). The high prevalence may be attributed to wetness and high humidity along the river bank which can encourage oocysts survival and increase their viability. The finding of higher prevalence in males agreed with the report of Maikai *et al.* (2009), this may be due to the tendency of male animals to disperse to other herds from different cattle concentration. In this report prevalence of *Cryptosporidium* oocysts was higher in non diarrheic calves and this concurs with the findings of Ayinmode and Fagbemi (2010). This could be an indication of on-going infections. It has been reported that presence of microorganisms such as *Salmonella, Campylobacter, Yersinia, Escherichia coli,* rotavirus, helminthes and coccidia are highly associated with diarrhoea in livestock and they may occur as mixed infection (Adesiyun and Kaminjolo,

1994). Detection of *cryptosporidium* oocysts among calves in this study are of concern as the infected calves mingled with non infected animals and also interact with man in their various households. This can lead to spread of infection in man and animals. The faecal droppings can also contaminate the environment and the water body.

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RISK FACTORS ASSOCIATED WITH GASTROINTESTINAL NEMATODE INFECTIONS OF CATTLE IN NAKURU AND MUKURWEINI DISTRICTS OF KENYA

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Abstract

A study was carried out in Nakuru and Mukurweini districts of Kenya to identify the risk factors associated with gastrointestinal nematode (GIN) infection in cattle on 128 dairy farms between June 16th 2010 and August 30th 2010. Faecal samples were collected from the rectum of 419 heads of cattle that were above three months of age on the selected farms, refrigerated and delivered to the Department of Veterinary Pathology, Microbiology and Parasitology, Faculty of Veterinary Medicine, University of Nairobi, for GIN analyses (McMaster method) within 7 days.

Questionnaires were administered on every farm to collect individual animal and farm management data. Logistic regression analysis was carried out (univariable and multivariable), and a model developed using a backward elimination method.

The univariable analysis revealed that animal age, district, time to last deworming, frequency of manure removal, source of forages, and the type of dewormer used last as the factors associated with GIN infections in cattle. The final regression model indicated that animal age, farm district, time to last deworming, and the type of dewormer used last as the factors associated with nematode infections in cattle. The study concluded that grazing management and the deworming management, particularly among young animals, were the main factors associated with cattle GIN infections.

Key words: *Risk factors, cross-sectional design, cattle, gastrointestinal nematodes.*

FACTEURS DE RISQUE ASSOCIES AUX INFECTIONS AUX NEMATODES GASTRO-INTESTINAUX DES BOVINS DANS LES DISTRICTS DE NAKURU ET DE MUKURWEINI AU KENYA

Résumé

Une étude a été réalisée dans les Districts de Nakuru et de Mukurweini au Kenya dans le but d'identifier les facteurs de risque associés à l'infection aux nématodes gastro-intestinaux (NGI) chez les bovins dans 128 fermes laitières entre le 16 juin 2010 et le 30 août 2010. Des échantillons de matières fécales ont été prélevés dans le rectum de 419 bovins âgés de plus de trois mois sur les fermes sélectionnées, réfrigérés et remis au Département de Pathologie Vétérinaire, Microbiologie et Parasitologie de la Faculté de Médecine vétérinaire de l'Université de Nairobi, aux fins d'analyses des GIN (méthode McMaster) dans un délai de 7 jours.

Des questionnaires ont été distribués à chaque ferme afin de recueillir les données sur chaque animal et sur la gestion de la ferme. Une analyse de régression logistique a été réalisée (à variable unique et à plusieurs variables) et un modèle développé en utilisant une méthode d'élimination régressive.

L'analyse univariable a révélé que l'âge des animaux, le district, le temps écoulé depuis le dernier déparasitage, la fréquence d'enlèvement du fumier, la source des fourrages, ainsi que le type de vermifuge utilisé la dernière fois étaient des facteurs associés aux infections GIN chez ces bovins. Le modèle de

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régression final a indiqué que l'âge de l'animal, le district où se situe la ferme, le temps écoulé depuis le dernier déparasitage, et le type de vermifuge utilisé la dernière fois étaient des facteurs associés aux infections par des nématodes chez les bovins. L'étude a conclu que le mode de gestion des pâturages et du déparasitage, en particulier chez les jeunes animaux, étaient les principaux facteurs associés aux infections GIN chez les bovins.

Mots-clés *facteurs de risque, analyse transversale, bovins, nématodes gastro-intestinaux.*

Introduction

Gastrointestinal nematode (GIN) infections in ruminants are an important problem of the developing world, causing mortality and reduced productivity (Wanyangu and Bain, 1994; Gatongi *et al.,* 1997), particularly where nutrition and sanitation are poor (Sharkhuu, 2001; Faye *et al.,* 2003. The problem is greatest in tropical countries with good rainfall (Radostits *et al.,* 1994).

The epidemiology of GIN infections is determined by several factors influenced by parasite-host-environment interactions (Barger, 1989; Thamsborg *et al.,* 1996; Ng'ang'a *et al.,* 2004). The major risk factors can therefore, be broadly classified as parasite factors (anthelmintic resistance of the different species), host factors (genetic resistance, age and physiological status of the animal), and environmental factors (climate, stocking density and management). The importance of GIN infections will vary greatly from one year to the next and between geographical locations, depending on the prevailing climatic conditions and management (Wanyangu *et al.,* 1994). Moreover, stress, poor nutrition and concurrent disease may be associated with the release of hypobiotic larvae from the dormant state, leading to clinical GIN helminthosis. There is also a great variation in resistance between GIN species. While some studies have reported that goats are more susceptible than sheep to a similar challenge, others have reported that sheep usually suffer heavier worm burdens because of the difference in their grazing habits (Tembely and Hansen, 1996).

In order to control gastrointestinal nematodes, it is important to identify the risk factors associated with GIN infection. In Kenya, farmer's education, animal age category, deworming and grazing system have been identified as the main predictors of GIN infections (Odoi *et al.,* 2007). This paper describes a cross-sectional study that was carried out to identify the risk factors associated with GIN infections in dairy cattle of Kenya.

Material and Methods

Study area

The study was carried out in Mukurweini District of Nyeri County and Nakuru District of Nakuru County in Kenya between June 16th 2010 and August 30th 2010. Nyeri County is one of the five counties of Central Province and forms part of Kenya's central highlands (The Constitution of Kenya, 2010). Dairy farming is an important enterprise in Mukurweini District, with the farmers practicing zero-grazing methods, where pastures are cut and carried to the cattle.

Nakuru County is one of the 14 Counties of the Rift Valley Province and lies within the Great Rift Valley (The Constitution of Kenya, 2010). Dairy farmers in the area practice both zero-grazing, and semi-zero-grazing, where the cattle are housed but allowed to graze at certain times.

Study design

In Nakuru and Mukurweini Districts of Kenya, 64 farms were selected from each district to participate in the study. In Nakuru, a simple random selection was employed at the farm level using a sampling frame of the dairy farms provided by the District Livestock Production Officer. For logistical reasons, a purposive sampling method was used in Mukurweini, as the research was conducted alongside another project comparing smallholder dairy farms with and without biogas digesters (Dohoo *et al.,* 2012a; Dohoo *et al.,* 2012b). In that study,

biogas digesters were distributed to a group of smallholder dairy farmers considered representative of the various sub-districts and demographics of smallholder dairy farmers in the area, and the referent group of farmers was randomly selected. Due to the similarity of farming practices across smallholder dairy farms in the district (virtually all zero-grazing units), the Mukurweini sample of farms was considered a fair representation of the population in the district. In Mukurweini district, all cattle that were above three months of age on the selected farms were sampled for the study. In Nakuru District, where some farms were larger, a systematic random selection method was used to ensure that no more than 10 animals were sampled per farm. A total of 419 head of cattle were selected in the two districts (202 in Nakuru and 217 in Mukurweini).

Faecal samples from each animal on the selected farms were collected and analyzed for faecal egg counts (FEC) using a Modified McMasters technique (Ministry of Agriculture, Fisheries and Food, 1986), with a lower detection limit of 50 eggs per gram (epg).

A questionnaire on farm management was administered on every farm, in addition to individual data that were collected for every animal recruited. The potential risk factors studied included: age of the animal, parity (if a cow), body condition score (BCS - using a 1 to 5 scale, with 1 being thin and 5 being fat), bodyweight (using a heart girth tape), government or private veterinary service availability, frequency of deworming, timing and type of dewormers used last, availability of shelter, type of floor, type of bedding, manure removal frequency, and the grazing system practiced at the farm.

Statistical analysis

An animal was considered to have a GIN infection if the faecal egg count was 100 epg or higher (Hansen and Perry, 1994). This cutoff was selected because the McMasters egg counting method gave an output in multiples of 50 (each egg seen represented 50 eggs, and so one egg seen (representing 50 egg) could be a false positive due to the passing through of ingested eggs, or a very light level of parasitism.

Frequency tables showing the GIN infection status versus the risk factors were generated, and the percentage of the infected animals in each level of the risk factor (using the Pearson's chi square) was calculated to ascertain the univariable association between GIN infection and the potential risk factors.

A logistic regression model was fitted to determine significant factors associated with GIN infection (the outcome variable), while controlling for the effects of other factors and confounders, such as animal age (Dohoo *et al.,* 2009). Factors that were significant ($p< 0.10$) in the univariable analyses were eligible to be entered into the model. The logistic regression analysis used a backward elimination procedure and variables were considered significant and remained in the final model at $P<0.05$. The fitted model was examined for goodness of fit by plotting half normal plot with both rough and smooth envelopes to act as confidence intervals, and to examining Cook's statistics for influential observations (Collet, 1991). A mixed logistic regression model analysis with the herd and the district as random effects, controlled for clustering of animals within herds and herds within districts, respectively, was used to confirm the results of the logistic regression model.

Results

Univariable analysis of factors associated with gastrointestinal nematode infections

Breed, age, gender, district, source of forage, frequency of deworming, time of last deworming, and product used at last deworming were univariably significantly associated ($p<0.10$) with cattle GIN infections, as described in Table 1. These variables were eligible for entering into the final models used to predict GIN infections.

There was a higher prevalence of GIN infection among animals on the farms that got forage from sources where many other cattle could graze, either by cutting from roadsides and carrying to the animals, or by communal grazing, compared with other sources of forages with limited or no other cattle exposure ($p = 0.009$).

Table 1: Univariable results of factors associated with gastrointestinal nematode infection from 419 dairy cattle in Nakuru and Mukurweini districts between June 16th 2010 and August 30th 2010.

Explanatory variables	Levels	Totals	Proportions infected (%)	χ^2	p value
Animal breed	Friesian	305	19	7.8	0.02
	Ayshire	88	11		
	Guernsey	26	27		
Animal age	3- 12 months	146	22	12.2	0.001
	>12 months	273	10		
Animal gender	Female	401	13	5.99	0.014
	Male	18	33		
Body condition1	Poor	143	18	3.43	0.064
	Good	276	12		
District	Nakuru	202	20	11.6	0.001
	Mukurweini	217	8		
Time of last dewormer	>6 months	38	29	9.44	0.001
	3 -6 months	82	13		
	< 3 months	299	9		
Frequency of deworming	>6 months	63	29	18.7	0.002
	3 -6 months	193	13		
	< 3 months	163	9		
Source of forage	Cut from farm	125	11	13.6	0.009
	Cut from other farms	176	11		
	Cut on roadside	12	25		
	Grazing on farm	34	9		
	Communal grazing	72	26		
Product used at last deworming	Albendazole	74	18	11.0	0.012
	Ivermectin	65	12		
	Levamisole	107	21		
	Unknown	173	8		

Multivariable analysis of factor for gastrointestinal nematode infection

The final multivariable logistic regression model indicated that animal age, district the animals were in, time to last deworming, and product used at last deworming were associated with GIN infections in cattle, controlling for clustering within herds, and the confounding effects of other variables. A mixed logistic regression model analysis with the herd and the district as random effects, controlling for clustering of animals within herds and herds within districts, respectively, confirmed these findings. Based on this final model, a higher odds of GIN infection was found in younger versus animals > 12 months of age, and in animals in Nakuru versus Mukurweini district. The odds of an animal having a GIN infection increased as the time to last deworming increased, although > 6 months ago was not greater than 5 months ago, and the odds of an animal having a GIN infection increased if it was on a farm with deworming frequencies less often than every 6 months versus a quicker frequency of deworming. Animals last treated with albendazole or levamisole had a higher odds of GIN infection versus animals last treated with ivermectin or an unknown dewormer. Source of forage, body condition, breed and gender did not remain significant in the final models.

The half normal plot and Cook's statistic plots demonstrated good fit of the final model to the data.

Discussion

The results of this study showed that animal age, district, and deworming management were significantly associated with the prevalence of GIN infections in dairy cattle. The results agree with those of a previous study carried out on smallholder mixed farming systems in Central Kenya highlands, which identified animal age, farmer's education, and deworming and grazing system as the main factors associated with nematode infections (Odoi *et al.,* 2007). A study carried in Kiambu District of Kenya also showed that cattle between weaning and one year of age were more prone to GIN infections, and that the highest prevalence of infection was found among this group (Waruiru *et al.,* 2001). Older animals have more developed innate and adaptive immune systems to counter GIN infections (Male *et al.,* 2006).

The nematode infection risk was shown to be higher in male animals; however there was a confounding effect with the age of animals, where all the male animals encountered in both districts were calves, as the farmers did not prefer to keep older males. Therefore, gender was not significant in the final model.

In the univariable analysis, cattle with poor BCSs had higher GIN infection prevalences compared with those with higher scores. However, there was a possibility of a reverse causation bias, not uncommon with cross-sectional studies (Dohoo *et al.,* 2009).

Table 2: Multivariable results of factors associated with gastrointestinal nematode infections from 419 dairy cattle in Nakuru and Mukurweini districts between June 16th 2010 and August 30th 2010.

Factor	Odds Ratio	P value
Age		
Aged < 12 months (referent category)	1	-
Aged above 12 months	0.3172	<.001
District		
Nakuru (referent category)	1	-
Mukurweini	0.3384	0.001
Time to last deworming		
Last deworming < 1 month ago (referent category)	1	-
Last deworming 2 months ago	1.737	0.362
Last deworming 3 months ago	2.166	0.136
Last deworming 4 months ago	4.848	0.023
Last deworming 5 months ago	9.901	0.002
Last deworming > 6 months ago	7.081	0.001
Frequency of deworming		
Dewormed less often than every 6 months (referent category)	1	-
Dewormed every 3 -6 months	0.3541	0.056
Dewormed every 0-3 months	0.3247	0.025
Product used at last deworming		
Albendazole (referent category)	1	-
Ivermectin	0.6890	0.481
Levamisole	1.064	0.868
Last dewormer unknown	0.2058	0.001

It would be hard to conclude whether poor body condition was a clinical sign of the GIN infection or a predisposing factor using this kind of study, because the duration of the infection is unknown. Also, newborn calves are born in lean body condition for calving ease, and age was a significant factor in the final model, therefore, age would confound the relationship between BCS and GIN infection, hence BCS was not significant in the final model.

Time to last deworming was an important factor for nematode infection, with the infection prevalence being highest among dairy animals that had not been dewormed in the last 5 to 6 months compared with animals dewormed within the previous five months. While pre-patent periods of GINs can be less than a month (Georgi & Georgi, 1997), re-infection can take substantially longer, even up to 6 months, because the nematode lifecycle involves environmental contamination, ingestion, infection and deworming animals breaks this lifecycle, reducing the adult worms that shed eggs, thereby reducing egg counts in manure, and pasture contamination of infective larvae.

Cattle on farms that dewormed at a frequency interval greater than six months also recorded a higher risk of GIN infections. Most farmers in this study practiced a deworming frequency of less than 3 months, which explained the overall low prevalence of nematodes (Kabaka *et al.,* 2012). This finding is in agreement with reports from other authors, that under traditional free-range grazing systems there is continuous infection and re-infection from heavily contaminated pastures, rendering anthelmintic treatment of short-term value compared with the situation under zero-grazing (Waller, 2004).

An interesting observation was that cattle of farmers who could not recollect the last dewormer used recorded lower prevalence of nematode infections. This was probably because this group of farmers used the veterinary service providers and that is why they did not know which drug had been used on their animals. The veterinary service providers would likely know which dewormers would be most effective against GIN infections, leading to a lower prevalence of GIN infection.

The significantly higher GIN infection in animals on farms that sourced forages from the roadside or by communal grazing could be explained by the contamination of roadside and communal pastures with infective larvae from animals from multiple sources. Minimization of faecal contamination on forage sources can be an effective measure for the control of GIN infections.

Conclusion

The significant factors associated with GIN infection in cattle were the age of the animal, the district in which they lived, the time from last deworming, the frequency of deworming, and the kind of dewormer used last. A regular deworming interval of three to four months should be encouraged among the young stock which is the most vulnerable group, especially in high risk areas.

The overall GIN infection prevalence was lower in Mukurweini as compared with Nakuru district. The most salient difference in the two districts is the grazing system used, where farms in the former practice zero-grazing systems almost entirely, and thus housing with zero-grazing should be advocated as a long-term control strategy for nematodes.

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ANTIBIOTICS SUSCEPTIBILITY PATTERN OF ESCHERICHIA COLI STRAINS ISOLATED FROM BROILER AND LAYER CHICKENS WITH COLISEPTICEMIA IN SUDAN

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Abstract

This study was carried out to determine the susceptibility of *E coli* strains isolated from broiler and layer chicken with colisepticemia to antibiotics used in poultry industry in the country. A total of fifty seven *E. coli* strains isolated from 43 broiler and14 layer farms with colisepticemia in Khartoum and Gezera state were investigated for antimicrobial susceptibility to ten antibiotic agents of Veterinary and human significant .Antibiotic activity against the isolate were determined by Disc diffusion test. Antimicrobial resistance of isolates was found for Gentamycin (26%) and Ciprofloxacin (39%) as less resistant antibiotic, Lincomycin (98%) and kanamycin and amoxicillin (95%) as more resistant antibiotics. multiple drug resistances were observed in all isolates. Twenty nine different resistance patterns were demonstrated Results obtained confirmed the presence of antibiotic resistant to poultry pathogen in poultry farms in Khartoum and Gezera State. It is recommended that antibiotic use in the management of collibacillosis in the farms should be based on the result of susceptibility tests because other than poultry health problems transmission of resistant *E coli* to human can occur.

Key words: *antibiotic susceptibility. Colisepticemia, Escherichia coli, Chicken, Khartoum and Gezera, Sudan*

PROFIL DE SENSIBILITE AUX ANTIBIOTIQUES DES SOUCHES D'ESCHERICHIA COLI ISOLEES CHEZ DES POULETS DE CHAIR ET DES PONDEUSES AYANT UNE COLISEPTICEMIE AU SOUDAN

Résumé

A Khartoum et dans l'État de Gezera, cinquante-sept souches au total de *E. coli* isolées chez 43 poulets de chair et 14 poules pondeuses ayant un*E coli*septicemie ont fait l'objet d'une étude dans le but de déterminer leur sensibilité antimicrobienne à dix antibiotiques de signification vétérinaire et humaine. L'activité antibiotique contre l'isolat a été déterminée par un test de diffusion sur disque. Concernant la résistance aux antimicrobiens, l'étude a révélé que les isolats étaient moins résistants à la gentamicine (26%) et à la ciprofloxacine (39%), mais plus résistants à la lincomycine (98%) et à la kanamycine et l'amoxicilline (95%). Les résistances à plusieurs médicaments ont été observées pour tous les isolats. Vingt-neuf profils de résistance différents ont été identifiés. Les résultats obtenus ont confirmé la présence de pathogènes de poulets résistant aux antibiotiques dans les élevages avicoles de Khartoum et de l'État de Gezera. Il est recommandé de procéder à des tests de sensibilité et d'utiliser les résultats pour déterminer les antibiotiques efficaces dans la gestion des collibacilloses dans les fermes, car outre les problèmes de santé qu'elles causent aux poulets, la transmission de souches résistantes d'E. coli à l'homme est possible.

Mots-clés : *sensibilité aux antibiotiques. colisepticémie, escherichia coli, poulets, Khartoum et Gezera, Soudan*

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Introduction

As a result of the increase in production of poultry, egg and poultry meat have become important sources of protein in Sudan, resulting in an increased per capita consumption. Colisepticemia is one of the important *E.Coli* syndromes responsible for significant economic loss in poultry. *E coli* is an organism that usually cause secondary bacterial infection and may also be a primary pathogen in birds (Gross. 1994). Both broilers and layers are susceptible to the disease (Foley *et al.*,. 2000 and Barnes and Gross, 1997). In Sudan both incidence and severity of Colibacillosis have increased rapidly (Omer *et al.,* 2010). Biosecurity measures, vaccination and use of competitive exclusion products were used to prevent and control the disease in some areas but with limited value (Gomis *et al.,* 2003; La Ragione *et al.,* 2001). Presently antibiotic therapy is an important tool to control outbreaks of Colibacillosis (Freed *et al.,* 1993). Uncontrolled widespread use of antibiotic agents in poultry industry are a regular practice in Sudan where colibacillosis is common and severe on poultry farms. The permitted uses of antibiotic agents vary among countries and regions (Carlton, 2008). Antibiotic misuse and/or improper usage is an important factor that help the emergence, selection and spreading of antibiotic-resistant *E.coli* and other microorganisms in both veterinary and human medicine (Van Den Bogaard *et al.*, 2001 and Witte,1998). Previous studies showed that *E coli* of poultry are commonly resistant to one or more antibiotics especially if used over a long period (Allan *et al.,*1993). Recent reports showed an increased resistance to antimicrobial agents which are commonly used for treatment (Yang *et.al.,* 2004; Cormican *et al.,* 2001). The increased antibiotic resistance in avian pathogenic *E.coli* in the country is worrying and indicates that widespread use of antibiotics for treatment, prophylaxis and as feed additives may lead to serious problem for both human, animal health and the environment in view of the significance of Colibacillosis and appearance of drug resistant strains and difficulties in treatment of *E. coli* infection in poultry farms in Sudan.

Materials and Methods

Area of study

Khartoum and Gezera state which are considered the most important states in order to contain the largest number of poultry farms in Sudan.

Description of samples

Fifty seven outbreaks of colisepticmia 43 broiler and 14 layer farm) were investigated. Affected flocks were of different location, ages, breeds, type and farmproduction system design Fig 1, 2, 3 from each outbreak five affected bird were subjected to Pathological to Pathological and bacteriological examination.

Isolation and identification

Samples which include liver and heart were cultured on MacConkey Agar (Oxoid) and sheep blood agar. Bacterial colonies that were lactose fermenter were tentatively identified as *E. coli*. Presumptive *E coli* isolates were confirmed with different biochemical tests using (API bioMerieux).

Antibiotic susceptibility test

E coli susceptibility to 10 antibiotic agents was determined by the disk diffusion method (Kirby-Bauer,1966).Sensitivity to the 57 *E coli* strains were determine against ampicillin AMP (10ug), Gentamycin GM (10ug) lincomycin L(2ug), Doxycycline D (30ug), streptomycin S (10ug), kanamycin K (30ug), amoxicillin AMX(30ug), ciprofloxacin CP(5ug), tetracycline TE (30ug), and Nitrfuantion NT(300ug). The diameters of the zones of inhibition were interpreted with reference to NCCLS (2001).

Results

E coli was isolated in a pure culture from all outbreaks investigated in broiler chicken. Perhepatitis and percaditis was obsereved at necropsy, a significantly higher incidence of field outbreaks (53%) were found in Cobb breed reared in an open system farms fig (2&3). Mortality rate in broiler ranged from 1%- 12% highest rate was reported in an open system farms, and 1.9% in the layers. Highest

percentage of *E coli* isolated from broiler chicken showed resistance to lincomycin (100%), Amoxycillin(95%) Kanamycin(93%),Te tracycline(86%), Doxycyclin and Nitrofuration (81%) Ampicillin(79%),Streptomycin(51%)and Ciprofloxacin(42%).Low levels of resistance was against Gentamycin (23%) percentage of isolates to the antibiotic agents were shown in table(3).In layer chicken main lesions observed include perhepatitis, percaditis, salpingitis and egg peritonitis. Antibiotic susceptibility tests results revealed that highest percentage of *E coli* isolates was resistant to Ampicillin and Kanamycin(100%), Amoxycillin, Lincomycin and Tetracycline(93%), Nirofuration (86%), Doxycycline, Streptomycin and Gentamycin (36%) while low levels of resistance was against Ciprofloxacin (29%). Percentage of isolates to the antibiotic agents are shown in Table 4. Resistance of 81%, 51%and 42% broiler isolates to Doxycycline, Streptomycin and Ciprofloxacin, respectively, were significantly higher than those isolated from layer 36%,36% and 29%. All the 57 isolates showed multiple resistances to at least 6 antibiotic agents. Twenty nine different patterns of resistance were obtained from the antibiotic agents used in this study Table 4. The most common resistance patterns were Am/K/AMX/L/NT/TE/D/S (14%), 81.AM/K/AMX/L/NT/D/TE (10%).

Discussion

The present study demonstrates that *E coli* is the causative agent of the septicaemia and mortality of birds in commercial broiler and layer farms investigated in Khartoum and Gezera states. Clinical signs and lesions observed in the affected birds are considered as typical signs of Colisepticemia caused by highly virulent *E coli* strains as reported by (Krishanmohan Reddy and Koteeswaran, 1994). Mortality rate observed during this study were in agreement with Zanella *et al.* (2000), who reported 5-10% mortality due to *E. coli* infections. However, these findings disagree with Omer *et al.* (2008) who reported 1 % mortality rate of the disease in broilers. From this study it was observed that many factors might play a role in the occurrence of the outbreaks such as farm design, bad management and poor hygienic measures as observed in number of farms investigated. It was also observed that the breed of chickens might be one of the factors associated with the susceptibility and severity of Colibacillosis as Cobb breed was found more susceptible (53%) to the disease in broiler chickens. Multiple antibiotic resistances was observed in all of the 57 *E coli* isolates, such high incidence of multidrug resistance may most likely be due to indiscriminate use of antibiotic at the present time. Resistance to six or more antibiotic agents was found similar to findings of previous studies in Sudan and other countries (Bass *et al.,* 1999; Guerra *et al.* 2003). Yang *et al* (2004) Miles *et al* (2006)., Zahraei Salehi and Farashi Bonab. (2006) Omer *et al.*, (2010) in China noted that 80% of *E coli* isolated from the livers of chicken was resistant to eight or more antibiotic agents. The multidrug resistant *E coli* is continuously increasing which were also reported by Hussain *et al* (1982) and Nazir (2004). *E coli* isolates from both broiler and layer chicken was found resistant to Lincomycin, Amoxicillin Kanamycin, Tetracycline, Nitrofuration and Apmicillin. Similar result were reported by Rahman *et al.*, (2008) Omer *et al.,* (2010) and Daini and Adesemowo, (2008). These antibiotics were extensively used in the poultry industry. For this reason these antibiotics are inactive against pathogenic *E coli*. strains at the present time. Results showed that 84% and 88% of isolates was resistant to Ampicillin, and Tetracycline similar results were reported by Rahman *et al* (2008); Omer *et al.,* (2010), Ozawa *et al.,* (2008) and Daini and Adesemowo, (2008). Resistance to streptomycin in layers of 36% do not agree with the results recorded by Al-Ghamdi *et al.,* (2001) and Rahman *et al.,* (2008). During this study 42% of *E coli* isolated form broiler chicken were resistant to Ciprofloxacin while 29% were found resistant in layer chicken. These results do not agree with the results of a study conducted by Omer *et al.,* (2010) who reported 100% resistant to Ciprofloxacin in Kassala state. Different resistance patterns to the drug among organism isolated from different regions Khartoum and Gezera state and other area could be related to properties of pathogenic bacteria and to the difference

Table 1: Distribution of broiler chicken

State	District	Breed/age	Rearing system
Khartoum	Gabal Awlia	Cobb/21D	Open
		Ross/30D	Close
		Ross32d	Close
		Ross25d	Open
		Ross1m	Close
	Alkalakla	Cob/17D	Open
		Ross/25D	Open
		Cobb20d	semiclosed
	Taiba alhassanab	Hubbard/30	Close
	Al shigilab	Hubbard/9D	Close
		Cobb/23D	Open
	Alhalfaia	Cob/34D	Open
		Cob/39D	Open
	shambat	Cobb/26D	Open
		Cob/46D	Open
	Alkadaro	Cobb/25D	Open
		Cobb/39D	Open
	Omdurman	Cobb/22D	Close
	Hiltkoko	Cob/23D	Open
		Cobb32d	Clsed
	Soba	Ross/23D	Close
		Cobb/42D	Close
		Cobb/24D	Close
		Cobb20d	Open
		Hubbard21d	Open
		Cobb20d	Open
		Ross	Close
	AldikhiAnat	Cobb26d	Open
Gezera	Altee	Hubbard/36D	Close
	Butri	Cobb/24D	Open
		Cobb/21D	Open
		Cobb/15D	Open
		Cobb/25D	Open
		Hubbard 28d	Open
	Algadid althora	Cobb/21D	Open
		Cobb15d	open
	Albagiar	Ross/23D	Close
		Cobb/17D	Close
		Cobb26d	Open
		Cobb15d	Open
	Medanee	Cobb/29D	Open
	Hasahesa	Cobb/19D	Open

Table 2: Distribution of layer chicken

District	Town	Breed/age	Rearing System
Khartoum	Gabalawli	Hisex/4W	Close
	Gabalawli	Hisex/18day	Close
	Taiba Al hassanab	Hyline/2M	
Hisex/4W	open		
open			
	Alshigilab	Hisex/5W	open
	Omdurman	Hisex 74W	closed
	Al kabashi	Hyline 6M	open
	Soba	Hisex 5 m	open
	Soba	Hisex 36d	closed
	Al halfaia	Lohman 3m	Open
	alsalamania	Hisex 35w	Closed
Gezera	Algadid althora	Hisex/5M	open
	Butri	Hisex 4m	open

Table 3: Percentage of antibiotic susceptibility of isolated *E coli* strains from broiler chicken with coli septicemia in Khartoum and Gezer state Sudan

Antibiotic agent	Susceptible %	Resistant%
Ampicillin	21	79
Kanamycin	7	93
Amoxycillin	5	95
Lincomycin	0	100
Nitrofuration	19	81
Tetracycline	14	86
Doxycycline	19	81
Streptomycin	49	51
Ciprofloxacin	58	42
Gentamycin	77	23

Table 4: Percentage of antibiotic susceptibility of isolated *E coli* strains from layer chicken with coli septicemia in Khartoum and Gezer state Sudan

Antibiotic agent	Susceptible %	Resistant%
Ampicillin	0	100
Kanamycin	0	100
Amoxycillin	7	93
Lincomycin	7	93
Nitrofuration	14	86
Tetracycline	7	93
Doxycycline	64	36
Streptomycin	64	36
Ciprofloxacin	71	29
Gentamycin	64	36

Table 5: Percentage of antibiotic susceptibility of isolated *E coli* strains from Chicken with coli septicemia in Khartoum and Gezer state Sudan

Antimicrobial agent (ug)	% Avian isolates (n=57)	
	R	S
β-lactum		
Ampicillin (10)	84	16
Amoxcillin (20)	95	5
Aminoglucoside:		
Gentamycin (10)	26	74
Kanamycin (3)	94	6
Streptomycin (10)	47	53
Tetracycline:		
Doxycycline (30)	70	30
Tetracycline (30)	88	12
Nitrofurans:		
Nitrofurantion (300)	83	27
Lincosamidise:		
Lincomycin (2)	98	2
Quinolones:		
Ciprofloxacin (5)	39	61

in the rate and usage of antibiotics (Afshin Zakeri and Pedram Kashefi., 2012). 23% and 6% of broiler and layer isolates were found resistant to gentamycin different results were recorded by Rahman 2008 and Daini and Adesemowo (2008) who report 54% of *E. Coli*.isolated from Nigeria were resistant to gentamicin. Resistance against Gentamycin and ciprofloxacin,was 30% and 48% respectively indicating that these drugs may still be effective in the treatment of *E. coli* infections in the poultry farm. Isolation of ciprofloxacin resistant *E. coli* strains was significantly high in broiler farm 42%. This event might be due to extremely use of Ciprofloxacin for treatment of the disease in poultry because of its very good effect against *E. coli*. Pandey *et al* (1998) reported that the majority of E Coil strains were sensitive to gentamicin and ampicillin and were resistant to tetracycline which supports the present finding but there is some variation in the sensitivity tests results with the earlier reports because some antibiotics became resistant due to their commonly used in poultry feed and treatment of diseases as mentioned by Prasad *et. al* 1997 concluded that the highest sensitivity to Quinolone antibiotic is because they are recently introduce in poultry industry and of limited use by the poultry farmers which is also suggestive for the present study.

In conclusion finding of these study and other results were compared and clearly demonstrate the increasing problem of drug resistance in poultry population , high and increasing resistance to some common antibiotics in poultry farms, probably due to use of antibiotics as feed additives for growth promotion, prevention and treatment of diseases and use of inappropriate antibiotics for treatment of diseases. use of these drug may cause serious problems because they do not have curative effects, increase economic costs and cause human problems when antibiotic residues remain in the poultry meat and egg. Very little data is available on the epidemiology, prevalence or the mechanisms of antimicrobial resistance in animal feed pathogens in Sudan Thus, introduction of surveillance programs to monitor antimicrobial resistance in pathogenic bacteria is Strongly needed in

Table 6: Pattern of antibiotic susceptibility in broiler Not necessary need to be simplified

isolate	Resistance	Sensitive
B1	Amp, K, AMX, L, NT, D CP	G,S ,TE
B2	Amp, K. AMX L, NT, TE D CP	G,S
B3	Amp, K, AMX, L, NT, , D CP	G,S ,TE
B4	Amp, K, L, NT, , D, S CP	G , TE, AMX
B5	Amp, K, AMX, L, NT, , D, S CP	G, TE
B6	Amp, K, L, NT D, S	G, CP, TE, AMX
B7	Amp, K, AMX, L, NT D CP	G,S ,TE
B8	Amp, K, AMX , L, NT TE, D S	G, CP
B9	Amp, K, AMX, L , ,TE , D S	G, CP, NT
B10	Amp, K, AMX, L TE D, S	G, CP, NT, K
B11	Amp, AMX, L, TE ,D S, G	NT, K, CP
B12	Amp, K, AMX, L, , TE D ,S	G, CP, NT
B13	Amp, AMX , L, ,TE, D S, G	CP, NT, K,
B14	Amp, K, AMX, L, , TE, D CP G,	S, NT
B15	Amp, K, AMX, L, TE D, ,S	CP, G, NT
B16	Amp, K, AMX, L, TE D CP G,	S, NT
B17	AMX, L, NT, TE D CP,	G, Amp, K, S
B18/	Amp, K, AMX, L, NT TE, G	CP, S, D
B19	K, AMX, L, NT, TE D,	CP, G, S, Amp
B20	Amp, K, AMX , L, NT TE D, S	Cp, G
B21	Amp, K, AMX , L, NT, TE D, S	CP, G
B22	Amp, K, AMX, L, NT, TE D S CP	G
B23	K, AMX, L, NT, TE D S CP,	G, Amp
B24	Amp, K. AMX, L, NT, TE D CP,	G, S
B25	Amp, K. AMX, L, NT, TE D CP	G, S
B26	Amp, K, AMX, L, NT, TE, G	CP, S.D
B27/	Amp, K, AMX, L, NT, TE, D	CP, G, S
B28	Amp, K, AMX, L, NT, TE, D	CP, G, S
B29	K, AMX, L NT ,TE, CP, G	Amp, S, D
B30	Amp, K, AMX, L, NT, TE, D	CP, G, S
B31	K, AMX, L, NT, TE,	CP, Amp, D, S, G
B32	Amp, K, AMX, L, NT, TE, D	CP, G, S
B33	K, AMX, L ,NT, TE,	CP, Amp.D, S.G
B34	Amp, K, AMX, L, NT ,TE , G	CP, D, S
B35	Amp, K, AMX, L, NT, TE, CP,	G ,S D
B36	Amp, K, AMX, L, NT, TE, D	CP, G, S
B37	K, AMX, L, NT , TE, D, ,CP G	Amp, S
B38	K, AMX, L, NT, TE, D, ,S CP	G, Amp
B39	Amp ,K, AMX, L, NT, TE, D, S	CP, G
B40	Amp, K, AMX ,L ,NT, TE D, S	CP, G
B41	Amp ,K, AMX ,L, NT, TE, D, S	CP, G
B42	Amp, K, AMX ,L, NT, TE , S,	CP, G
B43	K, AMX ,L ,NT , TE , S , CP ,G	Amp, D

Table 7: pattern of antibiotic resistance of *E coli* isolated from layer

Isolate	Resistance	Sensitive
L1	Amp ,K ,S, AMX ,L D,CP ,G	TE,NT
L2	Amp,K,S,AMX,L,TE, NT,G	CP,D
L3	Amp,K,S,AMX,L,TE,NT,G	CP,D
L4	Amp,K,S,AMX,L,TE, NT ,CP	G,D
L5	Amp,K,S ,AMX,L,TE, NT	CP,D,G
L6	Amp, K ,S ,AMX,TE,NT.CP	G,D,L
L7	Amp,K,AMX,TE,NT.L	CP,G,D,S
L8	Amp,K,L,TE,D,G,S	CP,NT,AMX
L9	Amp,K,AMX,L,S,TE,NT	CP,G,D
L10	Amp,K,AMX,L,TE,NT,CP,D	SG
L11	Amp,K,AMX,L,TE,NT, ,S	CP,G ,D
L12	Amp,K,AMX,L,TE,NT,S,G	CP,D
L13	Amp,K,AMX,L,TE,NT,S,D	CP,G
L14	Amp,K,AMX,L,TE,NT,D	CP,G,S

the country because in addition to animal health problems, transmission of resistant clones and resistance plasmids of *E. coli* from food animals (especially poultry) to humans can occur. Resistance to existing antibiotics is widespread and is a major challenge for both veterinarians and physicians. Therefore, it is important to carefully select antibiotics, preferably after antibiotic sensitivity testing and sensible use of such antibiotics at optimum doseage for sufficient duration to ensure effective treatment and control of various diseases caused by *E. coli* in poultry. Because of the wide variations in drug resistance patterns of the *E. coli* isolates, it is highly recommended that antibiotic use in the treatment of Colibacillosis of poultry in the farm should be based on the result of 156 susceptibility tests.

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PREVALENCE OF COAT COLOUR PHENOTYPES AND ITS INFLUENCE ON MANGE INFESTATION OF WEST AFRICAN DWARF GOAT

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Abstract

Prevalence of coat colour phenotypes and its influence on sarcoptic mange infestation of West African Dwarf (WAD) goats reared extensively by subsistence farmers in South-west Nigeria was investigated from March to October, 2011. The total number of goats randomly sampled from different villages within the same ecological zone were 11,772 consisting of 8,384 females and 3,388 males of different ages. Three basic coat colours were identified, namely black, brown and white accounting for 24%, 9.22% and 4.10% respectively. They probably constituted the underlying base for the caramel (bargerface), agouti and spotting patterns, giving rise to twenty combinations which accounted for the remaining 62.68% in the breed. The distribution pattern was similar for males and females. The number of goats randomly sampled and clinically inspected for presence of mange lesions on different parts of the body was 7,902. Standard parasitological procedure used to confirm the presence of mange mite from skin scrapings revealed that 42 goats (0.53%) were infested by Sarcoptes scabiei var. caprae. Infested cases were ranked according to severity of infestation as localized and mild (1), localized and moderate (2), localized and severe (3), generalized and mild (4), generalized and moderate (5) and generalized and severe (6) based on information from literature. Only five colour phenotypes (black, black with white marking, bargerface, white/brown mixed and white) were infested by mange. Black goats predominated (50%) the infested group, followed by those with black with white marking (23.81%), while white goats were least (4.76%). Least-squares analysis of variance showed that ranked estimate was significantly affected by coat colour ($P<0.05$). Goats with black and black with white marking were most affected with generalized and mild infestation, ranking 3.97±0.34 and 4.31±0.50, respectively ($P>0.05$). Those with bargerface and mixture of brown/white had similar ($P>0.05$) estimates (2.60±0.66 vs 3.52±0.93) while white goats were least affected with localized and mild infestation (0.88±0.18). The effect of age was significant ($P<0.01$) while sex was not significant. Animals less than one year were more affected with generalized and mild infestation, ranking 4.10 ± 0.58 compared with older animals (>1 year) that ranked 2.23 ± 0.55 with localized and moderate infestation. It is concluded that black goats were most prevalent and more susceptible to mange infestation while white goats were least affected. Selection in favour of other colour combinations that were not infested could further control prevalence of mange in the region.

Key words: *Goat, coat colour distribution, influence on mange infestation*

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PREVALENCE DE PHENOTYPES DE LA COULEUR DE LA ROBE ET SON INFLUENCE SUR L'INFESTATION DE GALE CHEZ DES CHEVRES NAINES D'AFRIQUE DE L'OUEST

Résumé

La prévalence de phénotypes de la couleur de la robe et son influence sur l'infestation de gale sarcoptique chez des chèvres naines d'Afrique de l'Ouest (WAD) élevées en régime extensif dans le sud-ouest du Nigeria a fait l'objet d'une étude réalisée de mars à octobre 2011. Le nombre total de chèvres échantillonnées de manière aléatoire dans des villages différents au sein de la même zone écologique était de 11.772, dont 8.384 femelles et 3.388 mâles d'âges différents. Trois couleurs de robe de base ont été identifiées - noir, brun et blanc - représentant respectivement 24%, 9,22% et 4,10%. Elles ont probablement constitué la base de l'aspect caramel (visage Barger), des motifs agouti et tacheté, et ont donné lieu à vingt combinaisons représentant les 62,68% restant dans cette race. Le mode de répartition était semblable pour les mâles et les femelles. Le nombre de chèvres sélectionnées de manière aléatoire et soumises à un examen clinique en vue de rechercher la présence de sillons creusés par la gale sur différentes parties du corps était de 7.902. La procédure parasitologique standard utilisée pour confirmer la présence d'acariens à partir de grattages cutanés a révélé que 42 chèvres (0,53%) étaient infestées par Sarcoptes scabiei var. Caprae. Les cas infestés ont été classés suivant la gravité de l'infestation dans les catégories « localisée et bénigne » (1), « localisée et modérée » (2), « localisée et sévère » (3), « généralisée et bénigne » (4), « généralisée et modérée » (5) et « généralisée et sévère » (6), sur la base d'informations extraites de la littérature scientifique. Seuls cinq phénotypes (noir ; noir avec taches blanches ; visage Barger ; mélange blanc / marron ; blanc) étaient infestés par la gale. Les chèvres noires dominaient (50%) le groupe infesté, suivies de celles à prédominance noire avec taches blanches (23,81%), tandis que les chèvres blanches étaient les moins infestées (4,76%). L'analyse de la variance par méthode des moindres carrés a montré que cette estimation classée était significativement affectée par la couleur de robe ($P < 0,05$). Les chèvres noires et les chèvres noires avec taches blanches étaient les plus affectées, avec une infestation généralisée et bénigne, leur classement étant respectivement de $3,97 \pm 0,34$ et $4,31 \pm 0,50$ ($P > 0,05$). Les chèvres au visage Barger et celles au mélange marron / blanc avaient des estimations ($P > 0,05$) similaires ($2,60 \pm 0,66$ vs $3,52 \pm 0,93$), tandis que les chèvres blanches étaient les moins affectées, avec une infestation localisée et bénigne ($0,88 \pm 0,18$). L'effet de l'âge était significatif ($P < 0,01$) alors que le sexe n'était pas important. Les animaux âgés de moins d'un an étaient plus affectés, avec une infestation généralisée et bénigne, leur classement étant de $4,10 \pm 0,58$ par rapport aux animaux plus âgés (> 1 an) dont le classement était de $2,23 \pm 0,55$ avec une infestation localisée et modérée. L'on conclut que les chèvres au phénotype noir étaient les plus dominantes et les plus sensibles à l'infestation par la gale, tandis que les chèvres au phénotype blanc étaient moins affectées. La sélection d'autres combinaisons de couleurs qui n'étaient pas infestées pourrait contrôler davantage la prévalence de la gale dans la région.

Mots-clés : *chèvre, répartition de la couleur de robe, influence sur l'infestation de gale*

Introduction

The WAD goats are the most numerous trypanotolerant species of small ruminants reared by small holder farmers in South Western Nigeria. They have been adjudged the most prolific, possessing high frequency of kidding and ability to survive under poor management condition (Adebambo *et al.*, 1994; Odubote, 1994b). Litter size at birth was estimated at 2.07, 2.02 and 1.96 for basic white, basic brown and basic black goats respectively (Odubote, 1994a). The breed has disproportionately short legs with height at wither ranging from 35-55 cm (Devendra and Burns, 1983). Qualitative traits have been studied as possible indicators of genetic superiority or adaptability (Odubote 1994b; Ebozoje and Ikeobi, 1998; Ozoje and Mgbere, 2002; Adedeji *et al.*, 2011). Early discovery by Odubote (1994b) indicated that the WAD goat coat colour is very variable and irregular, including black, brown, pied and mixed colours. Basic black colour predominated (53.3%) while basic white and brown goats accounted for 6.8% and 39.9% respectively. Reproductive performance and preweaning growth (Ebozoje and Ikeobi, 1998) and body measurements (Adedeji *et al.*, 2011) have been studied in WAD goats based on coat colour variation. The former authors showed that prolificacy (litter size) and fecundity (number of kids per year) and preweaning growth rates were highly affected by coat colour, with indication that black does had the largest litter both at birth and at weaning. Mortality was higher among white does, suggesting that coat colour plays an important role in adaptation and survival of goat breed. Review by Kine (2005) also showed that dark-colored animals of various livestock species grow faster in the tropics and subtropics, and survival and growth are less in lighter colored animals. The genetic basis of coat colour inheritance has been unraveled by several studies (Odubote, 1984b; Adalsteinsson *et al.*, 1994; Sponenberg, 1995, Ozoje, 1998; Saldaña-Muñoz *et al.*, 2004; Nadeau *et al.*, 2007; Norris and Whan, 2008; Fontanesi *et al.*, 2010; Ren *et al.*, 2011). According to Adalsteinsson *et al.* (1994) and Sponenberg (1995) colour is due to melanin deposits in the hair which comes in two basic types - eumelanin and pheomelanin. Eumelanin is usually black, but sometimes brown. It is the pigment responsible for black and brown areas on goats, or rarely for dusky blue colour.

While it is true that both goats and sheep are trypanotolerant, small ruminants suffer from various types of ailments of which mange is one of them. Saroptic mange is zoonotic (Clauss *et al.*, 2004; Lau *et al.*, 2007) and chronic infestation with sarcoptes has been observed in goats in Ethiopia (Sertse and Wossere, 2007; Kassaye and Kebede, 2010), Pakistan (Aatish *et al.*, 2007) and the UK (Lusat *et al.*, 2009). The mange mite feeds on the surface or burrow within the skin making very slender winding tunnel from 0.1 to 1 inch long. Kusilika and Kambarage (1996) described mange as a contagious disease of animal caused by parasitic mites characterized by a variety of clinical signs depending on the species of mites. They are responsible for great economic losses due to damaged skin and wool, anaemia, poor physical condition, decreased milk and meat production and suboptimal lambing and growth rates in sheep (Soulsby, 1982; Fthenakis *et al.*, 2000). The mange mites produce a number of local and generalized disease conditions and diseased animals become more prone to other bacterial and viral infections (Blood *et al.*, 2007). Mange may occur in farm animals of any age, especially those kept under poor management condition (Abu-Samra *et al.*, 1984; Radostitis *et al.*, 2000; Sertse and Wossere, 2007). Mange mites spread through direct contact between sheep or from ewe to lamb, while sucking (Schmidt, 1994). Overcrowding of animals in houses, markets, dips and communal grazing land facilitates rapid spread of the parasites. Kids and lambs are more severely affected than adult animals. Moist conditions favour the proliferation of the mites. Clinically affected and carrier animals are source of infestation (Kusiluka and Kambarage, 1996). In sarcoptic infections, the main signs are irritation with encrustations, loss of hairs and excoriation from rubbing and scratching. In long standing cases, the skin becomes thickened and nodules may develop on the less well haired parts of the skin including the muzzle around the eyes and inside the ears (Urquhart *et al.*, 2003).

Although early reports by Jahnke (1982) indicated that mange is one of the major challenges facing small ruminants in Nigeria, with a more recent information that it occurs mainly during the rainy season (Odo, 2003), more current information on the risk factors and prevalence of infestation in the country is rare. There is therefore need to investigate the prevalence of coat colours and its possible influence, along with other factors (age and sex) on infestation of goats by sarcoptic mange mites.

Materials and methods

Location of study

This study was carried out in five Local Government Areas (LGAs) of Ogun State in South Western Nigeria. They are Abeokuta North (30 villages), Abeokuta South (15 villages), Odeda (22 villages), Ewekoro (18 villages) and Obafemi-Owode (31 villages). The villages chosen through simple random selection method were proportional to the total number of villages present in each LGA. The region is about 288 meters above sea level and falls within latitudes 6o54' - 7o54' N and longitudes 3o11' - 3o53' E. The climate is humid and located in the Derived Savanna vegetation zone. It receives a mean annual precipitation of 1,037 mm distributed from March to October, with a mean annual temperature of 34.7oC. Relative humidity averages 82% throughout the year thereby favouring lush vegetation.

Survey of animals and management system

Population survey of WAD goats was carried out during the rainy season from March to October, 2011 since previous study indicated that mange infestation occurs mainly during the early and late rainy seasons (Odo, 2003). Prior to the survey, pre- visit arrangements were made through the Ogun State Agricultural and Rural Development Programme (OGADEP) extension officers in charge of the zone. They sensitized the farmers about the visits following which animals were confined at various locations to facilitate the study. Various households in selected villages were visited to examine individual animals. Total number of goats examined was recorded based on coat colour phenotype, sex (male or female) and age. About 11,772, consisting of 8,384 females and 3,388 males were sampled for distribution of coat colours. Within the traditional setting, more females than males are retained for reproductive purpose while more males are sold for the meat hence the wide variation in number. The age was estimated by dentition (Saini *et al.*, 1993) and placed under two categories (<1 year and >1 year). Animals were classified into 20 categories based on coat colour phenotype namely: black, predominantly white with black marking, predominantly black with white marking, brown, Swiss marking, tan, white, buckskin, badger face, white and brown, spotted brown, benzoar, spotted white, lateral belly, black mash, lateral stripes, grey, black and brown, spotted black and peacock. Geographical Positioning System (GPS) navigator was used to obtain the coordinates of the villages and communities sampled during the survey. With regards to management system, animals were kept on free range and minimal housing provided to serve as shelter. With reference to herd size most farmer maintained <15 goats per house hold. They were fed with kitchen wastes, cassava peels or corn chaff in the morning before being left to roam the surroundings to forage for grasses and browse plants.

Examination of animals and parasitological procedure

For studies on mange infestation, 7,902 goats were sampled. They were clinically inspected for presence of mange lesions on different parts of the body such as head, face, neck, breast and tail. A total of 69 suspected cases were ranked according to severity of infestation as: localized and mild (1), localized and moderate (2), localized and severe (3), generalized and mild (4), generalized and moderate (5) and generalized and severe (6). They generally had signs of scales, crusts, alopecia and itching. Skin scrapings of about 2.5 cm2 of the affected lesions was carried out and placed in sample bottles according to the method described by Fthenakes *et al.* (2000). The samples were

preserved in 10% formalin and dispatched to laboratory for further confirmatory examination within 12h after collection. About 20 ml of 10% KOH solution was added to each sample. The scrapings were macerated or teased with a mounted needle and centrifuged at 1500 rpm for 5 minutes. The sediments were examined under the microscope for the identification of the causal agent. Only 42 out of 69 cases screened were positive for mange mite infestation.

Statistical Analysis

Excel spreadsheet (Microsoft Corp., Redmond WA, USA) was used as the database to summarize the data on coat colour distribution. Each category of coat colour phenotype was expressed as a percentage of the total population under consideration. The effects of coat colour phenotype as well as age and sex on the severity of mange infestation were investigated by analysis of variance of data using the Multivariate General Linear Model of Systat program, release 5.02 (Systat, 1992). Interactive effects of the factors examined in a preliminary analysis were not significant and hence dropped from the model indicated below:

$$Yijklmn = m + Ci + Sj + Ak + \sum ijkl$$

where Yijkl = severity of infestation, μ=overall mean, Ci=effect of ith coat colour (i=black or black with white marking or barger face or brown/white or white), Sj= effect of jth sex (j= male or female), Sj= effect of jth age group (j= <1 year or >1 year), ∑ijkl=random residual error normally and independently distributed with zero mean and variance, ∂2). The relationship between frequency of individuals per coat colour phenotype and severity of infestation was examined using Pearson's correlation and Bonferroni probabilities (Systat, 1992).

Results

Coat colour distribution by percentage were black (24%), predominantly white with black markings (17.28%), predominantly black with white markings (9.96%), brown (9.22%), Swiss marking (7.04%), bargerface (5.24%), buckskin (5.16%), tan (5.12%), white (4.10%), white and brown (2.43%), spotted white (2.24%), spotted brown (1.75%), grey (1.48%), black and brown (1.28%), benzoar (1.07%), spotted black (0.71%), lateral belly (0.63%), black mash (0.61), lateral stripes (0.54) and peacock (0.13%) listed in order of decreasing frequency. The distribution pattern was similar for both males and females.

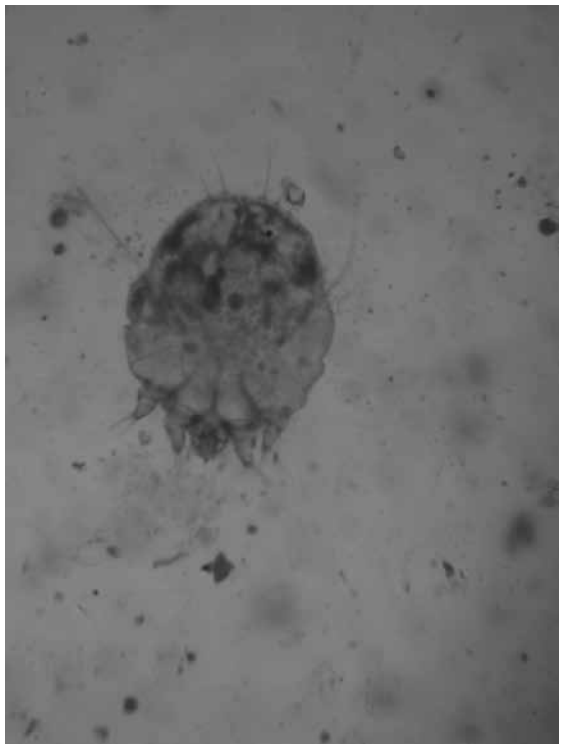

Fig. 1. Photomicrograph of Sarcoptes scabiei var. caprae

Out of 69/7902 suspected cases of mange mite infestation, 42/7902 goats (0.53%) were found to be positive following examination. Only one type of mite (Sarcoptes scabiei var. caprae) as shown in Fig. 1 infested goats in the area under study. Least squares means (±SE) of ranked estimates which were used as a measure of severity of infestation are presented in Table 1. The influence of coat colour on mange infestation was significant ($P<0.05$). Among 20 colour phenotypes examined for mange infestation (Table 2), only five (black, black with white marking, bargerface, white/brown combination and white) were infested by mange mite. Particularly, the black and black with white marking were most affected with generalized

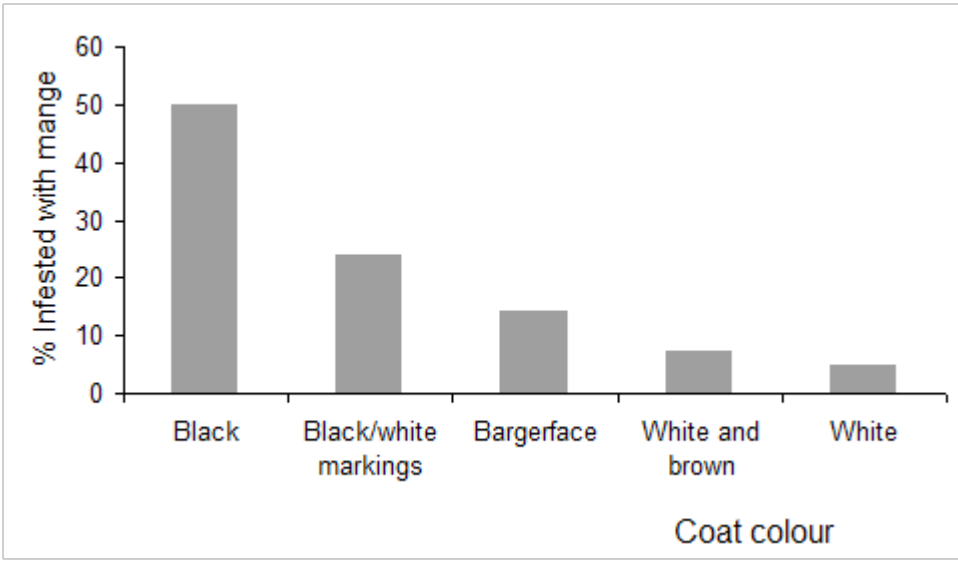

Fig. 2. Distribution of infested WAD goats based on coat colour phenotype between March and October, 2011

and mild infestation, ranking 3.97±0.34 and 4.31±0.50 respectively (P>0.05). bargerface and mixture of brown/white phenotypes had similar (P>0.05) estimates (2.60±0.66 vs 3.52±0.93) while white goats were the least affected with localized and mild infestation (0.88±0.18). In terms of frequency (Figure 2), black goats (50%) predominated the infested group, followed by black with white marking (23.81%), while the white goats had the least frequency (4.76%). Relationship between frequency of individuals within each colour phenotype and severity of infestation was positive but not significant (rp = 0.618; P = 0.267). The effect of age on infestation of goats by mange was significant (P<0.01) while the effect of sex was insignificant. Animals less than one year of age were more affected with generalized and mild infestation, ranking 4.10±0.58 compared with older animals (>1 year) that ranked 2.23±0.55 with localized and moderate infestation.

Discussion

Early discovery by Odubote (1994b) working with the same breed similarly indicated that the WAD goat coat colour is very variable and irregular, including black, brown, pied and mixed colours. There is clear evidence from the current study that more colour combinations have emerged over time, giving rise to differences in prevalence of various phenotypes compared with previous report. Between 1994 and 2011, frequency of the black goats has reduced to less than half from 53.3% (Odubote, 1994b) to 24% (current study). Similarly, the frequencies of the white and brown goats were lower than estimates by the forgoing author. This observation is expected since farmers do not have preference for a specific coat colour. All the phenotypes identified are in agreement with the colours (black, white, brown, bargerface, benzoar, Swiss markings, black with white markings and brown with white markings) recently identified by Adedeji *et al.* (2011) using a smaller sample size of 1119. It is obvious that more colour combinations are possible with a larger sample size as reported in the current study. The preponderance of black coat colour was attributed to better adaptation to the humid tropics (Odubote, 1994b; Ebozoje and Ikeobi, 1998); Their reduction in frequency over time could be due to dominance and or epistatic effects of other loci, in line with the observation of Sponenberg (1995) that dark brown is dominant to black which seems to be the more common type of brown in the Pygmy. Similarly, Ponzoni (1992) and Muñoz *et al.* (2004) suggested by their findings on sheep that white and brown are co-dominant and both are dominant to the other colours. White and spotted are

the same variable, blackbelly behaves as a heterozygote, black is a possible homozygote recessive and independent to white. About four polymorphic loci (A- agouti, B, S and C) have been identified in sheep (Ryder, 1980; Sponenberg, 1990) which interact to produce various colour phenotypes. Different shades of colours are also attributed to variation in size, density and distribution of pigment granules.

Sponenberg (1995) also reported three basic colors (black, dark brown/ mahogany and medium brown) in the pigmy goats. As solid colors, these are fairly rare in the breed, but they are the underlying base for the common caramel and agouti patterns, giving rise to basically nine colors within the Pygmy goat breed. These nine combinations are complicated by the addition of white spotting in some goats, which results in eighteen basic types, one spotted and one non-spotted. These observations corroborate most aspects of the current findings with the exception that white was the additional basic colour (besides black and brown) identified in the WAD goats of Nigeria. The inheritance pattern revealed by Sponenberg (1995) is such that most black-to-black matings produce black offspring. The author further noted that one pattern that can be superimposed over the basic color is the agouti pattern which is the mixture of white hairs into the base coat color. Caramel is the second major pattern called badgerface after a similar sheep color pattern consisting of a tan or cream body with dark belly, dark legs, dark marks on the face and a dark stripe down the back. The caramel pattern can be superimposed over the basic colors, and the result is caramels with black marks, dark brown marks, or medium brown marks. The mode of inheritance of coat colour is very complex as agreed by most authors (Ryder, 1980; Odubote, 1994b; Sponenberg, 1995; Muñoz *et al.*, 2004). More facts are emerging at the molecular level that a tandem duplication of a 190-kb portion of the ovine genome is responsible for the dominant white coat colour allele of domestic sheep (Norris and Whan, 2008). The authors further demonstrated that a single copy of agouti signaling protein with a silenced agouti signaling promoter occurs in recessive black sheep. The dominant white or tan (Awt) agouti signaling protein allele is responsible for the phaeomelanic phenotype in modern sheep breeds, while the most recessive allele, non-agouti (Aa) results in eumelanic (black/brown) phenotypes as reviewed by the foregoing authors.

Although a detailed investigation established low prevalence of sarcoptic mange in the area under consideration (Ogundiyi *et al.*, 2012), mange in goats with emphasis on risk factors in Nigeria has not been extensively investigated. This study clearly revealed that the severity of mange infestation differed significantly with coat colour phenotype and age of the animal. The observation that more black and black with white marking phenotypes were most severely infested than other phenotypes could be attributed to several reasons; It is possible that the condition around the black coat (which is more likely to trap and retain more heat from high radiation than other colours) was more favourable for increase in population size of the mites, leading to generalized lesions. It was reported that high temperature, humidity and sunlight favour mange mite infestations (Pangui, 1994). The number of white goats infested was quite low compared with other phenotypes. While the reason could partly be attributed to lower frequency of occurrence of white goats from this study, there are stronger indications from literature that mortality is higher among white goats, suggesting that coat colour plays an important role in adaptation and survival of goat breed (Ebozoje and Ikeobi, 1998). Review by Kine (2005) also showed that dark-colored animals of various livestock species grow faster in the tropics and subtropics, and survival and growth are less in lighter colored animals. Generally most of the phenotypes that were not affected had lower frequencies within the population. Black goats were more frequently and severely affected, pointing to increased susceptibility/survivability, although a positive but weak relationship existed between frequency of individuals per phenotype and severity of infestation.

That younger goats (<1 year) were more severely affected than older goats (>1 year) was expected in line with Kassaye and Kebede (2010) who similarly demonstrated

Table 1: Least squares means ±SE of severity of mange infestation measured by ranking

Source	Sub-Class	N	Mean±SE
Sex	Male	20	3.37±0.45
	Female	22	2.79±0.42
Age	<1 year	23	2.17±0.43[b]
	>1 year	19	3.99±0.45[a]
Colour	Black	21	3.97±0.34[a]
	Black with white marking	10	4.31±0.50[a]
	Badger-face	6	2.60±0.66[c]
	White and brown	3	3.52±0.93[bc]
	White	2	0.88±1.18[d]

[a,b,c] Means within a column with different superscripts differ significantly (P<0.01)
SE: Standard error

Table 2. Prevalence of sarcoptic mite infestation based on coat colour phenotype

Coat colour phenotype	Number of goats sampled	Number infested	% infested within colour phenotype	% based on total sample size
Black	2008	21	1.05	0.27
White with black marking	1296	0	0	0
Black with white marking	970	10	1.03	0.13
Brown	648	0	0	0
Swiss marking	622	0	0	0
Tan	467	0	0	0
White	368	2	0.50	0.03
Buckskin	356	0	0	0
Badger face	300	6	2.00	0.08
White and brown	204	3	1.47	0.04
Spotted brown	121	0	0	0
Benzoar	115	0	0	0
Spotted white	111	0	0	0
Lateral belly	75	0	0	0
Black mash	73	0	0	0
Lateral stripes	64	0	0	0
Grey	43	0	0	0
Black and brown	31	0	0	0
Spotted black	30	0	0	0
Total	**7902**	**42**	**-**	**0.53**

that there is a difference in mange mite infestation among different age groups, being higher in younger animals. Mukherjee and Dasgupta (2000) also reported higher prevalence of mange mite in young than the old age group. Other investigations with lice infestation also confirmed the same trend, being higher in animals <1 year age than >2 years age (Kassaye and Kebede, 2010). Earlier studies have established the fact that young and under-nourished animals are more susceptible to many diseases including ectoparasites (Noble and Noble, 1982). Young animals are heavily infested and the number decrease as the animal mature (Heath *et al.*, 1995; Radostitis *et al.*, 2000). These observations are likely to explain why animals <1year of age were more severely affected by mange than older animals.

Conclusion

Three basic coat colours were identified in West African Dwarf goat, namely black, brown and white accounting for 24%, 9.22% and 4.10% respectively. They probably constituted the underlying base for the caramel (bargerface), agouti and spotting patterns giving rise to twenty combinations which accounted for the remaining 62.68% in the breed. To implement effective control strategy against mange infestation, a good knowledge of factors or disease dynamics is required for planning by relevant stakeholders such as veterinary staff, livestock extension officers and farmers or animal breeders. The results of this study suggest that black goats and younger animals were more severely infested by mange. There are practical implications of these results for goat breeders. Conscious selection could be embarked upon to conserve the phenotypes that are more genetically superior in terms of productivity and adaptation since it is difficult to completely eradicate mange.

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f mange infestation measured by ranking

PREVALENCE OF LIVER FLUKE INFECTIONS AND OTHER GASTROINTESTINAL TRACT PARASITES IN SLAUGHTERED CATTLE IN DOUALA, CAMEROON

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Abstract

A survey of the prevalence of liver fluke and other gastrointestinal parasites in cattle at the Douala slaughter house was conducted between March and September 2011. Three hundred and twenty (320) cattle consisting of 100 females and 220 males were examined post-mortem. Liver, bile and stool samples were collected to determine the prevalence and intensity of liver flukes and other intestinal parasites of these ruminants. 10mls of bile collected from each cattle were concentrated by centrifugation at 3000rpm for two minutes, and the sediment examined under the microscope. Also, 0.2g of faecal material were collected, sieved and analyzed using the formol ether concentration technique. Results obtained showed that liver flukes had a general prevalence of 81.3% distributed as follows: 12.2% of the cattle were infected with *Fasciola Sp* only, 25.6 % with Dicrocoelium dendriticum only, and 43.4% with *Fasciola sp* and *D. dendriticum*. It was equally observed that more males 179 (81.4%) were infected than females 72 (72.0%), although the difference was not statistically significant. Males had a higher intensity of infection (300 epd and 410 epd) than females (265 epd and 365 epd) with *Dicrocoelium* and mixed infections respectively and these differences were statistically significant at $p \leq 0.035$ and 0.043. From the stool analysis, 73.8% of the animals were infected with *Trichostrongylus* sp having the highest prevalence of 47.3%,(151) followed by *Haemonchus sp*, 37.2% (119) while the least prevalence was observed in *Cooperia sp* 7.5 (24). Seven livers were totally condemned (declared unfit for human consumption) due to heavy infections. Educating the Fulani herdsmen on better field management, drug administration, and subsidizing the supply of better anthelminthic drugs will lead to improved cattle production and animal proteins.

Keywords: *Liverflukes, Gastrointestinal parasites, Slaughter house, cattle, Prevalence, Intensity, Douala, Cameroon.*

PREVALENCE DE DOUVES DU FOIE ET D'AUTRES PARASITES DU TRACTUS GASTROINTESTINAL CHEZ LES BOVINS ABATTUS A DOUALA (CAMEROUN)

Résumé

Une étude de la prévalence de douves du foie et d'autres parasites gastro-intestinaux chez les bovins à l'abattoir de Douala a été réalisée entre mars et septembre 2011. Trois cent vingt (320) bovins, dont cent (100) femelles et deux cents et vingt (220) mâles, ont été soumis à un examen post-mortem. Des échantillons de foie, de bile et d'excréments ont été prélevés pour déterminer la prévalence et l'intensité des douves du foie et d'autres parasites intestinaux de ces ruminants. 10mls de bile recueillis sur chacun des bovins ont été concentrés par centrifugation à 3000rpm pendant deux minutes, et le sédiment a été examiné au microscope. En outre, 0,2 g d'excréments ont été prélevés, tamisés et analysés en utilisant la technique de la concentration formaline - éther. Les résultats obtenus ont montré que les douves du foie avaient une prévalence générale de 81,3% répartie comme suit : 12,2% des bovins étaient infectés uniquement par Fasciola sp, 25,6% par Dendriticum Dicrocoelium seulement, et 43,4% par Fasciola sp et D. dendriticum. De plus, il a été noté que les mâles - 179 (soit 81,4%) étaient plus infectés que les femelles - 72 (soit 72,0%), mais la différence n'était pas statistiquement significative. Les mâles avaient une plus grande intensité d'infection (300 epd et 410 epd) que les femelles (265 epd et 365 epd), présentant respectivement des infections à Dicrocoelium et mixtes, et ces différences étaient statistiquement significatives à $p \leq 0,035$ et 0,043. L'analyse des matières fécales a révélé que 73,8% des animaux étaient infectés par Trichostrongylus sp qui avait la plus forte prévalence de 47,3%, (151), suivi de Haemonchus sp avec 37,2% (119) alors que la moindre prévalence a été observée pour Cooperia sp avec 7.5 (24). Sept foies ont été totalement condamnés (déclarés impropres à la consommation humaine) en raison de fortes infestations. La sensibilisation des éleveurs Fulanis à une meilleure gestion de terrain, l'administration de médicaments,

et le subventionnement de l'approvisionnement en médicaments anthelminthiques meilleurs conduiront à une amélioration de la production bovine et des protéines animales.

Mots-clés : *douves du foie, parasites gastro-intestinaux, abattoir, bovins, prévalence, intensité, Douala, Cameroun.*

Introduction

Livestock plays a crucial role in the economy of Cameroon. Cattle are considered one of the principal livestock, and their survival and development are necessary to ameliorate the worsening situation regarding the supply of animal products. Parasitism in cattle is a substantial problem plaguing livestock farmers across the nation. It has highly detrimental effects on the cattle industry. Gastrointestinal parasites of cattle are present in many tropical and subtropical countries (Keyyu *et al.,* 2006; Tasawar *et al.,* 2007; and Kanyari *et al.,* 2009). In chronic infections some of these parasites cause biliary cirrhosis in the livers of cattle and lead to production losses (Fabiyi, 1986, and Gargili, *et al* 1999). According to WHO/C.D.C (2002), there is worldwide loss in animal productivity to fascioliasis, and an estimate of over 3.2 billion U.S dollars loss is registered every year. Man is not usually considered to be a host of *Fasciola* sp. but outbreaks can be very devastating when they occur. The eating of water cress and lettuce cultivated in swampy areas appears to be a common source of human infections. An estimated 2.4 million people are infected with Fascioliasis worldwide and a further 180 million others are at risk of infection (WHO/CDC, 2002).

Cattle slaughtered in Douala and other towns in Cameroon come mainly from the savanna and Sahel regions of the country. The cattle herdsmen travel very long distances to the southern parts of the country with these cattle. The grazing herds are normally fed on natural pastures, characterized generally by annual grass species, and are only brought into their paddocks during the night. These livestock systems allow the possibility of infection and challenge by several parasites, especially in areas with frequent rainfall and mild temperatures (Adolf *et al.,* 2007). These parasites may not necessarily kill the animals but reduce production and restrict farmers far from international trade. The study was aimed at determining the prevalence and intensity of liver flukes and other intestinal parasites of cattle in the littoral region of Cameroon, which could be of production and zoonotic importance in cattle in the country.

Materials and Methods

Douala is situated around latitude 402' - 504'N and longitude 909' - 11 05'E. It is bounded to the west by the Atlantic Ocean and to the east by the River Wouri, (Figure 1). It is a marshy town with very high rainfalls and temperatures (3850mm and 28°C, respectively), with the equatorial rainforest on its outskirts. (Delancey and Mbuh, 2010). Douala is the most populated and industrialized town in Cameroon, being the capital of the littoral region and economic capital of Cameroon, with a population of over 2,510,283 people from about 130 ethnic groups (Delancey and Mbuh, 2010). All the cattle slaughtered in this town are transported from the far North, Adamawa and parts of the North-West regions of the country, where they are kept temporally around the slaughter house while waiting to be slaughtered. The animals were of local breeds and were slaughtered as per Islamic methods. The livers and biles of 320 cattle (220 males and 100 females) slaughtered between March and September 2011 were examined clinically and parasitologically. Collected bile samples were put into clean, airtight bottles, labeled and kept cool prior to transportation to the life science laboratory of the University of Buea for immediate examination. Bile samples not examined on the same day were stored in the refrigerator at 4°C for subsequent

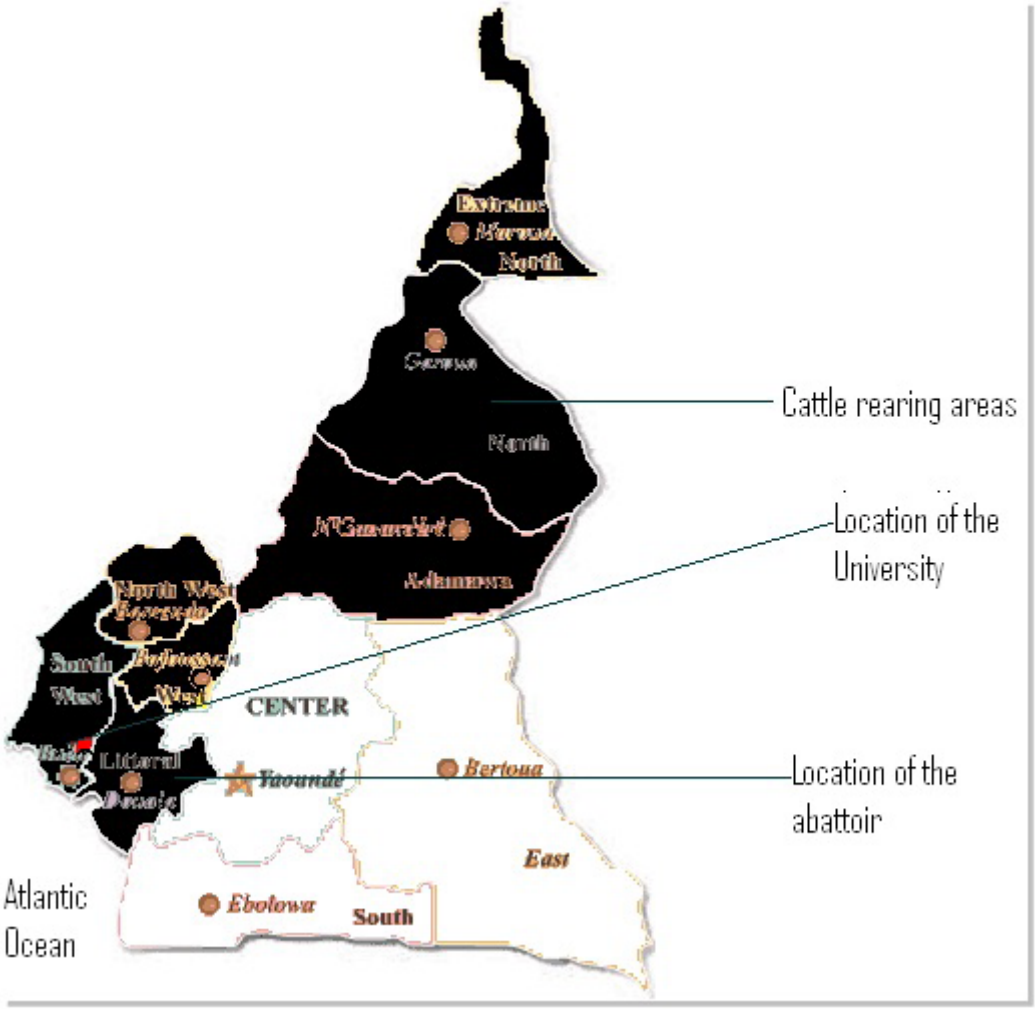

Figure 1: Map of Cameroon showing the cattle rearing areas The black areas indicate the cattle rearing areas

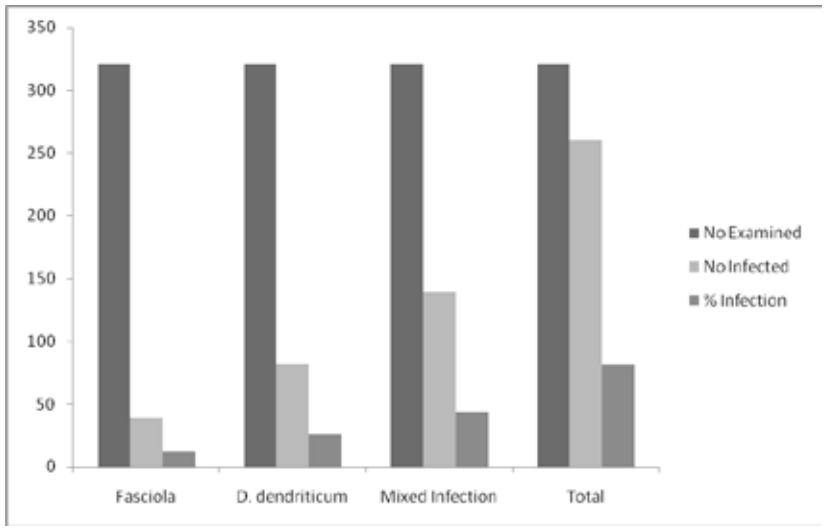

Figure. 2: The General Prevalence of Liver parasites

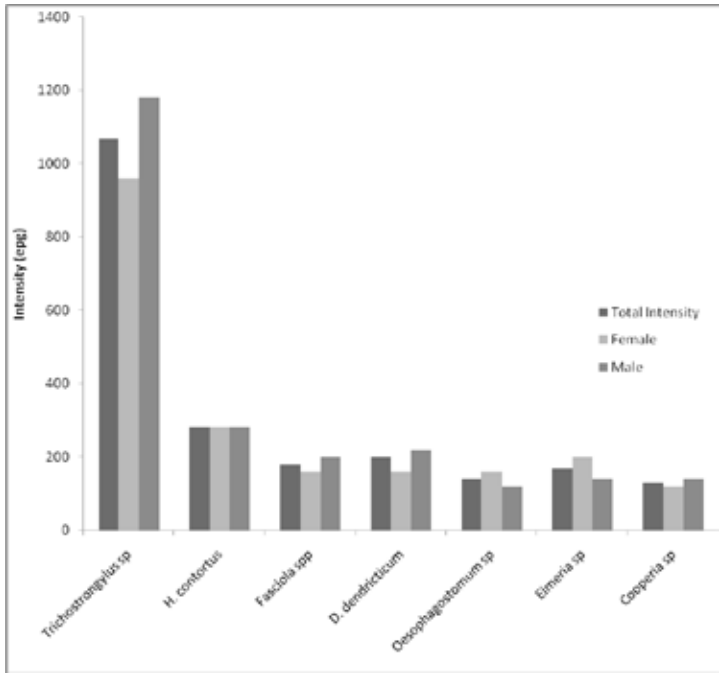

Figure 3: Intensity of Infection of the Various Parasite species from stool

Table 1: Sex-Related Prevalence of Single and Mixed Hepatic Parasites

Parasites	No Observed and percentage infection				Sig level (χ^2/ P value)
	No of Females examined	% female infected	No of Males examined	% Males infected	
D. dendriticum only	100	22 (22.0)	220	60 (27.3)	1.003/0.317
Fasciola spp only	100	16 (16.0)	220	23 (10.9)	1.975/ 0.16
Fasciola and Dicrocoelium	100	43 (43.0)	220	96 (43.6)	0.011/ 0.915
Total	**100**	**81 (81.0)**	**220**	**179 (81.4)**	**0.006/ 0.938**

Table 2: Intensity of Gallbladder Infection

Parasites	No of Positive cases		Mean E.p.d ± SE Total				Test
	Females	Males	Females	Males	Mean±SE	Prevalence	F-test/P-value
Fasciola spp alone	16	23	155±1	160±41	157±38	39(12.2%)	0.020/ 0.968
Dicrocoelium alone	22	60	265±48	300±54	283±59	82(25.6%)	0.875/ 0.035
Mixed infection	43	96	365±76	410±98	388±73	139(43.4%)	0.041/ 0.043

examination the next day. The sedimentation and centrifugation techniques as described by MAFF (1986) were used to detect the ova of liver flukes in the samples. Liver samples were examined according to the method described by Ogamba-Ongoma (1972). Stool samples were collected immediately the cattle were slaughtered and put in dry, clean, air-tight plastic containers. The samples were labeled and placed in cool boxes and transported to the University of Buea life Science laboratory for examination. The presence of fluke eggs and other gastrointestinal parasites in the intestines were determined by the formol-ether concentration technique of Christensen *et al.,* (1984). The number of eggs per gram of faeces for gastrointestinal parasites was determined by the modified McMaster method (MAFF, 1986). All samples collected were examined within 36hours.

The yellow areas indicate the cattle rearing areas, red, the location of the University while green colour shows the location of the abattoir.

Statistical Analysis

Data generated were computed using Microsoft excel and analyzed using the statistical package for social sciences (SPSS) version 16. A log transformation of egg counts was done to ensure normal distribution and the differences in prevalence with respect to sex were tested for statistical significance using the X^2 test. The differences in mean parasite egg/ml or epg of bile or stool respectively were tested for statistical significance using the student t-test.

Results

Characteristics of sampled animals

A total of 320 adult cattle were included in the study out of which 100 were females and 220 were males; all of them being local breeds. Out of this lot, 262 (82%) were from the Northern region of the country while 58 (18%) were from the North-West regions.

Two species of liver flukes, *Fasciola gigantica* (*F.gigantica*) and *Dicrocoelium dendriticum* (*D. dendriticum*) were encountered in this study. Parasite numbers obtained from one cutting surface of the liver of infected cattle ranged from 4 – 10 for *D. dendriticum* and 1 – 2 for *Fasciola* sp. Seven cattle (all males) had brownish and slimy biles with dark livers. The livers of these 7 cattle were declared unfit for human consumption by the veterinary officer at the abattoir..

Overall Prevalence of Liver Parasites

The prevalence of liver parasites in the study population is shown on Figure 2. 260 (81.3%) of the cattle were infected with at least one of the two species of the liver flukes. Out of these lot, 81 (81.0%) were females and 179 (81.4%) were males. Single infection with *Fasciola* had a prevalence of 12.2% while *Dicrocoelium* had a prevalence of 25.6%. Mixed infections with the two parasites had a prevalence of 43.4%

Sex Related Prevalence with liver flukes

Table 1 shows the sex-related prevalence of liver parasites. Out of the 100 females examined, *Fasciola* sp had a prevalence of 16.0%, *Dicrocoelium* a prevalence of 22.0%, and mix infections with the both parasites had a prevalence of 43.0%. Equally out of the 220 males examined, *Dicrocoelium* had a prevalence of 27.3%, *Fasciola* sp., a prevalence of 10.5%, and mixed prevalence of 43.6%. This gave an overall prevalence of 81.3%. The difference in prevalence between males and females was however not statistically significant at $P \leq 0.938$

Sex-related Intensity of Infection with liver flukes

Table 2 shows the mean egg count per drop of bile (egg/ml of bile). Female cattle had an intensity of infection of 155 eggs/ml of bile for *Fasciola* sp, 265 eggs/ml of bile for *D. dendriticum*, and 365 eggs/ml of bile for mixed infections, while the male cattle had an intensity of infection of 160 eggs/ml of bile for *Fasciola* sp, 300 eggs/ml for *D. dendriticum* and 410 eggs/ml for mixed infections. The difference between male and female intensity of infection with Fasciola was not statistically significant. Males had a higher intensity of infection than females with D. dendriticum alone, and mixed infections, and these differences were statistically significant at $P \leq 0.035$ and $P \leq 0.043$, respectively.

Gastrointestinal Parasites Faecal Culture

The overall proportions of infective larvae from cultures indicated that the common gastrointestinal parasites were *Trichostrongylus spp* (47.2%), *Haemonchus spp.* (37.2%), Fasciola spp(15.3%), *Dicrocoelium spp.,* (9.4%). *Oesophagostomum spp* (8.8%), *Cooperia spp* (7.5%) and other gastrointestinal parasites that were identified form their obvious cysts and egg morphologies included *Eimeria spp Trichuris spp* and *Strongyloides spp*.

Figure 3 shows the sex related intensity of infection of the different species of parasites present. *Trichostrongylus sp* had the highest mean intensity of infection (1070 epg of stool), from which females recorded 960 epg and males 1180 epg of stool. Generally, most animals had a moderate intensity of infection (<500epg of stool). However, there was no significant difference in intensity of infection of the various parasites with respect to sex at p =0.405.

Discussion

The present study exposed the endoparasites of productivity and zoonotic significance in cattle in Douala and it is clear that the animals were affected by a wide variety of parasites. These parasites included gastrointestinal nematodes, trematodes and protozoans. The study indicated a high prevalence and widespread distribution of flukes, gastrointestinal nematodes and a moderate prevalence and low intensity of protozoan infections.

Liver flukes and gastrointestinal parasites are highly prevalent in cattle raised in the cattle grazing areas of Northern and North-Western regions of Cameroon. The cattle owners preferred selling the male cattle to females because females were kept for reproductive purposes. The females were only sold when they became too old or when they began to show some clinical signs of infection. Liver flukes had a prevalence of 81.3% while gastrointestinal parasites had a prevalence of 73.8% in this study. These results are a little higher than those reported by Ndamukong *et al.,*. (2000) at the Buea Abattoir, where they obtained a prevalence of 67.4%, and Alicata (1999) in Hawaii where he registered 70% infection with Fasciola sp. and 76.3% with *F. hepatica* in the Kaudi abattoir.

Thrusfield (1986) and Castro *et al* (2004) had prevalences of 86.3% and 90.15% respectively with *Fasciola* while Kenyu *et al* (2006) recorded 67% prevalence of helminthe infections in traditional dairy farms. The high prevalences obtained in the present study may be a reflection of the management practices of the cattle owners. Most of the cattle are reared in domestic and peridomestic farms rather than organized farms. Cattle in Cameroon are reared wildly and fed repeatedly on the same pasture where they defaecate and the emerging larvae develop on the same pasture where they feed leading to repeated infection. This high prevalence of internal parasites of ruminants may also be due to the fact that cattle are allowed to forage on the same field for more than one season. Equally, since the northern region is generally a dry area, there might be high stocking density in communal grazing and watering areas which facilitates pasture contamination and ingestion of infective stages by grazing traditionally managed cattle. The research commenced at the beginning of the rainy season (March). During the dry season, there is scarcity of pasture and so these animals have to travel very long distances in Northern Cameroon to be able to feed, thereby accumulating a lot of parasites. This work shows clearly that the prevalence of liver flukes and other gastrointestinal parasites in cattle is a problem in Cameroon with little or no efforts are being made by the government to reduce infection. In Northern Cameroon, cattle are reared uphill with very few patches of water and grass, and so these cattle concentrate around few drinking spots increasing the chances of their infection. Adult cattle are trekked long distances to valleys, flood plains or swampy areas during the dry season, thus exposing them to a lot of parasites. Males showed a higher intensity of infection than females. This may be due to the fact that males are heavier feeders than females and exploit new forage sites where they can easily get infected especially by water sides where the metacercariae are encysted. Farmers should be educated on the importance of using own

pasture and dry season feed reserves as means to ensure safe feed for their cattle.

In conclusion, it is suggested that opening of schools in Northern Cameroon and including animal rearing in their curriculum will go a long way to reduce the prevalence of these parasites in these localities. Also, from the results of the study, it was observed that stool concentration was not a good method for analyzing liver flukes, since the prevalence in stool was far lower than that from bile concentration. Equally anthelminthic treatment on quarterly basis should be implemented to reduce the risk of re-infection. Appropriate education, control, and preventive strategies should be developed which can be easily implemented by the farmers.

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RELATIONSHIPS BETWEEN BODY CONDITION SCORE, MILK YIELD, INSULIN-LIKE GROWTH FACTOR-I CONCENTRATION AND RESUMPTION OF OVARIAN ACTIVITY IN BEEF COWS

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Abstract

A study was conducted to investigate the relationships between milk yield, body condition score (BCS), plasma concentrations of insulin-like growth factor (IGF-I) and the resumption of ovarian cyclicity in Sanga cows. Sixteen multiparous Sanga cows were grazed extensively on natural pasture. Cows were weighed monthly and scored for body condition weekly using a 9-point score (1= very thin and 9 = obese). Milk yield was determined daily. Blood samples were taken from cows once every week, from week 1 to 13 postpartum and processed for plasma. Resumption of postpartum ovarian cyclicity in cows was determined by measuring plasma progesterone concentrations from week 1 to 13 (90 days) postpartum. The cows were classified as having resumed ovarian cyclicity when progesterone concentration of ≥1.0 ng/mL was recorded in two consecutive weekly samples. Based on the resumption of ovarian activity, cows were classified as early cycling (≤ 45 days postpartum), late cycling (46-90 days postpartum) or non-cycling (no resumption by 90 days postpartum). The concentration of IGF-I was measured from week 1 to 10 in plasma samples. Results from the present study indicate that 37.5% of cows commenced ovarian cyclicity earlier, 31.25% commenced ovarian cyclicity later, while 31.25% failed to resume ovarian cyclicity within the period of study. BCS was significantly greater ($P < 0.05$) in early cycling (5.12) or late cycling (5.11) cows than non-cycling (4.69) cows. Milk yield was significantly ($P < 0.05$) higher in late cycling (1.09 ± 0.01 L/day) or non-cycling (1.10 ± 0.01 L/day) cows than in early cycling cows (1.02 ± 0.01 L/day). Early cycling (23.2 ± 1.26 ng/mL) or late cycling (19.5 ± 1.38 ng/mL) cows had greater ($P < 0.05$) plasma concentrations of IGF-I than non-cycling cows (14.7 ± 1.38 ng/mL). Cows had poor metabolic status. Higher plasma concentrations of IGF-I in the early postpartum period were associated with early resumption of ovarian cyclicity in cows.

Key words: *Body condition score, insulin-like growth factor-I, ovarian cyclicity, Sanga cow*

RELATIONS ENTRE LA NOTE D'ETAT CORPOREL, LE RENDEMENT LAITIER, LA CONCENTRATION DE FACTEURS DE CROISSANCE ANALOGUES A L'INSULINE-1 ET LE RETOUR DE L'ACTIVITE OVARIENNE CHEZ LES VACHES DE BOUCHERIE

Résumé

Une étude a été menée dans le but d'étudier les relations entre le rendement laitier, la note d'état corporel (BCS), les concentrations plasmatiques du facteur de croissance analogue à l'insuline (IGF-I) et le retour du cycle ovarien chez les vaches Sanga. Seize vaches multipares ont été intensivement mises en pâture dans un environnement d'élevage extensif sur des pâturages naturels. Les vaches étaient pesées chaque mois et leur note d'état corporel enregistré chaque semaine en utilisant un score de 9 points (1 = très maigre et 9 = obèse). Le rendement laitier était déterminé chaque jour. Des échantillons de sang étaient prélevés sur les vaches une fois par semaine, de la semaine 1 à la semaine 13 post-partum et traités pour le plasma. Le retour du cycle ovarien post-partum chez les vaches a été déterminé en mesurant les taux plasmatiques de progestérone, de la semaine 1 à 13 semaines (90 jours) post-partum. Le retour du cycle ovarien chez ces vaches était établi lorsque le taux de progestérone de ≥ 1,0 ng / mL était enregistré dans deux échantillons consécutifs par semaine. Sur la base du retour de l'activité ovarienne, les vaches ont été classées comme ayant un retour de cycle précoce (≤ 45 jours post-partum), un retour tardif (46-90 jours post-partum) ou une absence d'activité ovarienne (pas de retour de cycle à 90 jours post-partum).

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La concentration d'IGF-I a été mesurée de la semaine 1 à la semaine 10 dans les échantillons plasmatiques. Les résultats de la présente étude indiquent que 37,5% des vaches ont eu une reprise précoce de l'activité ovarienne, 31,25% ont eu un retour tardif du cycle ovarien, tandis que 31,25% n'ont pas eu de retour de cycle pendant la période d'étude. La note d'état corporel (BCS) était significativement plus élevé (P <0,05) chez les cas de retour précoce (5,12) ou les cas de retour tardif (5.11) que chez les vaches sans retour d'activité ovarienne (4,69). Le rendement laitier était significativement (P <0,05) plus élevé chez les vaches ayant eu un retour tardif (1,09 ± 0,01 l / jour) ou sans retour de cycle (1,10 ± 0,01 l / jour) que chez les vaches au retour de cycle précoce (1,02 ± 0,01 l / jour). Les vaches au retour de cycle précoce (23,2 ± 1,26 ng / ml) ou tardif (19,5 ± 1,38 ng / mL) avaient des concentrations plasmatiques d'IGF-I (P <0,05) plus élevées que les vaches sans retour de cycle (14,7 ± 1,38 ng / mL). Les vaches avaient un métabolisme faible. Les concentrations plasmatiques élevées d'IGF-I au début de la période post-partum ont été associées au retour précoce du cycle ovarien des vaches.

Mots-clés : *note d'état corporel, facteur de croissance analogue à l'insuline-I, cycle ovarien, vache Sanga*

Introduction

Reproductive efficiency is a major determinant of profitability in beef production enterprises (Wettemann *et al.,* 2003). Nutrition influences fertility in cattle directly through the supply of specific nutrients required for ovulation, fertilization, establishment of pregnancy and indirectly through its impact on the circulating concentrations of metabolic hormones and other nutrient-sensitive metabolites that are required for the success of these processes (Robinson *et al.,* 2006). The inhibition of luteinizing hormone (LH) pulse frequency and suppression of blood concentrations of glucose, insulin and insulin-like growth-factor-I (IGF-I) leads to low oestradiol concentration preventing the induction of gonadothrophin surge necessary for ovulation to occur in cattle (Yavas and Walton, 2000; Diskin *et al.,* 2003; Peter *et al.,* 2009).

Insulin-like growth factor-I is a potential mediator of nutritional effects on reproduction (Zulu *et al.*, 2002a). Concentrations in peripheral blood of lactating beef and dairy cows have been directly related to energy status, with higher concentrations being positively associated with body condition (Beam and Butler, 1999) and nutrient intake (Thissen *et al.,* 1994). Plasma concentrations of IGF-1 in postpartum cattle have been correlated with reproductive function. Increased plasma concentrations in early lactation have been associated with earlier resumption of ovarian function in beef (Nugent *et al.,* 1993; Roberts *et al.,* 1997) and dairy cows (Beam and Butler, 1997; Zulu *et al.,* 2002b; Obese *et al.,* 2011; Tamadon *et al.,* 2011).

The Sanga, a cross between a humped Zebu (White Fulani or Sokoto Gudali) and humpless cattle (West African Shorthorn or the N'dama) is one of the most prominent cattle breeds used for meat and milk production in smallholder peri-urban production systems in Ghana (Okantah *et al.,* 1999). Postpartum anoestrous interval and calving intervals in the Sanga cow have been reported to be prolonged (101 and 444 days, respectively), a consequence of poor management practices, inadequate nutrition and prolonged suckling (Obese *et al.,* 1999). Metabolic hormones and nutritionally-related metabolites could mediate the effect of nutrient intake on reproductive function (Wettemann and Bossis, 2000). Information is however, limited on the levels of circulating IGF-I and their relationship with the resumption of ovarian cyclity in Sanga cows managed within the pasture-based systems in Ghana. The objectives of this study were to evaluate the relationships between body condition score, milk yield, plasma concentrations IGF-I, and the resumption of cyclical ovulations in Sanga cows.

Materials and methods

Location of study

The study was conducted at the Animal Research Institute's Katamanso station located at Lat 05° 44' N and Long 00° 08' W in the Accra Plains of Ghana. The area has a bimodal rainfall pattern with the major wet season occurring from April to July and the minor season from September to November. The remaining months constitute the dry period. Annual rainfall and temperatures range between 600-1000 mm and 20°C to 34°C respectively.

Management of Animals

Sixteen multiparous Sanga cows were used in the study. They were housed in an open kraal and grazed from 05.00 h to 10.00 h and 13.00 h to 16.00 h daily mainly on natural pastures. *Panicum maximum, Sporobolus pyramidalis* and *Vertiveria fulvibarbis* constitute the dominant grass species in the grazing area, while thickets (mainly browse species) with *Griffonia simplicifolia, Baphia nitida* and *Milletia thoningii* were present (Oddoye *et al.,* 2002). The animals were not given any supplementary feed before or after grazing. Water was provided twice daily; morning and evening. Cows were milked twice a day; morning and evening during the rainy season and once a day during the dry season. Partial milking was practiced. In this system of milking, calves were separated from their dams in the evening and brought to suckle for a few minutes to stimulate milk let-down before milking. Milk was collected from two quarters of the udder, while that in the other two quarters were reserved for the calves. Mating was natural, with service bulls running freely with females all year round. Calves were weaned naturally (between 6 and 9 months of age). The animals were treated against ecto-parasites mainly ticks, fleas and mange mites using a pour-on acaricide (Flumethrin 1 % m/v) once a month during the dry season and fortnightly in the wet season. Treatment against endo-parasites was done using an anti-helminth (Albendazole 10 %) once a month during the dry season and fortnightly in the wet season. They were treated against diseases as the need arose and vaccinated against Contagious Bovine Pleuropneumonia once a year. Cows and their calves were weighed monthly, using a scale. Body Condition Score was determined weekly, using the 9-point score (1= very thin and 9 = obese; Nicholson and Butterworth, 1986).

Blood Sampling

Blood samples were collected from cows once every week, from week 1 to 13 postpartum at 08:00 h by jugular venipuncture into a 10 mL heparinised vacutainer tube (BD Vacutainer Systems, Plymouth, UK). They were then placed on ice immediately after collection, and plasma was separated by centrifugation at 1800×g for 15 min at 4 °C. Plasma was stored at -20 °C, until assayed for IGF-I and progesterone. Concentrations of IGF-I in plasma samples were measured weekly from week 1 to 10 postpartum, while progesterone concentrations were measured weekly from week 1 to 13.

Progesterone and IGF-I Assays

Resumption of postpartum ovarian cyclicity was determined by measuring the progesterone concentrations in plasma samples from cows. Plasma progesterone concentrations were measured using a commercial Progesterone ELISA kit (Gesellschaft fur Biochemica und Diagnostica mbH, Wiesbaden, Germany). The progesterone ELISA assay had a sensitivity of 0.3-0.7 ng/mL. Cows were classified as having resumed ovarian cyclicity when plasma progesterone concentration of ≥1 ng/mL was recorded in two consecutive weekly samples (Tamadon *et al.,* 2011). Based on the resumption of ovarian cyclicity, cows were classified as early cycling (resumed cyclicity ≤ 45 days postpartum), late cycling (resumed cyclicity 46-90 days postpartum) or non- cycling (no resumption by 90 days postpartum).

Plasma concentrations of IGF-I were measured in duplicate samples using the chloramine- T radioimmunoassay (RIA) method described by Gluckman *et al.* (1983). Interference by binding proteins was minimized by acid-ethanol cryoprecipitation method validated for ruminants by Breier *et al.* (1991). The assay sensitivity was 0.05 ng/mL and the intra and inter assay coefficients of variation (CV) were 6.3% and 7.9%, respectively.

Table 1: Resumption of ovarian cyclicity in Sanga cows

Oestrous cycle groups	No. of cows	Percentage of cows resuming ovarian cyclicity
Early cycling (≤ 45 days postpartum)	6	37.50
Late cycling (46-90 days postpartum)	5	31.25
Non- cycling (within 90 days postpartum)	5	31.25
Total	**16**	**100**

Table 2: Milk yield and body condition score in oestrous cycle groups

Parameter	Oestrous cycle groups (Least squares means ± SE)			Overall Mean	P-Value
	Early cycling (≤ 45 days)	late cycling (46-90 days)	non-cycling (within 90 days)		
Milk yield (L/day)	1.02 ± 0.01[b]	1.09 ± 0.01[a]	1.10 ± 0.01[a]	1.07 ± 0.01	<0.001
Body condition Score	5.12 ± 0.10[a]	5.11 ± 0.11[a]	4.69 ± 0.11[b]	4.97 ± 0.11	0.005

Means within each row with different supercripts (a,b) are significantly different (P<0.05)

Statistical Analysis

The effects of BCS, daily milk yield, and plasma concentrations of IGF-I on resumption of ovarian activity were analyzed using the repeated measures analysis of variance procedure of Statistical Analysis System (SAS, 1999). The model included as fixed effects treatment (oestrous cycle groups), time (weekly observations), and treatment x time (oestrous cycle groups × week) interactions. Where the model was significant, means were separated using the PDIFF procedure in SAS.

Results

Resumption of ovarian cyclicity in the Sanga cows

Results on the percentage of cows resuming ovarian cyclicity (Table. 1) indicate that 37.5% commenced ovarian cyclicity earlier (by 45 days postpartum), 31.25% commenced ovarian activity later (within 46-90 days postpartum), while 31.25% failed to commence ovarian cyclicity by 90 days (13 weeks) postpartum.

BCS, Daily milk yield and plasma IGF-I concentration

The BCS was significantly greater (P < 0.05) in early cycling (5.12) or late cycling (5.11) cows than non-cycling (4.69) cows (Table 2). There was no effect of time, or treatment x time interaction (P > 0.10) on BCS. The overall BCS averaged 4.97 ± 0.11. BCS in early cycling and non-cycling cows declined slightly between week 2 and 7 postpartum before remaining relatively steady as lactation progressed. The BCS, however, remained steady in late cycling cows during the period of study (Figure 1).

Mean daily milk yield was significantly (P < 0.05) higher in cows that either resumed ovarian cyclicity late (1.09 ± 0.01 L) or did

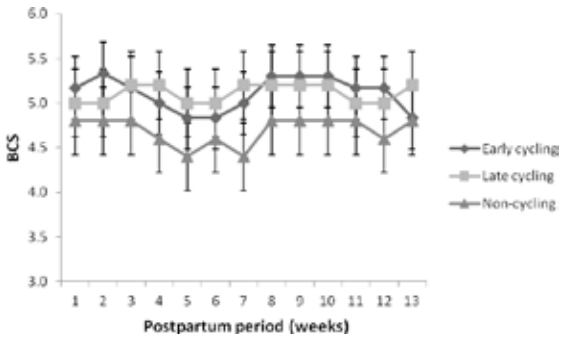

Figure 1: Least Squares means ± (SE) of body condition score in Sanga cows

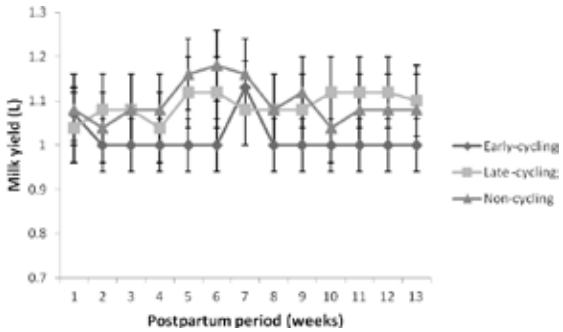

Figure 2: Least squares means (± SE) daily milk yield in Sanga cows

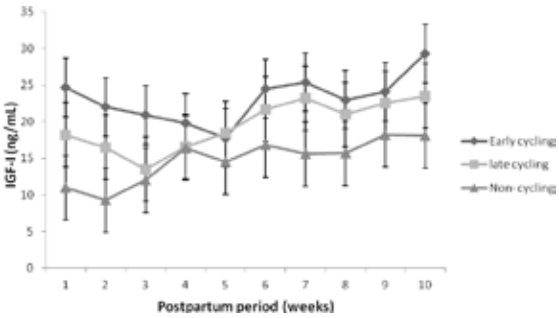

Figure 3: Least squares means (± SE) plasma IGF-I concentrations in Sanga cows

not resume ovarian cyclicity within 90 days postpartum (1.10 ± 0.01 L) than in cows that resumed cyclicity earlier (1.02 ± 0.01 L) (Table 2, Figure 2). The overall mean daily milk yield averaged 1.07 ± 0.01L. There was no interaction (P > 0.10) between treatment and time (oestrous cycle group x week) with respect to milk yield.

Cows that resumed ovarian cyclicity earlier (23.2 ± 1.26 ng/mL) or late (19.5 ± 1.38 ng/mL) had greater (P < 0.05) plasma concentrations of IGF-I than non-cycling cows (14.7 ± 1.38 ng/mL) from week 1 to 10 postpartum (Figure 3). The plasma concentrations of IGF-I declined initially to lower levels within the first two and three weeks postpartum in the three oestrous cycle groups. After week 3, levels increased slightly in late cycling and non-cycling cows peaking at week 10 postpartum. In early cycling cows, the IGF-I concentrations continued to decline until week 5, and then started rising until week 10 (Figure 2). Generally, the concentrations were higher over the postpartum period considered in the early and late cycling cows, than in the non-cycling cows. Neither time nor treatment x time interaction (P > 0.10) affected plasma concentrations of IGF-I.

Discussion

The study evaluated the relationships between BCS, milk yield, IGF-I, and the resumption of ovarian cyclicity in Sanga cows. In the present study, 37.5% of the cows had resumed ovarian cyclicity within 45 days postpartum, 31.25% had delayed resumption of ovarian cyclicity (resumption between 46-90 days postpartum), while 31.25% of cows did not resume ovarian cyclicity with the 90-day (13 weeks) period of study (Table 1). Obese *et al.* (1999) reported in an earlier study that 32.9% of Sanga cows resumed ovarian cyclicity by day 60 postpartum in smallholder farms in the Accra Plains. Thirty-one percent of the Sanga cows failed to resume ovarian cyclicity within 90 days postpartum (13 weeks of lactation) in the present study. Poor nutrition coupled with prolonged suckling period in this management system could account for this extended postpartum anoestrous period. Indigenous cows in Ghana, including the West African Shorthorn, N'dama and Sanga depend mainly on natural pasture, with little or no feed supplementation for most of their nutrient requirements. Feed intake is dependent on the availability of forage, which is influenced by season. Especially the dry season, there is scarce and poor quality forage (Okantah *et al.,* 1999). This reduces rumen efficiency and digestibility of the feed resulting in the inability of lactating cows to meet their nutritional requirements. They tend to lose weight and condition during lactation, Also cows are allowed to suckle their young until they are weaned naturally between 6 to 9 months of age in this study. Prolonged suckling and poor nutritional status delays resumption of ovarian function in cattle postpartum by reducing LH secretion and pulsatility (Jolly *et al.,* 1995; Williams *et al.,* 1996 Stagg *et al.,* 1998; Wetteman and Bossis, 2000; Crowe, 2008).

The BCS of an animal reflects its nutritional status and is useful for predicting reproductive performance. BCS at key periods in lactation, as well as, BCS changes has been associated with the resumption of oestrous cycles and reproductive success in cows (Pryce *et al.,* 2001). The overall mean of BCS 4.97 suggest cows were in poor to medium body condition according to the 9 point score of Nicholson and Butterworth (1986). The inability of cows with low BCS to resume ovarian cyclicity compared with early or late cycling cows could be attributed to the fact that a decreased BCS after calving, a possible consequence of poor nutritional status, increases the risk of delayed ovulation (Montiel and Ahuja, 2005). The mean daily milk yield of 1.07 L recorded in this study agrees with the

value 1.06 L reported for the Sanga but lower than the 1.42 L recorded for the Friesian-Sanga crossbreds at the Animal Research Institute's Frafraha Station in the Accra Plains of Ghana (Darfour-Oduro *et al.,* 2010). Milk production is high in crosses of indigenous breeds with exotic breeds, especially the F1 generation compared with the indigenous breeds as a result of the advantage afforded by heterosis (Aboagye, 2002).

Plasma concentrations of IGF-I during the postpartum period in the present study were lower than the range of values 40.9 ± 0.5 to 53.3 ± 10.1 ng/mL obtained between calving and day 40 postpartum for suckled Angus x Nelore cows grazing natural pasture in Brazil (Schneider *et al.,* 2010). They were also lower than the range of values 80 to 110 ng/mL reported for suckled obese Japanese Black cows grazed and supplemented with timothy hay from calving to week 9 postpartum (Kawashima *et al.* 2008). Breed differences and nutritional status of cows may account for the above differences. The relatively lower IGF-I concentrations recorded for the cows in the present study could be due to their poor nutritional status, a consequence of grazing natural pasture with no feed supplementation. Nutrient intake especially protein and energy affects the systematic concentration of IGF-I in the blood of cattle (Thissen *et al.,* 1994). Also high dry matter and metabolisable energy intake increased plasma concentration of IGF-I in Holstein-Friesian cows in a pasture based system (Obese *et al.,* 2008).

The plasma concentrations of IGF-I during the postpartum period have been correlated with reproductive performance in cows (Patton *et al.,* 2007). Increased plasma concentrations of IGF-I during early lactation was associated with the earlier resumption of ovarian function in beef cows (Nugent *et al.,* 1993; Roberts *et al.,* 1997). Also work by Stagg *et al.* (1998), established that plasma concentrations of IGF-I increase linearly up to the day of first ovulation in suckler beef cows. In the present study, cows that resumed ovarian cyclicity early during the postpartum period had greater concentration of IGF-I than late cycling or non-cycling cows (Table 2). This corroborates earlier report by Kawashima *et al.* (2008) for beef cows. High levels of circulating IGF-I enhances the follicular cell responsiveness to LH which in turn increases follicular oestradiol production which is a pre-requisite for ovulation to occur (Diskin *et al.,* 2003; Peter *et al.,* 2009).

Plasma concentrations of IGF-I were low initially and increased as lactation progressed (Figure 3). This supports the observation that concentrations of IGF-I are low after calving and in early lactation when many cows experience negative energy balance, but then increase as lactation progressed and cows move towards a more positive energy balance (Lucy, 2000).

Conclusion

Sanga cows had poor metabolic and nutritional status characterized by the low plasma concentrations of IGF-I and poor body condition score a consequence of inadequate nutrition. Early resumption of ovarian cyclicity in cows was associated with higher plasma concentrations of IGF-I during early lactation.

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LA TUBERCULOSE CHEZ LES PETITS RUMINANTS EN ALGÉRIE

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Résumé

La tuberculose des petits ruminants est une maladie de répartition mondiale qui sévit le plus souvent de façon sporadique. C'est une maladie à évolution progressive, à déclaration obligatoire associée à son aspect zoonotique.

Notre étude a consisté à rechercher les lésions suspectes de tuberculose par inspection des carcasses ovines et caprines dans deux abattoirs (Boufarik et Hadjout) d'une part, et à identifier l'agent pathogène, d'autre part.

L'inspection de 966 carcasses de petits ruminants a révélé la présence de lésions suspectes de tuberculose sur 40 carcasses, soit une proportion de 4,14%. Rapportés à chacune des deux espèces animales, les taux de prévalence sont similaires (3,89% et 4,40% respectivement chez les caprins et les ovins). Nous avons pris en considération deux facteurs qui peuvent influer sur la prévalence de la maladie et qui sont l'âge et le sexe. Nous avons observé que les mâles sont les plus atteints chez les caprins des deux localités alors que chez les ovins, les femelles sont plus touchées dans une des deux localités. Concernant l'âge, les animaux âgés sont les plus affectés chez les ovins et les caprins.

L'examen microscopique direct a détecté 2 cas positifs chez les caprins sur un ensemble de 19 cas suspects, soit 10,5%, et aucun cas chez les ovins. Les résultats de la culture mettent en évidence 8 cas positifs soit 42,1% pour l'espèce caprine et aucun cas positif chez les ovins.

L'identification des cultures positives montre que 75% sont des mycobactéries typiques vs 25% de mycobactéries atypiques.

On peut en conclure que cette affection est toujours présente sans pouvoir donner de chiffre précis sur sa prévalence en raison de l'absence de dépistage systématique chez ces espèces, même dans les élevages à effectif important.

Mots clés: *Petits ruminants, tuberculose, bacilloscopie, culture, abattoir.*

TUBERCULOSIS OF SMALL RUMINANTS IN ALGERIA

Abstract

Tuberculosis of small ruminants is a disease which is spread worldwide, it affects animals sporadically. It's a disease that evolves slowly and it is obligatory to declare it, in association to its zoonotic aspects.

Our study is to look for suspicious lesions of tuberculosis by inspection of carcasses of sheep and goats at the slaughterhouses of Boufarik and Hadjout (located in Northern Algeria).

This first step is followed by microscopic examination at the service of tuberculosis and mycobacteria of the Pasteur Institute of Algiers to highlight the pathogen.

We inspected 966 carcasses of small animals, 40 had suspicious lesions of tuberculosis, a proportion of 4.14%. By animal species, the proportion of suspicious lesions is similar, 3.89% and 4.40% in goats and sheep respectively.

We considered two factors that may influence the prevalence of the disease, age and sex.

We observed that males are the most affected in goats of the two places. However, in sheep, females are most affected in one of the two places. Regarding age, older animals are most affected in sheep and goats respectively.

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Microscopic examination detected 2 positive cases on a set of 19 suspected cases, or 10,5%, and no positive sampling in sheep. The culture results highlight 8 positive cases, or 42.1% for goats whereas in sheep no sampling was positive.
The identification of positive cultures showed that 75% are typical mycobacteria vs 25% atypical mycobacteria. It can be concluded that this affection is still present but we're unable to give precise figures on its prevalence due to the absence of routine screening in these species, even in herds with a large effective.

Keywords: *Small ruminant, tuberculosis, bacilloscopy, culture, slaughterhouse.*

INTRODUCTION

La tuberculose est classée par l'Office International des Epizooties (OIE) parmi les maladies de la liste B, en raison de graves problèmes socio-économiques et de santé publique qu'elle pose aux pays affectés et son impact sur les échanges internationaux d'animaux.
Mycobactérium bovis et *Mycobactérium caprae* sont les agents responsables de la tuberculose chez les petits ruminants. Ces agents ont été isolés chez ces espèces mais également chez les bovins (Sahraoui et al., 2009) et les humains dans plusieurs pays d'Europe tel que la France, l'Allemagne, l'Espagne, l'Autriche et la Croatie. La transmission à l'homme à partir des animaux, et notamment des petits ruminants, a été rapportée par plusieurs auteurs (Cousins, 2001). Même si la prévalence semble limitée, elle constitue cependant un véritable réservoir de germes et mérite une attention particulière en raison des risques qu'elle représente pour la santé humaine et animale (Perrin et Héraud, 2002).
En Algérie, la situation de la tuberculose des petits ruminants est peu connue. Aucune donnée sur le plan épidémiologique n'a, à ce jour, été publiée. La surveillance de la maladie se fait essentiellement dans les abattoirs, car le dépistage n'est pas réalisé chez ces espèces. Par conséquent, le véritable statut de la population des petits ruminants à l'égard de la tuberculose reste inconnu et les résultats de la présente étude permettent des comparaisons avec d'autres études.

MATERIEL ET METHODES

Cadre et période de l'étude :
Cette étude a été menée durant une période de 5 mois, de janvier à mai 2010 dans deux abattoirs situés au nord de l'Algérie, à savoir l'abattoir de Boufarik qui se trouve à 35 km au sud ouest d'Alger et celui de Hadjout situé à 80 km de la capitale.

Inspection :
Au total, 477 carcasses ovines et 489 carcasses caprines ainsi que leurs abats ont été examinés dans les conditions habituelles d'inspection (anté et post-mortem).

Analyses bactériologiques :
Le traitement des échantillons et l'examen bactériologique ont été effectués au Service de la Tuberculose et des Mycobactéries de l'Institut Pasteur d'Alger (Algérie).

Bacilloscopie :
Nous avons d'abord procédé à la dissection des échantillons puis à l'examen direct par la coloration de Ziehl-Neelsen.

Culture :
Au moyen de mortiers stériles, une partie du prélèvement est finement broyée à l'aide d'un pilon. Le produit de broyage ainsi obtenu est décontaminé par la méthode de Thorel et Boisvert (1976)
Le culot final est ensemencé sur 4 tubes de milieu Löwenstein-Jensen (2 tubes additionnés de glycérol à raison de 0,6% et 2 tubes enrichis de 0,1% de pyruvate).

*Identification***:**
L'identification phénotypique des cultures déclarées positives a tenu compte de deux critères: la pigmentation et le délai d'apparition des colonies, celles qui sont pigmentées (jaune ou orange) et apparaissent dans les deux semaines qui suivent l'ensemencement sont

dites atypiques, alors que celles qui ne sont pas pigmentées (colonies blanches) et apparaissant à partir du 28ème jour sont dites typiques.

L'identification biochimique s'est basée sur trois tests : les tests de catalase, de nitrate et de niacine. Nous avons considéré les souches à catalase, nitrate et niacine négatives comme typiques et celles présentant une catalase, niacine positives et nitrate (+/-) comme atypiques (De Kantor, 1998).

Analyse statistique

L'analyse a porté sur les carcasses de 966 animaux dont 489 caprins (301 à Boufarik et 188 à Hadjout) et 477 ovins (218 à Boufarik et 259 à Hadjout). Parmi les caprins, nous avions 370 mâles et 119 femelles; et parmi les ovins, 277 mâles et 200 femelles. Nous avons, grâce à des tests de χ^2, comparé les dénombrements d'animaux abattus en fonction des classes d'âge et du sexe dans les deux localités puis cherché les liens éventuels entre la prévalence des cas suspects de tuberculose et les classes d'âge puis le sexe. Nous avons enfin testé l'indépendance entre la localisation des lésions et l'espèce.

RESULTATS ET DISCUSSION

1-Distribution d'abattage des animaux dans les deux abattoirs en fonction de l'âge et du sexe.

La distribution d'abattage des ovins et des caprins dans les deux abattoirs en fonction de l'âge et du sexe est rapportée dans le tableau I.

Tableau 1: Répartition des animaux abattus dans les deux abattoirs en fonction de l'âge et du sexe.

		Age						Sexe			
Abattoirs	**Espèce**	**< 1 an**		**1-3 ans**		**> 3 ans**		**Mâle**		**Femelle**	
		n	%	n	%	n	%	n	%	n	%
Boufarik	**Caprine (n=301)**	33	10,96	95	31,56	173	57,48	189	62,79	112	37,21
	Ovine (n=218)	24	11,01	73	33,49	121	55,50	64	29,36	154	70,64
Hadjout	**Caprine (n=188)**	0	0	82	43,62	106	56,38	181	96,28	7	3,72
	Ovine (n=259)	0	0	98	37,84	161	62,16	213	82,24	46	17,76

Le traitement des données a révélé que :

- Le nombre d'animaux abattus dont l'âge dépasse 3 ans est prédominant de façon hautement significative ($p<0,001$) dans les deux abattoirs, avec respectivement pour les caprins et les ovins, 57,48% et 55,50% à Boufarik ; 56,38% et 62,16% à Hadjout. Aucun animal de moins d'un an n'a été abattu dans l'abattoir de Hadjout.

- L'abattage des mâles est significativement plus fréquent pour l'espèce caprine dans les deux abattoirs (62,8% et 96,3% respectivement pour Boufarik et Hadjout, $\chi^2=19,70$ et 161,04 ; $p<0,001$), alors que pour l'espèce ovine, l'abattage des femelles est prédominant à l'abattoir de Boufarik (70,6% ; $\chi^2=37,16$; $p<0,001$) contrairement à celui de Hadjout où l'abattage des males est majoritaire (82,2% ; $\chi^2=107,68$; $p<0,001$).

2- Prévalence des cas suspects de tuberculose des animaux dans les deux abattoirs (Boufarik & Hadjout).

La répartition des cas suspects de tuberculose des animaux est rapportée dans le tableau II.

Tableau 2: Répartition des cas suspects de tuberculose des animaux.

Espèce animale	**Animaux inspectés**	**Carcasses suspectes**	**Prévalence (%)**
Caprine	489	19	3,89
Ovine	477	21	4,40
Total	**966**	**40**	**4,14**

Sur les 966 carcasses ovines et caprines inspectées dans les deux abattoirs, 40 ont présenté des lésions suspectes de tuberculose, soit une prévalence de 4,14%. Ce taux est parfaitement similaire ($\chi^2=0,163$; $p=0,69$) dans les deux espèces avec 3,89% et 4,40% respectivement chez les caprins et les ovins.

Cette prévalence est plus faible que celle rapportée par Sahraoui et al (2011) pour une étude réalisée dans la même région et qui est de 6,03% dans l'espèce caprine, mais plus élevée par rapport à celles rapportées par Kulo et Seme (2007) pour une étude réalisée au Togo portant sur un effectif de 9855 carcasses ovines et 4398 carcasses caprines, et qui sont de 0,15% et 0,07%, respectivement chez les ovins et les caprins.

3- Facteurs de variation de la tuberculose des petits ruminants:

La distribution des cas suspects de tuberculose en fonction du sexe et de l'âge chez les deux espèces est rapportée dans le tableau 3.

Tableau 3: Distribution des cas suspects de tuberculose en fonction du sexe et de l'âge chez les ovins et les caprins.

Espèce animale	Sexe						Age								
	Mâle			Femelle			< 1 an			1-3 ans			> 3 ans		
	Animaux inspectés	Carcasses suspectes	%	Animaux inspectés	Carcasses suspectes	%	Animaux inspectés	Carcasses suspectes	%	Animaux inspectés	Carcasses suspectes	%	Animaux inspectés	Carcasses suspectes	%
Caprine (n=489)	370	16	4,32	119	3	2,52	33	0	0	177	2	1,13	279	17	6,09
Ovine (n=477)	277	7	2,53	200	14	7,00	24	0	0	171	0	0	282	21	7,45
Total (n=966)	647	23	3,55	319	17	5,33	57	0	0	348	2	0,57	561	38	6,77

Le traitement des résultats fait ressortir que les lésions suspectes sont comparables pour les deux sexes chez les caprins (χ^2=0,784; p=0,376) et plus fréquentes chez les femelles pour les ovins (7,00% vs 2,53% pour les mâles; χ^2=5,52; p=0,016). De plus, ces lésions ont été rencontrées beaucoup plus chez les animaux âgés de plus de 3 ans pour les deux espèces avec 7,45% chez les ovins (χ^2=15,19; p=0,001) et 6,09% chez les caprins (χ^2=8,57; p=0,014). La relative et plus faible proportion des lésions rencontrée chez les chèvres pourrait s'expliquer par le faible taux d'abattage des femelles conséquent à la réglementation. En ce qui concerne l'espèce ovine, les brebis âgées sont les plus touchées (7,00 contre 2,53% pour les mâles âgés), cette situation peut être la conséquence de leur affaiblissement dû aux lactations successives les rendant immunodéprimées (Thorel, 2003). Par ailleurs, Duarte et al. (2007), sur une étude réalisée au Brésil, ont rapporté que les femelles sont plus touchées que les mâles pour les deux espèces.

L'absence de lésions observée chez les jeunes animaux à l'abattoir de Boufarik pourrait s'expliquer par la nature progressive et chronique de la maladie.

4- Localisation des lésions

La localisation des lésions suspectes de tuberculose par rapport aux organes cibles est rapportée par espèce dans le tableau 4:

Tableau 4: Localisation des lésions suspectes de tuberculose par rapport aux organes et par espèce.

Organes	Espèce caprine		Espèce ovine	
	Lésions suspectes	*Pourcentage*	*Lésions suspectes*	*Pourcentage*
Poumons	3	14,3	13	43,3
Ganglions	7	33,3	6	20,0
Foie	9	42,9	10	33,3
Plèvre	2	9,5	1	3,3
Total	**21**	**100,0**	**30**	**100,0**

Les lésions suspectes de tuberculose sont surtout localisées au niveau hépatique (42,9%) chez les caprins sans que cette différence ne soit vraiment significative (χ^2=6,24; p=0,101) alors que chez les ovins l'atteinte pulmonaire est vraiment dominante (43,3%, χ^2=10,8; p=0,013). L'atteinte ganglionnaire est assez importante (33,3% et 20,0%, respectivement chez les caprins et les ovins).

La localisation diversifiée des lésions sur les organes pourrait s'expliquer par la généralisation progressive de la maladie à partir du complexe primaire chez les petits ruminants (Thorel, 2003). Selon Gonthier et al. (2010), le complexe primaire est essentiellement pulmonaire chez les petits ruminants avec souvent une généralisation progressive (Gonthier et al., 2010). Pour ce qui est de la prédominance de l'atteinte pulmonaire de l'espèce ovine, elle pourrait s'expliquer par la transmission de la maladie par voie respiratoire. En effet, Kulo et Seme (2007) sur une étude réalisée au Togo, ont rapporté que 2/3 des lésions sont localisées au niveau pulmonaire parmi 12 cas suspects de tuberculose et 1/3 au niveau mammaire ce qui est en faveur des risques épidémiologiques et sanitaires accrus (aspect zoonotique de la maladie par contamination des humains par le lait contenant des bacilles).

5- Analyses bactériologiques :

Les résultats des analyses bactériologiques, réalisées à partir des prélèvements suspects de tuberculose, sont rapportés dans le tableau 5.

Tableau 5: Résultats des analyses bactériologiques des cas suspects de tuberculose.

Espèces	Examen bacilloscopique				Culture bactérienne			
	négatif		positif		négative		Positive	
	n	%	n	%	n	%	n	%
Caprine	17	89,5	2	10,5	11	57,9	8	42,1
Ovine	21	100	0	0	21	100	0	0

A l'examen bacilloscopique, 2 prélèvements sur les 19 porteurs de lésions suspectes parmi les caprins se sont révélés positifs (soit 5,0% du total des 40 cas ou 10,5% de la population caprine suspecte), alors que chez les ovins aucun prélèvement n'a été trouvé positif. La faible proportion de résultats positifs à l'examen direct est liée à la faible sensibilité de cette méthode de diagnostic car la charge bactérienne doit être $\geq 10^4$ BAAR/ml (Carbonelle et al., 2003). De plus, elle n'est pas spécifique car toutes les mycobactéries sont des BAAR.

Sur les 40 échantillons mis en culture, seuls huit issus des prélèvements de l'espèce caprine ont donné une culture positive, soit 20.0% du total des cas ou 42,1% des caprins porteurs. L'absence

de cultures positives sur les ovins présentant des lésions à l'inspection peut s'expliquer par la présence d'autres affections causées par d'autres germes non tuberculeux appartenant toujours à la famille des Actinomycétales, tel que nocardia (Teklu, 2004).

Les résultats de l'identification des souches isolées sont rapportés dans le tableau 6.

Tableau 6: Résultats de l'identification des souches isolées.

Souches	Culture positive (n=8)	
	Nombre	Pourcentage
M. bovis	6	75
M. atypique	2	25

L'identification des cultures positives par les critères phénotypiques, le délai d'apparition et de pigmentation des colonies ainsi que les tests biochimiques montrent que le pourcentage des Mycobactéries à M. bovis (75%) est supérieur à celui des Mycobactéries atypiques (25%) sans que l'on puisse conclure à une différence vraiment significative vu le trop faible nombre de cas (n=8 ; χ^2=2,00; p=0,157)

Compte tenu que les caractères phénotypiques et biochimiques des souches de M. bovis et M. caprae sont similaires, seul le typage moléculaire permettrait de les caractériser.

CONCLUSION

Pour la première fois, nous avons déterminé la prévalence des cas suspects de tuberculose chez les petits ruminants dans deux abattoirs en Algérie. Les statistiques d'abattage ne nous donnent pas de résultats précis sur ce sujet. C'est pourquoi nous recommandons de compléter l'examen post mortem par des examens de laboratoire en cas de suspicion. Dans notre étude, ces derniers ont confirmé la présence de la tuberculose caprine et nous n'avons pas pu confirmer la présence de la tuberculose ovine dans cette même région.

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DONNÉES ETHNOBOTANIQUES ET CHIMIQUES DES PLANTES MÉDICINALES UTILISÉES EN AFRIQUE DANS LES TRAITEMENTS TRADITIONNELS VÉTÉRINAIRES.

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Résumé

Les savoirs traditionnels sur les plantes utilisées dans les traitements des personnes et des animaux en Afrique tropicale constituent une approche méthodologique de recherche industrielle de nouvelles molécules actives d'origine végétale.

Une méthode basée sur des enquêtes ethnobotaniques réalisées de janvier 2010 à mai 2011 a été appliquée pour recueillir des informations sur une quarantaine de plantes réparties dans huit pays d'Afrique de l'Ouest et du Centre auprès de 181 tradipraticiens.

Une recherche documentaire a été faite pour confirmer l'identité scientifique et ethnopharmacologique des plantes étudiées.

Des expérimentations ont été faites pour savoir les groupes chimiques des substances actives de chacune des plantes par des essais, dosage, et réalisations au laboratoire.

Les résultats trouvés présentent des groupes de plantes par propriétés thérapeutiques similaires. Les résultats prédictifs des essais cliniques demandent des recherches complémentaires d'isolement des molécules en vue de la configuration spatiale de leurs formules chimiques.

Mots clés : *savoirs traditionnels, plantes thérapeutiques, constituants chimiques, activités thérapeutiques, développement pharmaceutique. Afrique tropicale*

ETHNOBOTANIC AND CHEMICAL DATA OF HEALING PLANTS USED IN AFRICA IN THE VETERINARY TRADITIONAL TREATMENTS

Abstract

The traditional knowledge on vegetables used in the treatment of people and animals in tropical Africa establish a methodological approach of industrial research for new active molecules of vegetable origin.

A method based on ethno botanic inquiries conducted from January, 2010 till May, 2011 was applied to collect information on about forty vegetables distributed in eight countries of western Africa and the Center with 181 traditional practitioners

Document retrieval was made to confirm the scientific identity and pharmacological ethnology of the studied vegetables.

Experiments were made to know the chemical groups of active substances of each of the vegetables by essays, dosage and tests in the laboratory.

The result presents groups of vegetables by similar therapeutic properties. The predictive results of the clinical trials for additional research for isolation of molecules with the aim of the spatial configuration of their chemical formulae.

Keywords: *traditional knowledge, therapeutic vegetables, chemical constituents, therapeutic activities, pharmaceutical development, tropical Africa*

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Introduction

La médecine traditionnelle en Afrique constitue un moyen d'utiliser l'arsenal thérapeutique des plantes pour faire face aux problèmes de santé humaine et animale. En effet, en Afrique, le recours aux recettes de la médecine et de la pharmacopée traditionnelle connaît un regain d'intérêt et on estime que 70 à 80% des africains consultent les tradithérapeutes pour se soigner. Plusieurs raisons expliqueraient cette situation dont celles relatives au coût et surtout à la qualité des médicaments chimiquement définis. L''approche thérapeutique des tradipraticiens par les plantes est limitée par les difficultés d'identification, de préparation et dosage, de conservation, et surtout par celles du diagnostic de la maladie. Cependant, la médecine traditionnelle est basée sur des connaissances empiriques transmises de génération en génération et elle ne fait pas souvent l'objet d'étude scientifique. De ce fait l'on peut assister à des contres indications, intoxications par surdosage, des cas d'insuffisances rénales ou hépatiques. Par conséquent il est utile de s'intéresser aux potentialités thérapeutiques des plantes d'usage traditionnel afin de justifier leur utilisation et de trouver des formulations efficaces et accessibles à la population.

Une bonne connaissance des savoirs traditionnels et des caractéristiques chimiques et pharmacologiques des plantes médicinales impliquées dans le traitement des maladies vétérinaires en Afrique tropicale contribuera à élargir le répertoire des plantes utiles susceptibles de répondre efficacement aux traitements traditionnels vétérinaires à des coûts beaucoup plus accessibles.

La présente étude s'inscrit dans le cadre la valorisation des plantes médicinales de la flore d'Afrique de l'ouest et a pour but de faire connaître les plantes impliquées dans le traitement traditionnel vétérinaire. Une bonne connaissance de ces plantes et la documentation sur les différentes études réalisées sur celles-ci permettrait de justifier non seulement leur utilisation scientifique mais aussi l'orientation éventuelle de la recherche de nouvelles molécules.

Méthodologie

La méthodologie est basée essentiellement sur des enquêtes ethnobotaniques. Ces enquêtes ont été menées auprès de 181 tradipraticiens du Bénin, du Burkina Faso, du Cameroun, du Mali, de Mauritanie, du Niger, du Sénégal et du Togo. Les informations recherchées ont concerné les noms vernaculaires, les indications thérapeutiques et les différentes maladies aussi bien humaines que vétérinaires. Le questionnaire du guide d'entretien a porté entre autres sur l'indication thérapeutique de la plante, l'organe (partie de la plante) utilisé et le mode d'utilisation. Les noms vernaculaires dans les différents dialectes sont recueillis. L'indentification des plantes recensées est faite sur place et confirmée au Laboratoire de Botanique et Ecologie Végétale de l'Université de Lomé.

La récolte des informations a concerné une quarantaine de plantes réparties inégalement sur le Bénin, le Burkina Faso, le Cameroun, le Mali, la Mauritanie, le Niger, le Sénégal et le Togo.

Au cours d'une deuxième phase du travail, une recherche documentaire a été systématique. Un herbier a été constitué dont la conservation a été faite à l'abri de la lumière et de l'humidité à une température de 25° C et à un taux d'humidité de 25%. L'identification a été faite au laboratoire de Botanique de l'Université de Lomé par la méthode d'extraction alcoolique.

Les plantes qui ont servi d'expérimentations au laboratoire ont été récoltées de préférence : les racines au moment du repos végétatif (octobre-décembre); les feuilles justes avant la floraison, les fleurs au moment de leur plein épanouissement et les graines lorsqu'elles ont perdu la majeure partie de leur humidité naturelle.

Le principe a consisté à la séparation des substances présentes en mélange à l'aide d'un support.

Essais :

Ils ont concerné le taux de cendres (degré de propreté), la teneur en eau et porté à la dessiccation et la contamination microbiologique (champignons surtout).

Dosage

Le degré de l'activité thérapeutique des extraits de chaque plante sur les agents pathogènes et cellules animales a été mesuré en se référant aux propriétés thérapeutiques décrites par les tradipraticiens et recueillies lors des entretiens ethnobotaniques.

Réalisations :

Elles ont procédé par dosage chromatographique en phase gazeuse (CPG) pour les substances volatiles et par dosage chromatographique en phase liquide (CPL) pour les substances fixes. La spectrophotométrie a été utilisée pour les molécules absorbées dans l'ultra violet. Et pour les constituants mineurs des étapes de purification se sont avérées nécessaires et n'ont pas été réalisées faute de logistique de laboratoire. Quant aux constituants chimiques inconnus (voir résultats), il convient d'identifier l'empreinte digitale chimique de la drogue à l'aide des techniques les plus appropriées pour obtenir un maximum de précisions. Ce que nous n'avons pu réaliser faute de logistique de laboratoire. .

L'ensemble des travaux a couvert la période de janvier 2010 à mai 2011.

Données ethnobotaniques et chimiques de l'étude

Les plantes du Tableau I présentent des propriétés que d'autres auteurs ont trouvées lors de leurs investigations dans la lutte contre les ectoparasites. L'espèce Zanthoxylum chalybeum a des propriétés insecticides de même que celles de l'espèce Zanthoxylum gillettii trouvées par JIROVETZ L *et al.* Il faut préciser que ces deux espèces appartiennent à la famille des Rutaceae. Les grands groupes chimiques de ces plantes ont été alors déterminés et définissent les activités pharmacologiques décrites.

Les plantes à propriétés anthelminthiques (Tableau 2) sont d'usage courant dans les pays de l'étude. L'évaluation scientifique structurale doit permettre de connaître la structure chimique des composants des extraits végétaux actifs déterminés.

Les savoirs traditionnels ont constitué un référentiel d'orientation scientifique des investigations ethnobotaniques sur les effets anthelminthiques de certaines plantes de la flore tropicale en Afrique (6;7). Ces investigations ont permis de démontrer les effets pharmacologiques sur les helminthes (8). Des effets similaires ont été obtenus également par des extraits végétaux actifs d'autres plantes (1).

Les tableaux IIIa et IIIb montrent que les indications thérapeutiques des extraits des plantes décrites se rapportent à des effets thérapeutiques de type ".inhibition de la prolifération microbienne". En effet, les extraits de certaines espèces de plantes de la famille des Combretaceae ont révélé à l'analyse au laboratoire la présence des substances telles que les tanins, les saponines, les flavonoïdes qui ont montré un effet antimicrobien sur des agents pathogènes tels que Candida albicans, Staphylococcus aureus.

Les propriétés pharmacologiques des plantes présentées par le Tableau 4 montrent des effets divers de type nutritionnel et métabolique.

Conclusion

L'utilisation empirique des plantes en médicine traditionnelle africaine constitue de fait une hypothèse de son innocuité. C'est à partir de ce postulat que cet article a présenté par les Tableaux 1, 2, 3, et 4 le mode d'emploi de certaines espèces de plantes dans le traitement de certaines pathologies par les populations africaines. Et c'est à partir de ce mode d'emploi que l'on a pu réaliser les tests spécifiques d'identification, de pureté, de dosage et d'informations relatives aux constituants chimiques actifs de 39 plantes (données de la pharmacologie et des usages thérapeutiques

Au total, on peut retenir qu'une espèce végétale peut biosynthétiser plusieurs milliers de constituants chimiques différents. C'est l'intérêt que présente cet article de servir de point de départ à la synthèse ou hémi synthèse de nouveaux principes actifs pour l'industrie du médicament.

Balandrin *et al.* ont montré dans leurs travaux que les constituants chimiques isolés des matières végétales sont des matières premières innovantes pour l'industrie pharmaceutique et des produits de santé. On estime que plus

Tableau 1: Plantes anti-ectoparasites

N°	Nom scientifique de la plante	Usage traditionnel	Probables principes actifs	Propriétés pharmcologiques
1	*Calotropis procera (Ait.) Ait.f. (Asclepiadaceae)*	Utilisation des feuilles dans la lutte contre les ectoparasites au Sénégal.	Non connus	Importante activité insecticide des extraits de feuilles
2	*Tagetes minuta L. (Compositae))*	Utilisation de l'huile comme anti-mouches en Afrique de l'Ouest	Carvone, linaoöl	L'huile obtenue est un repoussant des mouches volantes
3	*Tephrosia vogueli Hook f. (Fabaceae)*	Utilisation des feuilles dans la lutte contre les insectes au Cameroun	Rotenon, tephrosin, deguelin (rotenoïdes), isolés des feuilles	Les rotenoïdes sont toxiques pour les insectes, les batraciens, les mollusques
4	*Azadirachta indica A. Juss (Meliaceae)*	Utilisation des feuilles et des graines dans la lutte contre les insectes au Mali, Niger, Mauritanie, Burkina Faso	Azadirachtin, isolé des graines	Propriétés insecticides et repoussantes de la substance active
5	*Zanthoxylum chalybeum Engl. (Rutaceae)*	Utilisation des écorces et des fruits au Niger dans la lutte contre les glossines dans les élevages de dromadaires	Palmatine (alcaloïde)	La palmatine est inhibitrice de la motilité de Trypanosoma lewis in vitro
6	*Balanites aegyptiaca (L.) Delire Zygophyllaceae)*	Utilisation du fruit dans la lutte contre les mollusques en Afrique de l'Ouest	Saponines	Les substances obtenues sont des molluscides

Tableau 2: Plantes anthelminthiques

N°	Nom scientifique de la plante	Usage traditionnel	Probables principes actifs	Propriétés pharmcologiques
1	*Dryopteris spp. (Aspidiaceae)*	Utilisation des rhizomes dans la lutte contre le helminthiases au Cameroun	Acyphloroglucines isolés du rhizome	Action efficace sur les helminthes et les tenias
2	*Carica papaya L. (Caricaceae)*	Utilisation des jeunes pousses et l'huile des grains dans la lutte contre les helminthiases au Nigeria	Papaïne isolée du latex	La papaïne est un excellent vermicide contre les ascaris et les oxyures
3	*Chenopodium ambrosioides L. (Clenopodiaceae)*	Les feuilles sont utilisées pour traiter les helminthiases animales au Niger, Mali, Burkina	Ascaridiol obtenue à partir des parties aériennes	L'Ascaridiol traite les ascaris et les ankylostomes
4	*Azadirachta indica A. Juss (Meliaceae)*	Utilisation des feuilles et des graines dans la lutte contre les insectes au Mali, Niger, Mauritanie, Burkina Faso	Azadirachtin, isolé des graines	Propriétés insecticides et repoussantes de la substance active

N°	Nom scientifique de la plante	Usage traditionnel	Probables principes actifs	Propriétés pharmcologiques
5	*Vernonia amygdalina Del. (Compositae)*	Les feuilles sont utilisées pour traiter les helminthiases animales dans plusieurs pays d'Afrique	Sesquiterpène lactones: vernodaline, vernolide, vernodalol et les Glycosides stérols. Vernoniosides A1, A2, A3	L'activité anthelminthique des sequiterpènes lactones a été démontrée
6	*Diospyros mespiliformis (Ebenaceae)*	Utilisation des écorces dans le traitement des helminthiases dans les pays d'Afrique de l'ouest et du centre	Hydroxyquinones (plumbagone, dyospyrine, dyspyrol)	Ces substances possèdent de nombreuses activités insecticides

Tableau 3 a : Plantes antimicrobiennes

N°	Nom scientifique de la plante	Usage traditionnel	Probables principes actifs	Propriétés pharmcologiques
1	*Kigelia africana (Lam). Benth. (Bignoniaceae)*	Utilisation des fruits et des écorces dans le traitement des plaies, ulères et abcès au Sénégal	Non connus	Les extraits de fruits et d'écorces ont montré une forte activité antimicrobienne sur Bacillus subtilis, Staphylococcus aureus
2	*Lepidium sativum L. (Cruciferae)*	Utilisation des graines dans le traitement des plaies chez les dromadaires et chevaux au Niger	L'huile essentielle volatile contient le Benzyllisothiocyanate isolé des grains	L'activité antimicrobienne de cette huile sur les bactéries et les champignos est bien connue.
3	*Euphorbia balsamifera Ait. (Euphorbiaceae)*	Utilisation du latex comme cicatrisant en Mauritanie	Triterpenoïdes	Les proprités cicatrisantes sont bien connues
4	*Cochlospermum tinctorium (Cochlospermaceae)*	Les racines sont utilisées pour traiter la diarrhée néonatale des veaux au Mali	Tanins obtenus à partir des racines	Les effets anti diarrhéiques des tanins ont été bien établis et les extraits ont montré une activité antimicrobienne
5	*Guiera senegalensis J.F. Gmel (Combretaceae)*	Utilisation des extraits des feuilles et des bourgeons dans le traitement de la lymphagite épizootique et les troubles abdominaux chez les animaux en Afrique de l'Ouest	Non connus	Les extraits obtenus à partir des feuilles ont des propriétés antiphlogistiques, antitussives et anti diarrhéiques

N°	Nom scientifique de la plante	Usage traditionnel	Probables principes actifs	Propriétés pharmcologiques
6	*Ageratum conizoïdes L. (Compositae)*	Utilisation de cette herbe au Cameroun pour traiter les mammites et le prolapsus vaginal chez les animaux	Non connus	L'huile obtenue de cette herbe a montré une activité antimicrobienne avec une action significative sur Staphylococcus aureus

Tableau 3 b : Plantes antimicrobiennes

N°	Nom scientifique de la plante	Usage traditionnel	Probables principes actifs	Propriétés pharmcologiques
1	*Mikana cordata (Burm.f.) B.L. (Combretaceae)*	Utilisation des feuilles dans le traitement des diarrhées profuses en Afrique de l'Ouest	Mikanolide, Dihydromikanolide isolées des parties aériennes	Les expérimentations ont montré que ces deux substances inhibent la croissance de Saphylococcus aureus et Candida albicans
2	*Khaya senegalensis (Meliaceae)*	Utilisation de l'écorce au Mali, Niger, Burkina chez les bovins et les chevaux dans le traitement de la fièvre, des tendinites et des troubles digestifs	Stérols (β-stérols) et coumarines (scopoletine)	β-stérol a montré une activité antipyrétique similaire à celle de l'acide acétylsalicylique de même que les coumarines
3	*Eucalyptus spp. (Myrtaceae)*	Utilisation des feuilles au Mlai, Burkina et Niger pour traiter la fièvre	Eucalyptol	L'huile d'Eucalyptus ont des propriétés antiseptiques
4	*Psidium guajava L. (Myrtaceae)*	Les feuilles sont utilisées dans le traitement de la diarrhée et de troubles digestifs	L'huile essentielle est riche en: cinéol, tanins, 4 acides triperniques, ursolique et l'acide oléanolique	Activité antimicrobienne à large spectre
5	*Plumbago Zeylanica L. (Plumbaginaceae)*	Utilisation des racines en Afrique de l'Ouest dans le traitement des maux d'yeux	Naphthoquinones, plumbagine, plumbagol isolés à patir des racines	La plumbagine possède des propriétés de la vitamine K et celles antimicrobiennes
6	*Solanum incarnum L. (Solanaceae)*	Utilisation du fruit au Cameroun dans le traitement du panaris interdigité chez les animaux	Purine isolée du fruit	Action sur les bactéries et les champignons

Tableau 4 : Plantes médicinales à usage divers

N°	Nom scientifique de la plante	Usage traditionnel	Probables principes actifs	Propriétés pharmcologiques
1	*Ricinus communis L. (Euphobiaceae)*	Utilisation de l'huile essentielle dans le traitement de la constipation en Mauritanie	Acide rünoléique	Propriété purgative connue
2	*Cassia abbreviata (Caesalpiniaceae)*	Utilisation de l'écorce au Mali, Niger, Burkina chez les bovins et les chevaux dans le traitement de la fièvre, des tendinites et des troubles digestifs	Anthraglycosides	Les anthraglycosides agissent comme des laxatifs
3	*Tamarindus indica L. (Caesalpiniaceae)*	Utilisation des pulpes et des feuilles au Sénégal pour traiter la constipation	Acides	Action laxative
4	*Combretum glutinosum (Combretaceae)*	Utilisation des feuilles au Mali, Burkina, Niger, Mauritanie dans le traitement de la rétention urinaire	Non connus	L'action diurétique de la décoction des feuilles a été cliniquement vérifiée en médecine humaine
5	*Agropyrum sp. (Graminae)*	Utilisation de la plante au Mali pour traiter la rétention urinaire	Acide glycolique obtenu à partir des rhizomes	La diurèse est attribuée à l'acide glycolique
6	*Securidaca Longepedon (Polygalaceae)*	Utilisation des tiges, des écorces dans la prévention et le traitement des morsures de serpents	Une protéine définie a été isolée des racines	Cette protéine a la même composition et similaire au poison de Naja nigricollis, ces deux protéines se disputent les mêmes récepteurs
7	*Atriplex spp. (Chenopodiaceae)*	La plante est utilisée dans le traitement des carences en sels minéraux dans les élevages nomades	Plante très riche en NaCl	-

de 500 000 arbres des régions tropicales ne sont pas exploitées et donc les substances bio actives dont dispose la recherche à ce jour sont énormes pour la découverte de nouveaux médicaments.

Toutefois, la qualité pharmaceutique est l'un des éléments essentiels qui garantit l'efficacité, mais aussi l'absence de toxicité des médicaments. C'est pourquoi, il était important et indispensable de définir de façon très rigoureuse l'identité et la qualité de la matière première végétale qui sert à la préparation de ces médicaments. Des études complètes à l'aide d'un appareillage de haute précision sont alors nécessaires pour l'extraction par filtration ou par fermentation des constituants actifs, la caractérisation de leurs formules chimiques, leur triage et l'obtention de principes actifs sûrs ou d'excipients stables.

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SEROLOGICAL AND PATHOLOGICAL ASSESSMENT OF LYMPHOID ORGANS IN CHICKS FED WITH GRADED LEVELS OF FERMENTED AND UNFERMENTED CASSAVA DIETS.

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Abstract

Despite the myriad of investigations in literature on cassava utilization in domestic chickens, its effects on the avian specific humoral immune system still remains elusive. This study investigated the effects of graded levels of fermented (F) and unfermented (UF) cassava diets on the lymphoid organs and its subsequent humoral immunological response in domestic chicks.

One hundred day-old Yaffa White cockerels were randomly divided into five groups (A to E) of twenty birds each. The replacement of maize with 0%, 20%, 30%, fermented or unfermented cassava root meal was on a quantitative basis in each group fed for 5 weeks. All other ingredients remained constant.

The mean body weight in different cassava treated groups (CTGs) showed no significant difference (P>0.05) compared with the control. The bursa and thymic weights showed both decrease and increase significant difference (P<0.05). There was significant decrease in the IBD antibody titers of CTGs compared with the control at week 5. Macroscopically, mild to moderate enlargement and atrophy of bursa of Fabricius, with petechiation of cortical surfaces of the thymus were observed. Microscopically, lesions such as interfollicular edema, interfollicular fibrosis, follicular atrophy, lymphofollicular hypoplasia, dystrophic epithelium, thickened splenic capsule, splenic lymphoid hypoplasia, thymic lymphoid hypoplasia, diffuse medullary thymic haemorrhages and Myoid cells proliferation were the consistent histopathological changes in all the CTGs. In conclusion, this study demonstrated that cassava inclusion feeds could cause subtle sublethal effects to the lymphoid organs of domestic chickens, and consequently lead to vaccine failure when it is administered for a long period of time.

Keywords: *Lymphoid organs, fermented and unfermented cassava, serology, pathology*

ÉVALUATION SEROLOGIQUE ET PATHOLOGIQUE DES ORGANES LYMPHOÏDES DES POUSSINS NOURRIS AVEC DES DEGRES PROGRESSIFS DE REGIMES DE MANIOC FERMENTE ET NON FERMENTE

Résumé

En dépit de la multitude de recherches documentaires sur l'utilisation du manioc dans l'alimentation des poulets domestiques, ses effets sur le système immunitaire humoral spécifique à l'espèce aviaire restent difficiles à déterminer. Cette étude a examiné les effets de régimes de manioc fermenté (F) et non fermenté (UF) aux degrés progressifs sur les organes lymphoïdes et la réponse immunologique humorale subséquente sur les poussins domestiques.

Des coqs blancs Yaffa âgés de cent jours ont été répartis de manière aléatoire en cinq groupes (AE) de vingt oiseaux chacun. Le remplacement du maïs par de la farine de manioc fermenté ou non fermenté - 0%, 20%, 30% - a été effectué sur une base quantitative dans chaque groupe pendant 5 semaines. Tous les autres ingrédients sont restés constants.

Le poids corporel moyen dans les différents groupes traités avec du manioc (CTG) n'a montré aucune différence significative (P> 0,05) par rapport au groupe témoin. Les bourses séreuses et les poids des thymus ont montré une différence significative au niveau de la diminution et de l'augmentation (P <0,05). On a noté une diminution significative des titres d'anticorps IBD des CTG par rapport au groupe témoin à la semaine 5. Macroscopiquement, un élargissement et une atrophie variant entre « bénins

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» et « modérés » - de la bourse de Fabricius, avec pétéchies des surfaces corticales du thymus ont été observés. Au microscope, des lésions dont un oedème interfolliculaire, une fibrose interfolliculaire, une atrophie folliculaire, une hypoplasie lymphofolliculaire, un épithélium dystrophique, un épaississement de la capsule splénique, une hypoplasie lymphoïde de la rate, une hypoplasie lymphoïde thymique, des hémorragies médullaires thymiques diffuses et une prolifération des cellules myoïdes sont les changements histopathologiques constants observés chez tous les CTG. En conclusion, cette étude a démontré que l'inclusion du manioc dans l'alimentation est susceptible de provoquer des effets sublétaux subtils sur les organes lymphoïdes des poulets domestiques, et par conséquent conduire à l'échec des vaccins lorsque ceux-ci sont administrés sur une longue période de temps.

Mots-clés : organes lymphoïdes, manioc fermenté et non fermenté, sérologie, pathologie

Introduction

Cassava *(Manihot esculenta Crantz)* covers about 80 million hectares in 34 African countries and requires little production skills or inputs, is drought tolerance and produces reasonable yields under adverse conditions (Treberge, 1985). Cassava roots are known to be rich in carbohydrates, but low in proteins and amino acids (Essers *et al.* 1994). It is also widely recognized as a cheap source of food energy and a possible replacement for scarce and costly grains. This is the basis for its agronomic and economic advantages which the crop enjoys over grain crops (Oyenuga, 1968).

Moreso, it has been tipped to play a major role in the food security of many African countries in the future (Prudencia and Al-Hassan, 1994).

However, contradictory reports abound in the literature over the use of cassava in humans (Banea-Mayambu *et al.* 2000; Oluwole *et al.* 2002; Adamolekun *et al.* 2010) and various animals species such as goats (Akinsoyinu, 1992; Soto-Blancoa and Gorniak, 2010), pigs (Soniaiya and Omole, 1983), cattle (Szylit, 1977; Thang *et al.* 2010), rabbits (Oso *et al.* 2010), fish (Ufodike and Matty, 1983; Ng and Wee, 1989) and wild life (Tewe, 1984; Adamolekun *et al.* 2010). In the investigations of these workers, low level of cassava utilization was adjudged to be a good replacement of energy, but high level of cassava caused deleterious effects. This intoxication has been attributed to widely ranging levels of cyanogenic glycoside, namely linamarin and lotaustralin, found in cassava root (Cenn, 1979).

In poultry, different adverse effects of cassava have been documented in the literature. These include increased feed consumption (Tewe, 1983), watery droppings, abnormal behavioral response of higher feed spillage (Iyayi and Fayoyin 2005), lower egg production and egg quality, reduced shell thickness and hatchability of eggs (Ngoka *et al.* 1982), growth depression (Vogt, 1966; Panigrahi *et al.* 1992), and mortalities characterized by postmortem lesions such as poor feathering, collapsed air sacs, enlarged and necrotic caeca (Ofuya and Obilor, 1993; Tathawan *et al.* 2002).

Despite the myriad of investigations in the literature on cassava utilization in domestic chickens, its effects on the avian specific immune system still remains elusive. In this study, we investigated the effects of graded levels of fermented and unfermented cassava feeds on the lymphoid organs and its subsequent humoral immunological response in domestic chicks.

Materials and Methods

Preparation of fermented and unfermented cassava

The preparation of fermented and unfermented cassava chips was performed according to the method of Khajarern *et al.* (1982). Briefly, fresh tubers of sweet cassava (with lower hydrocyanic acid content, Garcia and Dale, 1999) were washed with water to remove soil and other extraneous materials. The tubers were cut into smaller pieces, then soaked in water in a tightly covered plastic bowl and left to stand for three days for fermentation to occur, after which the macerated cassava was extracted, then sun-dried for 5 days. For unfermented cassava, tubers were sliced into smaller pieces after being washed, then sun-dried for 5 days

Estimation of hydrocyanide content

The fermented and unfermented cassava samples were submitted to the laboratory of International Institute of Tropical Agriculture (IITA) Ibadan, Oyo-State, Nigeria, for hydrocyanide content estimation. Analysis was carried out according to the method of Padmaja (1989).

Experimental design

One hundred day-old Yaffa White cockerels (100) were obtained from a local hatchery. The birds were randomly divided into five groups (A-E) of 20 birds each.

The replacement of maize with fermented and unfermented cassava root meal was on a quantitative (Weigh/Weight) basis. All other ingredients remained constant (Table1). The control group (A) was fed with 100% of maize as the energy source. Group (B) was fed with 20% (weight/weight) substitution of maize with fermented cassava and group (C) was fed with 30% (weight/weight) substitution of maize with fermented cassava. Group (D) was given 20% (weight/weight) substitution of maize with unfermented cassava and group (E) was given 30% (weight/weight) substitution of maize with unfermented cassava.

Each group was raised in deep litter pens with dry wood-shavings as litter material and exclusively separated from one another. All birds were subjected to standard management and health practices. Feed and water were provided *ad libitum* during the experimental period that lasted for 5 weeks. Infectious bursa disease (IBD) vaccinations were administered at 7th and 14th day old to all the birds in both cassava-treated and control groups to determine the level of humoral response in these birds. The experimental procedures were approved by the committee on ethics of the use of experimental animals of the college of Veterinary Medicine, Federal University of Agriculture, Abeokuta Nigeria.

Blood collection.

Three millimeters of blood was collected from three randomly selected birds per week through the heart. Blood samples were allowed to stand at room temperature for approximately 2 hrs for the blood to clot. The resulting serum samples were decanted and stored at -20^{0}C until serological analysis was performed for Infectious bursa disease antibody titre estimation.

Body and Lymphoid organ weights measurement.

Five birds were selected weekly for weight estimation using a weighing scale. Three birds were humanely euthanized (cervical dislocation) and necropsy was performed on them. The bursa of Fabricius, thymus and the spleen were extracted and weighed on a PB153-L Mettler Toledo balance (Switzerland).

Enzyme Linked Immunosorbent Assay

The enzyme linked immunosorbent assay method was performed, according to the manufacturer's recommendation (Flock check IBD, IDEXX®). Briefly, dilutions of test sera were made in the sample diluents buffer (1:500).

Fifty microliters (50µl) of each sample including the positive and negative controls was added into the infectious bursa disease virus (IBDV) coated plates and incubated for 1 hour at room temperature. The plates were then emptied and washed thrice. Goat anti-chicken horseradish peroxidase (HRP) conjugate was then added to the wells and allowed to incubate for 20 minutes at room temperature. The wash cycle was repeated three times at 15 minute intervals. Fifty microliters tetramethylbenzidine (TMB) substrate was finally added to the wells at room temperature for colour development and 50µl of stop solution was added after 15 minutes. The optical densities were determined using ELISA plate reader at 650nm.

Interpretation of optical density results

Upper limit of negativity (ULN) was determined by adding 0.0155nm to mean O.D value (0.0845) of negative control sera. Any serum with OD value greater than ULN (0.1nm) is regarded as containing IBDV antibody.

Histopathology and Morphometric analysis of the lymphoid organs.

All the extracted bursa of Fabricius, thymus and spleen were routinely fixed in 10% neutral buffered formalin, then dehydrated in graded levels of alcohol, embedded in paraffin wax, sectioned at 5µm and stained with heamatoxylin

Table 1: The formula employed per 100kg feed is as follows.

	Control (A)	20%F(B)	30%F(C)	20%UF(D)	30%UF (E)
Maize	48.20	38.60	33. 80	38.60	33.80
Soya Meal	9.64	9.64	9.64	9.64	9.64
Groundnut cake	12.00	12.00	12.00	12.00	12.00
Fish meal	3.20	3.20	3.20	3.20	3.20
Wheat offal	12.80	12.80	12.80	12.80	12.80
Palm kernel meal	8.00	8.00	8.00	8.00	8.00
Lime stone	2.40	2.40	2.40	2.40	2.40
Bone meal	2.40	2.40	2.40	2.40	2.40
Methionine	0.24	0.24	0.24	0.24	0.24
Lysine	0.24	0.24	0.24	0.24	0.24
Premix	0.80	0.80	0.80	0.80	0.80
Fermented Cassava	0.00	9.60	14.40	0.00	0.00
Unfermented cassava	0.00	0.00	0.00	9.60	14.60
Total	**100**	**100**	**100**	**100**	**100**
Chemical analysis					
Energy (kcal/kg)	2473.02	2405.38	2428.18	2411.21	2428.18
Crude protein (%)	17.62	16.89	17.14	16.90	17.14
Crude fibre (%)	3.96	4.72	3.84	3.78	3.84

Table 2: Hydrocyanide content of fermented and unfermented Mannihot esculenta

Mannihot esculenta	Hydrocyanide content (mg/kg)
Fermented	37.89
Unfermented	40. 01

and eosin (H & E). Histopathological alterations were observed under the Olympus light microscope. The histopathological lesions were scored according to their severity in the three lymphoid organs examined. These include; (-) no lesions were observed, (+) slight or mild lesions was present, (++) moderate lesions and (+++) severe or marked lesions.

The histological sections of the bursa of Fabricius were subjected to morphometric analysis consisting of measurements taken from 15 randomly selected follicles per bursa. The measurements were cortical thickness and medullar diameter. The morphometric analysis was performed using electronic Micrometrics TM SE Premium software (version 2.8 Accuscopes micrometric 2000-2009). The cortical thickness of each follicle was the mean of four measurements at cardinal points. The smallest medullar axis was considered the medullar diameter (Silva *et al.* 2003).

Statistical analysis of data

Data were presented as mean ± standard error of mean (SEM). One-way analysis of variance (ANOVA), Duncan multiple range tests and the least significant difference test were used to compare the means for significant differences at $P< 0.05$.

Results

The hydrocyanic acid content in the fermented and unfermented cassava roots are depicted in Table 2. The content of the hydrocyanide was slightly higher in the unfermented cassava than the fermented root.

Throughout the period of this study, there was no clinical manifestation of diseases, except excessive aggressive behaviour manifested by the birds in all the cassava treated groups (CTGs), evidenced by high feed spillage.

The body weights and the weights of bursa of Fabricius, thymus and spleen are depicted in Table 3.

Throughout the five weeks of this investigation, there was no significant difference ($P>0.05$) in the mean body weight of chicks in different CTGs and the control. Despite the fact that the body weights of these birds were not significantly different in all the groups, the weights of birds in group E (30% UF) at weeks 4 and 5 (287.0±52.00 and 349.00±46.7, respectively) had lower body weights compared with other groups.

At week 2, 3 and 4, there was significant increase ($P<0.05$) in the bursa mean weights of the CTGs compared with the control especially in group B, C, D and E. Moderate significant decrease ($P<0.05$) was observed in the CTGs (except in group C) at week 5.

The mean thymic weights showed significant increase ($P<0.05$) in groups B, C and D, with slight significant decrease ($P<0.05$) in group E compared with control at week 3. The splenic mean weights showed no significant difference throughout the period of this study.

Table 3: Weekly Body weights and organ weights from chicks treated with varying levels of fermented and unfermented cassava and the control group.

Weeks	Groups	Bow (g)	Buw (g)	Tw (g)	Sw (g)
1	A	101.46±18.0	0.47±0.08	0.40±0.23	0.10±0.02
	B	103.30±27.6	0.39±0.25	0.39±0.22	0.16±0.09
	C	114.94±11.5	0.53±0.22	0.42±0.06	0.08±0.02
	D	109.70±25.7	0.37±0.13	0.25±0.09	0.11±0.04
	E	114.16±10.1	0.53±0.11	0.64±0.24	0.11±0.01
2	A	170.20±31.20	0.71 ± 0.02^{a}	0.49±0.33	0.18±0.05
	B	186.60±47.60	0.96 ± 0.06^{a}	0.58±0.10	0.15±0.06
	C	164.44±48.51	1.23 ± 0.20^{bc}	0.83±0.11	0.29±0.02
	D	153.00±33.11	1.39 ± 0.03^{c}	0.83±0.29	0.20±0.04
	E	182.00±36.31	1.02 ± 0.13^{b}	0.69±0.23	0.48±0.34
3	A	232.40±33.7	0.96 ± 0.66^{a}	0.29 ± 0.10^{ab}	0.50±0.25
	B	293.80±53.9	1.23 ± 0.87^{b}	0.42 ± 0.19^{b}	0.88±0.52
	C	244.40±34.4	1.00 ± 0.26^{a}	0.47 ± 0.23^{b}	0.86±0.06
	D	267.0±56.00	1.19 ± 0.26^{b}	0.40 ± 0.15^{b}	1.08±0.39
	E	256.20±38.6	1.23 ± 0.34^{b}	0.25 ± 0.13^{a}	0.19±0.04
4	A	323.40±25.3	0.40 ± 0.11^{a}	1.49±0.66	0.71±0.27
	B	308.0±66.00	0.84 ± 0.35^{b}	0.87±0.38	0.56±0.19
	C	327.0±55.50	0.59 ± 0.04^{a}	0.94±0.28	0.59±0.16
	D	356.40±88.9	1.07 ± 0.38^{bc}	1.53±0.72	0.71±0.49
	E	287.0±52.00	0.60 ± 0.10^{a}	1.07±0.26	0.75±0.28
5	A	376.80± 50.5	0.85 ± 0.61^{a}	2.21 ± 1.19^{ab}	1.24±4.20
	B	397.00±37.2	0.53 ± 0.05^{b}	1.19 ± 0.20^{a}	0.89±0.11
	C	396.00± 50.0	1.04 ± 0.71^{bc}	2.80 ± 0.24^{b}	1.15±0.32
	D	366.00±27.7	0.61 ± 0.27^{b}	1.31 ± 0.29^{a}	0.77±0.22
	E	349.00±46.7	0.39 ± 0.16^{d}	1.85 ± 0.52^{ab}	1.02±0.45

Bow = Body weight, Buw =Bursa weights, Tw =Thymus weights, Sw =spleen weights.

[a, b, c, d] Means with the same superscripts are not significantly different, $P<0.05$ level along the columns

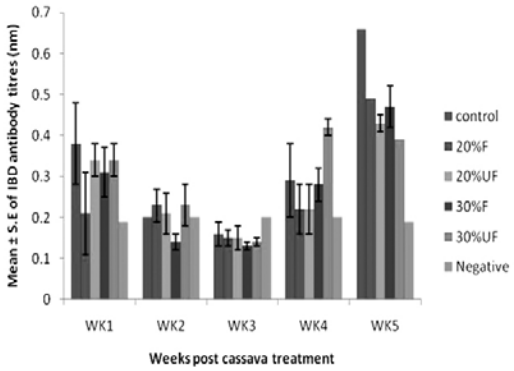

Fig. 1: Bar-chart showing the mean antibody titres of fermented and unfermented cassava treated chicks from week 1 to 5

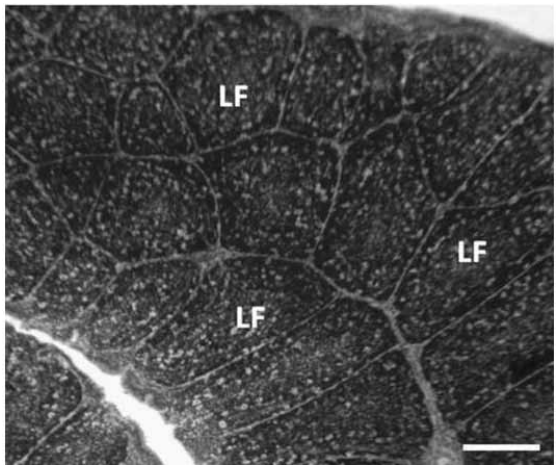

Fig. 2; Photomicrograph of the control Bursa of Fabricius at week 1 showing well formed lymphoid follicles (LF). H &E. Bar = 150µm

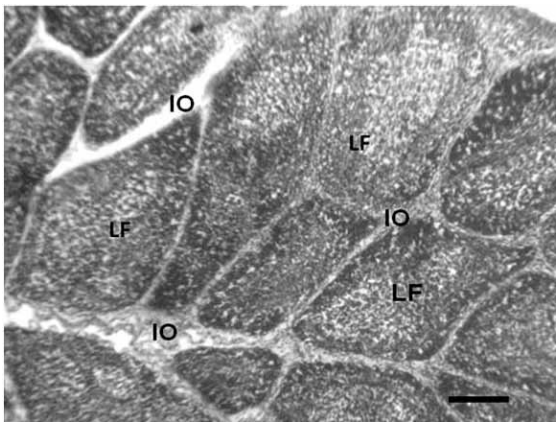

Fig. 3; Photomicrograph of the bursa of Fabricius showing depleted lymphoid follicles (LF) and mild interstitial oedema (IO) at week 1 in 30% unfermented cassava treated group. H &E. Bar = 80µm.

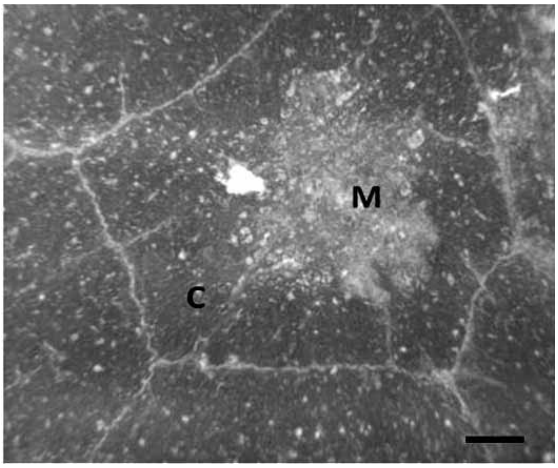

Fig. 4; Photomicrograph of the control thymus showing well formed cortical (C) and medulla (M) areas of the lymphoid follicles at week 2. H &E. Bar = 50µm

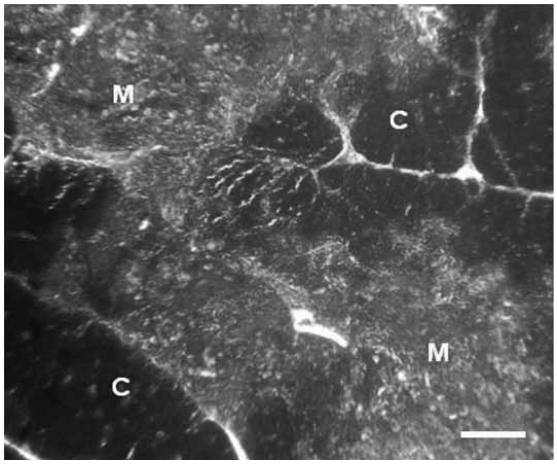

Fig. 5; Photomicrograph of the thymus showing haemorrhages in the medulla (M) and mild lymphoid depletion of the cortical areas (C) at week 2 in the 20% unfermented cassava treated group. H &E. Bar = 50µm.

Elisa Interpretation

At weeks 1, 2 and 3, there was no significant ($P>0.05$) changes in the infectious bursa disease antibody titers in all the cassava treated groups compared with the control. The antibody titers in the entire group showed gradual decline after the first and the second vaccinations. At week 4, the IBD antibody titers were at variance between the CTGs and the control group. At week 5, there was significant decrease ($P<0.05$) in the antibody surge in all the CTGs compared with the control. (Fig.1)

Pathological changes and Morphometric analysis of the lymphoid organs.

Grossly, mild to moderate enlargement of bursa of Fabricius was observed in 11 (18.3%) out of 60 chicks and 14 (23.3%) with petechial haemorrhages on the cortical surface of the thymus in the CTGs.

Microscopically, various lesions were observed in the bursa of Fabricius, thymus and the spleen in all the CTGs and the severity of these lesions is shown in Tables 4 to 6. The histopatological changes were similar, irrespective of the treatment groups with fermented or unfermented cassava, but there were variations in the degree or severity of lesions between treated groups.

The microscopic changes included interfollicular edema, interfollicular fibrosis, follicular atrophy, lymphofollicular hypoplasia, dystrophic epithelium, thickened splenic capsule, splenic lymphoid hypoplasia, thymic lymphoid hypoplasia, diffuse medullary thymic haemorrhages and Myoid cells proliferation (Figs.2 to 10).

Table 7 shows the morphometric measurements of the cortical thickness and medullary diameter in CTGs and the control group. Throughout the period of the experimental study, there was mild to moderate significant reduction ($P<0.05$) in the cortical thickness of bursa of Fabricius in all the CTGs compared with the control group.

The medullary diameter in the CTGs showed mild to marked significant decrease ($P<0.05$) compared with the control group, especially at weeks 4 [group C=82.47±24.45, group E= 82.17±40.95, group A (control) = 136.67±27.43] and week 5 [group B= 36.17±29.10, group C= 47.33±28.18, group A (control) = 133.50±35.76].

Discussion

This present study has demonstrated that inclusion of graded levels of cassava in poultry diets (up to 30% fermented and unfermented) might not cause adverse effects on the body weights of domestic chicks, since no significant difference was observed in the body weights of CTGs compared with the control, throughout the period of the study.

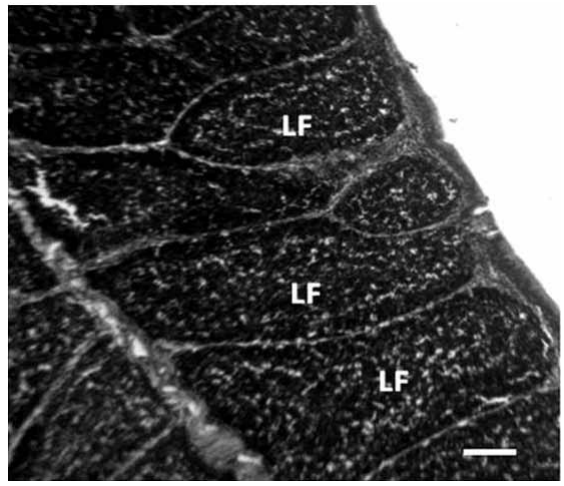

Fig. 6; Photomicrograph of the control Bursa of Fabricius at week 4 showing well formed lymphoid follicles (LF). H &E. Bar = 150µm.

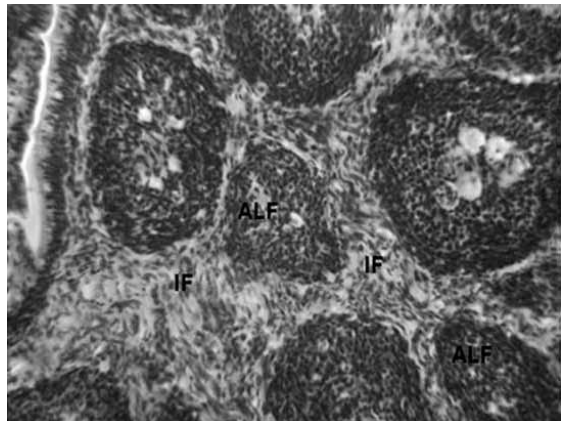

Fig. 7; Photomicrograph of the 20% fermented cassava treated Bursa of Fabricius at week 4 showing moderate interstitial fibrosis (IF) and atrophy of the lymphoid follicles (ALF). H &E. Bar = 150µm

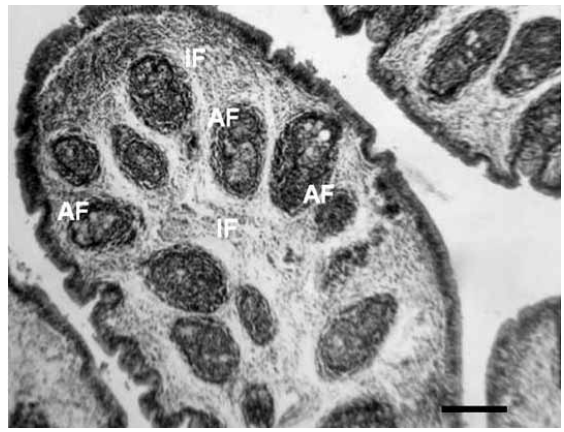

Fig. 8; Photomicrograph of the 30% unfermented cassava treated Bursa of Fabricius at week 5 showing marked interstitial fibrosis (IF) and severe atrophic follicles (AF). H &E. Bar = 150µm.

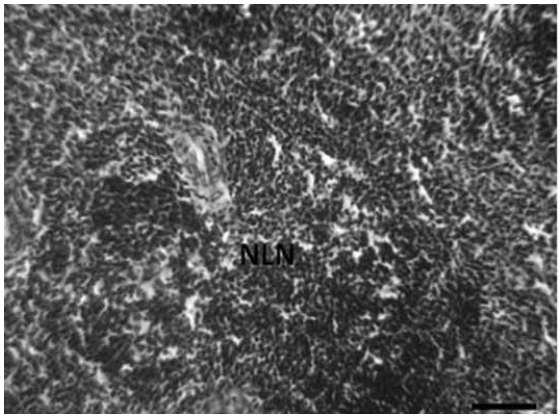

Fig. 9; Photomicrograph of the spleen in the control chicks at 4 week showing normal lymphoid nodules (NLN). H &E. Bar = 50µm.

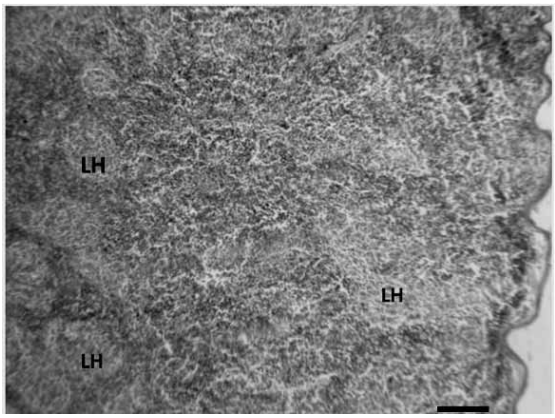

Fig. 10; Photomicrograph of the spleen at week 4 in the 30% fermented cassava treated group showing severe lymphoid depletion (LH) of the lymphoid nodules. H & E. Bar = 80µm

This is in agreement with the report of other workers (Panigrahi, *et al.* 1992; Tathawan, *et al.* 2002).

However, the study also demonstrated that cassava inclusion diets can cause subtle sublethal effects to the lymphoid organs of domestic chickens, even with the lowest cassava inclusion diet (20% fermented diet with lowest content of hydrocyanic acid).

This was demonstrated at week 5 in which antibody titers of CTGs were significantly decreased ($p<0.05$) compared with the control group. This was also corroborated by the severe histopathological changes observed in this group at this time (Fig. 8)

Table 4: Histopathological changes in the Bursa of Fabricius of domestic chicks fed with 20% and 30% fermented and unfermented Mannihot esculenta

Weeks	**Week1**					**Week2**					**Week3**					**Week4**					**Week5**				
Groups	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
Lesions																									
Interfollicular oedema	-	+	+	++	-	-	+	++	++	++	-	+	++	+	+	-	+	+	++	+	+	+	+	+	+
Interstitial fibrosis	-	+	+	+	++	-	+	++	+	++	+	++	++	++	++	-	++	++	++	++	+	++	+++	+++	+++
Follicular atrophy	-	-	-	-	++	-	-	+	++	++	-	+	++	+	++	-	++	++	+++	++	-	++	+++	+++	+++
Lymphoid hypoplasia	-	+	+	++	++	-	-	+	++	++	-	++	++	++	++	-	++	+++	+++	+++	+	++	++	++	+++
Dystrophic epithelium	-	-	-	-	++	-	-	+	+	++	-	++	++	++	++	-	++	++	++	++	-	++	++	+	++

- = Absent, + = mild, ++ = moderate, +++ = severe/ marked.

Table 5: Histopathological changes in the Thymus of domestic chicks fed with 20% and 30% fermented and unfermented Mannihot esculenta

Weeks	Week1					Week2					Week3					Week4					Week5				
Groups	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
Lesions																									
Lymphoid hypoplasia	-	-	-	-	++	-	-	+	+	++	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+
Thymic haemorrhage	-	-	-	++	+	-	-	+	++	+	-	+	-	++	+	-	-	-	+	+	-	-	-	-	+
Myoid cell hyperplasia	-	-	-	-	+	-	+	+	+	++	-	+	+	+	+	-	+	+	+	+	-	+	+	++	++

- = Absent, + = mild, ++ = moderate, +++ = severe/ marked.

Table 6: Histopathological changes in the Spleen of domestic chicks fed with 20% and 30% fermented and unfermented Mannihot esculenta

Weeks	Week1					Week2					Week3					Week4					Week5				
Groups	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
Lesions																									
Thicken capsule	-	-	-	-	+	-	-	+	+	+	-	-	+	+	+	-	+	++	+++	+++	-	+	++	++	++
Lymphoid hypoplasia	-	-	-	+	+	-	-	+++	+++	++	-	-	+	+++	++	-	+	++	+++	+++	-	+	++	++	++

- = Absent, + = mild, ++ = moderate, +++ = severe/ marked.

Table. 7: Morphometric analysis (cortical thickness and medullary diameter) of the bursa of Fabricius in chicks treated with fermented and unfermented cassava and control groups.

Parameters	Groups	Week1	Week 2	Week 3	Week 4	Week 5
Cortical thickness(μm)	A	20.75±5.28^{a}	20.58±5.00^{a}	21.21±3.70^{a}	22.66±5.35^{a}	21.08±6.48^{a}
	B	15.71±4.80^{b}	14.67±2.95^{b}	13.61±3.99^{bc}	13.33±8.30^{bc}	14.41±4.92^{b}
	C	14.83±4.40^{b}	15.25±4.85^{b}	12.05±3.94^{bc}	13.75±4.72^{bc}	12.96±5.40^{bc}
	D	15.54±3.20^{b}	14.46±4.00^{b}	15.71±3.20^{b}	17.36±4.18^{b}	11.50±6.29^{bc}
	E	14.41±3.50^{b}	18.04 ±5.50^{ab}	17.83±6.55^{b}	21.08±7.21^{a}	15.58±4.77^{b}
Medullary diameter (μm)	A	91.67±26.88^{a}	118.00±33.60^{a}	132.50±33.63^{a}	136.67±27.43^{a}	133.50±35.76^{a}
	B	80.33±45.04^{b}	86.17±29.73b^{c}	70.50±29.79^{bc}	106.00±34.77^{bc}	36.17±29.10^{bc}
	C	98.33±31.17^{a}	112.67±32.93^{b}	96.17±23.47^{bc}	82.47±24.45^{cd}	47.33±28.18^{bc}
	D	84.25±27.79^{b}	112.00±48.04^{b}	109.50±37.31^{bc}	91.50±49.29^{cd}	75.58±29.00^{b}
	E	98.33±31.17^{ab}	92.67±43.65^{bc}	49.67±23.70^{bcd}	82.17±40.95^{cd}	71.21±31.05^{b}

$^{a, b, c, d}$ Means with the same superscripts are not significantly different, P<0.05 level along the columns.

The gross changes observed in all the CTGs were similar, irrespective of the content of hydrocyanic acid and the quantity of cassava in the diets. The mild to moderate enlargement or increase in weights of the bursa of Fabricius at week 2, 3 and 4 might have been due interfollicular edema and fibrosis which were more prominent at this time (Table. 4). However, some of the histopathological changes, notably the severity and extent of bursa and spleen lymphoid hypoplasia, interfollicular edema and fibrosis, follicular atrophy, and thymic haemorrhages, varied slightly with increasing hydrocyanic acid content in the feeds. This is more prominent in the 30% UF cassava diet at week 5 and might be attributed to high level of hydrocyanic acid in the diet in this group.

There was a positive correlation between the specific humoral immune response to IBD vaccination and the histopathological changes observed in CTGs in this study.

Lesions such as lymphoid hypoplasia, interstitial edema and follicular atrophy might have been responsible for the suppression and reduction in the activity of the bursa of Fabricius to produce antibodies. This is more prominent at week 5.

In the literature, the pathophysiology of hydrocyanic acid has been attributed to cyanide inhibition of cytochrome oxidase (an enzyme responsible for the utilization of oxygen) resulting in an energy deficit within the target tissues, thereby causing tissue anoxia (USEPA, 1989). Moreso, Kamalu (1995) also affirmed that intact linamarin inhibits Na+K+ATPase causing electrolyte imbalance within cells, and this phenomenon is aggravated by free radicals generated by the hypoxia created by cyanide released from linamarin, which cause lipid peroxidation and cell membrane damage. These assertions might have been responsible for the histopathological changes, especially interfollicular edema and depopulation of the lymphoid organs in this study and the subsequent decrease in the antibody titres in the CTGs.

The gradual decline in the IBD antibody titers from week 1 to 3 in all the groups might have been due to waning of the maternally-derived antibody (MDA) or the mopping up of MDA by the IBD vaccinations at weeks 1 and 2.

Although, the immunological surge of antibody titer in CTGs and the control were at variance at week 4, the fermented diets (20%F and 30%F) showed better response compared with the unfermented feeds (20%UF and 30%UF) at week 5 (Fig 1).

It is also possible that T cell functions might have been compromised in this study, due to the histopathological changes such as lymphoid hypoplasia, medullary haemorrhages and Myoid cells proliferation in the thymus. Unfortunately, this was not verified and further studies might be necessary in other to determine the effects of cassava inclusion feeds on the specific cell mediated immunity.

The independent differences in the histological distinctions/functions between cortical and medullary regions of the lymphoid follicle in the bursa of Fabricius have been demonstrated by Azevedo and Betti (1993). This informed the separate determination of medullary diameter and cortical thickness of the lymphoid follicle of the bursa of Fabricius in this investigation, and observed possible different responses to the toxic principle in the cassava inclusion diets in both regions. In this study, both regions of the lymphoid follicle showed significant decrease ($P<0.05$) (Table 7) which might possibly indicate an impairment of the whole bursal function in cassava treated chicks. This is in agreement with the studies of Silva *et al.* (2003) in which *Senna occidentalis* was fed to chicks and showed reduction in both cortical thickness and medullary diameter of the lymphoid follicles.

In conclusion, this study has demonstrated that cassava inclusion in feeds can cause subtle sublethal effects to the lymphoid organs of domestic chickens, even with the lowest cassava inclusion diet (20% fermented) as demonstrated by the low specific humoral immunity of the IBD antibody titers and histopathological evaluations of the lymphoid organs. Therefore, prolonged feeding of cassava inclusion diets might be detrimental to the immune system and possibly cause outbreaks of diseases in poultry.

Despite the myriad of studies describing the effects of cassava in domestic chickens and other animal species, to the best of our knowledge, this study appear to be the first investigation describing humoral and histological adverse effects of cassava inclusion diet in lymphoid organs of domestic chickens.

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BLOOD PROFILE OF WEST AFRICAN DWARF GOATS FED PANICUM MAXIMUM SUPPLEMENTED WITH NEWBOULDIA LAEVIS LEAVES

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Abstract

Sixteen male West African dwarf goat kids of between 9 to 15 months old were used in a 90-day feeding trial to determine the effect of supplementing *Newbouldia laevis* with *Panicum maximum* on the *haematology* and blood biochemical constituent. The goats were randomly divided into four groups of four animals per group. Each group was individually fed one of the four treatment which include 100 %PM, 75 %PM: 25 %NL, 50 %PM: 50 %NL and 25 %PM: 75 %NL in a completely randomized design. White blood cell count, haemoglobin, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, total protein, albumin, globulin, glucose, cholesterol, alkaline phosphate transaminase and alanine phosphate transaminase were significantly ($P<0.05$) influenced by the treatments while Pack cell volume, Red blood cell count and mean corpuscular volume were not significantly ($P>0.05$) influenced. Although some of the blood parameters differ significantly they were within the normal range for normal healthy goats therefore it could be concluded that supplementing *Newbouldia laevis* with *Panicum maximum* had no adverse effect on the blood profile of the experimental goats.

Keywords: *Blood profile, West African Dwarf Goats, Panicum maximum and Newbouldia laevis*

PROFIL SANGUIN DES CHEVRES NAINES D'AFRIQUE DE L'OUEST NOURRIES AU PANICUM MAXIMUM ET UN COMPLEMENT DE FEUILLES DE NEWBOULDIA LAEVIS

Résumé

Seize chevreaux nains d'Afrique de l'Ouest âgés de 9 à 15 mois ont été utilisés dans un essai d'alimentation de 90 jours dans le but de déterminer l'effet de la supplémentation de *Newbouldia laevis* (NL) avec *Panicum maximum* (PM) sur l'hématologie et la composante biochimique du sang. Les chevreaux ont été répartis de manière aléatoire en quatre groupes de quatre animaux par groupe. Chaque groupe était nourri individuellement avec l'un des quatre traitements qui comprenaient 100% PM, 75% PM: 25% NL, 50% PM: 50% NL et 25% PM: 75% NL dans un dispositif complètement aléatoire. La numération leucocytaire, l'hémoglobine, l'hémoglobine corpusculaire moyenne, la teneur corpusculaire moyenne en hémoglobine, la teneur totale en protéines, l'albumine, la globuline, le glucose, le cholestérol, la transaminase de phosphatase alcaline et la transaminase de phosphate alanine étaient significativement ($P <0,05$) influencés par les traitements, tandis que l'hématocrite, la numération érythrocytaire et le volume globulaire moyen n'étaient pas significativement ($P> 0,05$) influencés. Même si quelques-uns des paramètres sanguins différaient considérablement tout en étant dans la fourchette normale pour des chevreaux sains normaux, on peut conclure que la supplémentation de *Newbouldia laevis* avec *Panicum maximum* n'a eu aucun effet négatif sur le profil sanguin des chevreaux soumis à cet essai alimentaire.

Mots-clés: *profil sanguin, chèvres naines d'Afrique de l'Ouest, Panicum maximum et Newbouldia laevis*

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Introduction

Goats contribute to the health and nutrition of several million people in developing countries. Rearing of goats provides important supply of animal proteins of high biological value, plus essential minerals and fat – borne vitamins, which are particularly significant for vulnerable groups' namely, pregnant and nursing mothers and young children (Momoh *et al.,* 1998).

Nutritional management is the primary concern of livestock enterprise as a properly fed animal is able to withstand environmental stresses and challenge disease causative organisms. In recent years, there has been a growing interest in many tropical countries to identify potentially important feed sources among shrubs and trees for inclusion in ruminant diets to provide green fodder that is high in protein to supplement the available low protein forage. This has been recognized as one of the most effective means of improving animal performance in smallholder livestock production (Blair, 1989).

The feeding patterns of goats is characteristic of the native husbandry practices whereby they scavenge for food to meet their daily nutrient requirements (Daramola *et al.,* 2005). However, due to scarcity of green fodder for these natural browsers, particularly in the dry season, attempts have focused on the utilization of the abundant but unconventional foliages in this eco-zone which tend to be green all-year-round. With a large proportion of plants being used for the nourishment of various domestic animals (Rehm and Epsig, 1991), naturally occurring browse and tree species thus are vital components in the diets of sheep and goats, with goats particularly dependent on them to meet their nutrient requirement.

Panicum maximum is considered one of the most valuable fodder plants, with high leaf and seed production and very palatable to livestock. There is a need to supplement *Panicum maximum* with other forages that have high nutritive value and available all year round. *Newbouldia laevis* is a boundary tree that can be used as supplement to feed livestock. Leaves of the tree are noted for high crude protein, essential vitamins, minerals and amino acids (Makkar and Becker, 1997: Gidamins *et al.,* 2003). Quantitative information on the productivity of small ruminants in sub-Saharan Africa on the utilization of leaves from shrubs and trees is scant in literature (Taiwo *et al.,* 2004).

Haematological and biochemical determinations in animals have been well documented by Oduye and Adadevoh (1976) and Taiwo and Anosa (1995). According to Karesh and Cook (1995) examining blood for their constituents is used to monitor and evaluate disease prognosis of animals. Much of the available information on the haematology and biochemistry for goats in the humid tropics has been on disease prognosis. Thus, there is paucity of information on blood parameters of goats offered foliages from unconventional plants as feed.

The potential for improved small ruminant production in Nigeria appears high given the wide distribution of evergreen browse and trees species. Therefore, there is need to investigate the abundant forage species of the tropics for their utilization as food for optimum productivity by these small ruminants.

Materials and Method

Experimental site

This study was carried out at Azol Farms, Obantoko, Odeda Local Government Area of Ogun state, Nigeria located at an altitude of 169m, latitude 7° 10' 50"N and longitude 3° 26' 37"E. The site is located in the rain forest vegetation zone of the South-Western Nigeria on Latitude7° 13' 49.46"N, longitude 3° 26' 11.98"E and altitude 76 m above the sea level. It receives a mean annual precipitation of 1,037mm, mean annual temperature of 34.7°C and relative humidity of 82 % (Google Earth 6.0).

Experimental animal and management

Sixteen (16) West Africa Dwarf goat bucks of between 9 to15 months old, purchased from villages around Abeokuta, were used for the experiment. The animals were quarantined for 3 weeks; treated against endo and ecto parasites and vaccinated against peste des petit ruminant using PPR vaccine in order to improve their health status and

suitability for the experiment. The animals were managed under intensive system and fed at 4 % of their body weight in accordance with the recommendation of NRC, (1985) and Devendra and Mcleroy (1982).

Plant materials and experimentation

Fresh *Newbouldia laevis* leaves were harvested around villages in Abeokuta while *Panicum maximum* was harvested from rangelands within the University environment. Both were air dried, chopped and mixed together in varying levels according to the treatment below. Water was provided *ad libitum.*

Treatment 1: 100% *Panicum maximum*

Treatment 2: 75% *Panicum maximum* and 25% *Newbouldia laevis*

Treatment3: 50% *Panicum maximum* and 50% *Newbouldia laevis*

Treatment 4: 25% *Panicum maximum* and 75% *Newbouldia laevis*

Experimental design

The sixteen experimental animals were randomly divided into four groups of four animals per group. Each group was randomly allotted into the four treatments in a Completely Randomized Design.

Data collection

10 ml of blood sample were collected from jugular vein of each animal using hypodermic needle and syringe at day 0, and day 50 of the experiment. 5 ml of blood sample was released into sample bottles containing ethyl dimethyl tetra acetic acid (EDTA) as anti-coagulant. The bottles were agitated thoroughly to ensure proper mixing of the blood with EDTA to prevent coagulation. The remaining 5 ml of blood samples were left in the syringe without anti-coagulant to harvest serum. Packed cell volume, white and red blood cell counts, serum glucose, total protein, alkaline phosphate transaminase (ALT) and alanine serum transaminase (AST) were determined from blood samples. Blood samples were analyzed immediately after collection for packed cell volume (PCV) and haemoglobin (HB) concentration as described by Jain (1993). Red blood cells (RBC), white blood cell (WBC) as well as the differential WBC counts were determined using the Neubauer haemocytometer after appropriate dilution (Lamb, 1981).

Statistical analysis

All data generated were subjected to one-way analysis of variance as contained in SPSS 2006. Significant differences among treatment means were determined using the new Duncan multiple range test (Duncan, 1955).

Results

The results of chemical composition of the *Panicum maximum* and *Newbouldia laevis* fed to the experimental animals were presented in Table 1. *Newbouldia laevis* leaf has the following values; 92.80, 18.72, 32.46, 24.46 and 8.38% for DM, CP, CF, EE and Ash, respectively while the DM, CP, CF, EE, and Ash and for *P. maximum* were 97.14, 11.75, 30.70, 5.87 and 8.94%, respectively.

The proximate composition of the experimental diet is presented in Table 2. The crude protein, ether extract and tannin were significantly ($P<0.05$) differed across the diets. Goats on 25% PM and 75% NL had the highest values (16.86 and 19.99) for CP and EE, respectively, while least value (11.75 and 5.87) was observed in animals fed 100%PM. However, DM and Ash were not significantly different ($P>0.05$) across the diets.

Table 3 shows the haematological parameters of West African Dwarf (WAD) goats at the commencement of the experiment. This serves as a reference to monitor the changes in the haematological profile of WAD goats as affected by the treatment. There were no significant differences ($P>0.05$) in all the parameters considered. However, goats on 100% PM had highest value for RBC, HB, PCV, MCV and MCHC compared with others. The highest mean value (26.35) for WBC was recorded for goats on 75%PM: 25%NL while those on 25%PM: 75%NL having the least value (14.20).

Table 1: Percentage Chemical composition of the *Newbouldia laevis* and *Panicum maximun*

Parameter	*Newbouldia laevis* (%)	*Panicum maximum* (%)
Dry matter	92.80	97.14
Crude protein	18.72	11.75
Crude fibre	32.46	30.70
Ether extracts	24.46.	5.87
Ash	8.38	8.94

Table 2: Proximate analysis of *Panicum maximum* substituted at different levels with *Newbouldia laevis* leaf

Parameters	100%PM	75%PM25%NL	50%PM50%NL	25%PM75%NL	SEM
Dry matter	97.14	96.06	94.97	93.89	11.89
Crude protein	11.75^{b}	13.49^{ab}	15.24^{ab}	16.86^{a}	3.74
Crude Fibre	30.72	31.16	31.59	32.03	4.35
Ether Extract	5.87^{c}	10.58^{b}	18.22^{a}	19.99^{a}	3.12
Ash	8.94	8.80	8.66	8.53	2.11
Tannin content	0	28.75^{c}	57.50^{b}	86.25^{a}	13.94

$^{a, b, c}$: means on the same row with different superscripts are significantly different ($p>0.05$).

Table 3: Effect of experimental diet on haematological parameters of West African Dwarf (WAD) goats at the beginning of the experiment

Parameters	T1 100%PM	T2 75%PM25%	T3 50%PM50%NL	T4 25%PM75%NL
Pack cell volume (%)	29.8±0.99	25.40 ±1.13	27.05± 3.04	23.25±5.73
Haemoglobin (g/L)	11.15±14.85	9.50 ± 3.04	10.45 ± 17.68	8.95± 15.15
Red blood cell (x1012/L)	18.25±1.75	16.38 ±0.04	17.49 ±2.71	15.67±2.97
White blood cell (x10/L)	24.10±8.49	26.35± 9.40	21.85±2.62	14.20±2.97
MCH (pg)	6.05±0.21	5.75 ±0.21	5.95 ±0.07	5.56±0.35
MCV (Fl)	16.40± 0.99	15.60 ±0.45	15.60 ± 0.64	14.80± 0.85
MCHC (g/l)	37.35±37.48	37.35±0.71	38.45± 21.9	14.20± 2.97

PM: Panicum maximum **NL:** Newbouldia laevis **MCH:** mean corpsular haemoglobin
MCV: Mean corpuscular volume **MCHC:** Mean corpuscular hemoglobin concentration

Table 4 shows the effect of varying levels of *Panicum maximum* (PM) and *Newbouldia laevis* (NL) leaves on the haematological parameters of WAD goats at the end of the experiment. Varying levels of PM and NL leaves have significant effect ($P<0.05$) on Hb, WBC, MCH and MCHC. Highest mean value (29.60) for WBC was observed for goats on 100% PM followed by goats on 75%NL: 25%PM (16.30) and 50%PM: 50%NL (14.65) while least value (14.55) was observed for goats on 25%NL: 75%PM. Highest value (10.90) for haemoglobin concentration was observed in goats fed 100% PM followed by those fed 50%PM: 50%NL (9.8) and goats on 75% NL: 25%PM (9.5) with goats on 75%NL: 25%PM had the least value (8.8).

Highest mean values (5.9) and (43.0) were observed for MCH and MCHC respectively for goats fed 100% PM. The least value for MCH (4.9) was observed in goats fed 75%NL: 25%PM while the least value (35.0) for MCHC was observed in goats fed 25%NL: 75%PM.

The treatment had no influence ($P>0.05$) on RBC, PCV and MCV. However, highest PCV values (27.5) was observed in goats fed 50%PM: 50%NL with goats on 75% NL: 25%PM having the least value (23.6). Goats fed

50%PM:50%NL had the highest value (19.95) and least values (17.64) was observed in goats fed 75%NL25%PM. Highest value (14.55) for MCV was observed in goats fed 25%NL75%PM with least value (13.4) recorded for goats fed 25%PM: 75%NL.

Table 5 shows the biochemical parameters of WAD goats at the beginning of the experiment. There were no significant differences (P>0.05) in biochemical parameters considered. However, the mean values (6.80) and (4.45) for total protein and albumin were highest in goats on 75%NL: 25%PM with least value (6.45) and (3.90) observed in goats fed 50%NL: 50%PM.

For globulin, highest mean value (2.85) was observed for goats on 100%PM while least value (2.4) was recorded for goats fed 75%NL: 25%PM. Goats on 50%NL: 50%PM had the highest mean values (51.0) and (65.70) for glucose and cholesterol level with least values (43.0) and (42.7) observed for goats on 100%PM. Highest mean AST value (52.0) was recorded in goats fed 75%PM: 25%NL with goats on 50%NL: 50%PM having the least value (27.50). Goats fed 75%NL: 25%PM had the highest mean value (24.5) for ALT with goats on 50%NL: 50%PM having the least value (8.0).

Table 6 shows the observed biochemical parameters of WAD goats as affected by the treatments at the end of the experiment. All the parameters considered were significantly (P<0.05) affected with the varying level of *Panicum maximum* and *Newbouldia laevis* leaves. Highest mean values (7.0, 6.6) (3.7, 3.83) was observed for total protein and globulin in goat fed 100%PM and 75% PM: 25%NL while least values (5.5, 4.7) (2.9, 3.0) was observed in goats fed 50%PM: 50%NLand 25%NL: 75%PM respectively. Albumin value (3.30) was highest in goats on 100%PM followed by goats fed 75 %PM: 25 %NL and 50 %PM: 50 %NL with goats on 25% PM: 75% NL having the least value. Highest glucose value (54.0) was observed in goats fed 100% followed by goats on 25% PM: 75% NL and those fed 75% PM: 25% NL with those on 50%PM: 50%NL having the least value (39.0). Cholesterol value (103.0) was highest in goats fed 100%PM followed by those animals on 50%PM: 50%NL and 25% PM: 75% NL while the least value (56.8) was observed in goats fed 25%PM: 75%NL. AST value (81.0) was highest in goats fed 100%PM followed by those on 75% PM: 25% NL with animals on 50%PM: 50%NL and 25%PM: 75%NL having the least values (41.0 and 47.0). Highest means value (17.0) (16.0) for ALT was observed in goats fed 50%PM: 50%NL and 75%PM: 25%NL followed by goats on 100%PM and least value (4.0) observed in goats fed 25%PM: 75%NL.

Discussion

The crude protein value (18.72) of *Newbouldia laevis* in this study was higher than the value (11.75) observed for *Panicum maximum*. *Newbouldia laevis* had higher crude protein value 18.73% than those reported by Ogunbosoye and Babayemi (2010), NRC (1975) and NRC (1981) having 9.89 %, 11 % and 14% respectively for weaner kids. It was also higher than 15.57 % reported by Ikiomaya and Imasuem (2007). The higher level of crude protein found in *Newbouldia laevis* may be attributed to maturity stage of the plant at the time of cutting (Ogunbosoye and Babayemi, 2010).

The crude fibre observed for both *Newbouldia laevis* and *Panicum maximum* were comparable. The crude fibre value in this study was lower compared with that obtained by Ogunbosoye and Babayemi (2010), Aganga and Tshwenyane (2004) and Duke and Atchely (1984).

Higher mean value (24.46) was obtained for ether extract in *Newbouldia laevis* with *Panicum maximum* having a lower value (5.87) which is an indication of availability of higher energy level for the WAD goats (Babayemi and Bamikole, 2004; Odedire and Babayemi, 2008). The ether extract of the diet was also higher than that reported by Ikiomaya and Imasuem, (2007) and Ogunbosoye and Babayemi (2010). Ether extract is the lipid component and the energy derived from it is utilized by the animal for body maintenance and production. The higher value of ether extracts in *Newbouldia laevis* is an indication of availability of higher energy for the animal to use as reported by (Babayemi and Bamikole, 2004 and Odedire and Babayemi, 2008). This is an important form of storage energy in plants and utilized by

Table 4: Effect of experimental diet on the haematological parameters of WAD goats at the end of the experiment

Parameters	T1 100%PM	T2 75%PM25% NL	T3 50%PM50%NL	T4 25%PM75%NL
Pack cell volume(%)	25.30 ± 0.00	27.00 ± 1.98	27.50 ±2.97	23.60 ± 0.00
Haemoglobin (g/L)	10.90± 0.00a	9.50 ±11.31^{ab}	9.80 ±4.34^{ab}	8.80±0.00^{b}
Red blood cell (x1012/L)	18.27 ±0.00	18.67 ±2.5	19.05 ±0.29	17.64±0.00
White blood cell(x10/L)	29.60±0.00^{a}	14.55±1.20^{b}	14.65 ±2.61^{b}	16.30± 0.00^{b}
MCH (pg)	5.90± 0.00^{a}	5.05 ±0.71 ^{b}	5.10 ±1.34^{b}	4.90± 0.00^{b}
MCV (Fl)	13.90± 0.00	14.55 ± 0.92	14.45 ±1.34	13.40±0.00
MCHC (g/l)	43.00± 0.00^{a}	35.00 ±16.26^{b}	35.75 ±23.34^{b}	37.20± 0.00^{b}

PM: Panicum maximum **NL:** Newbouldia laevis **MCH:** mean corpsular haemoglobin
MCV: Mean corpuscular volume **MCHC:** Mean corpuscular hemoglobin concentration

Table 5: Effect of experimental diet on biochemical parameters of WAD goats at the beginning of the experiment.

Parameters	T1 100%PM	T2 75%PM25NL	T3 50%PM50%NL	T4 25%PM75%NL
Total protein(g/dl)	6.80 ± 0.14	6.55 ±1.06	6.45 ±0.21	6.85 ± 0.64
Albumin (g/dl)	3.95± 0.49	4.05 ±0.35	3.90 ±0.42	4.45±0.21
Globulin (g/dl)	2.85± 0.35	2.50 ±1.41	2.55 ±0.21	2.40±0.42
Glucose (mg/dl)	43.0 ± 8.49	48.0 ±9.89	51.0±2.83	48.0±7.70
Cholesterol (mg/dl)	42.70± 7.14	61.75±12.80	65.70±4.10	61.00±9.19
AST (u/l)	33.50±14.85	52.00 ±35.36	27.50±3.54	47.50 ±31.82
ALT (u/l)	19.00 ± 8.49	11.00±0.00	8.00±0.00	24.50 ±10.61

AST: Alanine Serum Transaminase
ALT: Alkaline Phosphate Transaminase

Table 6: Effect of experimental diet on biochemical parameters of WAD goats at the end of the experiment.

Parameters	T1 100%PM	T2 75%PM25NL	T3 50%PM50%NL	T4 25%PM75%NL
Total protein(g/dl)	7.0± 0.00^{a}	6.6 ±0.87^{a}	5.5 ±0.00^{b}	4.7 ± 0.00^{b}
Albumin (g/dl)	3.30± 0.00^{a}	2.76 ±0.40^{b}	2.60 ±0.00^{b}	1.70±0.00^{c}
Globulin (g/dl)	3.70± 0.00^{a}	3.83 ±0.40^{a}	2.90 ±0.00^{b}	3.00±0.00^{b}
Glucose (mg/dl)	54.0± 0.00^{a}	50.0 ±3.46^{b}	39.0±0.00^{c}	51.0±0.00^{ab}
Cholesterol (mg/dl)	103.9±0.00^{a}	73.1±20.96^{b}	76.7±0.00^{b}	56.8±0.00^{c}
AST (u/l)	81.0 ± 0.00^{a}	68.0 ±6.93^{b}	41.0 ±0.00^{c}	47.0 ±0.00^{c}
ALT (u/l)	10.0 ± 0.00^{b}	16.0±3.46^{a}	17.0±0.00^{a}	4.0 ±0.00^{c}

AST: Alanine Serum Transaminase
ALT: Alkaline Phosphate Transaminase

animals for body maintenance and production. *Panicum maximum* had higher ash content 8.94 as compare to 8.38 observed in *Newbouldia laevis*. The Ash content of *Newbouldia laevis* was comparable to 8.46% reported by Ogunbosoye and Babayemi (2010) and was higher than 2.49 % reported by Ikiomaya and Imasuem (2007). Variability in the nutrient content of browse has been attributed to within species differences, plant parts, season, harvesting regime, location, soil type and age (Norton, 1994).

According to Karesh and Cook (1995) examining blood for their constituents is used to monitor and evaluate disease prognosis of animals. Mean PCV value observed in this study was within the reported range of 22-38% reported by Merck (2011), 22-38% reported by Lazzaro and Saanendoh (2005) but lower than the values of 36.9% and 35.5% for clinically healthy WAD goat and sheep, respectively (Taiwo and Ogunsanmi, 2003). Also, it is lower than at reported by Ikiomaya and Imasuem (2007). Aikhuomobhogbe and Orheruata (2006) asserted that lower PCV results in anemia reduced oxygen carrying-capacity of blood, increased pulse rate and consequently heart failure. Hence, it can be asserted that the experimental animals were within the normal range that can maintain the animal of normal PCV. Haemoglobin was also within the normal range of 7-15g/l reported by Daramola *et al.,* (2003), and Merck (2011). All observed values for haemoglobin were lower than 11.40g/l reported by Tambuwal *et al.,* (2002). Dietary treatments support relatively normal haemoglobin indicating that the experimental animals are capable of supporting high oxygen carrying capacity. The significantly different haemoglobin was within the reported range for goats Sirois (1995) indicating the absence of microcytic hypochromic anaemia occasioned by iron deficiency and improper utilization for the formation of Hb.

Animals on 25%PM: 75%NL have least values for PCV and Hb, this might be an indication that feeding *Newbouldia leavis* at the level higher than 75% could predispose the animals to pernicious anemia.

Although, RBC count was not significantly different (P>0.05) in this experiment, it was higher than the range reported by Tambuwal *et al.,* (2002) for Red Sokoto goat and those reported by Jain (1993). RBC aids in the characterization of anemia Merck (1979). Olayemi *et al.,* (2006) reported values lower than the observed values in this present experiment. The significantly Hb concentration and the non-significant RBC was in line with the previous work in which a Sericea lespedeza diet containing 22.2g CT/kg DM and intake of 1.03 g/kg DM studied in kiko crossbred male kids Solaimon *et al.*, (2011) and in contrast with the work of Olafadehan (2011) who reported insignificantly Hb concentration and the significant RBC concentration. The relatively lower PCV and RBC in goats fed 25%PM: 75%NL may be attributed to the presence of antinutritional factors particularly condensed tannins, which have been reported to have antinutitional effect (Robins and Brooker, 2005; Rubanza *et al.,* 2005).

Mean WBC observed in this study was higher than the normal physiological range of 4-13 x 103U/L, 4.0- 12.0 x103 mm-3and 15.39 x 103 for goat as reported by Merck (2011), Jain (1993) and Olayemi *et al.*, (2000), respectively.

The total protein and albumin observed for animals on 100%PM and 75%PM: 25%NL was within the range of 6.1-7.5g/dl reported by Merck (2011) but animals on 50%PM: 50%NL and 25%PM: 75%NL had lower values than the reported value. Although CP was highest in goats fed 25% PM: 75%NL but it had the lowest total protein level, which may be an indication of underutilization of the protein in the diet. Price and Butter (1980) reported that tannins have adverse effects which include depressed feed intake, binding dietary protein, binding with endogeneous protein and direct toxic effects. Significantly lower total protein and albumin of goats fed 50%PM: 50%NL and 25%PM: 75%NL is an indication of the relatively poor protein quality of the combination and of course, the level and availability of the dietary protein Olafadehan (2011). All the experimental goats were within the range of 2.7-4.4 g/dl for globulin (Merck, 2011). Goats on 50%PM: 50%NL were not within the reported range (Žubčić, 2001 and Merck, 2011) for glucose. However, other were within the reported range for healthy goats indicating that the animals were able to

utilize the energy content in the diet, animals on 50%PM:50%NL had a decreased glucose level but were still within the range indicating the goats are clinically healthy (Žubčić, 2001), thus appears plausible to infer that the observed depressed serum glucose is not due to tannic acid intoxication, but that the dietary energy was sufficiently utilized (Ologhobo *et al.,* 1992 and Zhu *et al.,* 1992). Cholesterol is conventionally used for diagnosing human and domestic animal hepatic damage Silanikove and Tiomkin (1992). Normal cholesterol value reported by Merck (2011) ranges between 65-136 mg/dL, experimental goats were within the reported range except those on 25%PM: 75%NL.

Enzymes are protein catalyst present mostly in living cells and are constantly and rapidly degraded although, renewed by new synthesis (Coles, 1986). Goats on 100%PM and 75%PM: 25%NL were within the reported range of 66-230 u/L for AST (Merck, 2011), while animals on 50%PM: 50%NL and 25%PM: 75%NL were lower than the reported range. The ALT values for goats on 25%PM: 75%NL and 50%PM: 50%NL were within the reported range of 15-52u/L for ALT as reported by Merck (2011) while those on 100%PM and 25%PM: 75%NL were lower than the reported range. Differences in serum biochemical parameters may be caused by nutrition, environment and hormonal factors Chineke *et al.* (2002). Normal enzyme level in serum is a reflection of a balance between synthesis and their release, as a result of the different physiological process in the body (Zilva and Pannall, 1984). According to Carola *et al.* (1990), transaminase enzymes are those mostly responsible for the synthesis of non-essential amino acids through the processes in the body.

Conclusion and Recommendation

Although some of the blood parameters differ significantly but were within the normal range for normal healthy goats therefore it can be concluded that supplementing *Newbouldia laevis* with *Panicum maximum* had no adverse effect on the blood profile of the experimental goats.

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EFFECTS OF IN VITRO MULTI-ENZYME TREATMENT OF FIBRE CONTENT ON UN-DECORTICATED SUNFLOWER SEED MEAL

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Abstract

A multi-enzyme mixture containing xylanase, glucanase and cellulase activity produced from *Penicillium fumiculosum* was applied to undecorticated sunflower seed meal (USFM) to degrade its fibre content and improve other chemical constituents. USFM was treated with different concentrations of the multi-enzyme solution at different pH and time. Proximate composition of the test ingredient was carried out before and after the enzymatic treatment in four replicates. Proximate evaluation showed that USFM contained 204.5, 336.2, and 192.2 crude protein, ether extract and soluble carbohydrates respectively. Phosphorus was the most abundant mineral followed by potassium and calcium. Amino acid profile showed that lysine was more abundant in USFM compared with soybean meal. Treatment with enzyme showed that crude protein increased by 2.78% while ether extract remain unchanged. Also, a 16.27% reduction was recorded in the Crude fibre value when USFM was incubated with enzyme. Crude fibre degradation was improved as temperature increased while at temperature beyond 45^{0}C, there was a reduction in the effectiveness of the enzyme. The highest degradation of crude fibre was recorded at pH 5 while an increase in the length of incubation led to improvement in crude fibre degradation and crude protein availability. It was concluded that the use of multi-enzyme in USFM enhances and improves utilization of proximate constituents.

Keywords: *Sunflower meal, Multi-enzyme, Fibre degradation*

EFFETS DU TRAITEMENT MULTI-ENZYMATIQUE IN-VITRO DE LA TENEUR EN FIBRES SUR LA FARINE DE GRAINS DE TOURNESOL NON DECORTIQUEES

Résumé

Un mélange multi-enzymatique contenant une xylanase, une glucanase et une activité cellulase produit à partir de Penicillium fumiculosum a été appliqué à une farine de graines de tournesol non décortiquées (USFM) pour dégrader sa teneur en fibres et améliorer les autres constituants chimiques. L'USFM a été traitée avec différentes concentrations de la solution multi-enzymatique à pH différent et à des moments différents. La composition immédiate de la matière d'essai a été effectuée avant et après le traitement enzymatique à quatre répétitions. Une évaluation immédiate a montré que l'USFM contenait respectivement 204,5, 336,2, et 192,2 protéines brutes, extrait à l'éther et hydrates de carbone solubles. Le phosphore était le minéral le plus abondant, suivi du potassium et du calcium. Le profil en acides aminés a montré que la lysine est plus abondante dans l'USFM par rapport à la farine de soja. Le traitement avec l'enzyme a montré que les protéines brutes ont augmenté de 2,78% tandis que l'extrait d'éther est resté inchangé. En outre, une réduction de 16,27% a été enregistrée pour la teneur en fibres brutes lorsque l'USFM a été incubée avec l'enzyme. La dégradation des fibres brutes s'est améliorée avec l'augmentation de la température, tandis qu'une température supérieure à 450° C a engendré une réduction de l'efficacité de l'enzyme. La plus forte dégradation des fibres brutes a été enregistrée au pH 5 alors qu'une augmentation de la durée d'incubation a conduit à une amélioration de leur dégradation et de la disponibilité de protéines brutes. Il a été conclu que l'utilisation d'une formule multi-enzymatique dans l'USFM renforce et améliore l'utilisation de constituants immédiats.

Mots-clés : *farine de tournesol, multi-enzymatique, dégradation de fibres*

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Introduction

The gradual increase in the world's poultry production concomitantly increases the need for ingredients to supply protein for diets. Even when the cereals constitute about 60-70% of the diet in order to supply the energy requirements of poultry, oilseeds, which primarily contribute to the dietary protein requirements of birds, is next in the category of importance. Whereas soybean meal (SBM) is the major protein source for the world's poultry production, other oil seeds such as cottonseed, rapeseed and sunflower are frequently used as alternatives (Waldroup, 1983; Swick, 1996). These oilseed meals are prominent crops in some areas of the world where substantial amounts are produced and where they contribute to the production of poultry meat and eggs in cost effective ways. However, most of these oilseed meals, including SBM, contain anti-nutritional factors (Ravindran and Blair, 1992; Ferket and Middleton, 1998) which place some constraints on their usage in poultry diets. For example, lectins, some of the oligosaccharides and oestrogenic compounds in SBM (Liener, 1980; Coon *et al.,* 1990; Irish and Balnave, 1993), gossypol in cottonseed meal, and glucosinolates and erucic acid in rapeseed meal are the most widely recognized anti-nutritional factors. So far, however, no report has tried to documented the presence of anti-nutritional factors in Sunflower meal (SFM). Although chlorogenic acid has been identified as a toxic constituent, its concentration in SFM does not lead to toxicity as judged by the lack of growth-retarding effects (Millic *et al.*, 1968). However, is interesting to note that the presence of high fibre in the un-decorticated seed meal in the tropics especially in Nigeria where decortication of sunflower seed meal ,imposes additional cost on the already high cost of feed.

On the other hand, the use of enzymes in domestic chickens has become more common in recent years (Lazaro, *et al.,* 2003; Mathlouthi *et al.,* 2004). Enzymes may be targeted at carbohydrates (carbohydrases), proteins (proteinases) or lipids (lipases) (Lesson and Summers, 1997). In poultry diets, enzymes are employed to increase the digestibility of feed ingredients and reduce the incidence of wet droppings which may result from the presence of non-starch polysaccharides (NSPs) (Choct *et al.,* 1995; Acamovic, 2001). In addition, enzymes reduce viscosity in the diet and digesta, enhance digestion and absorption of nutrients especially fat and protein, improve apparent metabolisable energy value of the diet, increase feed intake, weight gain and feed to gain ratio and reduce beak impaction and vent plugging (Pirgozliev *et al.,* 2007), decrease the size of gastro intestinal tract, alter the population of microorganisms in the gastro intestinal tract, reduce water intake and water content excreta. They also reduce the production of ammonia output of excreta including nitrogen and phosphorus, reduce the output of bile salts in digesta and improve the nutritive value of feedstuffs hence minimizing both manure production and wastage of dietary nutrients. (Annison and Choct 1991, Bedford 1996, Bedford and Morghan 1995 and Marquardt *et al.,* 1996). More recently, a multi-enzyme approach has come into enzyme supplementation of plant-based diet for poultry. Multi-enzyme products have a variety of activities and their effect in degrading cell walls and releasing nutrients for utilization may be synergistic. However, the presence of high fibre in the pericarp of the sunflower seed imposes limitation to its use. Also the chemical composition of any material to be used as feed resource for poultry will determine its success in utilization by the species of livestock to be fed. Most studies have focused on the use of specific enzymes on sunflower meal for poultry. Only very few studies have been conducted to date on the use of multi-enzyme (complex) activities on undecorticated sunflower seed meal sunflower for chickens. This present investigation therefore, was undertaken with the objective of assessing the effect of treatment of undecorticated sunflower seed meal with multi-enzyme complex on proximate composition and its effect on degredation on its fibre content.

Materials and Methods

Description of the Enzyme

Rovabio® Excel AP is a concentrated powder with endo-1,4-<beta>-xylanase (No. EC3.2.1.8), endo-1,3(4)- <beta>-glucanase (No. EC 3.2.1.6) and cellulases as the main active substances, obtained from a fermentation broth of *Penicillium fumiculosum*. Minimum activities include: 1400 units endo-1,4- <beta>-xylanase and 2 000 units endo-1,3 (4)- <beta>-glucanase per gram.

In-vitro Treatment of undecorticated sunflower seed meal

One kg of USFM was mixed with 100g of enzyme. The enzyme-substrate mixture was packed in a bag. The bag was incubated at 410^{0}C in a hot air oven for 36 hours. Before and after incubation, the USFM was analyzed for its proximate constituents, fibre fractions (neutral detergent fibre, acid detergent fibre, cellulose and hemi-cellulose) and its mineral composition.

In-vitro Determination of Optimum Condition for Treatment

Enzyme-substrate ratio was determined according to the method of Ahmad *et al.,* (2004). 3g of undecorticated sunflower seed meal (USFM) was treated with 1, 2, 3, 4, 5 or 6ml of enzyme solution. The USFM was placed in a 100ml flask and enzyme solution (enzyme solution was prepared by dissolving 1g of enzyme in 100ml of double distilled) water was added to each of the flask. Water was added to standardize the volume up to 1000ml. Flask were incubated in four replicates for 32 hours in an oven at 20, 25, 30, 35, 40, 45 and 50^{0}C. After incubation, the materials from each flask were used to determine the effect of the treatments on fibre degradation and protein availability. At optimized substrate enzyme ratio, the effect of the increasing pH, the effect of increasing temperature was also studied by incubating USFM for 24hours at 20, 25, 30, 35, 40, 45 and 50^{0}C. Lastly, the effect of the length of incubation was also studied on the degradation of the fibre content of USFM.

Proximate Analysis and Chemical Composition

The samples of undecorticated sunflower seed meal and enzyme treated USFM (and all other chemical analysis were done) in four replicates were analysed for their proximate constituents according to A.O.A.C. (2000) procedure. Dry matter (934.01g/kg) was determined by drying at 80^{0}C for 48 h (967.05) ash was measured in a muffle furnace at 510^{0}C for 18 h. Crude protein (6.25 N) in the samples was determined by LECO FP-200 Analyser (St. Joseph, MI, USA-AOAC method 986.06), oil (as ether extract) was extracted with petroleum spirit (b.p. 40 to 60_C) by the Sixhlet method (AOAC, 920.39).

Fibre Analysis

The method of Van Soest *et al.* (1991) was used to determine the neutral detergent fibre (NDF) and the acid detergent fibre (ADF). Insoluble hemicellulose was calculated as loss in weight of ADF residue after treatment with sulphuric acid. The loss in weight of the above residue upon ashing was used to calculate the lignin content.

Mineral Composition

From the triple digested sample, calcium, magnesium and iron were determine using a Perkin Elmer atomic absorption spectrophotometer (Model 5000, Perkin Elmer, USA), while a flame photometer (Elico, India) was used for the determination of potassium and sodium. Total phosphorus was assayed at 630nm following the APHA, (1980) method using a spectrophotometer (Model Spectronic 20D, Milton Roy, USA) which standard methods were used for magnesium (Mg), Iron (Fe), Potassium (K) and Sodium (Na).

Results

Proximate, mineral and amino acid composition of Undecorticated Sunflower Meal

The proximate, mineral and amino acid composition of USFM is presented in Table 1. The results showed that USFM (gkg^{-1}) contained 925.9 dry matter; 204.5 crude protein; 215.1 crude fibre; 391.2 ether extract; 52.0 ash and 142.2 Nitrogen free extract. The results revealed that NDF was the most abundant of the fibre fractions

Table 1: Proximate, Fibre, Mineral and Amino Acid Composition of USFM and SBM (gkg^{-1} DM)

Parameters	Amino Acid Profile kg^{-1}		Parameters	Amino Acid Profile kg^{-1}	
	USFM	SBM		USFM	SBM
Dry matter	925.90	930.00	Aspartic acid	12.42	5.22
Crude protein	204.50	400.00	Glutamic acid	28.16	8.48
Ether Extract	336.20	193.00	Serine	3.72	2.50
Crude Fibre	215.10	75.00	Histidine	2.86	1.80
Total Ash	52.00	46.00	Glycine	6.86	2.10
Soluble			Threonine	3.73	1.93
Carbohydrates	192.20	216.00			
Fibre Fractions			Arginine	10.19	3.69
NDF	332.00	245.41	Alanine	5.68	2.01
ADF	112.00	98.00	Tyrosinie	2.44	1.84
ADL	73.60	45.07	Valine	7.73	2.30
Hemicellulose	239.40	142.50	Methionine	1.63	6.60
Cellulose	3.50	1.52	Phenylalanine	6.21	2.60
Minerals			Isoleucine	6.45	2.20
Ca	3.50	2.0	Leucine	8.97	3.93
P	8.00	6.0	Lysine	3.95	3.54
K	6.67	6.68	Tryptophan	0.90	0.67
Mg	0.74	0.72	Glutamine	ND	ND
Na	0.046	0.055	Proline	ND	ND
Zn (ppm)	267.00	260.00	Asparagine	ND	ND
Mn (ppm)	250.00	255.00	Cystine	ND	ND
Fe (ppm)	128.00	10.00	Cystiene	ND	ND

ND = Not Determined
NDF - Neutral Detergent Fibre
ADF - Acid Detergent Fibre
ADL - Acid Detergent Lignin

having 332.0 gkg^{-1}. ADF, ADL, hemicellulose and cellulose values were 112.0, 73.6, 239.4 and 3.5 gkg^{-1} respectively. Analysis also revealed that of all the minerals analysed, phosphorus was most abundant (8.0gkg^{-1}) followed by potassium (6.67 gkg^{-1}) and calcium (3.5gkg^{-1}). Other minerals include magnesium 0.74, sodium 0.046 (gkg^{-1}), zinc 267ppm, Manganese 250 ppm and Iron 128 ppm. The composition of amino acid showed that USFM contained (g kg^{-1}) Aspartic acid (12.42), glutamic acid 28.16, serine 3.72, histidine 2.86, glycine 6.86, threonine 3.73, arginine 10.19, alanine (5.68), tyrosine (2.44), valine (7.73) and methionine (1.63). Other amino acid identified include phenylalanine (6.21), isoleucine (6.45), lysine (3.95) and tryptophane (0.9).

Also, chemical assay of soybean meal (SBM) showed that SBM contained (g kg^{-1}) 930.00 dry matter, 400.00 crude protein, 193.00 ether extract, 75.00 crude fibre, 46.00 total ash and 216.00 soluble carbohydrates. The results showed that NDF was the most abundant of the fibre fractions having 245.41 g kg^{-1}. ADF, ADL, Hemicellulose and cellulose values were 98.00, 45.07, 142.50 and 1.52 g kg-1 respectively. Analysis also showed that of the minerals analysed for, phosphorus was most abundant (6.0 g kg^{-1}) followed by Potassium (6.68 g kg^{-1}) and Calcium (2.0g kg^{-1}). Other minerals include magnesium (0.72), sodium 0.055 (g kg^{-1}), zinc (260ppm), Manganese (255ppm) and Iron (10.00ppm).

The composition of amino acid showed that SBM contain (g kg^{-1}) Aspatic acid 5.22, glutamic acid 84.89, serine 25.00, histidine 18.00, glycine 21.00, threonine 19.3, arginine 36.90, alanine 20.10, tyrosine 18.40, valine 23.00 and methionine 66.00. Other amino acid identified include phenylalanine 26.00, isoleucine 22.00, leucine 39.90, lysine 3.54 and thryptophane 6.70.

Effect of Enzyme Treatment on Proximate Composition, Fibre Fractions and Mineral Composition of USFM

Effect of enzyme treatment on proximate composition, fibre fractions and mineral composition of USFM is shown in Table 2. The result showed that the moisture content of the test ingredient reduced from 8.5 to 7.0%. An increase of 2.78% was obtained in crude protein content due to enzyme treatment. Ether extract values remained unchanged after the treatment. A reduction of 16.27% in crude fibre content of USFM was observed after treatment. The soluble carbohydrate content of the test ingredient increased by 16% of its original value. Enzyme treatment resulted in a reduction of 36.47, 16.60, 28.13, 17.41 and 16.85% in NDF, ADF, ADL, hemicellulose and cellulose and cellulose, respectively. Enzymic treatment of USFM affected the major minerals while the trace minerals were not affected. Calcium and phosphorus increased by 11.42 and 14.87 fold.

Table 2: Effect of Enzyme Treatment on Proximate Composition, Fibre Fractions and Mineeral Composition of Undecorticated Sunflower Seed Meal Treated with *Penicillium fumiculosum* (gkg^{-1})

Parameters	**Before Treatment**	**After Treatment**
Moisture (%)	8.50±0.05	7.0±0.54
Crude Protein	204.50±0.12	210.20±0.18
Ether Extract	336.20±0.22	336.00±0.22
Crude Fibre	215.10±0.20	180.09±0.45
Total Ash	52.01±0.56	54.01±0.21
Soluble Carbohydrates	192.20±0.24	222.10±0.25
Fibre Fractions		
NDF	332.00±0.14	210.9±0.14
ADF	112.00±0.24	93.4±0.36
ADL	73.60±0.26	52.9±0.18
Hemicellulose	239.40±0.23	197.7±0.43
Cellulose	3.50±0.44	2.91±0.45
Minerals		
Ca	3.50±0.24	3.90±0.24
P	8.00±0.54	9.19± 0.26
K	6.67±0.22	6.65±0.25
Mg	0.74±0.34	0.74±0.10
Na	0.046±0.22	0.047±0.11
Zn (ppm)	267.00±0.42	267.00±0.24
Mn (ppm)	250.00 ±0.21	250.00±0.25
Fe (ppm)	250.00 ±0.21	128.00±21

NDF - Neutral Detergent Fibre
ADF - Acid Detergent Fibre
ADL - Acid Detergent Lignin

Table 3: Effect of Temperature on Crude Fibre Degradability and Crude Protein Availability on Enzyme Supplemented Undecorticated Sunflower Seed Meal (%)

Temperature (T^0C)	Crude Fibre	Crude Protein
20	209.20 ± 0.12	215.40 ± 0.51
25	209.40 ± 0.51	215.00 ± 0.20
30	210.32 ± 0.62	215.00 ± 0.19
35	216.30 ± 0.71	195.40 ± 0.10
40	201.20 ± 0.80	180.00 ± 0.01
45	172.10 ± 0.11	176.10 ± 0.22
50	161.40 ± 0.20	175.10 ± 0.21

Table 4: Effect of Crude Fibre Degradability and Crude Protein Availability on Enzyme Supplemented Undecorticated Sunflower Seed Meal (%)

PH	Crude Fibre	Crude Protein
2	189.41 ± 0.11	190.47 ± 0.21
3	194.21 ± 0.20	192.82 ± 0.63
4	191.12 ± 0.21	206.12 ± 0.72
5	201.11 ± 0.25	218.12 ± 0.71
6	201.10 ± 0.41	205.14 ± 0.10
7	190.12 ± 0.18	192.85 ± 0.60
8	180.24 ± 0.21	190.41 ± 0.15

The effect of temperature on the crude fibre and crude protein content of USFM is shown in Table 3. Crude fibre values consistently increased as the temperature increase up to 40^0C where the highest crude fibre degradation occurred. On the other hand, CP availability consistently reduced. The effect of pH on the degradation of crude fibre and the availability of protein is presented in Table 4. The degradation of crude fibre was highest at pH5 and reduced beyond that pH while the availability of crude protein. Though the amount crude protein librated at pH 6 was the same for pH 5, numerically enzyme was more active at pH 5.

Discussion

The proximate composition of USFM presented in this study showed that the test ingredient is a rich source of crude protein, crude fibre and ether extract. The crude protein value recorded here is at variance with the report of Biobaku and Dosunmu, (2003) and Adeniji and Ogunmodede, (2006). These researchers reported a crude protein of 17.92 and 28.00%, respectively. The crude protein recorded in this study was 204.5 g kg^{-1} which is lower than the value reported by Adeniji and Ogunmodede (2006). The result of the crude fibre reported here agrees with the report of Rezaei and Hafezian, (2007). However, ether extract value of 336.5 g kg^{-1} reported here was higher than 254.2g kg^{-1} reported by Biobaku and Dosunmu, (2003) and 110.0g kg^{-1} reported by Adeniji and Ogunmodede, (2006).

NDF was the most abundant fibre fractions in USFM followed by hemicellulose and ADL. The mineral compositions were within the values reported by Biobaku and Dosunmu (2003). Phosphorus was the most abundant mineral in USFM. These suggest that apart from the test ingredient rich in fibre, it is also rich in inorganic phosphorus. It has been documented that about two-thirds of the phosphorus present in cereals and legumes is in a complex form that includes phytic acid and phytases (myo-inositol hexakis-dihydrogenphosphate, IP6). This compound chelates cations for animal (Cowleson, *et al* 2006; Bryden *et al.,* 2007; Pirgozliev *et al*., 2007). The most abundant amino acid in USFM is glutamic acid followed

Table 5: Effect of Length of Incubation Crude Fibre Degradability and Crude Protein Availability on Enzyme Supplemented Undecorticated Sunflower Seed Meal (%)

Length of Incubation (Hr)	Crude Fibre	Crude Protein
4	161.00 ± 0.01	93.47 ± 0.21
8	169.10 ± 0.21	102.12 ± 0.10
12	175.41 ± 0.40	112.45 ± 0.57
16	185.22 ± 0.51	191.02 ± 0.61
20	182.81 ± 0.60	212.75 ± 0.10
24	192.34 ± 0.11	215.40 ± 0.25
28	198.12 ± 0.10	219.40 ± 0.40
32	116.00 ± 0.11	220.20 ± 0.11

by aspartic acid. The test ingredient is also rich in sulphur amino acid and deficient in lysine (which is low in the test ingredient when) compared with soybean meal (Senkoylu *et al.,* 2004). It has been reported that sunflower meal has similar amino acid availability to soybean meal (Green and Kiener, 1989; Villamidae and San Juan, 1998). The variations recorded in the proximate composition have been attributed to the differences in variety, method of processing, age of the birds and feed formulation techniques employed in the various studies (Senkoylu and Dale, 1999). Some studies have indicted that high oil-sunflower meal was used (Senkoylu *et al.,* 2004) while others used high fibre hulled sunflower seed cake (Adeniji and Ogunmodede, 2006). In this study, undecorticated sunflower seed meal was used.

After incubation with the enzyme for 36 hours, proximate analysis showed an increase of (0.57%) in crude protein. This increase is in line with a slight increase obtained by Ahmad *et al.,* (2004) when sunflower oil meal was incubated with *Arachnoitus spp*. The ether extract values remained unchanged after treatment apparently because the enzyme had no activity for lipid metabolism. A 16.27% reduction in crude fibre is also noteworthy. This reduction may have resulted due to biodegradation caused by the enzyme. Also, 41^{0}C temperature chosen corresponds with the body temperature of chickens (Ahmad *et al.,* 2004). This observation is also in line with the results of the same author when they treated sunflower oil meal with enzyme produced from *Aranchnoitus spp*. On the other hand, CP availability consistently showed no increase up till 35^{0}C and thereafter reduced. This indicates that the enzyme was active on the test ingredient. The result also confirms the stability of the enzyme over a range of temperatures. The reduction in the effectiveness of the enzyme at 45 and 50^{0}C and on crude fibre and crude protein confirms the earlier report of Ahmad *et al.,* (2004). Specific enzyme may remain active at variable temperatures according to the report of Milagres *et al.,* (1993) who reported an optimum 40^{0}C for xylanase (made from *Penicillium anthienellum*, 60^{0}C for endoglucanase (Sharma *et al.,* 1990). 50^{0}C for maximum saccharification of sugarcane bagasse and wood from cellulose produced from *P. Fumiculosum* and 55^{0}C for cellulose produced from *Aranchnoitus spp* (Ahmad *et al.,* 2004). The 40^{0}C recorded in this study is close to 41^{0}C temperature of the chicken hence its effectiveness.

The activity of the enzyme at 5.0-6.0 showed that the enzyme is very stable at the pH and its effect may possibly be good if incorporated into chicken feed.

The degradation of crude fibre and availability of crude protein as a result of inclusion of USFM showed that these two proximate constituents increased with time. Earlier report by Ahmad *et al.,* (2004) showed that beyond 36 hours the rate of activity of enzyme from *Arachnoitus spp* decline because of increase sugar concentration. This study was not extended beyond 32 hours. However, the study established the fact that the more the incubation time, the more the effect of the enzyme on the crude fibre and the more the availability of crude protein.

Conclusion

Since sometimes decortication of sunflower seed may add extra cost on the total cost of production of feed ingredient especially in tropical environments, multi-enzyme treatment of undecorticated sunflower seed meal has profound positive effect on its proximate constituent despite its high fibre content.

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UTILIZATION OF WHEAT OFFAL-CARRIED PINEAPPLE WASTE IN THE DIET OF WEST AFRICAN DWARF GOATS (WAD)

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Abstract

This study investigated the processing of wheat offal-carried pineapple waste meal (WCPW) and its utilization in the diet of West African Dwarf (WAD) goats with a view to ascertaining the inclusion level for optimal performance. The WCPW was obtained by evaluating six different combinations of wheat offal (WO) and pineapple waste (PW). The WO and PW mixing ratio 1:2 (weight/weight) was found to be optimal as feedstuff for WAD goats using keeping quality and nutrient content as criteria. Four dietary treatments (control diet (0%), 20%, 30% and 40% WCPW) were fed to growing WAD goats of both sexes in a completely randomized design. The proximate, mineral and vitamin composition of all treatment diets were determined. Performance variables such as feed intake, average daily gain, feed efficiency, nitrogen balance and retention, apparent digestibility coefficient and blood counts of the goats were evaluated. Chemical composition shows that the mineral, vitamin and crude protein (CP) contents of WCPW containing diets were more than sufficient than that required by goats for maintenance. The total feed intake (462.13 g/day), feed efficiency (9.90%), average daily weight gain (45.54%), the apparent digestibility coefficient of crude protein, nitrogen balance (2.63 g/day) and retention (53.46%) of goats fed 20% WCPW were significantly higher ($p<0.05$) than the values obtained for goats fed other experimental diets. This indicates that animals fed 20% WCPW had the best performance characteristics. Although, the analysis of blood cells (red blood cell, white blood and packed cell volume counts) were significantly different ($p<0.05$) among the goats fed experimental diets, the counts fell within the normal physiological range for goats. The study demonstrated that WCPW can be included in the diet of WAD goats without any adverse effect and dietary inclusion of WCPW up to 20% was optimal for growth performance of WAD goats.

Keywords: *Pineapple waste, WAD goats, Wheat offal*

UTILISATION D'UNE COMBINAISON D'ABATS DE BLE ET DE DECHETS D'ANANAS DANS LE REGIME ALIMENTAIRE DE CHEVRES NAINES D'AFRIQUE DE L'OUEST (WAD)

Résumé

Cette étude s'est penchée sur le traitement d'un mélange d'abats de blé et de déchets d'ananas (WCPW : wheat offal-carried pineapple waste meal) et son utilisation dans l'alimentation des chèvres naines d'Afrique de l'Ouest (WAD : West-African Dwarf) en vue de déterminer le niveau d'inclusion nécessaire pour une performance optimale. Le mélange WCPW a été obtenu en évaluant six combinaisons différentes d'abats de blé (WO) et de déchets d'ananas (PW). Le ratio du mélange WO et PW de 1:2 (w / w) a été jugé comme étant une alimentation optimale pour les chèvres WAD en utilisant comme critères la qualité et la teneur en éléments nutritifs. Quatre traitements diététiques (régime témoin (0%), WCPW à 20%, à 30% et à 40%) ont été administrés aux WAD des deux sexes en croissance, selon une répartition complètement aléatoire. La composition immédiate, minérale et vitaminique de tous les traitements a été déterminée. Les variables de performances telles que la consommation alimentaire, le gain moyen quotidien, l'efficacité alimentaire, le bilan et la rétention d'azote, le coefficient de digestibilité apparente et la numération globulaire des chèvres ont été évalués. La composition chimique montre que la teneur en minéraux, en vitamines et en protéines brutes (PB) des régimes contenant le WCPW étaient plus que suffisante par rapport à celui nécessaire pour l'entretien des chèvres. La consommation alimentaire totale

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(462,13 g / jour), l'efficacité alimentaire (9,90%), le gain pondéral moyen quotidien (45,54%), le coefficient de digestibilité apparente des protéines brutes, le bilan d'azote (2,63 g / jour) et la rétention d'azote (53,46%) des chèvres nourries au WCPW à 20% étaient significativement plus élevés (p <0,05) que les valeurs obtenues pour les chèvres nourries avec d'autres régimes expérimentaux. Ceci est une indication que les animaux nourris avec le WCPW à 20% avaient les meilleures caractéristiques de performance. Bien que l'analyse des cellules sanguines (globules rouges, globules blancs et hématocrite) ait été significativement différente (p <0,05) chez les chèvres nourries aux aliments expérimentaux, les numérations se situaient dans la fourchette physiologique normale des chèvres. L'étude a démontré que le WCPW peut être inclus dans l'alimentation des chèvres WAD sans causer aucun effet indésirable, et son inclusion dans l'alimentation jusqu'à 20% était optimale pour la croissance des chèvres WAD.

Mots-clés : *déchets d'ananas, chèvres WAD, abats de blé*

Introduction

Feed accounts for between 50 and 80% of the total cost of production depending on the type of livestock (Odeyinka and Ajayi, 2004). Olomu (1984) estimated the proportion of feed cost of the total cost of production of a ruminant under intensive management as 55%. One of the major problems confronting the small ruminant production is the non-availability of feed all year round to meet the maintenance and productive requirements of the animals. Babayemi (2007) stated that in the tropics, ruminants are raised mainly on grasses, which are poor in nutrients and digestibility coupled with scarcity during the dry season. Poor productivity and high mortality of stock, which characterize this industry result mostly from not feeding the right quantity and quality of feeds to the various livestock species (Odeyinka *et al.,* 2003). The unprecedented increase in the cost of conventional ingredients (e.g. maize) used in compounding livestock feed has necessitated intensive investigations into the use of agricultural and agro-industrial by-products (Hamzat and Babatunde, 2001). Some of the agro-industrial wastes include the following: citrus molasses, cull onions, citrus pulp, coffee-husk and pulp, cassava waste, sugar cane molasses, shrimp waste meal, pineapple waste (Babatunde, 1998).

Food and Agricultural Organization (2004) ranked Nigeria among the leading pineapple producing countries with about 800,000 metric tonnes since year 2001. Therefore, efforts at finding better use for the pineapple waste generated from such huge quantities may be important in terms of preventing environmental pollution and waste of potential animal feed resource. Lamidi *et al.,* (2008) reported that broiler chickens could tolerate up to 10% pineapple waste (PW) in their diets without any deleterious effect. Taiwo *et al.* (2011) concluded that PW could be included in the diet of sheep up to 45% without adverse effect. A possible loss of essential nutrients from seepage during the drying of wet PW was observed in the study reported by Taiwo *et al.* (2011), where drying took about 14 days. Therefore, a more efficient processing method seems desirable. One strategy is to use dry feed materials to absorb the exudates from PW. Such dry feed materials previously utilized as effective absorbents are maize offal/bran, wheat offal/bran, brewers' dried grains and dewatered rumen contents (Makinde and Sonaiya, 2007); and the most effective absorbent was wheat offal (WO). Consequently, mixing PW and wheat offal may provide a more efficient alternative to existing methods in terms of reducing nutrient loss and quick drying. Makinde *et al.* (2011) investigated this procedure and confirmed its effectiveness resulting in an optimal combination of WO and PW (referred to as wheat offal-carried pineapple waste – WCPW) at ratio 1:2 (weight/weight) after sun drying for 4 h. This WCPW had approximately 88% DM, 16% CP and 10%

CF thus can serve as supplement feed source for ruminant animals (Makinde *et al.* 2011). Therefore, the general objective of this study was to evaluate the nutritive value of WCPW as animal feed and specifically the performance of West African Dwarf (WAD) goats on diets with graded levels of WCPW.

Materials and Methods

Experimental station and period

The experiment was carried out at the Sheep and Goat Unit of Obafemi Awolowo University Teaching and Research Farm Ile-Ife, Osun State, Nigeria at altitude of 240 m above sea level, 7° 28^{0}N and 4° 23^{0}E. Ile Ife ecologically typifies the hot and humid tropical forest zone. The experiment lasted 26 weeks between mid February and August 2011.

Processing of wheat offal-carried pineapple waste meal (WCPW)

The wheat offal-carried pineapple waste meal was processed as reported by Makinde *et al.* (2011). Briefly, wheat offal (WO) collected from Eagle Flour mills, Ibadan, Nigeria was thoroughly hand-mixed with fresh wet pineapple waste (PW; skins, peelings and the pulp peelings) collected in polyethylene woven sacks from the Lafia Canning Factory of Fumman Agricultural Products Nigeria Ltd, Moor Plantation, Ibadan, Nigeria. Six sets of the WO and PW mixture (1:1, 1:1.5, 1:2, 1:2.5, 1:3, 1:3.5) were evaluated based on capacity to dry to ≤ 10 - 12% moisture content in 4 h (Makinde and Sonaiya, 2007 and 2010). The criterion for this decision was to select the mix with the highest PW content that dried to ≤ 10 - 12% moisture content in 4 h. Moisture content > 12% had been reported not desirable for long term preservation (Rozis, 1997). The mixtures were sun-dried by spreading thinly on black polythene sheets (0.7 mm thickness) in two replicates each on the concrete roof (20.5 m high) of the Faculty of Agriculture, Obafemi Awolowo University, Ile-Ife, Nigeria. These were ground with a plate/burr mill after drying and the proximate composition determined according to the methods of AOAC (2000). Based on the selection criterion, the mix-ratio 1:2 (WO: PW) of the wheat offal-carried pineapple waste meal (WCPW) was found to be the optimum combination (Table 1).

Animals, feeding and management

The experiment utilized twenty weaner WAD goats of both sexes in a feeding trial for 16 weeks. The animals were between 5 and 7 months old, weighed between 4.0 and 10.5 kg and randomly allotted to four treatments in a completely randomized design. Four concentrate diets were compounded comprising 0, 20, 30 and 40% levels of inclusion of the 1:2 (WO: PW) wheat offal-carried pineapple waste meal (WCPW) (Table 2). The diets were fed to West African Dwarf (WAD) weaner goats as supplements to a basal ration of guinea grass (*Panicum maximum*). The goats were fed based on 3% of their body weights. Each animal was weighed before the commencement of the study and subsequently weekly throughout the experimental period. The goats were confined in slated floor pens in an open–sided house constructed from wood and wire gauze, with asbestos roof and concrete floor. The goats were fed *ad libitum* with free access to water and routine management and vaccination schedules carried out. Feed intake, feed efficiency, weight gain, nutrient digestibility, nutrient utilization and blood parameters were performance evaluation variables.

Measurement of digestibility

Two digestibility trials were carried out between 8 - 9th and 14 - 15th weeks during which the goats were housed individually in metabolism cages designed for the separate collection of faeces and urine, and fresh feed and water provided daily. Faeces were collected each morning before the feed was served. A 10% sample of faeces voided per day was dried in a forced-draught oven at 70°C for 24 hours. Faecal samples stored daily were bulked, thoroughly mixed, ground and sub-samples taken for chemical analysis. A 5 ml sample of the urine collected was preserved in of 5% (v/v) glacial acetic acid and stored at 4°C in a deep freezer for chemical analysis. Apparent nutrient digestibility was determined according to the following:

Apparent digestibility (%)
= Nutrient in feed – Nutrient in faeces x 100/Nutrient in feed

Table 1: The proximate composition of different blends of wheat offal and pineapple waste

	Feed ratio (Wheat Offal: Pineapple Waste)					
Parameter	**1: 1**	**1: 1.5**	**1: 2.0**	**1: 2.5**	**1: 3.0**	**1: 3.5**
Dry matter	91.70	90.80	88.30	85.30	85.00	84.70
Crude protein	15.55	15.40	16.20	15.70	16.00	16.20
Crude fibre	7.61	7.96	9.96	9.97	9.74	10.00
Ether extract	9.54	8.15	10.10	10.06	11.50	9.46
Ash	4.22	5.70	5.89	6.27	6.34	6.74
NFE	63.00	62.70	57.50	57.30	56.30	57.80

NFE; Nitrogen Free Extractives
Source: Makinde *et al.,* (2011).

Table 2: Gross and chemical composition of the experimental diets

Parameter (%)	**0%WCPW**	**20% WCPW**	**30% WCPW**	**40%WCPW**
Gross composition of diets				
Corn bran	40.00	20.00	10.00	-
WCPW	-	20.00	30.00	40.00
Palm Kernel Cake	53.00	53.00	53.00	53.00
Groundnut Cake	4.50	4.50	4.50	4.50
Bone Meal	1.50	1.50	1.50	1.50
Salt	0.50	0.50	0.50	0.50
Vitamin premix	0.50	0.50	0.50	0.50
Chemical composition of diets				
Dry Matter	92.78	92.95	92.78	93.23
Organic Matter	92.99	92.73	92.53	92.03
Crude Protein	16.38	18.20	17.75	17.75
Crude fibre	10.77	9.12	9.62	11.44
Ether Extract	11.87	12.59	10.05	9.88
Ash	7.01	7.27	7.47	7.97
Nitrogen free extract	46.75	45.77	47.89	46.19

WCPW: Wheat offal carried pineapple waste

Chemical composition and blood analysis

The proximate composition, mineral and vitamin content of WCPW was determined at the Animal Sciences Department, Soil Science and Land Management Department and Central Science Laboratory, Obafemi Awolowo University, Ile-Ife according to procedure of AOAC (2000). Fecal and urine samples were analyzed for proximate and nitrogen content, respectively. Blood collected from the goats at the jugular vein into EDTA bottles was analyzed for packed cell volume (PCV), red blood cell (RBC) and white blood cell (WBC) counts (Baker and Silverton, 1976).

Statistical analysis

Data were analyzed as completely randomized block design using the General Linear Models procedure of SAS® (2008) for analysis of variance (ANOVA). The main effects were the diets and replicates. The replicates per treatment were considered as blocks in order to increase the precision of the experiment. Differences between means were resolved by Duncan's multiple range test of the SAS® (2008) statistical package. Statistical significance was established when probability was less than 5% level of significance.

Results

Table 2 shows gross and chemical composition of the experimental diets. The gross composition of diets indicates the inclusion levels (0, 20, 30 and 40%) of WCPW to replace corn bran in the experimental diets. The crude protein content of diets containing WCPW was higher than that of thee control diet while the values of other proximate components were similar across all the experimental diets.

Table 3 shows the mineral and vitamin composition of WCPW as compared with NRC (1975, 2007) values for sheep. Macro and micro mineral content of WCPW were comparable and exceeded NRC values. Similarly, vitamin A and E contents of WCPW were higher than the NRC values.

Table 4 shows the performance of the WAD goats on WCPW diets. The total feed intake was significantly different ($p<0.05$) for goats on different diets. Goats fed 20%WCPW had the highest feed intake overall and for Panicum followed by goats on 0%WCPW, which

Table 3: Mineral and Vitamin composition of wheat offal-carried pineapple waste (WCPW)

Mineral	WCPW	NRC (1975)
Macro mineral (%)		
Calcium	0.48	0.21 – 0.52
Phosphorus	0.55	0.16 – 0.37
Magnesium	0.38	0.04 – 0.26
Sodium	0.16	0.04 – 0.01
Sulphur	0.16	0.14 – 0.26
Micro mineral (ppm)		
Iron	65.60	30 - 50
Cupper	11.68	5.00
Manganese	140.00	20 - 40
Zinc	282.50	N/A
Vitamin (I.U)		
A	8000	5000
E	2300	1400

N/A: Not available

Table 4: Performance characteristics of experimental goats over a sixteen-week period

Parameter	0%WCPW	20%WCPW	30%WCPW	40%WCPW	SEM	P value
ADFC (g/day)						
Concentrate	117.98^{a}	117.20^{b}	109.44^{c}	112.10^{b}	0.66	<0.0001
Panicum	303.53^{b}	344.93^{a}	292.66^{c}	287.80^{c}	2.07	<0.0001
Total Intake	421.51^{b}	462.13^{a}	402.10^{c}	399.90^{c}	2.11	<0.0001
AILW (kg)	6.68	6.61	6.43	6.18	0.02	<0.0001
AFLW (kg)	10.51^{b}	11.06^{a}	9.85^{c}	9.63^{d}	0.02	<0.0001
TWG (kg)	3.83^{b}	4.45^{a}	3.42^{c}	3.45^{c}	0.02	<0.0001
ADG (g/day)	39.21^{b}	45.54^{a}	35.00^{c}	35.24^{c}	0.10	<0.0001
FE (%)	9.66^{b}	9.90^{a}	8.60^{d}	8.82^{c}	0.66	<0.0001

^{a,b,c,d} means on the same row with different superscripts are significantly different ($p<0.05$), WCPW: Wheat offal carried pineapple waste, ADFC: Average daily feed consumption, AILW: Average initial live weight, AFLW: average final live weight, TWG: Total weight gain, ADG: Average daily weight gain, FE: Feed efficiency.

Table 5: Apparent digestibility coefficient of experimental goats

Parameter (%)	0%WCPW	20%WCPW	30%WCPW	40% WCPW	SEM	P value
Dry Matter	61.77a	60.74b	57.73d	60.20c	0.38	<0.0001
Organic Matter	87.37a	83.34d	85.29c	85.98b	0.57	<0.0001
Crude Protein	51.27c	53.92a	52.70b	50.23d	0.28	<0.0001
Crude Fibre	60.97c	65.13a	63.21b	52.52d	0.30	<0.0001
Ether Extract <0.0001	64.81d	68.32c	70.11b	72.36a	0.46	<0.0001
Ash	44.50d	56.36a	49.22b	43.15c	0.27	<0.0001
NFE	83.64d	90.41b	91.42a	86.04c	0.68	<0.0001

[a, b, c, d:] Means within each row with different superscript are significantly different ($p<0.05$)
WCPW: Wheat offal carried pineapple waste

Table 6: Mean nitrogen utilization of goats fed experimental diets

Parameter (%)	0%WCPW	20%WCPW	30%WCPW	40% WCPW	SEM	P value
Nitrogen Intake (g/day)	4.64[c]	4.93[a]	4.85[b]	4.85[b]	0.01	<0.0001
Nitrogen excretion faecal	1.33[c]	1.41[b]	1.41[b]	1.47[a]	0.03	<0.0001
Urinary	0.93[b]	0.89[c]	1.02[a]	0.96[b]	0.01	<0.0001
Total	2.26[b]	2.30[b]	2.43[a]	2.43[a]	0.02	<0.0001
Nitrogen loss (%N intake)						
Faecal	28.73[b]	28.46[d]	28.87[b]	30.30[a]	0.02	<0.0001
Urinary	20.10[b]	18.09[d]	21.04[a]	19.80[c]	0.02	<0.0001
Total	48.83[b]	46.55[c]	49.91[a]	50.10[a]	0.05	<0.0001
Nitrogen balance (g/day)	2.38[c]	2.63[a]	2.42[b]	2.42[b]	0.01	<0.0001
Nitrogen retention (%)	51.19b	53.46[a]	50.17[c]	49.88[c]	0.08	<0.0001

[a,b,c,d] means on the same row with different superscripts are significantly different ($p<0.05$)
WCPW: Wheat offal carried pineapple waste

Table 7: Blood analysis of experimental goats

Parameter (%)	0%WCPW	20%WCPW	30%WCPW	40% WCPW	SEM	P value
PCV (%)	35.60[a]	32.80[b]	32.00[b]	28.80[c]	1.23	<0.0001
RBC (106)	11.00[b]	12.00[a]	10.20[c]	9.70[d]	0.29	<0.0001
WBC (103)	9.27[c]	11.66[a]	7.88[d]	10.74[b]	0.26	<0.0001

[a, b, c, d] Means within each row with different superscripts are significantly different ($p< 0.05$)
WCPW: Wheat offal carried pineapple waste; PCV: Packed cell volume; RBC: Red blood cell; WBC: White blood cell.

was higher than goats on 30 and 40%WCPW. The final live weight for goats on 20%WCPW was significantly higher ($p<0.05$) than goats on 0%WCPW followed by 30 and 40%WCPW, in that order. Similar trend was followed for total weight and average daily weight gained. Goats fed 20%WCPW were superior to goats on other diets in all performance variables.

Table 5 shows the apparent nutrient digestibility coefficients. Digestibility coefficients of dry matter and organic matter were highest ($p<0.05$) for 0%WCPW and least

for 30%WCPW and 20%WCPW, respectively. For NFE, digestibility was highest ($p<0.05$) for 30%WCPW and least for 0%WCPW. Utilisation of ether extract was highest ($p<0.05$) for 40%WCPW and least for 0%WCPW. Comparatively, 20%WCPW had the highest digestibility score when all the nutrients are considered.

Table 6 shows that there was no significant difference ($p>0.05$) in nitrogen intake of goats fed 30%WCPW and 40%WCPW but higher ($p<0.05$) than for goats on 20%WCPW and 0%WCPW, which were equal. However, the % total nitrogen loss (nitrogen in faeces and urine) of goats fed 30 and 40%WCPW was significantly higher ($p<0.05$) than goats fed other diets and 20%WCPW had the least loss. The nitrogen retention percentage was highest ($p<0.05$) in 20%WCPW than other diets.

The analysis of blood components packed cell volume (PCV), red blood cell (RBC) and white blood cell (WBC) counts (Table 7) showed that there were significant differences ($p<0.05$) in each of these parameters among the animals fed experimental diets. Goats fed 20% WCPW scored most satisfactorily in a combination of the blood parameters. Packed cell volume was highest in 0%WCPW followed by 20%WCPW and lowest in the 40%WCPW diet. Further, goats on 20%WCPW had the highest RBC scores ($p<0.05$) followed by 0%WCPW, 30%WCPW and 40%WCPW, in that order. Similarly, goats fed 20% WCPW had highest scores ($p<0.05$) for WBC but were followed by those fed 40%WCPW, 0%WCPW and 30%WCPW, in that order.

Discussion

The main objective of this study was to evaluate the nutritive value of WCPW as animal feed and especially the effect on the growth performance of West African Dwarf (WAD) goats on diets with graded levels of WCPW. Results indicate a good potential for WCPW as animal feed and for inclusion in concentrate diets for growing small ruminants.

The gross and chemical composition of the experimental diets in Table 2 signifies comparable potential feeding value of diets with WCPW against the control diet (0%WCPW). However, the crude protein content of diets containing WCPW was higher than that of control diet (0%WCPW) while other proximate components were similar except crude fiber and ether extract (Table 2). Crude fiber decreased then increased as the WCPW content increased, apparently due to increasing fiber content contribution by WCPW. In contrast, ether extract decreased as the content of WCPW increased. Nevertheless, all diets met the minimum nutrient requirements for growing WAD goats (NRC, 1985). This suggests suitability of WCPW in the diets of small ruminants.

Further, the mineral and vitamins A and E content of WCPW (Table 3) met and even more than exceeded that recommended by NRC (1975, 2007) for sheep. In practice, mineral and vitamin requirements for goats are usually approximated from sheep values because of lack of data on goats (NRC, 1985). This result further underscores the feeding value of WCPW.

It appears that inclusion of WCPW beyond 20% depresses feed intake (Table 4). However, feed intake of goats in this study was higher than that reported by Olosunde (2010) for goats fed diets with sun-dried pineapple waste. This could be due to the mixing of wheat offal with pineapple waste, which probably resulted in improved palatability of the diets. In addition, Butterworth and Mossi (1985) and Akinlade *et al.* (2005) observed that the use of concentrate as a supplement for ruminants can improve dry matter feed intake. This result probably indicates that there is no adverse effect on intake when WCPW replaces corn bran in concentrates for WAD goats. Goats on 20%WCPW were superior in growth probably due to superior feed intake and feed efficiency compared with goats on the other diets. Masafu (2006) describes feed intake as a measure of diet appreciation, selection and consumption by an animal. Nevertheless, all animals on all diets were in a positive weight balance, suggesting nutritional adequacy of diets. The average daily weight gain range (35.00 – 45.51g/day) in this study was higher than that reported for WAD goats fed conventional protein supplements (palm kernel cake, soybean meal, brewers' dried grains and cotton seed cake)

by Arigbede (2007) but similar to the results of studies by Alikwe (2011) who fed soybean meal and dried poultry waste as supplements. Arigbede (2007) recorded 19.83 – 33.36 g/day and Alikwe (2011) 22.10 – 54.30 g/day. Similarly, the feed efficiency values obtained in this study were higher than those obtained for WAD goats by Arigbede (2007) and Alikwe (2011). These results indicate the potential of WCPW as a good substitute for conventional protein supplements in WAD goat diets.

Goats fed 20%WCPW were superior to others in overall utilisation of nutrients (Table 5), which was reflected in better growth performance. This was probably due to highest feed intake (Table 4) and digestibility scores in crude protein and crude fibre. It is possible that inclusion of WCPW at 20% favoured increased activities of fibre degrading bacteria in the rumen. Feed intake and digestibility are regarded as major factors that determine potential animal performance (Beever, 1993). The superior scores for digestibility and intake by goats on 20%WCPW indicates better acceptability, consumption and utilization than other diets. Although, neutral detergent fiber (NDF) and lignin were not determined, the depression in dry matter digestibility for goats on 20%WCPW compared with 0%WCPW may be due to increase in NDF and lignin because of higher feed intake. Bakshi and Wadhwa (2004) observed that voluntary dry matter intake and dry matter digestibility are dependent on fiber, especially neutral detergent fiber and lignin. Norton (1994) also reported increased digestibility with reducing NDF values. Although, the digestibility coefficients for all the diets were lower than what was obtained by Olosunde (2010) who fed sun-dried pineapple waste to sheep, goats in this study had higher growth rates (Table 4). These results further underline the potential of WCPW as feedstuff for WAD goats.

Nitrogen balance has been described as a good indicator of the protein value of a diet when the amino acid supply is balanced with the energy supply (Babayemi and Bamikole, 2006). A positive nitrogen balance indicates that the protein requirement for maintenance in experimental animals was adequately met by the dietary treatments. All the diets had positive nitrogen balances, which indicates adequacy in protein requirement for maintenance. Animals fed 20%WCPW diet had the highest ($p<0.05$) N balance (2.63) and N retention percentage (53.46%). This indicates that the optimum level of inclusion of WCPW was at 20% and that at higher inclusion levels efficiency of protein utilization decreased. All the nitrogen balance values obtained in this study (2.37 – 2.63 g/day) are similar to 2.23 – 3.30 g/day reported by Ogunmoye (1995) for WAD goats fed soybean-based diets. Nitrogen retention as a percentage of nitrogen intakes ranged between 49.88% (40%WCPW) and 53.46% (20%WCPW). However, these were lower than values obtained by Arigbede (2007) and Alikwe (2011) probably because they fed conventional protein sources. Nevertheless, all the values were similar to the range (32.6 to 58.3%) reported by Ndemanisho *et al.*, (1998) and Babayemi and Bamikole (2006) for WAD goats fed concentrate diets.

The PCV values, red blood cell (RBC) and white blood cell (WBC) counts obtained in this study were within normal physiological ranges (27.0 – 45.0%, 9.0 - 15.0 x 106 and 4.0 – 12.0 x 103, respectively) reported by Jain (1993). This probably shows that feeding WAD goats diets with WCPW up to 40% inclusion did not adversely affect their physiological well-being.

The study demonstrated that dietary inclusion of WCPW up to 20% was optimal for growth performance of WAD goats. Further studies that could enhance the utilization of pineapple waste in WAD goat diets will contribute to the pool of alternative feed ingredients and reduction in environmental pollution from pineapple waste by pineapple processing industries.

Impact

Combinations of wheat offal and pineapple waste using simple techniques for quick recycling resulted in a potential animal feedstuff. This feedstuff was satisfactorily utilized by WAD goats, which are commonly found and reared by smallholder farmers and householders in most rural communities in

Nigeria and many other developing countries in sub-Saharan Africa. This may be important as a supplemental feed source for ruminant animals during the dry season when forages are in short supply. Recycling pineapple waste would reduce the extra cost incurred by pineapple canning industries in disposing the waste and ultimately reduce environmental pollution from it.

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THE PROTECTIVE EFFECT OF WALNUT (*TETRACARPIDIUM CONOPHORUM*) LEAF AND ONION (*ALLIUM CEPA*) BULB RESIDUES ON THE EXPERIMENTAL *PSEUDOMONAS AERUGINOSA* INFECTION IN *CLARIAS GARIEPINUS* JUVENILES

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Abstract

The study assessed the effect of Walnut Leaf (WL) and Onion Bulb (OB) residues on disease resistance of *Clarias gariepinus* juveniles against infection with the bacteria pathogen *Pseudomonas aeruginosa*. *Clarias gariepinus* juveniles were fed with diets containing 0 (control), OB2 (0.5%), OB3 (1.0%), OB4 (1.5%), OB5 (2.0%), WL6 (0.5%), WL7 (1.0%), WL8 (1.5%) and WL9 (2.0%). Biochemical (serum total protein, albumin, globulin, albumin: globulin ratio) and haematological indices of the fish were investigated. Fish were exposed to 0.5ml of 107 *Pseudomonas aeruginosa* of 24h old culture with the percentage mortality and relative level of protection recorded for 4 weeks post – infection. The results demonstrated that the fish fed with treated diets showed increased in biochemical and *haematological indices* ($P<0.05$) compared with the control. The challenge infection showed an improvement from treated groups with percentage mortalities and relative level of protection highest in WL8 (3.33%, 90%) and OB2 (3.33%, 90%) and least (33.33%, 0%) in control respectively. The results suggest that walnut leaf residue at 1.5% inclusion for one month could be a potential, less expensive and promising dietary supplement that would positively affect growth, haematology and make *C. gariepinus* more resistant to *Pseudomonas aeruginosa*

Keywords: *Pseudomonas aeruginosa, Clarias gariepinus, Onion bulb*, Walnut leaf Mortality, *Haematology*

L'EFFET PROTECTEUR DES RESIDUS DE FEUILLES DE NOYER *(Tetracarpidium Conophorum)* ET DE BULBES D'OIGNON *(Allium Cepa)* CONTRE L'INFECTION EXPERIMENTALE AUX *Pseudomonas aeruginosa* CHEZ DES *Clarias gariepinus* JUVENILES

Résumé

L'étude a évalué l'effet des résidus de feuilles de noyer (WL) et de bulbes d'oignons (OB) sur la résistance des *Clarias gariepinus* juvéniles à l'infection aux bactéries pathogènes *Pseudomonas aeruginosa*. Des *Clarias gariepinus* juvéniles ont été nourris avec des régimes contenant 0 (témoin), OB2 (0,5%), OB3 (1,0%), OB4 (1,5%), OB5 (2,0%), WL6 (0,5%), WL7 (1,0%), WL8 (1,5%) et WL9 (2,0%). Les indices biochimiques (la teneur totale en protéines sériques, l'albumine, la globuline, le ratio albumine/globuline) et hématologiques des poissons ont été étudiés. Les poissons ont été infectés avec 0,5 ml de 107 *Pseudomonas aeruginosa* issues d'une culture de 24 h ; et le taux de mortalité et le niveau relatif de protection ont été enregistrés pendant 4 semaines après l'infection. Les résultats ont montré une augmentation des indices biochimiques et hématologiques ($P <0,05$) chez les poissons nourris avec les régimes traités par rapport à ceux nourris avec le régime témoin. L'infection d'épreuve a montré une amélioration chez les groupes traités ; le pourcentage de mortalité et le niveau relatif de protection étaient élevés respectivement chez les poissons nourris aux régimes WL8 (3,33%, 90%) et OB2 (3,33%, 90%) et faibles (33,33%, 0%) chez le groupe témoin. Les résultats font penser que les résidus de feuilles de noyer à une inclusion de 1,5% pendant un mois pourrait être un supplément alimentaire potentiel, moins cher et prometteur, susceptible d'avoir des effets positifs sur la croissance et l'hématologie et augmenter la résistance des *C. gariepinus* aux bactéries *Pseudomonas aeruginos*.

Mots-clés : *Pseudomonas aeruginosa, Clarias gariepinus, bulbe d'oignon, feuille de noyer, mortalité, hématologie*

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Introduction

Disease outbreaks were recently identified as the major constraint to aquaculture production with consequent adverse effects on the industry's economic development (Yunxia *et al.*, 2001). Attempts to control or prevent such devastating outbreaks using conventional antimicrobials and other chemotherapeutants have been generally unsuccessful (Jadhav *et al.,* 2006). The uncontrolled and repeated uses of antibiotics to treat bacterial infections have in some cases led to the development of antibiotic- resistant pathogens (Flores *et al.,* 2003; Food and Agriculture Organization, 2006). Considering the potential threat of diseases on human and animal health, issues associated with the use of antibiotics, disease management aspect should therefore focus on environmental-friendly, preventive methods such as the use of immunostimulants and natural products with antimicrobial properties.

Immunostimulants, which have been effective in enhancing immune responses in salmonids, carp, channel catfish and giant fresh water Macrobrachium rosenbergii include lavamisole (Siwicki *et al.,* 1990), glucans (Yano *et al.,* 1989; Robertson *et al.,* 1990; Niki *et al.,* 1991; Mishra *et al.,* 2004) and chitin (Sakai *et al.,* 1992; Anderson and Siwicki, 1994). Das *et al.,* 2009 reported that immunostimulants could be administering orally or in the feed.

As an hypothesis, onion (*Allium cepa*) bulb and walnut (*Tetracarpidium conophorum*) leaf, as plant immunostimulants, can be used as a growth promoter and in health management in African catfish (*Clarias gariepinus*) in which it could increase body weight gain, feed intake and feed efficiency. Onion (*A. cepa*) bulb and walnut (*T. conophorum*) leaf potentially display broad spectrum activities against bacterial agents (Gram positive and Gram negative) both in vitro and as well as in vivo studies (Abd-Elallatif and Ebraheem, 1996) and also antihelmintics and anti-fungal properties. The aim of the current study was therefore to evaluate the possible protective effect of walnut leaf and onion bulb residues as a potential antimicrobial in the farming of *Clarias gariepinus* against an experimental challenge infection using *Pseudomonas aeruginosa*

Materials and Methods

Plant collection and identification

Onion bulbs were purchased from Bodija market in Ibadan, Nigeria. Walnut leaf was obtained from a farm at Oka -Akoko, Nigeria. They were authenticated at the herbarium of the Forestry Research Institute of Nigeria (FRIN), Ibadan, where a voucher specimen was deposited under FHI 107515.

Preparation and Extraction of Plant Materials

Onion extraction

The onions bulbs were washed with distilled water and allowed to air dry at ambient temperature (25oC) for one hour. The dry outer coverings of the onions were manually peeled off, washed and extracted as described by Azu and Onyeagba, (2007). 200g of the fresh onion bulbs were blended into fine powder and soaked in 100ml of 95% ethanol for 24hrs. The pulp obtained was left in a clean, sterile glass container, shaken vigorously to allow for proper extraction, filtered using a sterile muslin cloth after which the residue was obtained, air-dried and stored (4°C) until required.

Walnut leaf extraction

The extraction was as described by Ajaiyeoba and Fadare (2006). The air – dried walnut leaf were ground with a hammer mill to fine powder. 200g of the powder of walnut leaves was soaked in 100ml of 80% methanol for 72 hours. Walnut leaf were properly mixed with methanol, filtered using a sterile muslin cloth after which the extract was obtained, air – dried and stored at (25°C) until required.

Preparation of Experimntal Diets

The proximate composition of the experimental diet was 40.0%crude protein, 15.9% ether extract, 15.7% ash, 7.4% moisture, and 20.9% NFE. Nine experimental diets were prepared by incorporating walnut leaf and onion bulb residues at the following inclusion levels; 0 (control), 0.5%, 1.0%, 1.5% and 2.0% respectively. Feed ingredients such as fishmeal, soyabean, maize, starch, vegetable oil, Di calcium

phosphate (DCP), salt and vitamin- mineral premix were added and the dry ingredients were mixed thoroughly in a mixer. Water was added and the resulting dough was pelleted. The pelleted diets were sun –dried and stored in airtight containers at room temperature to prevent mycotoxin formation until required.

Culture Of Pathogen

P. aeruginosa was collected from the Laboratory stock of the Department of Microbiology, University of Ibadan, Nigeria. The pure cultures were sub-cultured on Nutrient slants and preserved in the refrigerator at 4°C until required for the study

Collection of Blood and Serum

This was carried out before and after the experiment at the haematological laboratory of Veterinary Pathology Department, University of Ibadan within 30 minutes of sampling. a distance of 3 – 4cm from the genital opening of each fish was punctured and wiped with dry tissue paper to avoid contamination with mucus. The needle was inserted at right angle to the vertebral column of the fish, which was gently aspirated during penetration. The blood was taken under gentle aspiration until about 1cm3 had been obtained. Thereafter the needle was gently withdrawn and the blood gently transferred into heperarinized plastic containers. The samples were then mixed gently but thoroughly. The haematology were taken according to the methods of Blaxhall and Daisley (1973) Plasma was obtained from blood samples by centrifugation and then drawn into 1cm3 plastic syringe transferred into a universal bottle in refrigerator to be later used for biochemical analysis.

Challenge Test

270 *Clarias gariepinus* (30 from each treatment) were challenged by intraperitoneal route with 0.5ml of 107 *Pseudomonas aeruginosa* of 24h old culture. The challenged fish were kept under observation for 30 days. The mortalities were recorded and the relative level of protection (RLP) among the challenged fish was determined as described by Azza and Abd-El-Rhman (2009), Ibrahem *et al.,* (2010)

$$\text{RLP} = 1 - \frac{[\text{percentage of mortality in treated group}] / \times 100}{[\text{percentage of mortality in control group}]}$$

Statistical Analysis

Challenge test, haematology and biochemical analysis results from the experiment were subjected to one-way analysis of variance (ANOVA) using SPSS (Statistical Package for Social Sciences 2006, version 15.0). Duncan multiple range test was used to compare differences between individual means.

Results

Challenge test of *Pseudomonas aeruginosa* injected by intraperitoneal route and relative level of protection among *Clarias gariepinus* treated with onion bulb and walnut leaf was given in Table 1. Mean post challenge test haematological parameters of African Catfish *Clarias gariepinus* Juveniles fed treated onion bulb and walnut leaf (Table 2). Mean post challenge test plasma biochemistry parameters of African Catfish (*Clarias gariepinus*) Juveniles fed onion bulb and walnut leaf are shown in Table 3. Post challenge test blood serum of African Catfish *Clarias gariepinus* Juveniles fed onion bulb and walnut leaf are given in Table 4

Discussion

A properly functioning immune system is critical in maintaining the fitness and health of an organism; thus the opportunity to determine the effect of exposure to bacteria on the performance and immunity of the fish species and on their natural habitats (AraKoosh, *et al.,* 2005). The results showed that infected fish in the present study were weak in the 1st week of the experiment with decrease in feed intake and weight gain (see Table 1). However, feed intake and weights increased in the second week. The first mortality was recorded in the control experiment on the 5th day. The observed symptoms of this infection included lack of appetite, swimming abnormalities,

Table 1: Challenge test of *Pseudomonas aeruginosa* injected by intraperitoneal route and relative level of protection among *Clarias gariepinus* treated with onion bulb and walnut leaf

	Control	OB2	OB3	OB4	OB5	WL6	WL7	WL8	WL9
Weight:									
Initial	45.12±2.47	46.67±1.75	50.35±2.94	42.43±2.69	46.24±2.07	47.97±3.25	41.19±0.83	44.19±1.01	37.42±1.06
Week 1	44.22±1.99	46.57±1.04	49.95±2.80	43.05±3.56	45.89±1.29	48.08±3.46	40.94±0.37	44.37±0.62	36.80±3.29
Week 2	47.68±1.83	51.14±0.94	54.62±2.90	46.30±4.62	47.69±0.77	53.63±2.40	45.68±1.10	48.99±0.54	41.22±1.52
Week 3	50.33±1.23	52.41±0.39	56.76±2.64	48.28±2.81	50.65±0.69	54.47±2.27	48.74±1.08	51.15±0.21	41.76±1.04
Week 4	50.66±3.69	55.87±5.18	59.77±0.38	53.92±1.07	52.08±0.64	57.91±0.54	52.96±1.47	55.93±1.00	43.46±1.30
Weight gain	5.54±1.22[a]	9.30±3.43[d]	9.42±2.56[e]	11.49±1.62[g]	5.84±0.43[b]	9.94±2.71[f]	11.77±0.64[h]	11.74±0.01h	6.04±0.30c
Number of fish injected	30	30	30	30	30	30	30	30	30
Mortality (N)	10[b]	1[a]	4[a]	2[a]	3[a]	3[a]	2[a]	1a	3a
Mortality (%)	33.33[e]	3.33[a]	13.33[d]	6.67[b]	10.00[c]	10.00[c]	6.67[b]	3.33a	10.00c
Relative level of protection	0[a]	90[e]	60[b]	80[d]	70[c]	70[c]	80[d]	90e	70c

Key: Mean followed by the same letter is not significantly different ($p > 0.05$)

blotted appearance, skin alteration, anorexia, shaking head, and mouth tumidity. These syndromes were similar to those reported by Zhang *et al.,* (2009).

Results of the challenge test shown in Table 1 revealed that the mortality rate following challenge with P. aeruginosa was reduced in the groups of fish fed with onion bulb and walnut leaves incorporated diets. OB 2 and WL 8 showed highest rate of survival as compared with the control; the values were 3.33%, 90% for percentage mortality and relative level of protection (RLP) in OB 2 and WL 8 respectively and the control value was 33.33%, 0% for percentage mortality and relative level of protection. It can be inferred from the challenge study that the increased protection against the pathogen could be due to the enhancement in the defence system as evidenced from the increase in different immune parameters such as lymphocytes and white blood cell in post-challenge fish.

The findings of this present study is in accord with the work of Shalaby *et al.,* (2006) who reported that diets with Allium sativum and chloramphenicol showed decrease in the mortality rate of *O. niloticus* challenged intraperitonealy with *A.hydrophila.* Das *et al.,* (2009) reported that mortality following challenge with *A. hydrophila* was decreased in the group of fish fed with Euglena incorporated diets compared with the control. The report also by Ibrahem *et al.,* 2010 that the showing relative level protection (RLP) after challenge infection using *Aeromonas hydrophila* was higher in treated groups (41.67% and 33.33% for vitamin c and insulin respectively) than the control (0%) supported the findings of the present study.

Similarly in their study, Aly *et al.,* (2008) reported that the group that was treated with probiotics (*Bacillus subtilis* and *Lactobacillus acidophilus*) showed higher levels of protection against the test pathogens than the control (without probiotics). Sharma *et al.,* 2010 also reported that the challenge test with *A. hydrophila* proved that increased per cent survival rate was highest (42.85%) in the treatment containing 2 g /kg

Table 2: Mean Post Challenge Test Haematological Parameters of African Catfish (*Clarias gariepinus)* Juveniles fed treated onion bulb and walnut leaf

Parameters	Pre – challenge	Post challenge			
		CONTROL	OB2	OB3	OB4
PCV (%)	12.50±2.50	29.00±0.00[a]	37.00±3.00[a]	28.50±2.50[a]	26.00±1.00[a]
Hb (g/dl)	4.10±0.45	9.30±1.00[ab]	11.95±0.75[b]	9.10±0.90[ab]	8.30±0.50[ab]
RBC x1012/ l	1.07±0.05	2.75±0.06[a]	4.05±0. 35[a]	3.19±0.66[a]	2.63± 0.02[a]
WBC x109/ l	15,250±5.50	15,250±5.50[a]	18,500±2.10[a]	17,375±8.25[a]	14,250±0.14[a]
Platelet (m/µl)	133,000±1.10	131,000±5.00[a]	195,000±3.50[a]	190,500±5.95[a]	140,000±3.40[a]
MCV (Fl)	118.17±0.38	105.50±2.30[a]	91.50±0.03[a]	91.75±1.15[a]	99.05±4.35[a]
MCH (Pg)	3.91±0.86	3.88±0.11[a]	2.96±0.50[a]	2.92±0.32[a]	3.16±0.0.21[a]
MCHC (g/dl)	34.00±0.04	32.00±0.00[a]	32.50±0.05[a]	32.00±0.00[a]	32.00±0.10[a]
Lym x109/ l	69.00±1.00	63.00±3.00[a]	85.50±2.50[b]	72.50±4.50[ab]	71.50±0.01[ab]
Hetero x109/ l	25.00±2.00	33.00±0.10[b]	13.50±3.50[a]	23.00±3.00[ab]	26.00±0.50[ab]
Mono x109/ l	3.00±0.00	2.00±0.50[a]	0.50±0.00[a]	2.00±0.50[a]	0.50±0.01[a]
Eos x109/ l	3.00±1.00	2.00±0.05[ab]	0.50±0.00[a]	1.00±0.01[ab]	1.00±0.00[ab]

	Post challenge				
	OB5	WL6	WL7	WL8	WL9
PCV (%)	31.00±1.00a	29.50±1.50a	29.00±1.00a	32.50±3.50a	26.10±0.10a
Hb (g/dl)	9.70±2.10ab	9.15±0.65ab	9.10±0.40ab	10.30±1.30ab	8.05±1.55a
RBC x1012/ l	3.34±0.58a	3.19±0.43a	2.87±0.55a	3.53±0.76a	2.44±1.11a
WBC x109/ l	17,550±2.50a	17,750±1.95a	15,725±1.38a	17,500±2.00a	16,750±4.45a
Platelet (m/µl)	111,000±1.00a	119,500±5.00a	125,500±2.50a	157,000±3.90a	148,500±6.00a
MCV (Fl)	91.95±0.20a	93.50±0.90a	104.20±1.60a	94.40±1.03a	125.05±4.00a
MCH (Pg)	2.88±0.13a	2.90±0.19a	3.27±0.49a	2.98±0.27a	3.80±1.09a
MCHC (g/dl)	31.50±0.05a	31.00±0.05a	31.50±0.05a	31.50±0.02a	31.00±0.10a
Lym x109/ l	71.50±1.50ab	65.50±4.50a	73.00±2.00ab	74.00±1.00ab	70.50±4.50ab
Hetero x109/ l	25.50±1.50ab	31.00±0.03ab	22.50±1.50ab	22.50±0.05ab	27.00±0.09ab
Mono x109/ l	1.50±0.10a	1.50±0.43a	1.50±0.02a	2.00±0.05a	1.50±0.01a
Eos x109/ l	1.50±0.02ab	2.00±0.00ab	3.00±0.71b	1.50±0.01ab	1.00±0.00ab

Key: Mean followed by the same letter is not significantly different ($p > 0.05$)
NOTE: PCV = packed cell volume, Hb =Haemoglobin, RBC = Red Blood Cell, WBC = White Blood Cell, MCV =Mean Cell Volume, MCH = Mean Cell Haemoglobin, MCHC = Mean Cell Haemoglobin Concentration, Lym =Lymphocytes, Hetero =Heterophil, Eos = Eosunophil, Mono = Monocytes

W. somnifera followed by 1 g /kg W. somnifera (14.28% survival) and 9.92% survival in the diet containing 3 g/ kg W. somnifera root when compared with the control which is also in agreement with the present study.

The decrease in mortality rate, in this study, with dietary onion bulb and walnut leaves after injection of bacteria, *P. aeruginosa*, is in agreement with previous studies conducted in *O. mossambicus* fed with diet containing *Ocimum sanctum* (Logambal *et al.,* 2000), L. rohita fed with the diet containing herb Achyranthes aspera (Rao *et al.,* 2006) and *O. mossambicus* treated with Eclipta alba leaf extract (Christybapita *et al.,* 2007). It may be deduced that the plants constituents may directly initiate activation of the innate defence mechanisms acting on receptors and triggering intracellular gene activation that may result in the production of antimicrobial molecules (Bricknell and

Table 3: Mean Post Challenge Test Plasma Biochemistry Parameters of African Catfish (*Clarias gariepinus)* Juveniles fed onion bulb and walnut leaf

Parameters	Pre – challenge	Post challenge			
		CONTROL	OB2	OB3	OB4
Total protein (g/dl)	3.10±0.14	4.40±0.10^{a}	4.95±0.35^{a}	4.50±0.20^{a}	3.90±0.85a
Albumin (g/dl)	0.95±0.35	1.15±0.50^{abc}	1.90±0.30^{cd}	2.80±0.50^{e}	1.10±0.00^{ab}
Globulin (g/dl)	2.15±0.49	3.25±0.05^{a}	3.05±0.25^{a}	1.65±0.15^{a}	2.30±0.90^{a}
A.G Ratio	0.50±0.28	0.30±0.00^{a}	0.60±0.50^{a}	1.70±0.50^{b}	0.50±0.40^{a}
	3.00±1.00	2.00±0.05^{ab}	0.50±0.00^{a}	1.00±0.01^{ab}	1.00±0.00^{ab}

	Post challenge				
	OB5	WL6	WL7	WL8	WL9
Total protein (g/dl)	4.25±0.55^{a}	3.90±0.50^{a}	3.90±0.30^{a}	4.35±0.45^{a}	4.80±1.20^{a}
Albumin (g/dl)	0.95±0.15^{a}	1.10±0.50^{ab}	2.00±0.30^{d}	1.45±0.15^{abcd}	1.85±0.45^{bcd}
Globulin (g/dl)	3.30±0.40^{a}	2.80±0.40^{a}	1.90±0.60^{a}	2.90±0.30^{a}	2.95±1.65^{a}
A.G Ratio	0.50±0.00^{a}	0.55±0.05^{a}	1.20±0.50^{ab}	0.50±0.10^{a}	1.00±0.40^{ab}

Key: Mean followed by the same letter is not significantly different ($p > 0.05$)

Table 4: Post Challenge Test Blood Serum of African Catfish (*Clarias gariepinus)* Juveniles fed onion bulb and walnut leaf

Parameters	Pre – challenge	Post challenge			
		CONTROL	OB2	OB3	OB4
AST(IU/l)	151.00±1.31	124.00±1.20^{a}	111.00±0.00^{a}	122.00±2.00^{a}	106.00±1.50a
ALT(IU/l)	68.00±8.49	38.50±1.50^{a}	32.00±0.00^{a}	31.50±0.50^{a}	25.50±0.50^{a}

	Post challenge				
	OB5	WL6	WL7	WL8	WL9
AST(IU/l)	115.50±2.50^{a}	105.50±2.50^{a}	115.60±3.00^{a}	122.50±0.20^{a}	109.00±0.01^{a}
ALT(IU/l)	28.00±2.00^{a}	28.50±2.50^{a}	32.50±0.10^{a}	27.50±0.50^{a}	33.00±0.02^{a}

Key: Mean followed by the same letter is not significantly different ($p > 0.05$)

Dalmo, 2005). The stimulation of specific and non-specific immune defence observed in the present study might be due to the presence of one or more components present in *Allium cepa* and *Tetracarpidium Conophorum*. The antimicrobial properties present in OB and WL might contribute to suppress the growth of bacteria and reduces the microbial load and inhibit pathogenic infection in the fish.

The values of Packed Cell Volume (PCV) and Red Blood Cell (RBC) generally increased in the treatments except WL9; OB4, WL9 respectively in post challenge test compared with the values obtained in pre challenge and the control of the post challenge test. The value of White Blood Cell (WBC) increased in the treated groups as compared with the control in post challenge test, although the differences were insignificant ($P > 0.05$) among the treatments. The results of the present findings supported the work of Das *et al.,* 2009 that also found increased WBC and RBC after 10 days challenge with Aeromonas hydrophila as compared with control. Das *et al.,* 2009 also reported decreased in Hb after 10 days challenge which agrees with present findings as decreased Hb was reported in the treatments except OB2, OB5 and WL8 as compared with control.

The values of lymphocytes recorded in post challenge test were higher than the one obtained in pre- challenge and the control of post challenge and the values were significantly higher (P< 0.05) in all the treated groups compared with the control in post challenge test. The value recorded in control were also lower than the one recorded in the pre – challenge, the reason for this might be due to lower immune functions against fish pathogen, *Pseudomonas aeruginosa*. This suggests that walnut leaf and onion bulb residues could enhance non – specific immune response. Also, the findings support the report of Dugena *et al.,* (2003) that fish fed medicinal plants harbour a variety of specific and non –specific defence mechanism against invading pathogens. The results of the present study revealed that increased in White Blood Cells (WBC) and lymphocytes following feeding of walnut leaf and onion bulb residues diets supported the notion of antimicrobial properties of walnut leaves and onion bulb (Ajaiyeoba and Fadare, 2006; Azu and Onyeagba, 2007) and traditional herbal medicines (Blumanthal *et al.,* 2000; Kumar and Anantharaja, 2007).

Results obtained from the post challenge test; there were lower levels of total protein in treated groups except OB2, OB3 and WL9 as compared with the control, there were no significant different (P > 0.05) among the treatments. The albumin level was reduced in OB4, OB5 WL6 and increased in OB2, OB3 WL7, WL8 and WL9 as compared with the control after the challenge test. The globulin were generally reduced in the treated groups except OB5 as compared with the control for post challenge test and the values obtained were insignificantly (P> 0.05) in the treated groups as compared with the control in post challenge test. The albumin and globulin ratio were higher in the treated groups as compared with the control in post challenge test and the variation of albumin and globulin ratio was insignificant (P> 0.05) among the treated groups as compared with the control.

Results of the present findings were in accord with Das *et al.,* 2009 that reported a reduction in values of total protein, albumin level and globulin content and there was no significant decrease in the globulin content of post challenge as compared with the control, this were applicable in the present research. Das *et al.,* 2009 reported increase in values of albumin and globulin ratio at 10 days post challenge with A. hydrophila as compared with the control which also support the present findings that revealed higher values in the treated groups compared with the control.

Increases in values of total protein, albumin, globulin level and albumin and globulin ratio of treated groups of post challenge test compared with pre – challenge and the control of post challenge are thought to be associated with a stronger innate immune response of fish. The reason for these results might be due to the presence of constituents in walnut leaves and onion bulb that have stimulatory effect on the immune mechanisms of *C. gariepinus*.

Transamination is considered to be important in assessing the state of the liver and some other organs (Verma *et al.,* 1981). Attention has been focused on the changes in AST and ALT activities which promote gluconeogenesis from amino acid as well as on the changes in aminotransferase activities in the liver (Hilmy *et al.,* 1981; Rashatuar and Ilyas, 1983). AST and ALT activities might be altered by a variety of chemical, biological and physiological factors or by a disturbance in the Kreb's cycle. Decreased activities of the Kreb's cycle cause a decrease in its intermediates, thereby letting AST and ALT compensate by providing a – Ketoglutarate (Salah El-Deen and Rogers, 1993).

The result of the present study in post challenge test showed that AST and ALT activities decreased in all the treated groups as compared with the pre – challenge and control of the post challenge test. This report agrees with those reported by Shalaby *et al.,* 2006 who found decrease in AST and ALT fed *Oreochromis niloticus* at different graded level of *Allium sativum* and chloramphenicol. The possible role of reduction of AST and ALT can be attributed to the presence of constituents of walnut leaves and onion bulb that may stabilize the cell membrane and protect the liver against deleterious agents and free – radical- mediated toxic damages to the liver cell. This is reflected in the reduction of liver enzymes. *Allium cepa* and *T. conophorum* helps the liver to maintain its normal function by accelerating

the regenerative capacity of its cells. Thus, from the present experiment, it is clear that dietary supplementation of walnut leaf and onion bulb residues at all inclusions have enhancing effects on innate immunity and disease resistance of P. aeruginosa.

Conclusion

In conclusion, walnut leaf and onion bulb residues appear to be useful tools that can be used to enhance growth, survival and non- specific immune functions against fish pathogens such as *Pseudomonas aeruginosa* and the inclusion of walnut leaf residue may significantly enhance the productivity in aquaculture industry.

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GROWTH PERFORMANCE AND SURVIVAL RATE OF *CLARIAS GARIEPINUS* JUVENILES FED DIFFERENT LEVELS OF AFLATOXIN – CONTAMINATED FEEDS

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Abstract

This experiment was conducted to evaluate the effects of different levels of aflatoxin- contaminated feed (0% toxigenic maize, 25% toxigenic maize +75% good maize, 50% toxigenic maize+50%good maize, 75% toxigenic maize +25% good maize and 100% toxigenic maize) on growth, survival, haematology and histology of *Clarias gariepinus* juveniles. Ten *Clarias gariepinus* juveniles with average weight of 42.81±0.01g were subjected to five treatments, with two replicates for each. The fish were fed twice daily at 5% body weight of 40% crude protein for six weeks. Growth performance indices such as Mean Weight Gain (MWG), Specific Growth Rate (SGR), Survival Rate (SR) and Feed Conversion Ratio (FCR) were determined. Haematological parameters such as Packed Cell Volume (PCV), Red Blood Cell (RBC), Haemoglobin (Hb) content and lymphocytes were evaluated. Data resulting from the experiment were subjected to descriptive statistics and ANOVA at P = 0.05. Results showed that highest mean weight gain (107.00± 10g), specific growth rate (0.03±00g), survival rate (60.00%) and feed conversion ratio (0.68±0.05) were observed with control diet. MWG, SGR, SR and FCR were significantly ($p<0.05$) higher in control diet compared with the other treatments. Also, packed cell volume, red blood cell (count) were higher in the control compared with other treatments, red blood cell counts were significantly different ($p< 0.05$) in control than other treatments. Lymphocytes were better in control and treatment 1 (25% toxigenic maize + 75% good maize) while decreased value were observed in the other treatments which indicate reduction in immune response of the fish. The control diet and treatment 1 were significantly ($p<0.05$) higher than other treatments but no significant difference ($p>0.05$) were recorded between control and treatment 1 (25% toxigenic maize + 75% good maize). Histology analyses of the liver and intestine of aflatoxin – contaminated feeds revealed severe degeneration and diffuse necrosis and mucosal erosion respectively. This study concludes that presence of aflatoxin contaminated feed affect the survival and growth performance of *Clarias gariepinus* juveniles

Keywords: *Growth, Aflatoxin- contaminated feeds, Clarias gariepinus, Haematology Survival*

PERFORMANCE DE CROISSANCE ET TAUX DE SURVIE DES JUVENILES *CLARIAS GARIEPINUS* NOURRIS AVEC DES ALIMENTS CONTAMINES AVEC DIFFERENTS NIVEAUX D'AFLATOXINES

Résumé

Cette expérience a été menée dans le but d'évaluer les effets des aliments contaminés avec différents niveaux d'aflatoxines (maïs toxigène 0%, maïs toxigène 25% +75% de bon maïs, 50% de maïs toxigène +50% de bon maïs, 75% de maïs toxigène +25% bon maïs et 100% de maïs toxigène) sur la croissance, la survie, l'hématologie et l'histologie des juvéniles *Clarias gariepinus*. Dix *Clarias gariepinus* juvéniles (42,81 ± 0,01 g) ont été répartis en cinq groupes de traitements différents, avec deux répétitions chacun. Les poissons étaient nourris deux fois par jour au poids corporel de 5% de la protéine brute à 40% pendant six semaines. Les indices de croissance tels que le gain pondéral moyen (MWG), le taux de croissance spécifique (SGR), le taux de survie (SR) et l'indice de consommation (IC) ont été déterminés. Les paramètres hématologiques tels que l'hématocrite (PCV), la numération érythrocytaire (RBC), la teneur en hémoglobine (Hb) et les lymphocytes ont été évalués. Les données résultant de cette expérience ont été soumises à des statistiques descriptives et

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à une analyse de la variance ANOVA à P = 0,05. Les résultats ont montré que le gain pondéral moyen (107,00 ± 10g), taux de croissance spécifique (0,03 ± 00g), taux de survie (60,00%) et indice de consommation (0,68 ± 0,05) les plus élevés ont été observés pour le régime témoin. Le MWG, le SGR, le SR et le RCF étaient significativement (p <0,05) plus élevés dans le régime témoin par rapport aux autres traitements. En outre, l'hématocrite et la numération érythrocytaire étaient plus élevées dans le traitement témoin par rapport aux autres traitements ; la numération des globules rouges étaient significativement différente (p <0,05) dans le groupe témoin par rapport aux autres traitements. Les lymphocytes étaient meilleurs dans le groupe témoin et le traitement I (25% de maïs toxigène + 75% de maïs bon) tandis qu'une baisse de la valeur a été observée dans les autres traitements, ce qui représente une indication de la diminution de la réponse immunitaire des poissons. Le régime témoin et le traitement I étaient significativement (p <0,05) plus élevés que les autres traitements, mais aucune différence significative (p> 0,05) n'a été enregistrée entre le traitement témoin et le traitement I (25% de maïs toxigène + 75% de bon maïs). Les analyses histologiques du foie et de l'intestin des juvéniles nourris avec des aliments contaminés par l'aflatoxine ont révélé respectivement une dégénérescence grave et une nécrose diffuse et une érosion des muqueuses. Cette étude a révélé que la présence d'aflatoxine dans les aliments a une incidence sur la survie et la croissance des juvéniles *Clarias gariepinus*.

Mots-clés : *croissance, aliments contaminés par l'aflatoxine, Clarias gariepinus, hématologie, survie*

Introduction

Malnutrition and infection are major obstacles for the survival, growth, reproduction and disease resistance of fish on a global scale. With the rise in global awareness of fish as a valuable source of protein, this has led to increased progress in aqua feeds with diets being specifically designed to meet the nutritional requirements of species, life cycle and health condition of fish (Rawling *et al.,* 2012). The rapid development of aquaculture in recent years has led to the emergence of diseases and problems associated with intensive farming. Serious infections such as fungal growth have been reported. These diseases have resulted in production losses and they remain a primary constraint substantial commercial development of aquaculture (Cerezuela *et al.*, 2012) .

In Africa, it is common that pelleted feeds are generally improperly stored. The improper storing, packing and transport facilities, in conjunction with the high temperature and humidity in these areas, are conducive to fungal growth and the potential for aflatoxin production (Chavez-Sanchez *et al.*, 1994).

Mycotoxins are toxic compounds produced as secondary metabolites of toxigenic moulds of *Aspergillus*, *Alternaria, Claviceps*, *Fusarium, Penicillium* and *Stachybotrys* genera occurring in food and feed commodities both during pre-and post harvest. When ingested, mycotoxins may cause a mycotoxicosis which can result in acute or chronic disease episode; some are carcinogenic, mutagenic, teratogenic, estrogenic, hemorrhagic, immunotoxic, nephrotoxic, hepatotoxic, dermotoxic, and neurotoxic (Dragan *et al.*, 2010). Under suitable temperature and humidity conditions, mycotoxins may develop on various foods and feeds causing serious risk to human and animal health. The principal classes of mycotoxins include a metabolite of *A. flavus* and aflatoxin B1 (AFB1), the most hepatocarcinogenic substance known, which has been recently proven to be genotoxic (Dragan *et al.* 2010). Aflatoxins can be found in maize, peanuts, tree nuts and dried fruits.

The extent of the damage produced by aflatoxins depends on the toxin concentration present in foods or feeds and also on the time period of exposure, as well as animal species susceptibility (Stewart & Larson, 2002). Within a given species, the magnitude of toxicity is influenced by age, sex, weight, diet, and exposure to infectious agents. Fry are more susceptible to aflatoxicosis than adults, and certain fish species are more sensitive than others (Royes & Yanong, 2002). Since the discovery of the nature

of aflatoxins, aflatoxicosis has been investigated predominantly in freshwater species, especially in rainbow trout, (*Oncorhynchus mykiss*) (Gallagher & Eaton, 1995) and, also, in American channel catfish (*Ictarulus punctatus*) (Gallagher & Eaton, 1995), Nile tilapia (*Oreochromis niloticus*) (Tuan *et al.*, 2002), Indian major carp (*Labeo rohita*) (Sahoo *et al.*, 2001; Sahoo *et al.*, 2003; Murjani 2003), mosquitofish (*Gambusia affinis*) (McKean *et al.*, 2006) but there is a dearth of information on fungal infection in African catfish, *Clarias gariepinus*, being one of the most suitable cultured species in Africa, especially Nigeria. Therefore, this study was undertaken to evaluate the effect of different levels of aflatoxin- contaminated feed on the growth, survival, haematology and histopathology of *Clarias gariepinus*.

Materials and Methods

Collection of samples and typed- strains

Samples of uninfected maize grains (Type ACR. 97TZL COMPI-W) and Typed-strains of toxigenic *Aspergillus flavus* were collected at the Pathological Unit, International Institute of Tropical Agriculture, Moniya, Ibadan, while other feeds such as Fishmeal, Soya meal, Groundnut cake, Methionine, Lysine, salt, Dicalcium phosphate, Vitamin premix were bought at Kesmas Feed Mill, Ibadan, Nigeria.

Isolation of fungi

Fungi were isolated from soil and Clean Up (CU) medium was used for isolating *Aspergillus flavus*. Pure culture were prepared and stored on 5-2 medium (5% V8 juice and 2% agar), pH 5.2 at 31^{0}C until required.

Determination of inoculum volume

Spores were harvested from five day old culture of isolates. A suspension of spores was obtained by dilution of spores in 1000ml of sterile distilled water in which 1ml of Tween 20 has been added for adhesion of spores to substrate. The desired spores concentration of 1×106 spores/ml was determined by spore counting using Hausser haemocytometer.

Analysis of aflatoxin in colonized maize grains

20g each of uninfected and infected maize samples was ground to extract aflatoxin with 100ml of 70% methanol (ratio 1:5) using a high speed blender (Waring Commercial, Springfield, MO, USA) for 3 minutes. The mixture was passed through Whatman Paper No 1,, the extract was collected in a 250-ml separatory funnel. The solution was extracted twice, firstly with 25ml methylene chloride partition by filtering through 40g of anhydrous sodium sulphate to remove residual water and secondly with 10mls of methylene chloride. Extracts were pooled in a propylene cup and evaporated to dryness in a fume hood. Residues were redissolved in 1ml of methylene chloride and either diluted or concentrated to allow accurate densitometry. Extracts and aflatoxin standards were spotted on thin- layer chromatography plates (Silica gel 60, 250µm). The plate was developed and separated in a developer; diethyl- ether-methanol-water (96:3:1) solvent mixture. Aflatoxins were quantified using scanning densitiometer, CAMAG TLC Scanner 3 with win CATS 1.4.2 software (Camag AG, Muttenz, Switzerland) according to the method described by Atehnkeng *et al.*, (2008).

Inoculation of maize grains with toxigenic Aspergillus flavus

1kg each of maize grains was weighed into ten autoclavable bags making a total of ten kilograms. These were soaked for two hours and autoclaved at 121^{0}C for 20minutes. Maize grains were cooled and transferred into incubating bags. These were inoculated with 200mls spore suspension of toxigenic strains of *Aspergillus flavus*. Maize grains were incubated in the screen house for ten days for colonization of aflatoxin. After colonization, spores were washed off with water containing Tween 20 and excess water was drained off. Maize grains were spread in glass house for ten days and later stored in cold room.

Experimental system

The experiment was carried out in ten plastic experimental tanks (50x34x27cm) for 6 weeks in the Wet Unit of Microbiology Laboratory of the University of Ibadan, Ibadan. The water level in each tank was maintained at volume of 35litres throughout the experimental period. Water in each tank was replaced every

three (3) days throughout the period of the experiment to maintain relatively uniform physiochemical parameters and also to prevent fouling that may result from food residues. The source of water was from University of Ibadan (U.I) water station.

Experimental procedure & feeding trials

Each treatment has two replicates, 10 fish per replicate with mean initial body weight of 42.81± 0.01g and uniform- sized fish was selected from 250 juveniles, weighed and distributed in experimental tank. The fish was acclimated for fourteen days in plastic aquaria before the experiment. The experiment lasted for 6 weeks during which the fish was fed at 5% body weight daily. The diet per day was divided into two; 2.5% given in the morning by 8.00 – 9.00 am and 2.5% in the evening by 5.00 pm. Measurement of the weight changes was performed weekly and the feeding rate adjusted weekly according to the new body weight.

Diet formulation

Five treatments were employed comprising treatment I=control (0% aflatoxin-infected maize) good maize, treatment II=25% aflatoxin- infected maize + 75% good maize, treatment III= 50% aflatoxin-infected maize+50% good maize, treatment IV=75% aflatoxin-infected maize+25% good maize, treatment V=100% aflatoxin-infected maize.

Maize grains were milled and required amounts of infected maize and uninfected maize for each treatment were weighed carefully before mixing thoroughly with other feed ingredients. After preparation feed ingredients were mixed together to formulate 40% crude protein diet. Each diet mixture treated separately was extruded through a 1/4mm diet mincer of Hobart A-200T pelleting machine to form a noodle like strand which was mechanically broken into suitable sizes for the *Clarias gariepinus* juveniles. The pelleted diets were sun dried, packed in labeled polythene bags and stored in a cool dry place until required (Table 1).

Each of the feed mixture was analysed for aflatoxin concentration at the Pathology Unit, International Institute of Tropical Agriculture, Moniya, Ibadan according to the method of Atenhkeng *et al.*, (2008).

Biological evaluation

Weight gain

= final body weight - initial body weight

Weight gain (%)

$$= \frac{100 \text{ (final body weight - initial body weight)}}{\text{Initial body weight}}$$

Specific growth rate (SGR)

$$= \frac{100(\log_e \text{final body weight} - \log_e \text{initial body weight})}{\text{Time (days)}}$$

Feed conversion ratio (FCR)

$$= \frac{\text{Dry weight of feed fed (g)}}{\text{Fish weight gain (g)}}$$

Survival rate (%)

$$= \frac{\text{Initial Number of Fish Stocked} - \text{Mortality} \times 100}{\text{Initial number of fish stocked}}$$

Haematological analysis

Haematological analysis of the fish was carried out at the haematological laboratory of Veterinary Pathology Department, University of Ibadan within 30 minutes of sampling. A distance of 3 – 4cm from the genital opening of each fish was punctured and wiped with dry tissue paper to avoid contamination with mucus. The needle was inserted at right angle to the vertebral column of the fish. The blood was taken under gentle aspiration until about 3ml had been obtained. The needle was gently withdrawn and the blood transferred into heparinized plastic containers and complete haematology was done as described by the methods of Blaxhall and Daisley (1973).

Histopathological analysis

Histopathological analysis of fish was carried out at the Faculty of Veterinary Pathology, University of Ibadan, using the methods of Culling (1974) and Drury *et al* (1967) for the organs or tissues. Representative samples were randomly selected from each treatment and dissected to remove the liver and small intestine which were then preserved in 10% formalin in labelled bottles. Sectioned parts were dipped in 70% ethanol,

Table 1: Gross composition of experimental diets (g/100g)

Ingredients	Control	25% toxigenic + 75% good maize	50% toxigenic + 50% good maize	75% toxigenic + 25% good maize	100% toxigenic maize
Fishmeal(g)	13.96	13.96	13.96	13.96	13.96
Soya beans(g)	27.92	27.92	27.92	27.92	27.92
Groundnut cake(g)	27.92	27.92	27.92	27.92	27.92
Uninfected maize(g)	27.00	20.25	13.50	6.75	-
Toxigenic maize(g)	-	6.75	13.50	20.25	27.00
Methionine(g)	0.10	0.10	0.10	0.10	0.10
Lysine(g)	0.10	0.10	0.10	0.10	0.10
Vitamin premix(g)*	1.00	1.00	1.00	1.00	1.00
Salt(g)	1.00	1.00	1.00	1.00	1.00
Dicalcium phosphate(g)	1.00	1.00	1.00	1.00	1.00
Total (g)	100.00	100.00	100.00	100.00	100.00

*vit-min premix (vitamin and minerals premix) each 2.5kg of premix contains; vitamin A, 12.5 million international unit (MIU); D3,2.5 MIU; E, 40g; K3, 2g; B1, 3g; B2, 5.5g; B6, 5g; B12, 0.25g; Niacin 55g; Calcium pantothenate 11.5g; Choline chloride, 500g; folic acid, 1g; Biolin, 0.08g; Manganese,120g; Iron,100g; Zinc,80g; Copper, 8.5g ; Iodine,1.5g ; Cobalt, 0.3g ; Selenium, 0.12g ; Anti- oxidant,120g.

Table 2: Aflatoxin content (ppb) in maize before and after compounding fish feed

Treatments	Aflatoxin content in maize before compounding feed (ppb)	Aflatoxin content after compounding feed (ppb)
Control	0.00	0.00
25% toxigenic A. flavus inoculated maize + 75% good maize	250.00	250.05
50% toxigenic A. flavus inoculated maize + 50% good maize	500.00	500.22
75% toxigenic A. flavus inoculated maize + 25% good maize	750.00	750.07
100% aflatoxin inoculated maize	1000.00	1000.03

95% alcohol, followed by absolute alcohol twice for 2 hours each at 62^{0}C-65^{0}C. Blocked samples were mounted on microtome holder and then sectioned using a rotary microtome at an angle of 45^{0}C and 5µm. Samples on slides were then dried in the incubator at 35^{0}C s overnight.

Series of slides were arranged and stained in a staining trough serially and the slides taken through absolute xylene for 2 minutes twice, then xylene and alcohol (50:50), followed by absolute alcohol for two minutes, followed by haemoxylene. They were then returned to water for blueing for 2-3 minutes and were dipped quickly inside acid-alcohol (absolute alcohol+10% HCL), and to water for 2 minutes, alcohol, eosin staining for counter staining, to absolute alcohol for two minutes three times, then xylene for 2 minutes. Two drops of D.P.X were mounted to make permanent slides. Slides were air-dried for 12 hours, stored, and viewed under the microscope.

Statistical analysis

Data resulting from this study were subjected to one way analysis of variance (ANOVA) using SPSS version 15, 2006 and Statistical Analysis System (1999). Duncan multiple range test was used to compare differences between individual means.

Table 3: Growth performance of *Clarias gariepinus* juveniles fed aflatoxin-contaminated feed for 42days

Parameters	Control	25% toxigenic + 75% good maize	50% toxigenic + 50% good maize	75% toxigenic + 25% good maize	100% toxigenic maize
Initial body weight (g)	43.00±0.00^{a}	42.70±0.20^{a}	42.8±0.80^{a}	42.78±0.18^{a}	42.75±0.05^{a}
Final body weight (g)	150.00±10.00^{b}	70.00±5.00^{a}	72.5±0.00^{a}	67.00±1.00^{a}	70±10.00^{a}
Mean weight gain (g)	107.00±10.00^{b}	27.30±4.80^{a}	29.7±0.80^{a}	24.22±0.82^{a}	27.23±9.98^{a}
Survival rate	6.00±0.00^{b}	2.00±0.00^{a}	5.00±1.00^{b}	3.00±0.00^{ab}	1.00±0.00^{a}
Survival rate (%)	60.00±0.00^{b}	5.00±0.00^{b}	25.00±5.00^{b}	15.00±10.00^{ab}	5.00±0.00^{a}
SGR	0.03±0.00^{b}	0.01±0.00^{a}	0.01±0.00^{a}	0.01±0.00^{a}	0.01±0.00^{a}
FCR	0.68±0.00^{a}	1.44±0.00^{ab}	2.19±0.00^{bc}	2.44±0.00^{c}	2.14±0.00^{bc}

Key: Mean followed by the same letter is not significantly different ($p > 0.05$)

Table 4: Haematological parameters of fish fed with varying levels of aflatoxin – contaminated feeds (mean±SEM)

Parameters	Control	25% toxigenic + 75% good maize	50% toxigenic + 50% good maize	75% toxigenic + 25% good maize	100% toxigenic maize
PCV (%)	26.00±2.88^{ab}	24.33±0.88^{ab}	25.00±2.08^{ab}	24.33±0.33^{a}	26.0±0.58^{ab}
HB(g/dl)	8.43±1.02^{ab}	7.6±0.58^{ab}	8.23±0.70^{ab}	9.1±0.29^{a}	8.4±0.23^{ab}
RBC(×12/L)	3.83±0.42^{a}	1.47±0.06^{c}	2.42±0.64^{abc}	3.09±0.26^{ab}	1.53±0.58^{c}
MCV (Fl)	70.24±12.20^{c}	165.58±1.33^{a}	120.34±31.71^{abc}	91.59±0.51^{bc}	170.71±10.24^{a}
MCH (Pg)	227.41±39.37^{b}	521.71±59.90^{a}	395.11±102.26^{ab}	294.38±11,80ab	549.45±5.65^{a}
MCHC (g/dl)	32.38±0.49^{a}	31.48±3.47^{a}	32.93±0.22^{a}	32.15±1.35^{a}	32.38±1.61^{a}
WBC x109/ l	12.63±2.65^{cd}	18.1±0.58^{bcd}	18.62±0.73^{bcd}	26.53±6.18^{a}	28.67±4.63^{a}
PLATELETS (m/µl)	180.67±8.97^{b}	344±1.15^{a}	191.33±13.13^{b}	264±34.83^{ab}	377±14.80^{a}
LYMPH x109/ l	75.33±0.89^{a}	75.67±0.89^{a}	64±3.06^{b}	58.67±5.24^{b}	57±0.58^{b}
NEUTRO x109/ l	22.33±0.33^{a}	20±1.0^{b}	33.33±2.40^{a}	38±5.51^{a}	41±0.58^{a}
MONO x109/ l	1.33±0.33^{c}	3.33±0.33^{a}	1.33±1.67^{c}	2.0±0.58^{ab}	1.67±0.67^{bc}
EOSINO x109/ l	1.0±0.58^{a}	1.0±0^{a}	1.0±0.58^{a}	1.33±0.89^{a}	1.33±0.33^{a}

$^{a,b,c,d:}$Values with different superscripts are significantly different at ($P<0.05$)

NOTE: PCV = packed cell volume, Hb =Haemoglobin, RBC = Red Blood Cell, WBC = White Blood Cell, MCV =Mean Cell Volume, MCH = Mean Cell Haemoglobin, MCHC = Mean Cell Haemoglobin Concentration, Lymph =Lymphocytes, Neutro =Neutrophils, Eos = Eosunophil, Mono = Monocytes

Results

Aflatoxin content (ppb) in maize before and after compounding fish feed was given in Table 2. Growth performance of *Clarias gariepinus* juveniles fed aflatoxin-contaminated feed for 42 days (Table 3). Haematological parameters of fish fed with varying levels of aflatoxin are given in Table 4. Histopathological changes in liver tissues and intestine of *Clarias gariepinus* are presented in Table 5 and Table 6 respectively

Discussion

In this study, the initial mean body weight of *C. gariepinus* was 42.81±0.01g and at the end of the experiment there was general increase in mean weight gain (see Table 2). However, the highest weight gain was recorded in control (107.00±10.00) compared with other treatments. The significant decrease in weight gain in treated groups suggested that the normal digestive and nutrient absorptive functions of the intestinal epithelium were affected. On growth parameters, the highest growth performance was observed in fish fed on control diet compared with the treated groups. Specific growth rate and condition factor are a reflector of the health status in fish (Ibrahem *et al.*, 2010). Specific growth rate (SGR) from the results revealed that control (0.03± 0.00) had better growth rate compared with the treated groups at the level of P>0.05, although they there were significant differences among the treatments ($p > 0.05$).

Feed Conversion Ratio (FCR) is used to assess feed utilization and absorption (conversion of feed to flesh). There were significant difference ($P < 0.05$) among the treatments with FCR best (0.68± 0.05) in control and very poor in treatment 4 (2.44 ± 0.24). The result suggests that control diet was better utilized by the *C. gariepinus* juveniles. The inability of *Clarias gariepinus* juveniles to

Table 5: Histopathological changes in liver tissues of *Clarias gariepinus* fed experimental diets for 42 days

Treatments	Composition toxigenic A. flavus in maize (ppb)	Histopathological changes
1	Control	Severe portal congestion and vacuolar degeneration
2	25% toxigenic + 75% good maize	Mild diffuse hepatic vacuolation
3	50% toxigenic + 50% good maize	Very severe degeneration and diffuse necrosis
4	75% toxigenic + 25% good maize	Very severe diffuse hepatic degeneration and necrosis
5	100% toxigenic maize	Very severe fatty degeneration and diffuse necrosis of hepatocytes

Table 6: Histopathological changes in intestine of *Clarias gariepinus* fed experimental diets for 42 days

Treatments	Composition toxigenic A. flavus in maize (ppb)	Histopathological changes
1	Control	Severe mucosal erosion of the muscularis layer appear highly thickened
2	25% toxigenic + 75% good maize	Severe mucosal erosion
3	50% toxigenic + 50% good maize	Moderate epithelial necrosis
4	75% toxigenic + 25% good maize	Mild epithelial degeneration and necrosis with cellular infilteration by mononuclear cells
5	100% toxigenic maize	Severe mucosal erosion of the muscularis layer appear highly thickened

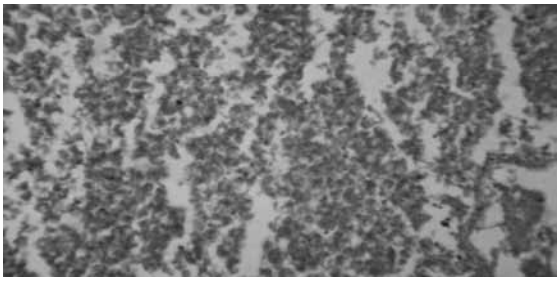

Plate 1: Histological section showing portal congestion and vacuolar degeneration in the liver of *Clarias gariepinus* fed control diet

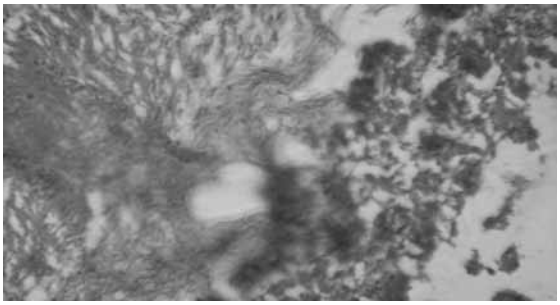

Plate 2: Histological section showing severe fatty degeneration and diffuse necrosis of hepatocytes in liver of *Clarias gariepinus* fed 100% toxigenic maize

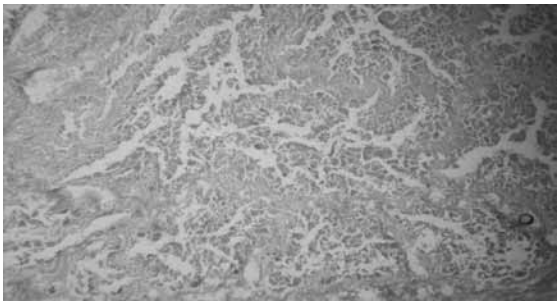

Plate 3: Histological section showing mucosal erosion of muscularis layer in intestine of *Clarias gariepinus* fed control diet

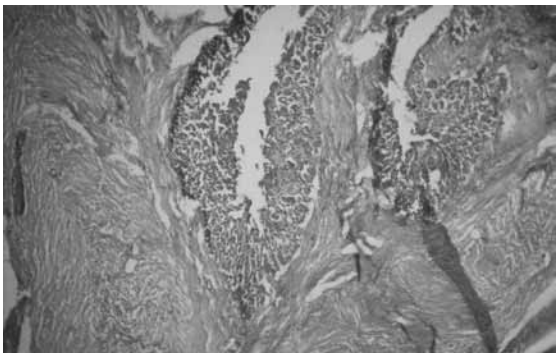

Plate 4: Histological section showing severe mucosal erosion with the muscularis layer appearing thickened in intestine of *Clarias gariepinus* fed 100% toxigenic maize

efficiently utilize the essential nutrients in their feed might have accounted for the observed significantly lower feed conversion ratio in the treated groups of the study.

These results were in accord with the report of Gbore *et al.*, (2010) who reported high weight gain and FCR in control compared with the other treatments. Also, Sepahdari *et al.*, (2010) reported highest weight gain, SGR and FCR, in their report which is in support of this present study. In addition, the report of Arsenia *et al.*, (2004) reported highest total biomass, total weight difference and gain in weight in the control (treatment 1) consistent with the present findings.

Survival rate of fish recorded in this present study were highest in control (60%) and was significantly higher ($p < 0.05$) than other treatments which is also in agreement with the report of Arsenia *et al.*, (2004) who recorded highest survival (100%) in the control. The report of Sepahdari *et al.*, (2010) is in agreement with the present study which reported better and highest survival rate in the control.

Haematology is widely used in clinical diagnosis and pathological conditions in aquatic and terrestrial animals. The application of haematological techniques is therefore valuable in fish biology in the assessment of fish health and stress response (Olukunle, 1996). Haematological indices are an index and a reflection of the effects of dietary treatments on animal in terms of the type, quality and amount of feed ingested and nutrients available to an animal to meet its physiological, biochemical and metabolic requirements (Gbore and Akele, 2010, Ewuola *et al.*, 2008a). In the present study, the Packed Cell Volume (PCV), Haemoglobin (Hb) content, Red Blood Cell (RBC) recorded decrease in values compared with the control and there were no significant difference ($p > 0.05$) in PCV and Hb content among treatments.

The values of haemoglobin, an iron – containing conjugated protein that performs the physiological function of transporting oxygen and carbon (IV) oxide were not significantly different ($p>0.05$) among the treated groups and the control treatment. Reduction in the haematological parameters of the treated groups might be a reflection of altered dietary protein metabolism including

digestibility and absorption of the nutrients in the intestine of *Clarias gariepinus* juveniles as well as biosynthesis in the body systems of *Clarias gariepinus* juveniles. These results are similar to the report of Adakole (2012) who reported that haematological values observed in *Clarias gariepinus* exposed to metal finishing company effluent were within the range of values obtained in this present study.

The values of lymphocytes recorded were highest in treatment 2 and there were no significant difference ($p > 0.05$) between the treatment 2 and the control but there were significant difference ($p < 0.05$) between the control, the treatment 2 and other treatments. Reduction in the value of treated groups compared with the control showed a decline in the immune response of *Clarias gariepinus* to aflatoxin – contaminated feds which may results in suppression of immune system and susceptibility to disease. Also, excessive levels or presence of this aflatoxin – contaminated feeds may cause metabolic burden of fish due to elevated liver glycogen deposition and prolonged hyperglycaemia which might suppress the immune functions and increase the susceptibility to infectious diseases

External clinical signs of abnormal swimming, feeble and stationary in one place, reduced appetite, skin bleach and Oedema (around the operculum) these clinical manifestation become pronounced as the level of aflatoxin- contaminated feeds increased. This result is in agreement with the report of Arsenia *et al.* (2004) who reported abnormal swimming, feeble and stationary in one place and reduced appetite, lesion on the body surface and fin and tail rot.

Severe histological alteration were observed in the liver of the experiment fish such as severe portal congestion and vacuolar degeneration, severe fatty degeneration and diffuse necrosis of hepatocytes. This suggests that there was an increase in the number of dead cells found in the liver of the fish as a result of high concentration of aflatoxin in the diets. This study is in agreement with the findings of Arsenia *et al.* (2004) who found extensive necrosis, acute cellular swelling or ballooning necrosis and chronic granulonetus inflammation in Nile tilapia fed with mould feed contaminated with aflatoxin. Also, the work of Sepahdari *et al.* (2010) agrees with the present study from their findings that hepatocytes degeneration confluent necrosis in beluga (Huso huso) fed different levels of aflatoxin, AFB1. Also, Jayabarathii and Mohamudha (2010) demonstrated liver generative reversible lesion in growing hen fed commercial poultry feed.

Alterations in the intestine revealed severe mucosal erosion of the mascularis layer appearing highly thickened and epithelial degeneration and necrosis with cellular infiltration by mononuclear cells were observed among the treatments and this observation is in agreement with the report of Gbore (2007) who observed progressive erosion of the intestinal mucosa in experimental pig fed with increased concentrations of dietary FB1. Also, the report of Ewuola *et al.*(2003) is in support of this study, that found progressive erosion of epithelial lining of the small intestine resulting from chronic exposure to *F. verticollioides* culture material containing 1.69 – 1.90mg fumonisin/kg in rabbits.

Conclusion

In conclusion, diets containing aflatoxin – contaminated feeds generally reduced nutrient utilization by adversely affecting proper nutrient digestion, absorption or metabolism as well as growth performance and survival in *Clarias gariepinus* juveniles. Presence of aflatoxin in formulated feed is detrimental to fish health and is economically unproductive because the functional differences could have economical effects on large farm production as it affect the productivity and reduced the economic gain.

Acknowledgements

We are grateful to Dr Ranajit Bandyopadhya and Dr Joseph Atehkeng of Pathological Unit, International Institute of Tropical Agriculture, Moniya, Ibadan, Nigeria for provision of typed strains of toxigenic *Aspergillus flavus* and analysis of aflatoxin concentration in the diets during the experiment.

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THE DIGESTIVE TRACT HISTOLOGY OF THE FARMED FINGERLING AFRICAN CATFISH (*Clarias gariepinus BURCHELL, 1822*).

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Abstract

The digestive trait histology of the African catfish fingerling was investigated. The histology revealed stratified squamous epithelium in the oro-pharyngeal wall, tongue and oesophagus. Their lamina propria contained collagen fibres. The oesophagus contained muscularis mucosae of skeletal muscle and large amount of adipose tissue in the submucosa. This adipose tissue suggests that the fingerling oesophagus may be involved in metabolite storage. The stomach was lined by simple columnar epithelium with mucin in the apical region. The stomach lamina propria of cardiac and fundic regions contained gastric glands but none in the pyloric region. The intestine was lined by simple absorptive columnar epithelium containing goblet cells and intraepithelial leukocytes. Myenteric plexus was seen between the inner circular and outer longitudinal smooth muscles of the tunica muscularis. Mucin histochemistry revealed the presence of acid and neutral mucins in the mucin containing cells but the oro-pharyngeal mucous cells contained only neutral mucin. The study concludes that the absence of acid mucin in the oro-pharyngeal wall would contribute to high mortality of fingerlings, since absent acid mucin may help protect against pathogens.

Key words: *Histology, Mucin, Fingerling, Farmed, African Catfish*

HISTOLOGIE DU TRACTUS DIGESTIF DES ALEVINS DE POISSON-CHATS D'AFRIQUE (*Clarias gariepinus BURCHELL, 1822*).

Résumé

Le tube digestif d'alevins de poisson-chat d'Afrique ont fait l'objet d'une investigation. L'histologie a révélé un épithélium pavimenteux stratifié qui tapisse la paroi oro-pharyngée, la langue et l'œsophage. Leurs lamina propria contiennent des fibres de collagène. L'œsophage contenait une muqueuse musculaire du muscle squelettique et une grande quantité de tissu adipeux dans la sous-muqueuse. La présence de ce tissu adipeux permet de penser que l'œsophage des juvéniles peut être impliqué dans le stockage des métabolites. L'estomac était tapissé d'un épithélium cylindrique simple avec de la mucine dans la région apicale. La lamina propria de l'estomac dans les régions cardiaque et fundique contenait des glandes gastriques mais aucune dans la région pylorique. L'intestin était tapissé d'un simple épithélium cylindrique absorbant contenant des cellules caliciformes et des leucocytes intra-épithéliales. Le plexus myentérique a été observé entre les muscles lisses circulaires internes et les muscles lisses longitudinaux externes de la tunique musculaire. L'histochimie des mucines a révélé la présence de mucines acides et neutres dans les cellules qui en contenaient, mais les cellules muqueuses oro-pharyngées ne contenaient que de la mucine neutre. L'absence de mucine acide dans la paroi oro-pharyngée peut contribuer à une forte mortalité des alevins, puisque cette mucine acide qui faisait défaut aurait contribué à les protéger contre les agents pathogènes.

Mots-clés : *histologie, mucine, alevin, élevé, poisson-chat d'Afrique*

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Introduction

Nigerians are large consumers of fish, with an annual average demand estimate at 1.4 million MT (Kudi *et al* 2008). However a demand and supply gap of at least 0.7 million MT exists nationally with import making up the short fall at a cost of 400 billion American dollars per year. Domestic fish production of about 0.5 million MT is mainly supplied by artisan fishermen (85%), and to a limited extent by fish farmers (15%), Adekoya and Miller, 2004; Emokaro *et al.* 2010. According to FAO (2007), this figure (0.7 million MT) makes Nigeria the largest importer of fish in the developing word. To take advantage of the large market created by this deficit, some Nigerians are increasing their participation in aquaculture, with many fish farmers focusing on catfish, as they can have a market value of two to three times that of other cultivable species like *Tilapia* and *Heterobranchus* (Emokaro *et al.* 2010). Most commercial aquaculture in Nigeria relies on supply of fingerlings from hatcheries (Adewumi and Olalaye, 2011). The problems associated with fish hatchery and fingerling survivability are well documented by Adewumi and Olalaye, (2011) and Faturoti *et al.* (1986).

Whereas the ontogenetic development of the digestive system of some teleost have been documented (Govoni *et al.* 1986; Camino and Bailey, 1995; Baglole *et al.* 1997; Cahu and Zambonino-Infante, 2001), there is dearth of information in available literature on the microscopic development of the farmed African catfish fingerling organs including those of the digestive system. Hence, this paper presents the histology and mucin histochemistry of the fingerling digestive tract. The information obtained will be of importance to fingerling nutritionist, pathologist and hatchery managers.

Materials and Methods

Twenty five fingerling African catfish sourced from a commercial aquaculture in Eastern Nigeria were used for the study (Fig.1). They weighed an average of 1.55g and measured a standard body length of 4.57cm. The fish were euthanized with chloroform. The body cavity was cut open through the ventral surface and the alimentary tract dissected out. The specimen under study – the digestive tract was excised and sections of oro- pharyngeal wall, tongue, oesophagus, stomach, intestines (proximal, middle, distal and rectum) were immediately fixed in 10% neutral buffered formalin.

The tissues were passed through graded ethanol, cleared in xylene, impregnated and embedded in paraffin wax. Sections 5 – 6µm thick were obtained with Leitz microtome model 1512. They were stained with haematoxylin and eosin for light microscopy examination (Bancroft and Stevens, 1977). Mucins were demonstrated using alcian blue (AB) at pH 2.5 (Steedman, 1950; Lev and Spicer, 1964) and periodic acid Schiff (PAS) procedure with and without prior digestion with diastase (Lillie and Greco, 1947; Ikpegbu *et al.* 2011). In addition, the PAS technique was employed in combination with AB for neutral and acid mucin (Bancroft and Stevens, 19771). Photomicrographs were taken with – Motican 2001 camera (Motican UK) attached to Olympus microscope.

Results

The tongue tunica mucosa was lined by stratified squamous epithelium containing PAS positive mucous cells (Fig.2). The lamina propria-submucosa contained collagen fibres. The skeletal muscles of the tunica muscularis were oriented mostly in longitudinal direction (Fig.2). Hyaline cartilage was present at the base of the tongue. Oro-pharyngeal wall epithelium was lined by stratified mucous epithelium. The lamina propria contained collagen fibres. The mucous cells were weakly PAS positive (Fig. 9), but AB negative (Fig.14)

Esophageal tunica mucosa was lined by stratified squamous epithelium containing both PAS and AB positive mucous cells lined the longitudinal folds, but the neutral mucin dominated (Figs.3, 10 and 16). The lamina propria-submucosa contained bundles of striated muscles in mostly longitudinal orientation. The tunica muscularis was of circularly oriented skeletal muscle . Tunica serosa was present with subserosal vascularization.

The stomach had three regions of cardia, fundus and pylorus. The cardiac stomach mucosal lining was of simple columnar epithelium (Fig.4). Each individual columnar

cell had a basal nucleus and neutral mucin in the apical cytoplasm (Figs.11 and 17). The epithelium also contained intraepithelial leukocytes. Gastric pits were also seen. The lamina propria contained gastric glands; beneath the glands were collagen connective tissue fibres. The tunica muscularis contained smooth muscle cells in an inner circular and outer longitudinal orientation. Myenteric plexus was observed between the two muscle layers of tunica muscularis. Tunica serosa covered the stomach outermost layer. Subserosal blood vessels were seen also. The fundic stomach was covered by simple columnar mucous epithelium. The gastric pits were narrower and deeper than that observed in the cardiac stomach. The lamina propria contained larger gastric glands than the cardiac stomach. The pyloric stomach was also covered by simple columnar epithelium. The gastric pits were very shallow. The pyloric region lamina propria contained no gastric gland (Fig 5).

The intestine was separated from the stomach by the pyloric sphincter (Fig.6). The intestine was divided into thick proximal, coiled middle and distal intestines, and straight rectum. The proximal intestine histology in low power presented mucosal folds that transversed the entire diameter of the central lumen. As shown in (Fig.7), an eccentric narrow lumen with simple mucosal fold was observed. All the folds were lined by a simple columnar absorptive epithelium that contained goblet cells and migratory leucocytes. The goblet cells contained both neutral and acid mucin (Fig.12) The core of lamina propria contained loose collagen fibres, lymphocytes and profiles of capillaries. Collagen fibres was contained in the submucosa. The tunica muscularis contained smooth muscle fibres arranged in an inner circular and outer longitudinal layers. Tunica serosa was seen. There was also subserosal vascularization.

The luminal tunica mucosa of the middle and distal intestines were modified into simple mucosal folds. These mucosal folds were finger-like to orange leaf shaped in-growths into the lumen on a transverse section (Fig.7). The mucosal folds contained covering epithelium and a lamina propria core. The epithelium was of simple absorptive columnar cells with most goblet cells seen in the distal segment. These goblet cells contained both acid and neutral mucins (Figs.13 and 15). The lamina propria core contained collagen fibres and blood vessels. The tunica muscularis contained smooth muscles of inner circular and outer longitudinal layers (Fig.7). Tunica serosa was of simple squamous cells.

Fig.1: Domesticated african catfish fingerling

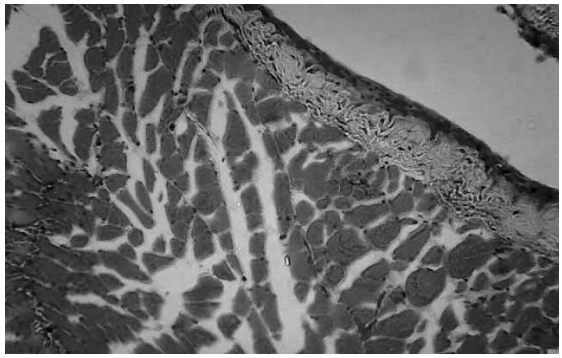

Fig.2: Section of fingerling tongue showing epithelium (EP), collagen fibres(CT) in the lamina propria/submucosa,and skeletal muscle (SKM) in the tunica muscularis. H. & E. X400.

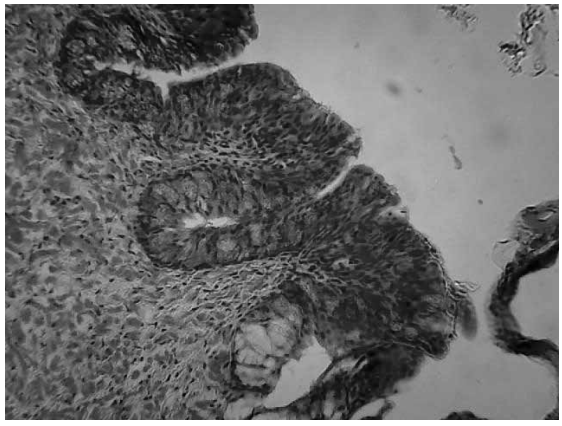

Fig. 3: Transverse section of fingerling oesophagus showing stratified squamous epithelium (EP) containing mucous cells (MC). Note lamina propria containing skeletal muscle (SKM). H. & E. x400

Rectum: Grossly, no intestino-rectal valve was seen. The lumen of the rectum on transverse section contained a central lumen and many eccentric lumina formed by isolate pockets of round shape muscularis mucosae. The covering epithelium was simple columnar with more goblet cells than other intestine segments. The goblet cells were both PAS and AB positive.

Histochemistry

The reaction of the mucins to histochemical procedure of PAS,AB and AB/PAS ,demonstrated the presence of polysaccharides and neutral mucin (red to magenta colour), acid mucin (blue to blue-green), and mixed acid / neutral mucin (purple when present in equal quantities but tended towards the colour of the predominant mucin i.e. more acidic mucin content was bluish while more neutral mucin content was red to magenta colouration to combined AB-PAS procedure.

The summary of the mucin histochemical reaction is presented in table 1.

Discussion

The stratified epithelium of the oro-pharyngeal, tongue and oesophagus has a protective function (Albrecht *et al.*, 2001; Diaz *et al.*, 2008). The collagen bundle seen in the lamina propria-submucosa region would serve to support, strengthen and preserve the entirety of gut wall against sudden and violent extension. The skeletal muscles may be involved in voluntary trituration. The mucous cells produce mucin which is involved in lubrication and defense against pathogens (Albrecht *et al.*, 2001; Micale and Mughia, 2011) The presence of mucous cells indicates production of mucin for lubrication of the organ against mechanical abrasion since the teleost digestive tract lacks salivary gland (Micale and Mughia, 2011; Elbal and Agullero, 1986). However, the absence of acid mucin in the oro-pharyngeal wall and little quantity in the oesophagus may expose the fingerlings to attack by pathogens (Grau *et al.*, 1992). This lack of protection by acid mucin may, in addition to other militating factors, contribute to high mortalities of fingerlings in hatcheries (Faturoti *et al.*, 1986; Ugwu *et al.*, 2011). The absence of taste buds in the tongue indicates that it may not be involved in food selection through gustation (Ezeasor, 1986).

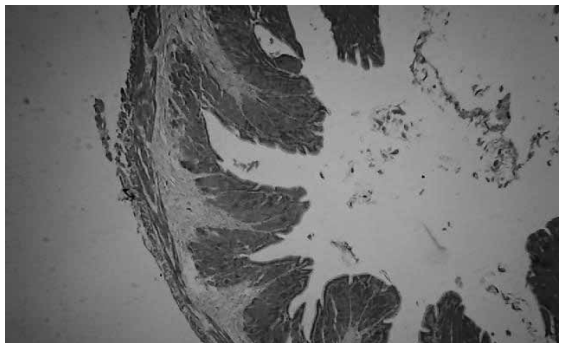

Fig. 4: section of fingerling cardia showing epithelium (EP), gastric glands (GG), tunica muscularis (TM), and stomach cavity (SC). H&E x100

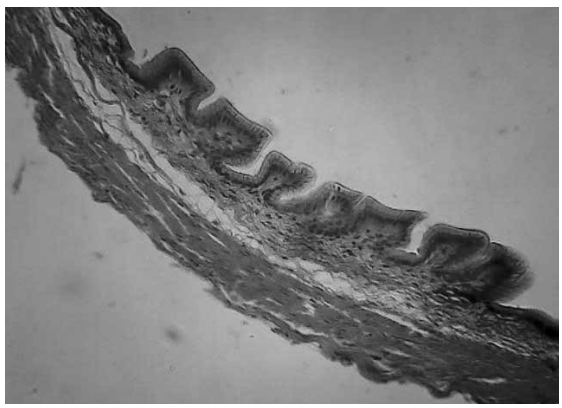

Fig. 5: Section of fingerling pyloric stomach showing simple epithelium (EP). Note the absence of gastric glands in the lamina propria (LP). Circular smooth muscle in the tunica muscularis (CM).H&E x 400

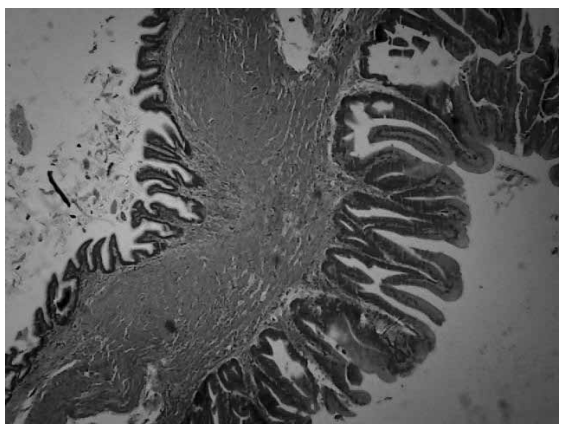

Fig.6. section of fingerling stomach/intestine junction showing pyloric end (PE), smooth muscles of the pyloric sphincter (PS), and the intestine end (IE). H.& E. X 100

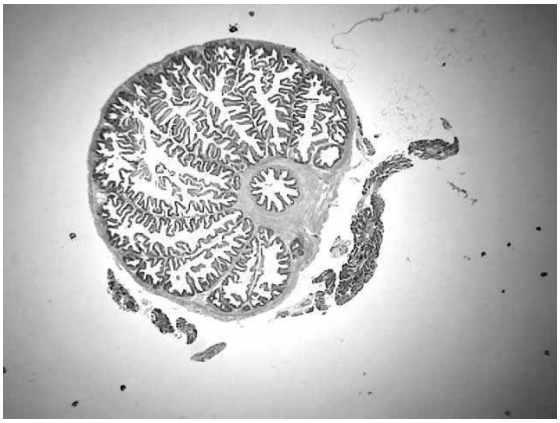

Fig.7: section of fingerling proximal intestine showing mucosal folds that traverse the central lumen (MF).note eccentric lumen (EL) formed by in growth of the tunica muscularis(TM). Note the pancreas (P) . H.& E. x40

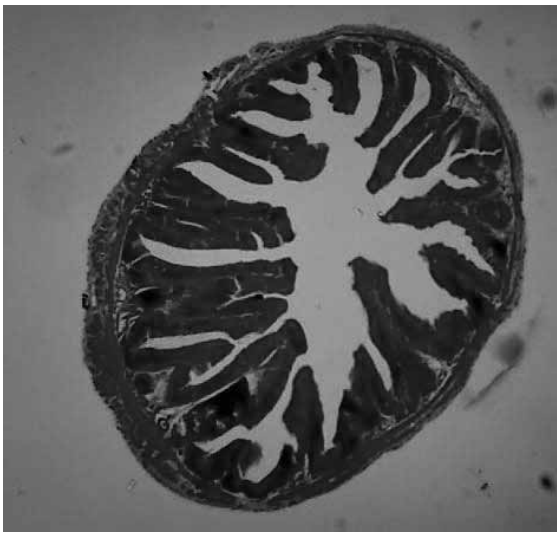

Fig.8. Section of fingerling middle intestine showing simple mucosal folds (MF). H. & E. x100

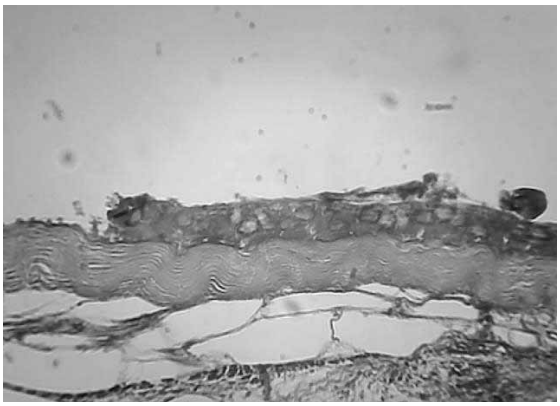

Fig.9. section of fingerling oro-pharyngeal mucosa showing poor PAS reaction of epithelial mucous cells (MC) .PAS x 400

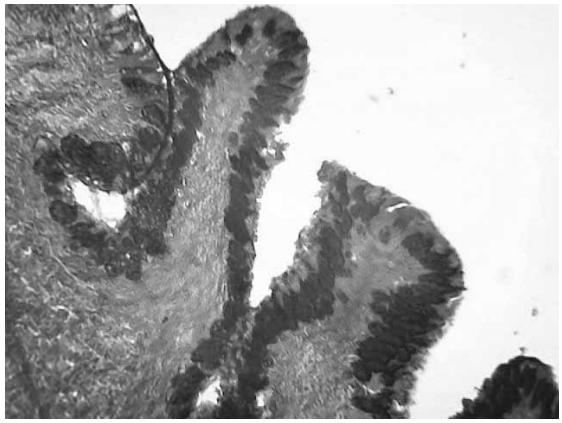

Fig.10. section of fingerling esophagus showing PAS positive mucous cells (MC), on the longitudinal folds. PAS X 400

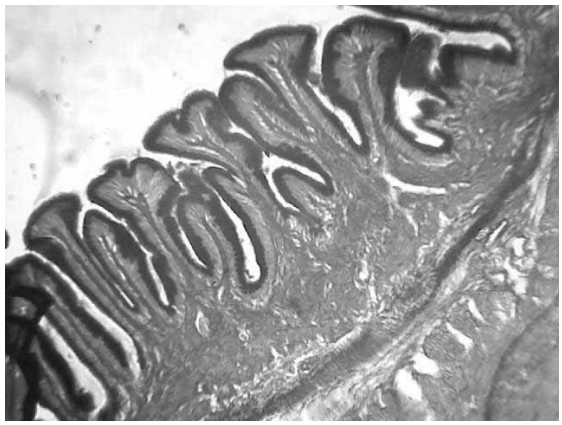

Fig.11: section of fingerling cardiac stomach showing PAS positive apical mucin (AM), in the columnar epithelium. PAS X400

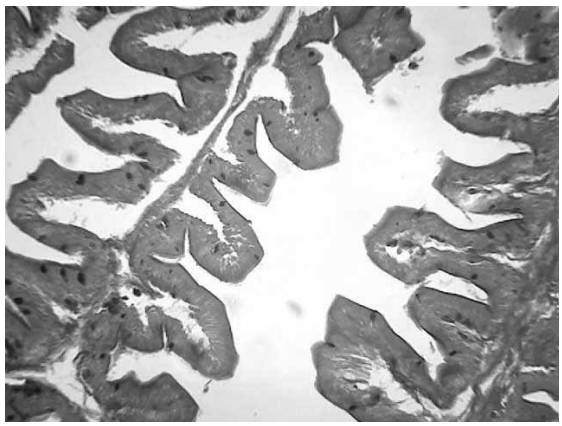

Fig.12: section of fingerling proximal intestine showing PAS positive goblet cells (GC).PAS X 400

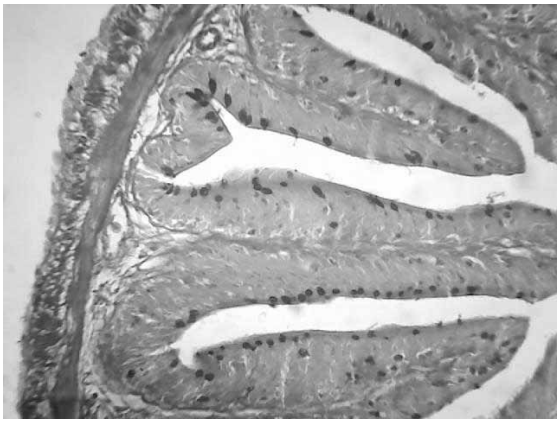

Fig.13. section of fingerling middle intestine showing abundant PAS positive goblet cells (GC). PAS X 400

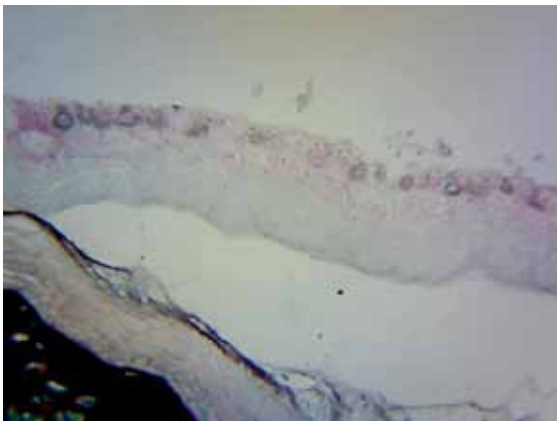

Fig.14. section of fingerling oro-pharyngeal wall showing AB negative mucous cells (MC).AB x 400

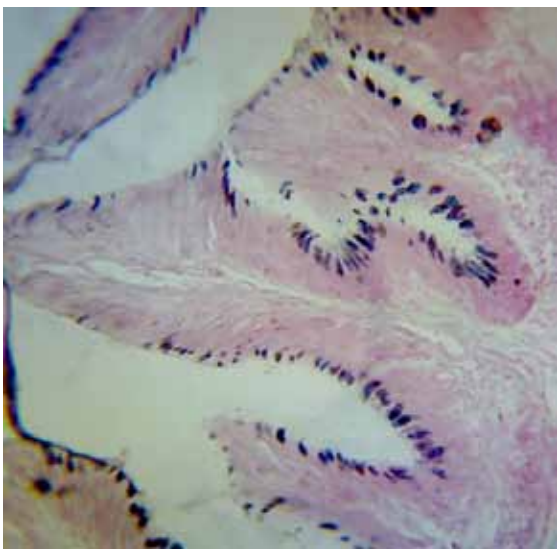

Fig.15. section of fingerling distal intestine showing AB positive goblet cells (GC).AB X 400

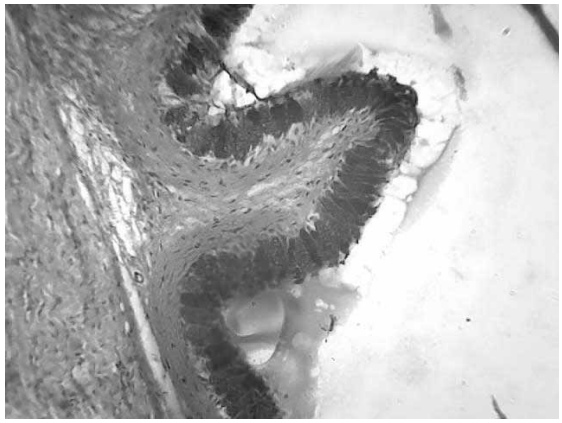

Fig.16. section of fingerling oesophagus showing mucous cells that contained neutral mucin dominant mucous cells(C). Note few acid mucin dominant cells (A) .AB/PAS X400

Fig.17. section of fingerling pylorus showing epithelia containing only neutral (N) mucin. AB/PAS X 400

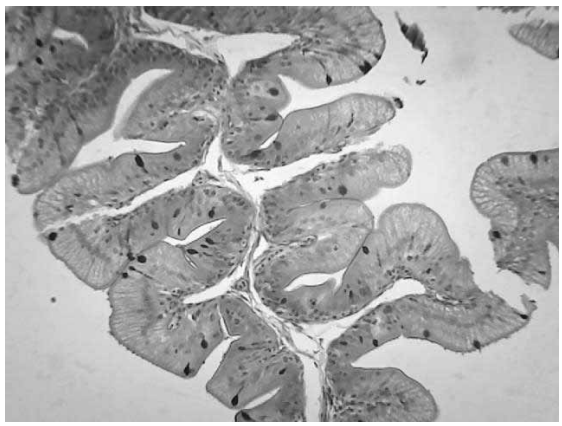

Fig.18. section of fingerling proximal intestine showing goblet cells that contained a combination of acid and neutral mucin (C), acid (A), and neutral mucin (N) .AB/PAS X400

Table 1: Mucin histochemical reaction of epithelial mucosubstances in different portions of the digestive tract of fingerling *Clarias gariepinus*.

Procedure	T	BC	E	S	PI	MI	DI
PAS	+	±	+	+	+	+	+
AB Ph 2.5	-	-	-	-	+	++	+
AB/PAS	AB	AB	AB	PAS	AB	AB	AB

KEY: AB, Alcian blue; PAS, Periodic Acid Schiff; AB/PAS, combined Alcian blue with PAS procedure after diastase treatment; BC, oro-pharyngeal wall; T, Tongue; E, Esophagus; S, Stomach; PI, proximal intestine; MI, Middle intestine; DI, distal intestine; R, Rectum; (-), no staining observed; (±) poorly stained (+), low; (++), medium; (+++), high; AB, AB dominance; PAS, PAS dominance.

The presence of mostly circularly oriented striated muscle in the oesophagus has been reported by other authors (Falk-Petersen and Hansen, 2001; Raji and Norouzi, 2010) and is associated with ability to voluntarily reject unwanted material (Jaroszewka *et al.*, 2008). The lymphocytes seen are involved in specific defense mechanism (Diaz *et al.*, 2008; Ezeasor, 1984; Banana-Khojasteh, 2009).

The stomach general microanatomy of the regions are similar except the absence of gastric glands in pylorus which may be attributed to the need for reduced gastric acid secretion that may interfere with the needed alkaline medium for digestive processes in the intestine by pancreatic enzymes (Ribeiro *et al.*, 1999). The gastric epithelium was of simple columnar absorptive type with apical neutral mucin that is associated with absorption of easily digestible molecules (Grau *et al.*, 1992).The cells of the gastric glands would probably be oxyntopeptic cells (Barrington, 1957; Yang *et al.*2010; Xiong *et al.*, 2011) producing both pepsinogen and hydrochloric acid). The collagen bundle in the submucosa is for support and strengthening.The pyloric sphincter containing circular smooth muscles serves as an involuntary valve regulating food passage into the intestine. This may help delay food in the stomach longer to achieve maximal gastric digestion. The sphincter also serves as valve to prevent reflux of feed from intestine to the stomach.

The intestinal transversing mucosal fold with villi seen in the proximal intestine is a specialization that increases the surface area for nutrient absorption, and agrees with with findings of Al-Hussaini *et al.*, (1949).The goblet cells produce mucin involved in epithelium protection (Neuhaus *et al.*, 2007), but the low quantity of acid mucin may not provide adequate protection; hence increased mortalities due to pathogen attack. The neutral mucin also helps in absorption of easily digestive substances such as disaccharides and short-chain fatty acids (Elbal *et al.*, 2004).

Conclusion

The study concludes that the absence of acid mucin in the oro-pharyngeal wall may contribute to high mortality of fingerlings, since the absent acid mucin may have helped protect against pathogens.

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LES FILIERES D'EXPORTATION DU BETAIL SUR PIED AU TCHAD

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Résumé

L'exportation du bétail sur pied au Tchad représente un des postes les plus importants de l'économie du pays, générant officiellement un chiffre d'affaires proche de 60 millions de dollars. Des études plus récentes arrivent cependant à une estimation de plus du double de cette valeur, qui correspondrait alors à la moitié des exportations tchadiennes annuelles. Ces chiffres confirment la place majeure qu'occupe le secteur de l'élevage au Tchad, dont 80% du cheptel appartient à des systèmes pastoraux et qui contribue à la subsistance de 40 % de la population rurale et à près de 20% du PIB du pays. En l'absence d'un recensement récent, on estime les effectifs de ruminants à près de 20 millions de têtes, 44% de bovins, 38% de petits ruminants et 18% de camelins, majoritairement présentes dans les zones agro-climatiques septentrionales saharienne et sahélienne (73%, vs. 27% en zone soudanienne). Malgré leurs faibles performances zootechniques, ces animaux font l'objet d'une demande en augmentation, essentiellement en raison de la forte croissance démographique et de l'urbanisation dramatique du Nigéria voisin, au sein d'un marché dont l'offre voit le rôle de plus en plus important que jouent les marchés de savane.

Notre étude analyse les différents types de marché, de la collecte du bétail à proximité des élevages jusqu'à leur commercialisation dans les grandes villes où il sera abattu, en détaillant la fiscalité inhérente à chacun de ces déplacements, et identifie les acteurs de ces différentes filières, tant exportateurs proprement dits qu'intermédiaires aux rôles clairement définis. Tout en privilégiant la description des circuits de commercialisation les mieux connus, et notamment ceux des bovins en direction du Cameroun et du Nigéria, elle aborde également une filière cameline moins étudiée dont les produits sont destinés principalement à la Libye voisine, mais également à l'Egypte après un transfert par le Soudan.

Mots-clés : *Exportation, Bétail sur pied, Tchad*

THE EXPORT CHANNEL OF LIVE CATTLE IN CHAD

Abstract

The export of live cattle is one of the most important positions in the country's economy, generating a turnover officially closer to 60 millions of dollar US. More recent studies, however, an estimate of more than double this value, which would correspond to half of Chadian exports annually. These figures confirm the prominent role the livestock sector in Chad where 80% of livestock owned pastoral systems and contributing to the livehoods of 40% of the rural population and nearly 20% of GDP countries. In the absence of the recent census, estimated the number of ruminants are nearly 20 million head, 44% of cattle, 38% of small ruminants and camels 18%, mainly present in northern agro-climatic zones Saharian and Sahel (73% vs. Sudanian 27%). Despite their low animal performance, these animals are subject to increasing demand, mainly due to high population growth and urbanization in neighboring Nigeria (in a market where supply sees the role increasingly important role of markets savannah).

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Our study analyzed the different types of market, the collection of nearby livestock farms until they are marketed in large cities where it will be shot, detailing the taxation inherent in each of those movements, and identifies the different industries actors, exporters themselves as intermediaries to clearly defined roles. While focusing on description of marketing channels best known, including those of cattle in the direction of Cameroon and Nigeria, it addresses a less studied camel industry whose products are intended primarily to neighboring Libya, but also Egypt after a transfer from Sudan.

Keywords: *Guardrail, Export, Live, Cattle, Chad.*

Introduction

Le secteur de l'élevage pastoral revêt un caractère stratégique pour le Tchad. Il représente une activité qui touche 40% de la population, en premier lieu les populations pastorales, agro-pastorales et de plus en plus d'agriculteurs qui, depuis quelques décennies, acquièrent du bétail. L'élevage s'intègre aujourd'hui dans l'économie du pays par la commercialisation de ses produits sur les marchés urbains et ruraux, et sa contribution au PIB hors pétrole a été estimée pour 2004 à 17% (INSEED, 2004). Le capital constitué par l'élevage représenterait un montant de près de 1000 milliards de francs CFA, engendrant un flux monétaire annuel de près de 137 milliards de francs CFA (Massuyeau, 2002). Mais le secteur de l'élevage reste difficile à évaluer du fait de la dispersion des activités pastorales sur un vaste territoire (1 284 000 km²) et de la faiblesse de l'outil statistique du pays. Le dernier recensement des effectifs de bétail date de 1976, et il s'avère difficile de donner aujourd'hui un ordre de grandeur exact du cheptel tchadien ; on estime cependant à 80 % la part du cheptel qui appartient à des systèmes pastoraux (Barraud *et al.,* 2001).

Malgré ces incertitudes, nous nous sommes efforcés dans ce travail de décrire au plus près les filières d'exportation de bétail sur pied vers les pays voisins dont la demande est en croissance constante, d'en décrire les circuits de commercialisation et les acteurs, et d'en quantifier les flux générés. Une partie de cette étude prend en compte la fiscalité inhérente à ces exportations et analyse les décisions de ces acteurs face aux taxes qui leur sont demandées, qu'elles soient nationales, comme c'est le cas depuis de nombreuses années, ou régionales, à l'instar du passeport mis en place en 1994 par la CEBEVIRHA(Commission économique du Bétail, de la Viande et des Ressources halieutiques) pour garantir la libre circulation du bétail sur pied dans la zone CEMAC (Communauté économique et monétaire de l'Afrique centrale), qui regroupe le Cameroun, le Congo, le Gabon, la Guinée équatoriale, la République Centrafricaine et le Tchad (CEBEVIRHA, 2003).

Les filières « bétail sur pied »

Les effectifs de ruminants au Tchad

On estime à près de 20 millions de têtes l'effectif global, 44% de bovins, 38% de petits ruminants et 18% de camelins (PSSP, 2005), répartis inégalement dans les différentes zones agro-climatiques du pays, tel que cela est présenté dans le tableau I.

Tableau I

En dépit de la pertinence de ces estimations, il demeure une grande incertitude sur les taux de croît appliqués aux dernières données de recensement, et donc sur les effectifs réels du cheptel tchadien. Il est tout aussi difficile de présenter des valeurs moyennes des paramètres zootechniques, qui restent faibles et conformes à l'environnement dans lequel évoluent les animaux : en 2002, le Projet d'Appui aux Systèmes d'Elevages Pastoraux (PASEP) rapportait des productions pondérales annuelles de 30 ; 50 et 5 kg de poids vif, respectivement pour les bovins, les camelins et les petits ruminants, ainsi qu'un âge à la première mise-bas de 4 ans pour les bovins et camelins et de 15 mois pour les petits ruminants, avec des productions laitières respectivement de 3 ; 6 et moins de

1 litre par jour. Les taux d'exploitation pour ces mêmes espèces, respectivement, étaient dans cette même étude rapportés à 13 ; 6 et 25%. Cependant, ces paramètres ne tiennent pas compte de la mobilité des troupeaux, alors qu'un travail de suivi comparatif des troupeaux sédentaires, transhumants et nomades au Sahel nigérien voisin a montré que les indices de productivité des systèmes d'élevage transhumants étaient de 25% supérieurs à ceux des troupeaux sédentaires (Colin de Verdière, 1995). Selon le MERA (2009), les effectifs disponibles ou exploitables étaient en 2002 de l'ordre de 817 000 têtes de bovins, 640 000 têtes d'ovins, 1,7 millions têtes de caprins et 71 000 têtes de camélidés.

La filière bovine

Comme on peut le voir sur la figure 1, la production de bovins est exportée sur pied principalement vers le Nigeria, le Cameroun, le Soudan et la RCA, bien que les exportations vers ce dernier pays soient en déclin en raison de l'insécurité qui y règne. Les camelins sont exportés vers la Libye et l'Egypte via le Soudan.

Estimation du chiffre d'affaire de la sous-filière

En 2002, selon le Ministère des Finances, l'exportation de bétail à partir du Tchad représentait 30% des exportations du pays, soit environ 37,5 milliards de FCFA. Cependant, une étude de terrain sur les circuits de commercialisation (Koussou et Liagre, 2003) a relevé qu'en 2000 seulement 240 000 têtes sur les 520 000 estimés avaient été déclarées, soit 46% des exportations, amenant à un chiffre d'affaires de 86 milliards de FCFA, proche de la valeur de 85,4 milliards rapportée par la Banque des États de l'Afrique Centrale qui se fonde sur une croissance marquée et régulière des exportations tchadiennes depuis 1994, qui auraient atteint 470 000 têtes de bovins en 2001 (BEAC, 2001).

En adoptant l'estimation haute du cheptel bovin et camelin, la valeur totale du bétail commercialisé pourrait même atteindre 220 milliards de FCFA, soit très nettement au-delà du chiffre estimé par la comptabilité nationale (Bonnet *et al.,* 2004). Dans le même sens, les chiffres obtenus par Liagre *et al.* en 2004 pour les abattages d'animaux tchadiens sur les trois principaux abattoirs de Lagos, Ibadan et Port-Harcourt (entre 613 000 à 800 000 têtes selon les hypothèses retenues) sont eux aussi supérieurs aux estimations d'exportation de 520 000 têtes la même année par le PRASAC (Pôle Régional de Recherche appliquée au Développement des Savanes d'Afrique Centrale). On peut même s'attendre à des volumes encore plus importants si l'on devait intégrer les abattoirs des autres centres urbains du sud Nigeria et les circuits du Nord passant par le Niger (Liagre *et al.,* 2004).

La part du cheptel camelin dans cette estimation atteindrait près de 3 milliards de FCFA, le chiffre d'affaire réalisé sur les ventes de dromadaires au marché d'Abéché s'élevant à près de 655 millions de FCFA, et le montant des transactions sur ce marché correspondant approximativement à 20% du total des exportations de camelins depuis le Tchad Oriental à destination de la Lybie et de l'Egypte. En appliquant à ces données une marge brute de 10% (Koussou et Duteurtre, 2002) on peut donc raisonnablement penser que le revenu dégagé par les exportateurs de bétail au Tchad varie entre 10 et 20 milliards de FCFA.

Circuits de commercialisation vers le Cameroun et le Nigeria

La filière bovine vers le Cameroun et le Nigeria

Selon Trueba (2000), le bétail sur pied exporté hors de la région vers le Nigeria et le Cameroun emprunte les principaux circuits suivants (du Nord au Sud, figure 2):

Le circuit Nord : c'est le principal circuit d'exportation du pays, particulièrement actif de septembre à avril. Il draine via Massaguet l'essentiel du bétail produit en zone sahélienne, et amène les troupeaux jusqu'aux portes de N'Djaména où ils traversent le fleuve Chari au pont de N'Guéli. Ils rejoignent ensuite, à pied, via les postes frontières de Kousseri (frontière Tchad-Cameroun) et de Gambourou (frontière Cameroun-Nigéria), le marché de Maïduguri au Nigéria. Une fois vendus, les animaux sont acheminés par camion jusqu'au marché de Kano qui approvisionne la capitale fédérale du Nigéria, Abuja.

Tableau I : Répartition du cheptel tchadien (en milliers de têtes et en %) par zone agro-climatique

Zones / Espèces	Bovins (%)	Camelins (%)	Ovins (%)	Caprins (%)
Saharienne	1	669	110	67
	(0,01)	(19,1)	(4,6)	(1,3)
Sahélienne	6 515	2 841	1 531	3 677
	(74,2)	(80,9)	(64,5)	(71,1)
Soudanienne	2 262	0	733	1 427
	(25,8)		(30,9)	(27,6)
Total	**8 778**	**3 509**	**2 374**	**5 171**

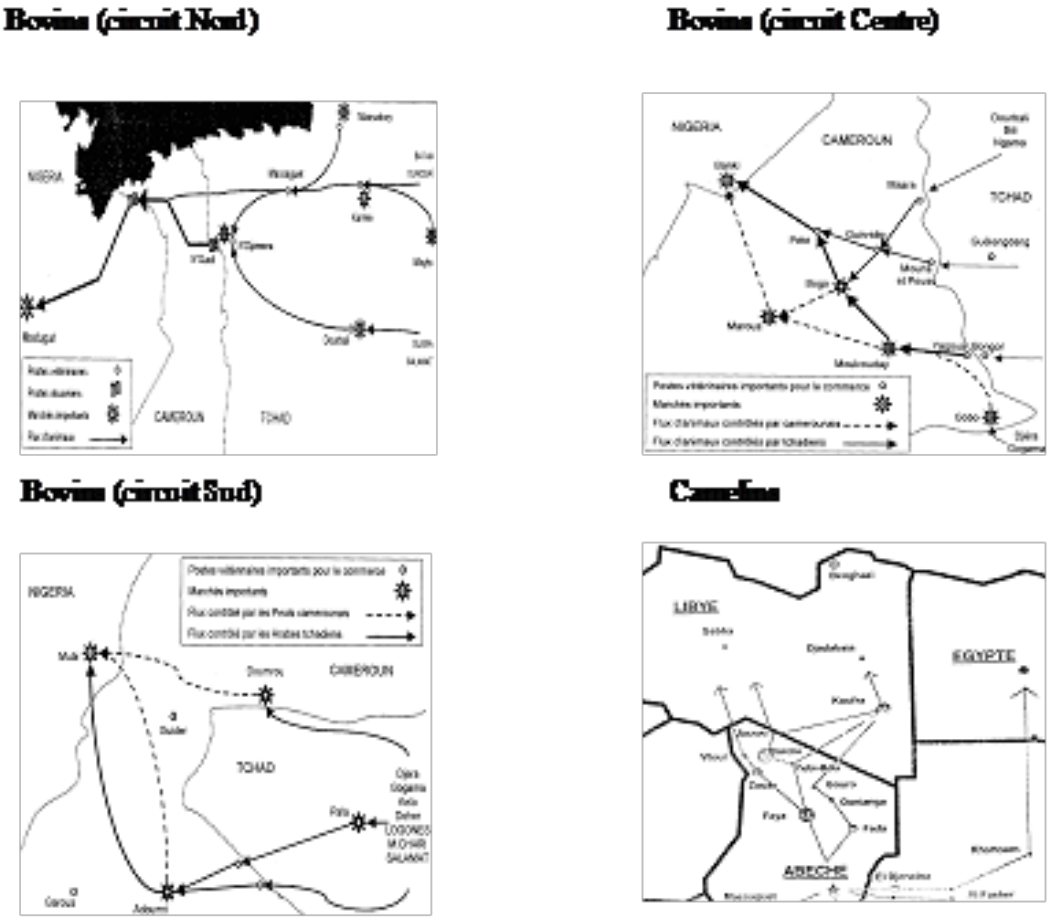

Figure 2 : Les circuits d'exportation

Le circuit Centre : il draine les animaux du Centre et du Sud du Tchad, essentiellement en saison des pluies. Les troupeaux venant du Sud longent le Chari jusqu'à Bousso et atteignent Bongor à l'Ouest, où ils passent à gué le Logone via le poste frontière camerounais de Yagoua. Après avoir traversé les marchés relais de Bogo et de Moulvouday au Cameroun, ils atteignent le marché de Banki au Nigeria. En saison sèche, le bétail qui ne bénéficie plus des eaux de surface entre Bousso et Bongor poursuit plus au nord jusqu'à Guelengdeng. Il y est rejoint par les troupeaux provenant des marchés du Centre.

Les animaux traversent le Logone pour rejoindre les postes frontières de Mourla ou de Mazra au Cameroun, avant d'être conduits sur les marchés de Bogo puis de Banki.

Le circuit Sud : il est alimenté par du bétail en provenance des marchés du sud du Tchad et de RCA, principalement en saison sèche. L'essentiel du bétail est exporté sur le marché de Mubi au Nigeria via les marchés de Pala au Tchad et d'Adoumri au Cameroun. Ils ne peuvent être conduits plus au sud à cause de la barrière sanitaire de Mbe qui interdit le passage aux animaux vers l'Adamaoua. Ce circuit depuis les grandes sécheresses des années 80 a connu un véritable essor, dynamisé par l'arrivée de nombreux éleveurs arabes du Batha dans la zone soudanienne et l'installation de commerçants de la même origine sur les différents marchés précédemment cités (Trueba, 2000). Cependant, certains troupeaux sont rachetés au Cameroun, sur les marchés d'Adoumri et dans une moindre mesure sur le marché relais de Doumrou, par des commerçants Peuls qui convoient le bétail soit sur le marché de Mubi, soit à l'abattoir de Garoua.

Un nouveau circuit semble aussi s'être récemment mis en place depuis la construction de la route goudronnée Doba-Ngaoundéré malgré la barrière sanitaire de la Bénoué.

La filière cameline vers la Libye

Elle tourne principalement autour du marché d'Abéché, à partir duquel les camelins sont exportés vers la Libye, mais aussi, en nombre moins important, vers l'Egypte via le Soudan. L'activité sur ce marché est saisonnière et dépend du passage des grands transhumants chameliers (à l'aller, en août, comme au retour, en octobre) qui à eux seuls en deux mois fournissent près du quart des effectifs annuels. Le reste de l'année, le marché est approvisionné essentiellement par des collecteurs qui vont chercher les dromadaires jusque dans les campements, ainsi que par des transhumants chameliers séjournant en saison sèche aux pieds des montagnes à l'Est d'Abéché ou plus à l'Ouest dans le Batha aux alentours d'Oum Hadjer ou dans la zone d'Am Dam.

Trois circuits principaux d'exportation ont été identifiés (Vigneau, 1998), le principal, qui représente près de 85% des exportations, allant d'Abéché à Sebha en Libye en passant par Faya ou par Yebi Bou. Le convoyage des animaux en Libye, long et dangereux, en particulier à cause du manque de points d'eau, se fait par camion. En revanche, les animaux exportés vers l'Egypte se déplacent à pied.

Quel que soit le circuit, au cours de ces déplacements, les exportateurs s'exposent à de nombreux risques, parmi lesquels Koussou et Duteurtre (2002) citent le manque de points d'eau le long des axes de commercialisation et l'insécurité due aux voleurs et aux coupeurs de route. A cela s'ajoutent le niveau élevé de fiscalisation de la filière, les fréquentes dévaluations du naira nigérian qui déstabilisent les cours du bétail, et les crédits douteux des commerçants camerounais ou nigérians qui ont pour habitude de payer à terme (1 à 2 mois).

Figure 1
Evolution de l'offre et de la demande

Liagre *et al.* (2004) notent une augmentation de l'activité du commerce intérieur du bétail de 22% entre 1997 et 1999 ; ce niveau s'est maintenu en 2000 avant de diminuer en 2001. Cette activité marque une croissance plus forte au Sud du Tchad en direction du Nigéria, dans des proportions de 145% en 1997 et 80% en 2001, alors qu'ils stagnent ou déclinent pour les zones sahéliennes. Cette augmentation serait essentiellement due à l'insécurité régnant au nord-ouest de la Centrafrique qui oblige les troupeaux à transiter par les marchés méridionaux du Tchad avant de rejoindre le Cameroun et le Nigeria. En conséquence, la part relative des marchés des savanes au sein des marchés tchadiens est en augmentation progressive : de 10% en 1997, elle est devenue supérieure à 20% en 2001, tant pour la circulation intérieure que pour l'exportation.

La demande régionale semble loin d'être satisfaite par la production de viande (CEBEVIRHA, 2003) alors que la croissance annelle de la demande en viande, estimée en 2005 à 4% par an, est appelée à croître en Afrique de l'Ouest de plus de 250% à l'horizon 2020 (Delgado *et al.,* 1999). Le marché nigérian constitue le principal

Tableau 2: Taxes officielles du commerce de bétail par tête de bovin

Taxes	Décomposition	Barème	Valeur
	Trypanocide		250
Fonds Elevage	Boucle d'oreille		250
	Vaccin bovipestique		1500
	Consultation		100
	Droits de sortie	8% de la valeur mercuriale	4800
	Taxe sur le Chiffre d'Affaires	3% de la valeur mercuriale	1800
Exportation	Taxe préférentielle cumulée	0,4% de la valeur mercuriale	240
	Redevance statistique	2% de la valeur mercuriale	1200
Taxe de marché			1500
Passeport			2500
Laissez-passer			100
Total			14240

moteur de cet ensemble : outre la taille du pays (plus de 60% des consommateurs de la région) et son fort taux d'urbanité (60% des nigérians sont des citadins), la demande intérieure nigériane en produits animaux a connu un bond significatif ces 20 dernières années, consécutivement à l'amélioration du pouvoir d'achat des populations, à la relative bonne tenue des cours mondiaux des produits pétroliers, et à la stabilité, même fragile, du naira. Selon la CEBEVIRHA (2003), la disponibilité apparente en viande y est passée de près de 230 000 tonnes en 1990 à plus de 325 000 tonnes en 1999, puis à 400 000 tonnes en 2001. Cette disponibilité ne résulte pas uniquement de la production domestique, même si le Nigéria concentre à lui seul plus de 50% du cheptel bovin de l'Afrique de l'Ouest : les résultats d'études récentes mettent en relief le poids des importations de bétail au Nigéria, en particulier celles du Tchad et du Niger (Balami, 1999), ces deux pays satisfaisant vraisemblablement à eux seuls 20 à 25% de la consommation apparente au Nigéria (Liagre *et al.,* 2004). Parallèlement, les prix de la viande et du bétail au Nigéria ont subi une forte augmentation ces dix dernières années, avec deux phases d'accélération, la première lors de la dévaluation du FCFA, la seconde à partir de 1999, date qui correspond à la relance de l'activité économique au Nigéria.

Organisation des filières et des marchés de bétail vif

Types de marchés

Quatre types de marchés à bétail peuvent être identifiés (Koussou et Liagre, 2004) : (1) Les marchés de collecte permettent aux collecteurs et aux petits commerçants d'acheter les animaux aux éleveurs et de constituer des lots qui seront acheminés jusqu'aux marchés de regroupement. Ils fonctionnent dans tous les principaux bassins d'élevage et le long des parcours de transhumance des zones sahélienne et soudanienne. Leur périodicité est hebdomadaire, bien que le fonctionnement d'un certain nombre soit plutôt rythmé par les saisons. (2) Les marchés de regroupement se concentrent au fur et à mesure que l'on se rapproche de la frontière ; leur activité dépend des saisons, puisqu'au Sud ils ne sont alimentés par les transhumants qu'en saison sèche alors qu'au Nord c'est en saison des pluies que le cheptel abonde. Le reste de l'année l'offre dépend donc en grande partie du cheptel des sédentaires. Ces marchés permettent le rassemblement des animaux collectés et la constitution des troupeaux destinés à être convoyés vers les marchés relais ou terminaux de consommation. Les collecteurs amènent les troupeaux aux représentants des grands commerçants qui procèdent aux allotements par âge, état corporel, sexe des animaux. C'est également le lieu de constitution de la taille

optimale de troupeaux qui peuvent être confiés à des convoyeurs. Généralement la taille des troupeaux ainsi constitués varie entre 50 et 75 bêtes pour 2 bergers. (3) Les marchés relais sont souvent très proches des frontières. Ils constituent des centres de régularisation et de remise en forme des animaux avant leur acheminement vers les marchés terminaux de distribution et de consommation. Ces différents marchés, distants de quelques centaines de kilomètres, sont organisés en réseau et fonctionnent 7 jours sur 7. Le transfert du bétail de l'un vers l'autre des marchés frontaliers permet de répondre aux fluctuations différentiées de la demande sur les marchés terminaux. (4) Les marchés terminaux, enfin, sont situés dans les grandes agglomérations. Les plus importants se rencontrent dans quelques villes du Nord Cameroun (Garoua, Maroua,…) mais surtout dans les villes tentaculaires du Nigéria (Port Harcourt, Abuja, Ibadan …). Ces marchés réceptionnent les camions provenant des marchés relais du Nord-est (Maïduguri, Banki et Mubi) et assurent la distribution aux bouchers. Ils sont également en réseau pour réguler l'offre par rapport à la demande, grâce à des zones de pacage permettant le stockage temporaire des animaux. Ils alimentent aussi les abattoirs principaux des centres urbains, dotés généralement d'infrastructures conséquentes (eau courante, plate forme de débarquement, boxes pour garder les animaux, aire d'abattage).

Les acteurs de la filière

A l'instar de Trueba (2000), on peut distinguer les exportateurs proprement dits des intermédiaires, au rôle clairement défini.

Les exportateurs

Les éleveurs frontaliers peuvent jouer un rôle dans l'exportation du bétail sur pied, même si le cheptel exporté ne représente pas de gros volumes. Ce sont soit des sédentaires vivant à proximité de la frontière qui s'appuient sur leurs liens familiaux pour vendre leurs animaux au Cameroun, soit des transhumants qui lors de leurs déplacements transfrontaliers vendent du cheptel sur les marchés qu'ils traversent. Ce sont surtout les collecteurs qui alimentent les marchés de collecte. Très nombreux, ils sont aussi capables de s'associer pour exporter du bétail qu'ils partent chercher jusque dans les campements. La plupart élèvent aussi leur propre cheptel, mais ils ne sont pas suffisamment influents et dépendent donc fortement des bonnes grâces des commerçants patentés dont ils utilisent le nom pour établir un passeport d'exportation. Les commerçants non patentés sont très actifs sur les marchés de regroupement, particulièrement dans les circuits Sud (Pala), où ils s'appuient sur un solide réseau au Cameroun pour circuler avec un simple laissez-passer sans avoir à présenter de passeport, et Centre (Bongor), où ils sont liés à des commerçants de Maroua pour établir les laissez-passer sanitaires dans les postes vétérinaires de Gobo et de Moulvouday. Les commerçants patentés, quant à eux, seuls officiellement habilités à exercer ce commerce, contrôlent les marchés relais qui constituent l'ossature des réseaux transfrontaliers.

On y distingue (1) les Arabes, qui gèrent la filière entre Pala, Adoumri et Mubi, (2) les commerçants de Bongor, qui contrôlent celle qui va de Bongor ou Guelengdeng jusqu'à Banki, (3) les commerçants de N'Djaména, pour la partie entre N'Gueli et Maïduguri, et enfin (4) les commerçants du BET (Borkou Ennedi Tibesti) qui se partagent la filière d'exportation des dromadaires vers la Libye. Tous ont développé un réseau d'influence pour contrôler les plus petits commerçants - en prêtant leur nom pour établir les passeports - et pour éviter les taxes douanières - en faisant passer une grande partie des exportations tchadiennes pour du cheptel camerounais. A titre indicatif, la filière officielle du circuit Nord est composée d'une trentaine de commerçants patentés qui eux mêmes prêtent leurs nom à une multitude de petits commerçants non patentés en quête de passeport, moyennant une rémunération moyenne de 15 000 FCFA / tête (Djefil, 2003). Aux côtés de tous ces exportateurs figurent les transitaires : 3 bureaux de transit sont agréés pour l'exportation du bétail, ils aident les commerçants à faire les formalités douanières au tarif de 50 FCFA par tête (Djefil, 2003).

Les intermédiaires

Les convoyeurs sont les personnes qui accompagnent les animaux sur les marchés relais ou terminaux. Ils travaillent toujours avec le même commerçant, avec lequel ils n'ont pas nécessairement de lien de parenté. Une fois les

animaux achetés, ils les marquent sur le marché d'origine et organisent avec d'autres convoyeurs des troupeaux d'une taille qui varie de 100 à 200 têtes En général, un homme convoie au maximum 25 têtes, et touche près de 65,000 CFA pour un trajet d'une durée moyenne de 1 mois. Les itinéraires suivis sont connus mais peuvent varier en fonction de la disponibilité en eau, de l'insécurité et du dispositif de contrôle, qu'ils tentent généralement de contourner. Sur les marchés, certains commerçants arabes patentés, les Damin, qui se sont installés depuis suffisamment longtemps au Cameroun et au Nigeria et ont changé de nationalité, sont nommés comme garants par leurs chefs de canton ou de tribu d'origine : ils accueillent les commerçants de leur groupe, leur assurent l'hébergement et l'alimentation pour leur bétail, et réallotent les animaux en lots indépendants dont ils négocient le meilleur prix de vente, tout en garantissant l'origine des animaux vendus et s'engageant à rembourser l'acheteur en cas de malversation. Ils prélèvent également pour le compte des vendeurs le paiement des taxes officielles qu'ils redistribuent à qui de droit par la suite. Ceux qui ont une patente prêtent parfois leur nom aux commerçants qui voudraient établir un passeport pour le bétail, et peuvent aussi faire bénéficier de leur nom les exportateurs tchadiens qui n'ont pas de passeport et qui voudraient se munir d'un simple laissez-passer camerounais. Leurs services sur le marché sont rémunérés de manière forfaitaire, allant de 1,000 à 1,500 FCFA / tête. Ne pouvant pas intervenir dans des transactions n'impliquant pas un commerçant de leur propre tribu ou clan, ils peuvent être très nombreux sur un marché : Koussou et Duteurtre (2002) ont ainsi avancé le chiffre de 70 garants sur le marché de Roro. On trouve également d'autres intermédiaires, comme les Dallala (ou Sabbaba, ou Samsara), qui servent de courtier pour les commerçants vendeurs, ou les Rakadja (ou Dealer), qui profitent de leur connaissance du marché (prix, commerçants) pour faire le lien entre différents commerçants d'un même marché : contrairement au courtier, ils cherchent à tirer vers le bas le prix d'achat afin de dégager leur propre marge en revendant l'animal à sa vraie valeur. Enfin, sur les marchés tchadiens et camerounais du circuit Nord, les Cherik, qui sont généralement des Haoussas ayant fondé une famille à l'étranger, assurent aux commerçants nigérians une information fiable sur la disponibilité et les prix du bétail sur les différents marchés de la filière.

Les flux de bovins

Trueba (2000) confronte les informations données par le chef du marché de Maïduguri, qui dénombre 250,000 têtes importées au travers du circuit Nord, à celles des douanes de N'Guéli qui rapportent un total de 167,000 animaux exportés, et en déduit que ce sont plus de 80,000 têtes qui traversent en fraude le Chari au niveau du lac à l'aide de pirogue, au tarif moyen de 10 000 FCFA pour une vingtaine de têtes. Ce circuit, traditionnellement emprunté par des animaux de race Kouri, est aujourd'hui celui de nombreux commerçants Kanembou. Malgré tout, le circuit de N'Guéli est le mieux contrôlé par les douanes. S'agissant du circuit Centre, les données fournies par le poste vétérinaire de Bongor font état de 10,000 têtes exportées en 2000, auxquelles il faut ajouter le bétail dont les passeports ont été faits dans des postes en amont (Haraze Magueige, Roro et Sarh), dont l'effectif a été estimé par Trueba (2000) à 20,000 têtes. En 2002, Koussou et Duteurtre évaluent, après observations sur le marché de Bongor et entretiens dans différents postes vétérinaires, le même flux à 145,000 têtes : il semble donc que 80 % du bétail exporté depuis le Tchad par ce circuit passe en fraude avant d'être régularisé comme du bétail appartenant à un commerçant camerounais lors de la délivrance du laissez-passer sanitaire au Cameroun. L'essentiel de la fraude aurait lieu en saison sèche et serait l'œuvre des gros commerçants, patentés ou non, les plus petits préférant obtenir le passeport officiel de la CEBEVIRHA en utilisant le nom d'un des commerçants patentés de Bongor. Ainsi, comme à N'Djaména, le marché est concentré dans les mains de quelques commerçants patentés (Trueba, 2000). Pour le circuit Sud, l'essentiel du bétail est exporté sans passeport par des commerçants arabes non patentés. Contrairement à ce qui est observé pour les autres circuits, rares sont les commerçants tchadiens qui cherchent à emprunter le nom d'un commerçant patenté pour exporter leur bétail. Ils préfèrent

généralement s'appuyer sur leur réseau de commerçants Arabes naturalisés camerounais ou sur celui des commerçants Peuls au niveau des marchés d'Adoumri et de Doumrou. En empruntant le nom et la nationalité des ces derniers, les exportateurs tchadiens peuvent aisément obtenir un laissez-passer dans l'un des postes vétérinaires bordant la frontière et récupérer tranquillement leur bétail, devenu camerounais, une fois au Nigéria, pays hors CEMAC. Les exportations par ce circuit sont estimées par Trueba (2000) à 125,000 têtes.

Taxes et autres frais prélevés lors de l'exportation du bétail sur pied

L'exportation de bétail est sujette à un certain nombre de taxes officielles (tableau II), comme la taxe pour le Fonds d'élevage perçue par les services vétérinaires des postes de sortie, la taxe à l'exportation demandée par les douanes et la taxe sur la vente du bétail, qui est un impôt général délibératoire prélevé par le trésor public sur les marchés. De plus depuis 1994, la CEBEVIRHA a mis en place un passeport pour le bétail qui est sensé garantir la libre circulation du bétail sur pied dans la zone CEMAC. Encore peu utilisé, il est délivré dans les postes de sortie, et y sont mentionnés l'identité du convoyeur, le pays d'origine, la destination, les postes de contrôle, le nombre d'animaux (au plus 50 têtes) et les espèces. A ces taxes s'ajoutent certains frais prélevés sur le territoire national par une multitude d'acteurs (garants, courtiers, sultans ou chefs de canton, mairies, Bureau National du Fret, Fonds d'Entretien Routier, chambre de commerce; de même, tout commerçant souhaitant convoyer du bétail est tenu d'établir un certificat sanitaire et un laissez-passer de circulation intérieure qui mentionnent le nombre d'animaux, l'espèce, le marché de départ et les postes de contrôle dans lesquels ils doivent faire viser leurs documents. Une fois au Cameroun, les commerçants tchadiens doivent encore s'acquitter d'un certain nombre d'obligations, en particulier du visa de transit et des taxes réclamées par les communes et les sultanats qu'ils traversent. Enfin, au Nigeria, il leur faut normalement payer une lourde taxe à l'importation (300,000 FCFA / tête de bovin) mais très peu suivent le canal officiel et la grande majorité préfère s'acquitter d'un simple visa de transit (autour de 5,000 FCFA / tête de bovin).

Tableau II

Selon Koussou et Duteurtre (2002), la somme des taxes officielles et des frais divers payés par les commerçants peut atteindre jusqu'à 25% du prix de vente d'un bovin, alors que les frais de convoyage ne dépassent pas 2% et la marge brute à peine 10%. Dans un tel contexte, nombre de commerçants adoptent des stratégies d'évitement à tel point que selon le PSSP (2005), seulement 35% des exportations réelles seraient déclarées. Le manque à gagner pour l'Etat tchadien serait d'alors de l'ordre de 3 à 4 milliards de FCFA (MERA, 2009).

Conclusion

Le trafic transfrontalier en Afrique de l'Ouest et en Afrique Centrale des animaux sur pieds occupe sans nul doute le premier poste des transactions régionales des produits du cru (hors brut pétrolier). La présente étude confirme que l'ordre de grandeur du chiffres d'affaires de la filière d'exportation de bovins sur pied, et dans une moindre mesure celle de camelins, est proche de 80 milliards de FCFA. Mais il semblerait que l'essentiel des exportations de bétail échappe à l'enregistrement et aux droits de douanes, avec un manque à gagner pour l'Etat qui serait de plusieurs milliards de francs CFA, soit 4 à 5% des recettes budgétaires. Cause ou conséquence, on constate également que, malgré sa place dans l'économie nationale et son rôle dans l'assurance de la sécurité alimentaire, l'élevage ne reçoit qu'une dotation très faible du budget de l'Etat, avec environ 1% du budget national. De tels chiffres ne peuvent laisser indifférents et justifient pleinement la volonté des pouvoirs publics de mener une étude économique approfondie afin de mettre en lumière l'importance de la contribution des systèmes de production pastoraux dans l'économie et la fiscalité nationales, tout en conduisant une réflexion sur le dispositif juridique et institutionnel à développer pour pérenniser les aménagements pastoraux publics.

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SHORT COMMUNICATION

A NOTE ON MILKING PRACTICES IN THE SMALLHOLDER DAIRY PRODUCTION SYSTEMS OF NAIROBI AND ITS ENVIRONS

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Small-scale dairy farming plays a significant role in many tropical and sub-tropical developing countries. It is faced with several disease challenges (Gitau *et al.* 1994; Gitau *et al.* 1999; Gitau *et al.* 2010) and more recently many welfare issues and concerns have been reported as impacting negatively on the industry (Aleri *et al.,* 2011; Aleri *et al.* 2012). Welfare implications reported include: poor feeding and management practices, inappropriate housing and some welfare diseases (mastitis, body injuries, lameness and anoestrus) (Nguhiu *et al.* 2009; Aleri *et al.* 2012). Losses due to mastitis are ranked highest in the dairy industry (Huijps *et al.* 2009). Its risk and predisposing factors are well elaborated (Nyman *et al.* 2007; Olde Riekerink *et al.* 2007; Moussavi *et al.* 2012). In Kenya, several risk factors have been reported (Abuom *et al.* 2011). However, data on milking practices used by the farmers are not available hence the need for the current study. The aim of this study was to determine status and educate the small-scale dairy farmers in Nairobi area on milking practices and its implications on mastitis.

Materials and Methods

Study area

This cross-sectional study was carried out in peri-urban areas of Nairobi, Kenya which included parts of Kiambu, Kikuyu and Kajiado Districts between July and October 2009. Nairobi is the capital city of Kenya and occupies an area of approximately 696 square kilometers. It lies between 01° 17′S latitude and 36° 48′E longitude. The study area has two main agro-ecological zones the lower highland with an altitude of between 1820 m and 2070m and the other upper midland with an altitude of between 1200 m and 1820 m above sea level. The peri-urban areas included in the study were located North, South and West of the City. Nairobi has an estimated population density of over 3017 persons per square kilometer. It has a high number of smallholder dairy production units whose increase is encouraged by availability of a ready market for milk in the city. In some of the less densely populated suburbs dairy cattle are also reared.

Study design and Data Collection

A total of 80 smallholder dairy units were included in the study. The study area was divided into 4 zones, which were named North, South, West and Central, with Nairobi suburbs as the Central zone. Each zone was further subdivided into 4 subzones. From each zone 20 smallholder units were selected. They were selected purposively by the help of local veterinarians and Animal Health Assistants with whom the farmers were more acquainted. In this study, a smallholder dairy unit was defined as one with a minimum of 3 and a maximum of 16 adult dairy cows. Data were collected using semi-structured questionnaires by interviewing the farmers. Data collected involved milking practices adopted by the farmers. Data were checked for any entry errors and thereafter entered in Microsoft Excel, (2003).

Results

General descriptions

A total of 80 smallholder units were evaluated in this study. The average number per farm was 5 adult cows and the median number was 4. The age of the cows ranged between 3-14 years, with an average of 7 years and median age of 6.50 years. The cows were of various breeds: 55.90% (171) were Friesian with average weight of 433 kg, 21.60% (66)

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were Ayrshire with average weight of 419 kg, 4.90% were Guernsey with average weight of 299 kg, 1.30% were Jersey with average weight of 388 kg, and 16.30% (50) were not pure but cross-breeds between any two of the pure breeds. These cross breds had an average weight of 340 kg. Milking was done twice/day approximately twelve hours apart and the daily milk yield in the evaluated farms ranged from 2.5 litres to 30 litres per cow. The average was 11.38 ± 5.91 litres per cow.

Milking areas

None of the farms evaluated in this study had a milking parlour. Instead, improvised cubicles were used as milking areas (Figure 1) in 76% of the farms. In the remaining 24% of the farms, cows were milked in their respective resting areas or cubicles. In 61% of the farms, the improvised milking cubicles measured less than 1m X 0.95m and in the remaining 39%, they measured more than 2m X 1m. In 49% of the farms, the cubicles in which milking was done had protruding objects such as nails and sticks.

Milking routines/practices

The routine practices carried out during milking in the farms that were evaluated are summarized in Table1. Tying of the cows' hind limbs during milking was routinely practiced in 82.5% of the farms. Udder cleaning before milking was done in all the farms and in half of them, warm water was used for cleaning while in the other half cold water was used. In a majority (82.5%) of the farms, the cows had their udders washed using a common udder towel. In 76.25% of the farms, the udder drying towels were grossly dirty. In 71.25% of the farms, these udder drying towels were rough in texture. In 96.25% of the farms, hand-milking was done. The stockmen milked the cows by pulling the teats in 76.6% of the farms, while in 23.4% they milked by squeezing the teats. Machine milking was done only in 3.75% of the farms (Figure 2). Lubrication of the teats with milking jelly before milking was routinely done in 82.5% of the farms, but no milking jelly was used in the remaining 17.5% of the farms. Cows in farms where no milking jelly was used prior to milking, kicked and were restless during milking periods.

Discussion

The results of this study indicated that the milking practices adopted by dairy farmers in the smallholder systems in Nairobi and its environs were unique and variable and majority were unconventional. The lack of conventional milking parlours for the farmers in this study was mainly due to lack of funds to set-up the milking parlours and probably the few number of cows per farm. This lack of infrastructure resulted in the adoption of unique milking parlours at the farms which were poorly constructed or the use of resting areas for milking which had hygiene implications. These observations have not been reported previously.

Figure 1: Areas used for milking in some of the farms evaluated for milking practices in the smallholder dairy production systems in Nairobi and its environs (July 2009 –October (2009). A- cow being milked in a cubicle and B- a stockman milking a cow in one of the cubicles with protruding sharp objects.

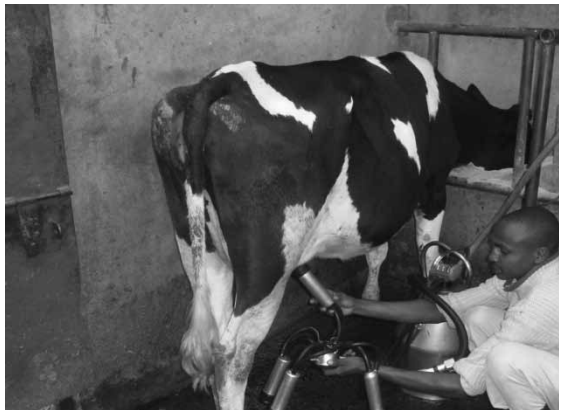

Figure 4.20: The use of milking machine in 3 out of the 80 farms evaluated for milking practices in the smallholder dairy production systems in Nairobi and its environs (July 2009 – October 2009). A-Portable type of milking machine; B- a stockman preparing to use the portable milking machine.

Table 1: Milking protocols and handling of the udder during milking in the 80 farms evaluated for milking practices in the smallholder dairy production systems in Nairobi and its environs (July 2009 – October 2009).

Milking practice	Number of farms	Percentage of farms
Machine milking	3	3.75
Hand milking	77	96.25
Warm water udder cleaning	40	50
Cold water for udder cleaning	40	50
Common washing cloth	66	82.50
Individual washing cloth	14	17.50
Dirty drying cloth	61	76.25
Clean drying cloth	19	23.75
Rough drying towel	57	71.25
Smooth drying towel	23	28.75
Use of milking jelly	66	82.50
No milking jelly used	14	17.50
Teat pulling technique	59	76.6
Teat squeezing technique	18	23.4

Washing of the udder and teats before milking as found in this study is in conformity with good udder hygiene practices (Radostits *et al.* 2003; Radostits *et al.* 2007). However, the use of cold water rather than warm water may be uncomfortable to the cows, and is contrary to the general recommendations that enhance the comfort of the cows during milking (DEFRA 2003). In addition to this, the use of the same piece of cloth or towel for washing and drying udders of several cows, was a poor animal welfare practice. It increases the risk of developing udder infection, which can be transmitted easily from one cow to another. This is contrary to recommended udder hygiene guidelines (Codes of Practice 2009; Radostits *et al.* 2003). Furthermore, these towels which were of rough texture in a majority of farms were always causing discomfort to the cows during use. The routine use of milking jelly to lubricate the teats before milking was a good animal welfare practice. However, failure to have germicidal teat dips for disinfecting the teats post-milking could

increase the risk of mastitis because of the likelihood of transmitting pathogens between animals by milkers' hands, dirty towels, shared udder cleaning and drying towels, as well as pathogens from the environment (Philpot 1979; Radostits *et al.* 2007).

There is need to adopt the practice of post milking teat dipping because of its ability to reduce environmental mastitis by 50% in some herds (Blowey and Edmondson 2010). The financial status of most of the smallholder farmers in this study makes it difficult for them to afford and maintain milking machines. Hand-milking in these farms is therefore, the only option. The teat-pulling hand milking technique that was employed by most of the stockmen in these farms was the cause of pain and discomfort during milking and was due to ignorance by the farmers.

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SHORT COMMUNICATION

FOODBORNE HELMINTH SAPRO-ZOONOTIC PARASITES IN EDIBLE LAND SNAILS FROM PLANTED AND NATURAL FOREST ECOSYSTEMS

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Snails are major rainforest floor invertebrates and serve as prey of vertebrates including snakes, small mammals and large birds. Snails are usually found in large numbers at night at waste dump sites, plantations (forest ecosystems), covered farmlands and woody debris (Ebenso, 2003a). The tropical rainforest is the most biologically diverse terrestrial ecosystem on earth (Gillespie *et al.,* 2004). The cost of non-market damage by snails to human health and ecosystems are high (Ebenso, 2003b). Snails are part of soil macrofauna and are herbivores and detritivores (Ebenso and Ologhobo, 2009).

The cost of habouring parasites in terms of human misery and economic loss is high. The highest costs are paid in the tropics and sub-tropics where parasites present a continual and unacceptable threat to the well-being of millions of people. Parasites are also a major cause of mortality and reduced reproductive success among domesticated and wild animals (Northrop – Clewes and Shaw, 2000). The increased demand for animal proteins in developing countries will lead to intensification of the production systems in which the risk of zoonotic infections needs to be assessed (Dorny *et al.,* 2009).

Zoonotic-parasites are animal parasites that can infect humans. Sapro-zoonotic parasites mean that parasites can infect humans from contaminated soil, vegetables or water (Youn, 2009). The vast majority of metazoan parasites of vertebrates are represented by two phyla, the acoelomate *Platyhelminthes* and the *pseudocoelomate Nematoda*. The most commonly used term to describe these parasites is "helminths." Helminths are common and ubiquitous parasites of man and the causative agents of a list of debilitating, deforming and fatal diseases of humans and animals (Northrop – Clewes and Shaw, 2000).

Foodborne parasites continue to circulate among backyard and free-ranging livestock reared in poor sanitary conditions, which survive in areas of low socio-economic level (Pozio, 2008). Humans get infected by eating raw or undercooked meat infected with cyst stages of these parasites. Children develop diseases by walking barefoot in sandy areas or playing in dirt or sand boxes that contain infected animal feaces (Labriola, 2009). It has been reported that gastropoda find mammalian feaces (manure) an attractive food source (Speiser, 2001), which together with the regular ingestion of contaminated soil, demonstrates the potential for internal pathogen carriage (Ebenso *et al.,* 2012).

In most parasitic infections, the infective third-stage larva from embryonated eggs marks the transition from free-living to the infections parasitic stage (Cantacessi *et al.,* 2010). In livestock, most of the zoonotic parasites are detected only by visual inspection during slaughter (Pozio, 2008). Some roundworms species have a natural reseviour among wildlife, hunters whose common habit leaving animal carcasses in the field after shining or removing and discarding the entrails increases the probability of transmission to new hosts (Pozio and Murrell, 2006). It is possible that the unique ability of zoonotic parasites to replicate in human host permits cycles of autoinfection, leading to chronic disease that can last for several decades (Genta, 1989).

As promising as micro livestock integration sounds, helminthosis will threaten the production and availability of meat to the

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general public (Olayemi, 2011). The aim of this study was to highlight the risk of consuming edible land snails (*Archachatina marginata*) harvested from the wild (forest ecosystem), especially as wildlife are reserviours of helminth parasites.

Materials and Methods

Study Area

The study was carried out at the Teaching and Research Farm (TRF), University of Uyo, located within the Niger Delta region of Nigeria and is characterized by a humid tropical forest climate where snails thrive well (Ebenso *et al.,* 2002), between latitude 5°2'16"N and longitude 7°55'16"E. It has a temperature range of 26-28°C and an annual precipitation range of 2000-3000mm, with relative humidity of 70-80% and photoperiod of 12hr light: 12hr darkness.

The three forest sites were two compartments of 17-year old planted forest at the TRF, of exotic *Gmelina arborea* and *Tectona grandis* trees and indigenous *Nauclea diderrichii* trees; while the reference site was a natural forest compartment at the TRF. These sites were not fenced, hence pets, livestock and people freely moved across.

Sample Collection

The 15 live samples of *Archachatina marginata* were randomly collected from the 3 forest sites at a distance of 30m apart at night (21:00-23:00Hrs) using flash light. Snails were transported in labeled sterile polythene bags to the laboratory. Within 24 hrs of samples receipt at laboratory, fresh fecal samples were taken from the snails for further analyses.

Coproscopy

Only 30 snails from forest samples voided feaces. Qualitative coproscopy involved methods of Bowman, (1999) for direct smear, flotation and concentration of eggs, and larvae culture cysts, while quantitative (enumeration and identification) involved methods of ILRAD, (2000) with the aid of Cornell-McMaster dilution egg counting technique.

Statistical Analysis

Data were collected on the possession of helminths in snails and analyzed using descriptive statistics according to methods of SAS (1999).

Results and Discussion

The results revealed *Oesophagostomum sp* (nodular worms) recorded a prevalence level of approximately 10%, (Table 2) this compares with high values by Krief *et al.,* (2010). These authors reported that the factors such a high prevalence remain unknown. These parasites can be lethal to humans. According to Storey *et al.,* (2000) patients are mostly children aged under 10 years. Clinical disease, due to encysted larvae, known as oesophagostomosis sometimes leads to death and is endemic in Africa (Polderman *et al.,* 1991). This nematode parasite is commonly found in ruminants and primates, including human, a high prevalence of this parasite infection has been reported in human populations, 1 million being estimated at risk (Storey *et al.,* 2000). The presence of this parasite remains asymptomatic in wild animals (Krief *et al.,* 2008). Human cases have been attributed to a zoonotic origin, non-human primates being proposed as a potential reserviour (Polderman and Blotkamp, 1995). However, in the TRF, primate populations have not been documented. There is possibility of transmission of parasite from human feaces from potential disease carriers. This assumption is supported by reports of Chapman *et al.,* (2006), that people being close to the forest are usually not using latrines.

Trichuris sp (whip worm) also recorded a prevalence rate of approximately 7% within the planted forest (Table 2). According to Areekul *et al.,* (2010) trichuriasis infection is common especially in tropical and sub-tropical regions where public sanitation and living conditions are substandard. About 10% of the world population may be infected. Reports of Stephenson *et al.,* (2000) indicate that children with this infection have dysenteric syndrome presenting with chronic mucous diarrhoea, rectal prolapse, anemia from blood loss and iron deficiency, clubbing of fingers, protein-energy malnutrition, and growth retardation.

Table 1: The distribution of positive samples of helminth zoonotic – parasites in faeces of A. marginata collected from planted and natural forest ecosystems

				Distribution of positive samples							
Coproxopic samples		**Positive samples**		**Crestoda**		**Nematoda**		**Protozoa**		**Trematoda**	
N	%	N	%	N	%	N	%	N	%	N	%
30.00	100.00	16.00	53.00	2.00	6.63	12.00	39.75	2.00	6.63	0.00	0.00

Table 2: Prevalence (%) of helminth zoonotic-parasites in faeces of A. marginata collected from planted and natural forest ecosystem

Zoonitic parasite	**Indigenous (N=6)**		**Exotic (N=4)**		**Natural (N=6)**	
	N	**%**	**N**	**%**	**N**	**%**
Crestoda						
Cysticercus sp	1	3.32	0	0	1	3.32
Nematoda						
Strongyloides sp	0	0	1	3.32	1	3.32
Haemonchus sp	1	3.32	0	0	1	3.32
Bunostomum sp	0	0	0	0	1	3.32
Trichuris sp	1	3.32	1	3.32	1	0
Oesophagostomum sp	2	6.64	1	3.32	0	0
Nematodirus sp	1	3.32	0	0	0	
Protozoa						
Eimeria coccidia	0	0	1	3.32	1	3.32

The parasitic load at the natural forest (Table 2) may be as a result of incidence of poor sanitary condition, as supported by reports of Chapman *et al.,* (2006).

Only the indigenous forest recorded *Nematodirus sp* prevalence of 3% of all forest sites in the present study (Table 2). The life-history of this parasite is unusual because larva develop to the infective third stage within the egg and these eggs hatch when temperature is above 10°C, host develop acute enteritis with watery diarrhoea accompanied by inappetence and weight loss (Denwood *et al.,* 2008). This parasite is the most pathogenic organism that infects sheep. Animals can die if the disease is not controlled by anthelmintic treatment (Armour and Coop, 1991).

The only parasite in the present study found in the natural (and did not occur in planted forests) forest was the *Bunostomum nematode* with a prevalence rate of 3% (Table 2). It is also called cattle hookworm; it causes a parasitic skin infection cutaneous larva migrans, frequently termed "creeping eruption." According to Labriola *et al.,* (2009), this skin infection has a worldwide distribution whenever humans have had skin contact with soil contaminated with infected animal feaces. Individuals may have severe inflammatory reactions cursing intense purities that anorexia develops and even become psychotic.

Strongyloides sp recorded a low 3% prevalence rate in the exotic forest (Table 2). According to Siddiqui and Berk, (2001) strongyloidiasis is difficult to diagnose because the parasite load is low and the larval output is irregular. Agi (1997) recorded 25% prevalence of the parasite in two Niger Delta communities. Symptoms include abdominal discomfort, nausea, anorexia and abdominal bloating (Berk *et al.,* 1987). The infection affects 30-100 million people world wide endemic in sub-Saharan Africa (Jorgensen *et al.,* 1996).

Haemonchus sp (Table 2) is a blood-feeding worm that causes anaemia and associated complications, ruminants are worst

affected. This gastric nematode according to Cantacessi *et al.,* (2010) is of socio-economic importance due to the production losses they cause in small ruminant livestock. Once eggs are ingested with vegetable by the host they can hatch within a day.

Emeria coccidia were the only non-metazoan parasite prevalent in the study area (Table 2). Infection of this coccidian parasite can cause a watery diahrroea, nausea and vomiting in humans (Dorny *et al.,* 2009).

Cysticercus sp (flatworm) is a metacestodes prevelant at 3% within the indigenous forest (Table 2). Humans may acquire infection by accidentally ingesting eggs eating contaminated food, and cause cysticercosis. This parasite may lodge in the brain and cause neurocysticercosis, one of the most important causes of acquired epilepsy in endemic areas (Dorny *et al.,* 2009). This parasite is associated with unhygienic environment, with poor disposal of human faeces.

Cooking is effective in killing these zoonotic parasites if the appropriate temperature (80-100°C) is reached in the core of the meat product (FDA, 2011).

Conclusion

Most of the edible land snails sold and consumed in Nigeria were picked from the wild (particularly forest ecosystem). This study revealed the transmission route of zoonotic foodborne parasites into the food chain, though prevalence rate may be low. Human infections and livestock production losses from foodborne are generally not monitored and are under recognized. Edible snails for consumption should be sourced from recognized farms, with zero-zoonotic parasite status. Properly processed and cooked snail meat is expected to kill parasites. The forest, whether natural or planted close to human habitation in rural areas are often used as toilets and refuse dumps. Snails inhabiting such forests mostly bear the helminth sapro-zoonotic parasites. Therefore, it is not advisable to collect snails from the forests bordering human settlements for food.

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SHORT COMMUNICATION

EVALUATION OF ANTIRABIES VACCINATION PROGRAMME FOR DOGS AT EDDIE VETERINARY CLINIC UYO AKWA IBOM STATE, NIGERIA.

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Rabies is an important zoonosis in Nigeria and other parts of the world. It is endemic in Nigeria and affects all warm blooded animals. It is 100% fatal in both man and animals (Acha and Szysfres 1980). Several authors (Bougler and Hardy,1960, Nuru,1973; Aghomo,1989 and Okoh,1989). have described the epidemiology of rabies in Nigeria. The disease is endemic in Akwa Ibom State as in other parts of Nigeria and dogs have been identified as the major source of human rabies (Ogunkoya *et al* 1984 and Taiwo *et al* 1998.)

Rabies vaccination campaigns have been carried out by the Federal ministry of agriculture as well as the Akwa Ibom State department of Veterinary services yearly via the print media and the Nigerian Veterinary Medical Association during the annual interactive forums with farmers.

The advocacy has been on compulsory registration of all dogs, vaccination of all dogs from 3 months of age, control of stray dogs, destruction of stray dogs, treatment of all dog bites as suspected rabid bite until proven otherwise. All these are in line with the recommendation of the World Health Organization (WHO, 1987)

Vaccination of dogs and cats are normally carried out through either the voluntary routine vaccination approach in which keepers of dogs and cats that are 3 months and above take them to the Veterinary Clinic for vaccination. Thereafter annual booster are recommended to maintain the immune status of the animals against rabies and various fees are changed. Also there is compulsory vaccination which is free and is sponsored by drug companies, Federal or State government.

Materials and Methods

Vaccination against rabies (antirabies) histories of all dogs registered between 2007-2011 were collected from registers and case notes of the preventive medication in the Veterinary Clinic (Eddie Vet Clinic Uyo) these included dates of registration, ages, sexes, date of Antirabies vaccination. A simple method was used in analyzing the data obtained retrospectively from the clinical records.

Since the actual number of dogs in Uyo Akwa Ibom State is unknown and no dog census has been conducted in recent years, the number of dogs which are three months and above registered in the Veterinary Clinic was taken as the base line. These dogs were classified by sex and vaccinated dogs for each year.

Vaccination coverage for each year was calculated as the proportion of registered dogs that were vaccinated.

Results

A total of 517 dogs aged 3 months and above were recorded in the clinic for 5 years (2007-2011), an average of 103 dogs a year. There were made up of 50 males and 53 females on the average. An average of 52 dogs or 67% of the annually registered dogs were vaccinated against rabies yearly. The annual vaccination actually varied between 57%-78%. The year with the highest number of vaccinated dogs was 2007 probably due to the fact that there was an aggressive antirabies campaign by the Nigerian Veterinary Medical Association & Federal Livestock department in collaboration with the Veterinary Department of the Akwa Ibom

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Table I: Showing Total Number of Dogs Registered With the Clinic

Year	No. of Dogs Reg. for the Year*	No. Vaccinated		Sex Registered	
		No.	%	Male	Female
2007	76	30	78	28	48
2008	54	18	64	16	38
2009	118	40	68	62	56
2010	121	71	57	80	41
2011	148	101	68	67	81
Total	517	260	235	253	264
Mean	103	52	67	50	53

*Represents total number of dogs registered in the clinic aged 3-6 months.

State Government. Thereafter, it decreased because the campaign was not sustained and strengthened, and besides people have to pay for these services instead of being free as at the time of the campaign where the charges were borne by the Federal Government.

Discussion

The prevailing vaccination coverage of the dogs at risk of rabies in Uyo of all dogs that are up to 3 months and above at Eddie Veterinary Clinic varied between 57%-78% (average is 67%). This is lower than the accepted lower value of 70% as recommended by the World Health Organization (WHO) in 1987 in order to effectively interrupt rabies transmission cycle in any community.

The predominance of females among the dogs vaccinated and even registered in the clinic is a reflection of the fact that most dog owners now keep their dogs for breeding purposes. Uyo is the capital of Akwa Ibom State and is one of the fastest growing state capitals with the influx of people from other parts of the world; there is need for security of lives and property. Thus, there is a demand for dogs for security purposes. Dog owners are capitalizing on this fact to breed dogs for sale not only to individual dog owners but to security companies.

The study has revealed that the voluntary vaccination approach, though it reduced government expenditure is less effective. There is need to back it up with a subsidy for vaccination costs, public health education on rabies through public enlightenment programmes on local radio stations, distribution of relevant extension leaflets to all who visit the veterinary and human clinics and hospitals for any ailments, potent enforcement of regulations on dog registrations, compulsory vaccinations of all dogs and cats, and periodic elimination of stray dogs from the town.(Adeyemi *et al* 2000).

A community-wide campaign combined with free vaccinations similar to the one carried out in Lima, Peru and which had drastic effects on stemming rabies in that city (Lombard *et, al;* 1988) may also be sponsored by the local government or any company to cover the whole of Uyo the capital city. This will effectively control rabies spread in the capital city.

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SHORT COMMUNICATION

DICEPHALUS AND DIPROSOPUS FOETUSES: GROSS AND RADIOLOGICAL OBSERVATIONS IN WHITE FULANI CATTLE IN NIGERIA.

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The White Fulani cattle are the most numerous and widespread of all Nigerian cattle breeds, representing about 37.2% of the national cattle population. Their population estimate in Nigeria, Cameroon and the Central African Republic is about 9,645,000 (Tawah and Rege 1996). The incidence and prevalence of congenital malformations in this breed of cattle are unknown.

Dicephalus (two-headed) and diprosopus (two-face) are congenital malformations uncommonly observed in ruminants (Vanderzon *et al.,* 1998; Shojaei *et al.,* 2006; Mukaratirwa *et al.,* 2006). Although few cases have been documented in humans (Spencer, 1992; Harma *et al.,* 2005) and in avian species (Mazzullo *et al.,* 2007a), they are extremely rare in horses and pigs (Mostafa *et al.,* 2005), and occasionally in cats and dogs (Camon *et al.,* 1992; Mazzullo *et al.,* 2007b). Previous reports have shown the incidence of congenital inherited defects in cattle to be 5.4 per cent (Omran, 1996). Leipold *et al.,* (1972) have also reported that identical twins are rare in cattle, with conjoined twins occurring approximately once in every 100,000 births. Various aetiological factors have been incriminated in domestic animals with congenital anomalies such as genetic defects with a complex mode of inheritance, nutritional factors and teratogens (Shojaei *et al.,* 2006; Mazzullo *et al.,* 2007b).

In Nigeria, few cases of congenital malformations in ruminants have been documented. These include annelia, scoliosis, umbilical hernia, cleft palate, atresia ani, acephalus, microcephaly, kyphoscoliosis and spinal bifida (Ibrahim *et al.,* 1987, 1990; Bello *et al.,* 2006). Other reports in ruminants include asymmetry in con-joined lambs (Willis, 1962).

Reports of dicephalus and diprosopus in White Fulani cattle, are rare in the literature. This survey, described gross and radiological observations of a dicephalus and a diprosopus in White Fulani foetuses in Nigeria.

Materials and Methods

In an abattoir survey of congenital malformations in White Fulani cattle in Abeokuta Ogun-State, Nigeria, two cases of malformed co-joined twins were observed in the central abattoir of Abeokuta metropolis where averagely 200 cattle were slaughtered per day. The survey was carried out between October 2008 and April 2009. Regrettably, there was no proper documentation of history of animals slaughtered in this abattoir; neither were there any adequate facilities for antemortem inspection. The histories of the two dams involved were unknown. They were purchased from itinerant Fulani herdsmen and brought to the abattoir for slaughter. These two foetuses were brought to the Department of Veterinary Pathology, Federal University of Agriculture, Abeokuta, Nigeria, for proper and thorough postmortem examinations. They were aged according to the method of Richardson (1980). Ventro-dorsal and dorso-ventral radiographs of diprosopus and dicephalic foetuses were obtained using X-ray machine (Siemens, Germany).

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Results

Case 1:

The diprosopus foetus was in good body condition, aged 4-months 9 days and weighed 7.5kg. The body of the foetus was hairless. There were two heads with two cerebral hemispheres which were set at an angle of about 140^0 to each other. There were two meninges with moderate cerebellar hypoplasia. There were maxillary and mandibular duplication resulting into two faces. The foetus showed two snouts, four orbits, four eyes, two oral cavities, two nostrils and four ears with two ears on the same base. There was agenesis of the rostra right aspect of the upper lip of the right head (Figure 1). Moderate cleft palate and absence of epiglottis were observed in the Buccal cavity of the right head. The two tongues were conjoined at the base just rostra to the single epiglottis. The oropharyngeal regions of each head merged to form a single laryngopharynx and oesophagus with partial duplication of the hyoid apparatus. Caudal to the larynx the foetus appeared normal. There were two separate cervical vertebrae which fused together at the thoracic inlet.

The radiography revealed two well outlined cranium fused medially by moderate soft tissues. Two distinct vertebral columns extended caudally from the foramen magnum of each cranium to the level of the thoracic inlet where they fused. Distal to the point of fusion, a single vertebra column ran through the entire thorax and the abdomen

Case 2:

Seven months after the diprosopus foetus, another case of female dicephalus was observed in the same abattoir. The foetus weighed 4.3 kg and aged 3-months 10 days. Two normal heads were present on two normal necks with a fused shoulder and thoracic region. Two forelimbs and two hind limbs were present. There were two vertebral columns along the entire body length. The two heads were perfectly formed showing four orbits, four nostrils, two mouth, four ears, and two oesophaguses (Figure 3). There were two lungs with two hearts, each supplying one half of the body. The two hearts were covered by one pericardial sac (Figure 4). The chambers of both hearts were well formed with the large vessels. In the thoracic cavity, there was one herniated diaphragm with two caudal vena cavae that fused to become one at the thoraco-abdominal junction. Each foetus had separate oesophagus which entered into separate rumens. Two reticulums, two omasums and two abomasums were observed. The right rumen was bigger (almost twice) than the left and herniated through the diaphragm into the thoracic cavity and formed a protrusion between the two necks. There were two spleens, each attached to each rumen, with the right spleen bigger than the left spleen. The left side of the proximal end of the right spleen twisted upward and revealed an outgrowth that budded (about 1cm) from the visceral surface of the splenic parenchyma (Figure 5).

The two duodena from each stomach fused distal to the pyloric sphincters. There was one pancreas and one well lobulated liver but with two gall bladders. Caudal to this point of fusion, all structures of the digestive and urogenital systems were single, although there was duplication of the vertebral column which formed kyphoscoliosis and eventually form two tails. The foetus had a single anus and vulva.

The radiograph revealed that the con-joined foetuses had two separate vertebral columns that ran through the entire length. Both vertebral columns showed dorsal curvature at the level of the cervical and lumbar vertebrae. Also, the con-joined foetuses had single common os-coxae and two separate tails (Figure 6).

Discussion

This abattoir survey described two cases of congenital anomalies (dicephalus and diprosopus) in White Fulani cattles in Abeokuta, South-Western part of Nigeria. Previous authors have documented dicephalus and diprosopus in cattles of different breeds, but report of these conditions within a short period of time (seven months) in a single breed of cattle, are rare in the literature. The gross morphological observations in the reports of Mcgirr *et al.*, (1987) and Vanderzon *et al.,* (1998) were similar to our findings. However, the presence of four forelimbs, herniated abomasum, abnormal ribs orientation, spinal bifida and common cavernous venous sinuous that joined the hearts were not observed in this report.

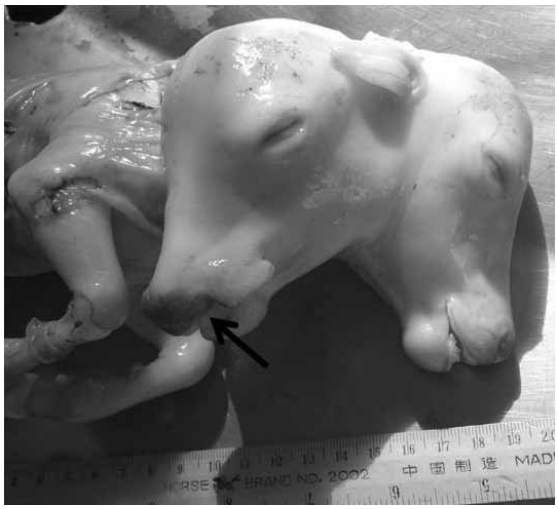

Fig. 1: Photograph of the Diprosopus foetus showing two face, two mouth, two snouts, four eyes, four ears and agenesis of the rostra left aspect of the upper lip of the right head (arrow).

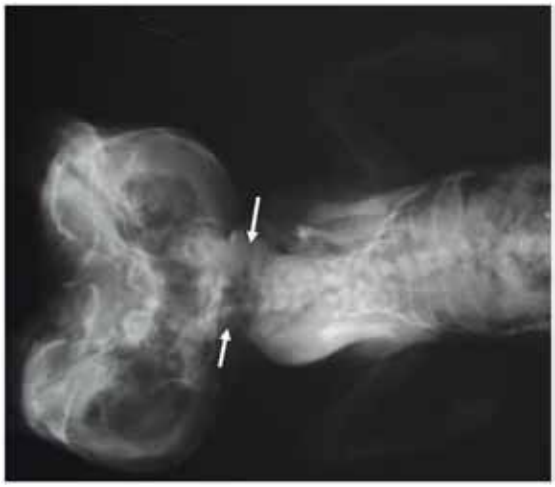

Fig. 2: Radiograph of the diprosopus foetus showing two cranium and two cervical vertebrae (arrows) which fused at the thoracic inlet.

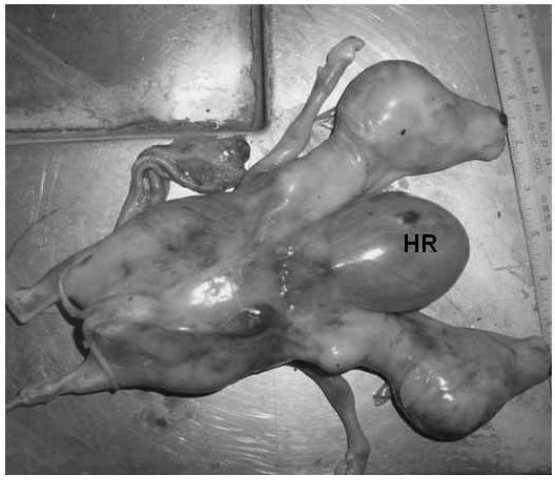

Fig. 3: Photograph of the dicephalic foetus with two heads, two vertebra columns with two tails and herniated rumen (HR).

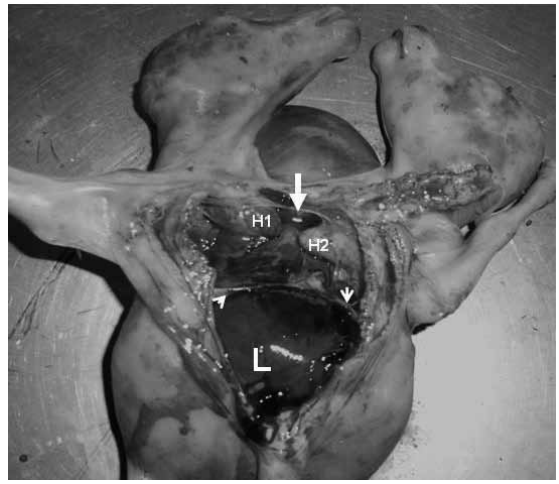

Fig. 4: Photograph of the dicephalic foetus showing one diaphragm (small arrows), one thoracic cavity, one liver (L) with two hearts (H1 and H2) covered by one pericardial sac (big arrow).

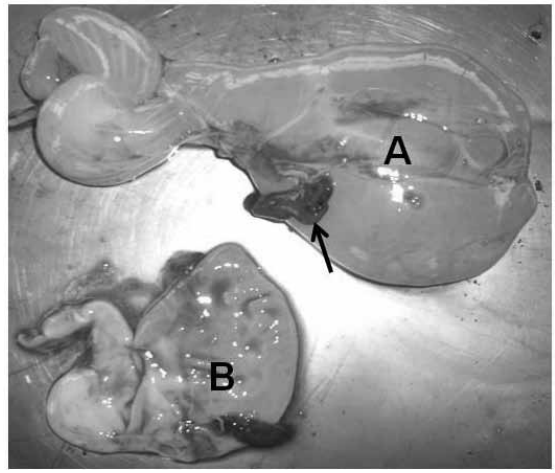

Fig. 5: Photograph of the dicephalic foetus showing the enlarged right complex stomach (A) with the budded outgrowth (arrow) on the spleen and the left small complex stomach (B).

The presence of one pericardial sac enclosing the two heart, herniation of enlarged right rumen, budded outgrowth on the visceral surface of the right spleen, kyphoscoliosis, cranial duplication of the caudal vena cava in the dicephalus foetus and agenesis of the rostral upper lip of the right head in the diprosopus foetus, to the best of our knowledge, are rare findings in the literature.

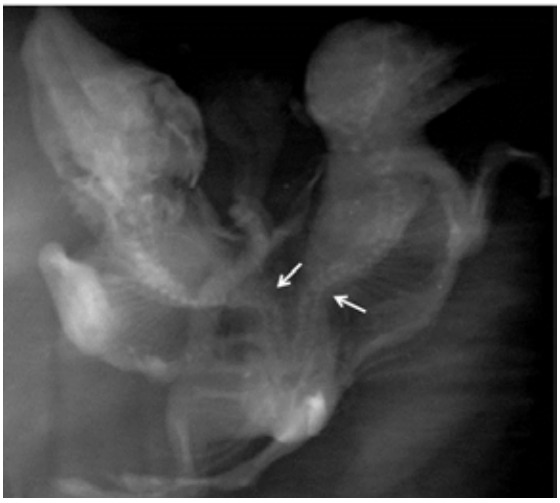

Fig. 6: A radiograph of the dicephalic foetus showing two vertebra columns with kyphoscoliosis (arrow) in the thoraco-abdominal region.

The incidence of these congenital anomalies in this breed of cattle remains unknown. Contrary to Leipold (1972) that showed the incidence of conjoined twins in cattle to be once in 100,000 births, this report revealed two anomalies in one abattoir within seven months, where an averagen of 200 head of cattle were slaughtered per day (i.e. approximately 42,000 cattle).

Conflicting reports abound in the literature as to the possible aetiopathogenesis of these anomalies (Easton, 1985). Some authors were of the opinion that they were due to genetic aberrations inherited from the dam (Fisher *et al.,* 1986, Mazzullo *et al.,* 2007b) while others incriminated malpositioning of the blastocyst which compresses the foetus during the crucial developmental stages of embryogenesis (Vanderzon *et al.,* 1998).

In this report, the cause of these anomalies is unknown. However, it is possible to speculate that teratogenic plants might have played a significant role, since Fulani herdsmen practice nomadic farming and most of their disease managements were basically on ethno-veterinary practices as suggested by Leeflag (1993).

Infectious agents such as Bovine viral diarrhea, Mucosa disease and Akabane viruses are also possible aetiologies (Hishinuma *et al.,* 1987), which unfortunately were not explored in these cases.

In humans, two theories have been proposed by Spencer (1992) to explain these anomalies. The first theory asserted that incomplete fission of a single embryonic disc occurred few days after fertilization and the second theory proposed that fertilized ovum divided completely into two embryonic discs and their unusual proximity results in secondary fusion. In this report the mechanisms of these anomalous formations cannot be explained, but it is possible that any of these conditions might have occurred through any of these pathways, since both showed duplication of the head and cervical vertebrae, but were only separated by soft tissues in the diprosopus foetus as revealed by the radiograph.

Various sequellae have been suggested in the literature with dams with congenital anomalies such as dystocia, uterine rupture, uterine prolapse, foetal and parturient cow death (Peter, 2004). In this report, despite the fact that these foetuses were not carried to term, yet the sequellae would have resulted into any of these conditions and cause great economic loss through decimation of animal population in the herd.

In conclusion, reports of dicephalic and diprosopus foetuses in White Fulani breed of cattle within a short period of seven months, are rare in the literature and the authors are unaware of any other report of dicephalic and diprosopus fetuses in this breed of cattle as it is documented in this report.

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

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

The Editor in Chief
December 2011

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

Aims and scope
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 livestock industry in Africa and to better utilization of animal genetic resources.

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

- Veterinary microbiology, epidemiology
- Marketing, Economics
- Infectious and non infectious disease
- Parasitology
- Genetic improvement and Biotechnology
- Animal production, nutrition and welfare
- Science and policy in animal health and production
- All aspects of honey bees, especially their social behavior, foraging and use of social and solitary bees for crop pollination activities
- Developments in beekeeping equipment and techniques
- Conservation biology:
- Global change and wildlife management
- Diseases and their impacts on wildlife populations
- Wildlife management in urban and agricultural environments
- Climate change impacts on animal resources in Africa
- Fisheries, aquatic fishery

Language
The language of submission should be either in English or French. The abstract is translated to the other three languages of the African Union , by the editors, after acceptance.
To be considered for publication in the BAHPA, any given manuscript must satisfy the following criteria:

- Originality. BAHPA does not accept manuscripts that have already been published elsewhere. However, studies that replicate results that are already in the literature may be considered for publication, as the independent confirmation of results can often be valuable, as can the presentation of a new dataset.
- Audience. Manuscripts submitted must be of broad interest to animal health and production professionals in general, they must capture and holds readers' attention.
- Usefulness. Manuscripts submitted must help researchers, trainers, educators and policy makers in all regions of Africa improve their effectiveness.
- Rigorous methodology. Manuscripts submitted must be based on valid and reliable information, documentation or sound concepts, empirically, logically and theoretically supported.
- Well written to ensure clear and effective presentation of the work and key findings. The BAHPA editorial staff does not copyedit the text of accepted manuscripts, it is therefore important for the work, as presented, to be intelligible. Perfect, stylish language is not essential but it must be clear and unambiguous. If the language of a paper is not clear, Academic Editors should recommend that authors seek independent editorial help before submission of a revision. Poor presentation and language is a justifiable reason for rejection.
- Experiments, statistics, and other analyses performed are described in sufficient detail. The research must have been performed to a technical standard to allow robust conclusions to be drawn from the data. Methods and reagents must also be described in sufficient detail so that another researcher is able to reproduce the experiments described Conclusions are presented in an appropriate fashion and are supported by the data. The results must be interpreted appropriately, such that all conclusions are justified. However, authors may discuss possible explanations for their results as long as these are clearly identified as speculations or hypotheses, rather than as firm conclusions. Inappropriate interpretation of results is a justifiable reason for rejection.
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Authors are invited to submit electronically their manuscripts via attachment only at bahpa@au-ibar.org (The use of an email submission speeds up the decision-making process, enables immediate distribution and allows authors to track the status of their own manuscripts) to the editor in a secured PDF and word format. Manuscript can be sent by post in case of unavailability of internet services (authors should be aware that in this case it will take longer time to be published).

Authors submitting articles to the BAHPA must follow the guidelines in this document. Submissions that deviate from these guidelines will be returned to the corresponding authors for changes and compliance. Your attention to and compliance with the terms and conditions described in the Authors Guidelines document is greatly appreciated! Adherence will increase the likelihood that your submission will be favorably reviewed, and will make the work of everyone involved – you, your reviewers, and your editors – easier.

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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.

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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. Please allow 3 months for the listing to be published.

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All manuscripts submitted to BAHPA should include the following features:

1. On page one of the manuscript, the following should be clearly written/inserted: the corresponding author, name of the institution, place where the work was carried out, 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 and References.
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 findings presented and the conclusion(s) reached. Up to six keywords should be provided. The abstract should contain the objectives, brief description of materials and methods, highlights of significant results, conclusions and recommendations.
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. Identify the statistical tests used to analyze the data. Indicate the prospectively determined P value that was taken to indicate a significant difference. Cite only textbook and published article references to support your choices of tests. Identify any statistics software used.
7. Results or experimental data should be presented clearly and concisely, in a non-repetitive way. Subheadings may be accepted
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9. State the conclusions, theories, implications, recommendations that may be drawn from the study.
10. Provide a paragraph of around 100 words only, explaining the importance of the manuscript's findings for a non-specialist audience. These points will be published at the end of the article in a box entitled 'Impact'.
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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.
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3. Use 1 inch margins on top, bottom, left and right margins,
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Examples of References

- Journal Articles: Ouyang D, Bartholic J, Selegean J, 2005. Assessing sediment loading from agricultural croplands in the Great Lakes basin. Journal of American Science, 1(2): 14-21.
- Books: Durbin R, Eddy SR, Krogh A, Mitchison G, 1999.

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

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