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BULLETIN OF ANIMAL HEALTH AND PRODUCTION IN AFRICA

VOL. 61 NO. 1 **CONTENTS** **MARCH, 2013**

ORIGINAL ARTICLE **PAGE**

1. Comparative Infectivity and Pathogenicity of Kano and Sokoto (Northern Nigeria) Isolates of Trypanosoma Evansi in Rabbits Jatau, *ID, Muhammad, ST, Umaru-Sule, B, Yusuf, KH, and Owolabi, YH* 7

2. Immunogenicity and Pathology of Formalin-Killed-Sepa Salmonella Enterica Paratyphi A Antigen in Pullet. *Ogunleye A. O., Ajuwape A. T. P., Alaka O. O., Adetosoye A. I*..... 17

3. Serological and Virological Study of Newcastle Disease and Avian Influenza in Chickens in Rural Areas in Senegal. *Kone P., Enede F., Rabeson F. A., Sene M., Feussom-Kameni Jean-Marc, Sow A., Gueye A., Alambedji R., Missohou A., Akakpo A. J* 25

4. Enquêtes Entomologique et Parasitologique sur les Trypanosomoses Bovines à la Station D'avétonou/Itra, au Togo. *Talaki E., Dao B., Kulo A., N'Féidé T*..... 33

5. Quantitative Analysis of Oxytetracycline Residue in Beef and Chicken Meat From Cities of Southwest Nigeria. Olatoye I O and Ogundipe G A T 39

6. Effect of Aqueous Extract of Euphorbia Hirta on Haematological and Biochemical Parameters of Sprague Dawley Rats. *Otsyina H R, Ansah C, Duwiejua M, Woode E, Aboagye FA and Aning KG*..... 49

7. Effet de la Fertilisation au Molybdène sur les Biomasses et le Nombre des Nodules D'arachis Glabrata à Différentes Périodes de Fauche dans L'ouest Cameroun. *Mboko A. V., Tendonkeng F., Matumuini F. N. E. , Zougou G. T., Boukila B. et Pamo E. T*..... 57

8. Effect of Sorghum - Barley Brewer's Spent Grain as a Feed Ingredient on Broiler Performance and Carcass Characteristics. Manu-Barfo P., Nortey T.N.N. and Naazie A. 67

9. Growth Response and Carcass Traits of Birds Fed Diets Containing Three Differently Processed Discarded Vegetable-Bovine Blood-Rumen Content Mixtures. *Ekunseitan, D. A., Balogun, O.O, Sogunle O. M., Yusuf O. A., Ayoola A. A., Ekunseitan, O. F.* 77

10. The Effect of Feeding Sorghum-Barley Brewer's Spent Grain (Sbbsg) on Performance and Egg Quality of Laying Hens. *Nortey T. N., Naazie A. and Manu-Barfo P*..... 91

11. Digestibilité in vivo des Feuilles Fraîches de Bananier (Musa Sp.) Traitées à la Mélasse Chez les Brebis Djallonké (Ovis Aries) *Boukila B., Tendonkeng F., Pamo T. E., Matumuini N.E. F., Mboko A. V., Miegoue E. et Allogo A. R*..... 103

12. Factors Affecting Virulence of E. Coli Isolates from Diarrheic Sheep and Goats. *El-Mahrouk A. M. O. M G. Agour and A.M. Montasser* 111

COMPARATIVE INFECTIVITY AND PATHOGENICITY OF KANO AND SOKOTO (NORTHERN NIGERIA) ISOLATES OF TRYPANOSOMA EVANSI IN RABBITS.

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Abstract

The present study was aimed at comparing the infectivity and pathogenicity of two isolates of Trypanosoma evansi in experimentally infected rabbits. Three groups each of five crossbred rabbits, two of which were challenged with approximately 2 x 106 T evansi of the Kano (KT) and Sokoto (ST) isolates, respectively, while the third group was the uninfected control (UC). The rabbits were monitored for parasitaemia, rectal temperature, clinical signs and changes in the packed cell volume (PCV), total leukocyte count and total plasma protein during the course of the infection. Impression smears of the brain, liver, lungs, heart, spleen, kidney, adrenal gland, bone marrow and aqueous humour were made, stained with Giemsa and examined for the presence of trypanosomes. The result revealed that all the infected rabbits developed intermittent fever and parasitaemia, with mean pre-patent periods of 3.6±1.8 and 7.8±3.1 days for the KT and ST groups, respectively. There was a decrease in mean PCV of the infected groups compared with the control. The KT group had the lowest PCV throughout the study compared with the ST and UC groups. There was a significant inverse correlation ($r = -0.7262$, $P < 0.05$) between parasitaemia and the PCV in the KT group, but not in the ST group ($r = -0.6070$, $P > 0.05$). Leukocytosis was higher in the ST group compared with the KT group all through the study, and was significantly higher ($P < 0.05$) at days 11 and 18 post infection. Trypanosomes were observed in 56% of the organ impression smears from the KT group as against 11% in the ST isolate. Both isolates were infective to the rabbits, but the KT isolate appears to be more virulent than the ST isolate.

Key words: *Trypanosoma evansi isolates*, pathogenicity, rabbits, Northern Nigeria

ETUDE COMPARATIVE DE L'INFECTIVITE ET DE LA PATHOGENICITE DES ISOLATS DE TRYPANOSOMA EVANSI CHEZ LES LAPINS DE KANO ET SOKOTO (NORD DU NIGERIA).

Resume

La présente étude avait pour objectif de comparer l'infectivité et la pathogénicité de deux isolats de Trypanosoma evansi chez des lapins expérimentalement infectés. Trois groupes, comprenant chacun cinq lapins croisés, ont été utilisés pour l'étude. Deux groupes ont été infectés respectivement avec environ 2 x 106 T evansi des isolats de Kano (KT) et de Sokoto (ST), tandis que le troisième groupe constituait le groupe témoin non infecté (UC). Les lapins ont été suivis pour établir la parasitémie, la température rectale, les signes cliniques et les changements de l'hématocrite (PCV), la numération leucocytaire totale et la protéine plasmatique totale pendant l'infection. Des prélèvements par empreinte directe sur le cerveau, le foie, les poumons, le coeur, la rate, les reins, les glandes surrénales, la moelle osseuse et l'humeur aqueuse ont été receuillis, colorés au Giemsa et examinés pour détecter la présence de trypanosomes. Le résultat a révélé que tous les lapins infectés ont développé une fièvre intermittente et une parasitémie, avec des périodes pré-patentes moyennes de 3,6 ± 1,8 et 7,8 ± 3,1 jours respectivement pour les groupes KT et ST. On a observé une diminution de la valeur moyenne de l'hématocrite (PCV) chez les groupes infectés par rapport au groupe témoin. Le groupe KT avait la plus faible PCV tout au long de l'étude par rapport aux groupes ST et UC. On a relevé une corrélation inverse significative ($r = -0,7262$, $P < 0,05$) entre la parasitémie et la PCV dans le groupe KT, mais pas dans le groupe ST ($r = -0,6070$, $p > 0,05$). La leucocytose était plus élevée

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dans le groupe ST par rapport au groupe KT tout au long de l'étude, et était significativement plus élevée (P <0,05) aux jours 11 et 18 post-infection. Des trypanosomes ont été observés dans 56% des calques d'organes chez le groupe KT contre 11% dans l'isolat ST. Les deux isolats étaient infectieux pour les lapins, mais l'isolat KT semble être plus virulent que l'isolat ST.

Mots-clés: isolats de Trypanosoma evansi, pathogénicité, lapins, Nord du Nigeria

Introduction

The disease caused by Trypanosoma evansi, commonly known as "surra" is an important and most widely distributed *trypanosomosis* occurring in wide range of hosts including man (Reid, 2002; WHO, 2005). *T. evansi* is transmitted mechanically through the oral parts of haematophagous insects, mainly from the genera Stomoxys and Tabanus (Luckins, 1999). Surra is a major constraint to the improved and sustainable production of camel in arid areas of Africa (Elamine *et al.,* 1999). Economic losses are usually due to mortality, reduced animal work output, reduced work tolerance, poor carcass quality, weight loss, reduced milk yield, low reproductive efficiency and cost of treatment (Seidl *et al.,* 1998). Anaemia is the main outcome of infection by *T. evansi*, and resistance to the development of anaemia as well as control of parasitemia, reflect the degree of tolerance to infection by the hosts (Trail *et al.,* 1991).

In Nigeria particularly in the Northern parts, surra is increasingly gaining economic importance due to the increasing value of camel as a source of meat (Falope, 1991) and draught animals (Mohammed, *et al.,* 1997; Abdullahi, 2006). The common practice of herding camels with sheep and goats by pastoralists in northern Nigeria may likely expose these animals to the risk of infection by *T. evansi* as experimental studies showed that sheep and goats are susceptible to the parasite (Audu *et al.,* 1999 and Shehu *et al.,* 2006). Despite its increasing importance, *T. evansi* is the least studied of all the animal trypanosomes in Nigeria. Consequently there are little available information with regards to the epidemiology and pathogenesis of *T. evansi* in Nigeria. More so, since there is considerable variation in the severity of disease caused by *T. evansi* infection in different geographical areas of its occurrence (Herrera *et al.,* 2004); it is therefore, conceivable to evaluate the pathogenicity of local strains of this parasite.

The aim of present study was to compare the infectivity and pathogenicity of isolates of *T. evansi* obtained from naturally infected camels from Kano and Sokoto states of North-western Nigeria in experimentally infected rabbits.

Materials and Methods

Experimental animals: Fifteen (3 males and 12 females), Chinchilla x New Zealand white cross bred rabbits weighing between 0.45kg to 0.875kg were used for the study. The rabbits were housed in a standard rabbit cages in the Department of Veterinary Parasitology and Entomology, Ahmadu Bello University, Zaria. The animals were allowed to acclimatise for 2 weeks during which they were screened and treated for gastrointestinal and haemoparasitic infections. They were fed throughout the experiment with commercial grower mash (Feed Masters®) and water was supplied ad libitum.

The *Trypanosoma evansi isolates*: The two isolates of T evansi namely Kano isolate (KT) and Sokoto isolate (ST) were isolated from naturally infected camels at slaughter at Kano and Sokoto municipals abattoirs respectively. The isolates were maintained by two syringe passages in albino rats in the Department of Veterinary Parasitology and Entomology Ahmadu Bello University Zaria before the commencement of the experiment.

Kano and Sokoto states are among the seven states of the north-western geopolitical zones of Nigeria. They are located within the Sudan savannah vegetation zones of Nigeria with distinct dry and wet seasons. Dry season starts from October through April to May or June in some parts. Wet season begins in most parts in May and last up to September or October with mean annual rainfall of between

510 – 1140 mm (Agabi, 1995).

Experimental grouping and infection: The rabbits were divided into 3 groups of 5 rabbits (1 male and 4 females) and identified as KT, ST and uninfected control (UC) groups. The three groups of rabbits were housed separately with feed and water given separately. Two donor albino rats each with high parasitaemia of the KT and ST isolates of *T. evansi* respectively were humanely sacrificed and their blood was collected into a separate labelled sample bottles containing salts of Ethylene diamine tetera acetate (EDTA) as anticoagulant. The parasitaemia in each of the collected blood was adjusted to 2 x 106 trypanosomes per milliliter (Herbert and Lumsden, 1976) by diluting with physiological saline solution. Each rabbit from KT and ST groups were inoculated intraperitoneally with 1ml of the expanded blood containing the KT and ST isolates, respectively. The experimental animals were allowed to pass through the course of the infection.

Collection of blood samples: Blood was aseptically collected from each rabbit by veinpuncture of the ear vein. For the first ten days post infection and then twice weekly, 0.1ml of blood was collected from each infected rabbit for parasite detection and estimation. At the day of infection and thereafter twice weekly until the end of the experiment, 1ml of blood samples meant for determination of packed cell volume, total plasma protein, total leukocytes counts were also collected in sample bottles containing 1mg of EDTA as anticoagulant.

Parasitaemia detection and estimation: Trypanosomes were detected using wet blood film and parasitaemia were estimated using the rapid "marching" method described by Herbert and Lumsden (1976). Parasitaemia was determined initially daily for 10 days and there after twice weekly.

Haematological examinations: Packed Cell Volume (PCV) and Total white blood cells count (WBC) were determined using the standard microcapillary and heamocytometric methods, respectively, as described by Jain (1986). Total plasma protein was determined using a refractometer method (Coulter Electronics, Hearts England) as described by Kerr (1989).

Monitoring of daily rectal temperature and clinical signs: The rectal temperature of each of the experimental animals was measured and recorded early in the morning using digital clinical thermometer daily for the first 11 days following infection and then twice weekly for the remaining period of the experiment. The infected rabbits were also monitored for clinical signs throughout the course of the experimental infection.

Preparation and examination of organ impression smears: At the end of the experiment two rabbits from each group were humanely sacrificed following chloroform anaesthesia after which brain, liver, lungs, heart, spleen, kidney, adrenal gland, bone marrow and aqueous humour were collected and used for the preparation of Giemsa stained organ impression smears as described by Adam et al. (1971). The smears were examined using light microscope at x 1000 magnification for the presence of trypanosomes.

Data analysis: The data were summarized as mean ± SDM and analyzed by one way analysis of variance (ANOVA), Tukey's multiple comparison tests and Pearson's correlation test using Graph pad software version 5.0 (Graphpad Prism, 2010). Values of $p < 0.05$ were considered as statistically significant.

Result

All the experimental rabbits infected with the KT and ST isolates of the *T. evansi* developed intermittent parasitaemia with mean pre-patent periods of 3.6±1.8 and 7.8±3.1 days, respectively. The parasitaemia was high and fulminating in the KT infected rabbits with peak mean parasitaemia of 105±21.0 x 106/ml of blood at 15 days post infection, while infection with ST was however characterized by low parasitaemia with highest peak parasitaemia of 21.33 ±20.84 x 106/ml of blood at day 18 post infection (Figure 1).

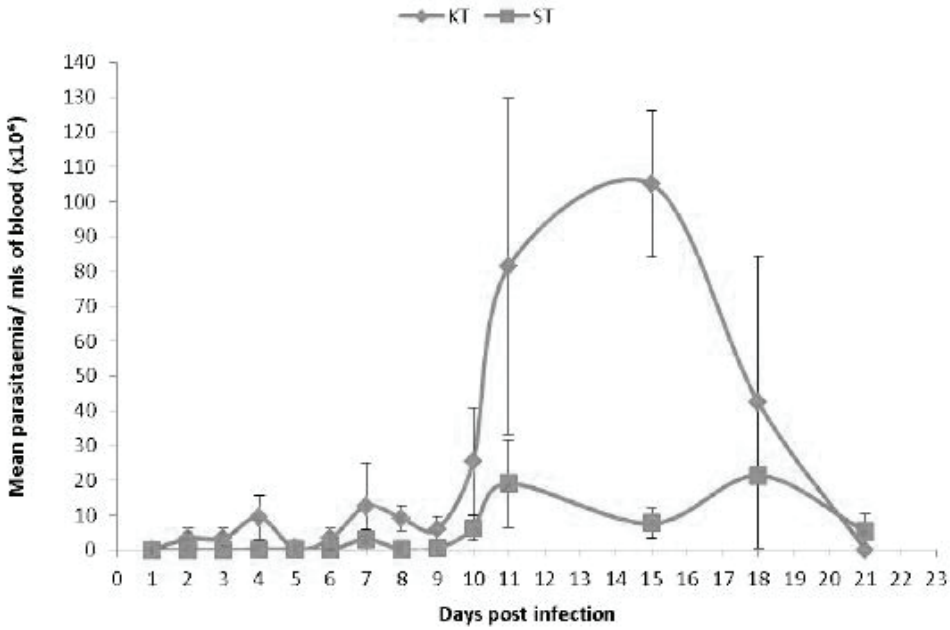


Figure 1: Mean parasitaemia values of rabbits experimentally infected with Kano (KT) and Sokoto (ST) isolates of Trypanosoma evansi.

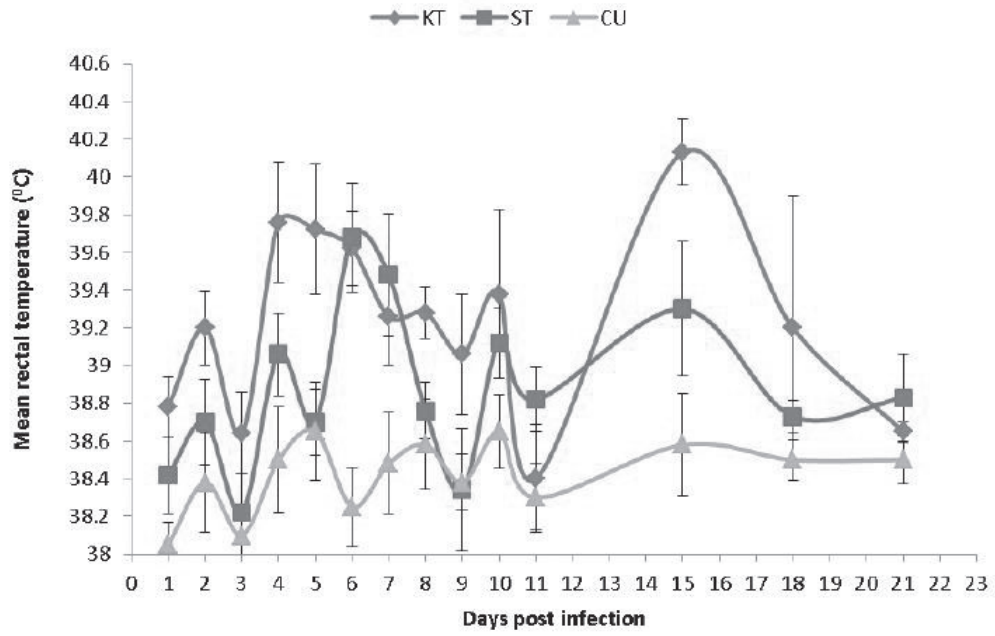


Figure 2: Mean rectal temperature of uninfected (CU) rabbits and those experimentally infected with Kano (KT) and Sokoto (ST) isolates of Trypanosoma evansi.

Intermittent rise in rectal temperature was observed in the infected rabbits during the course of the infection compared with the temperature of the uninfected rabbits. Peak temperature increase of 39.8±0.320C and 40.1±0.180C were observed at day 4 and 15 post infection, respectively in the KT infected rabbits. The highest mean rectal temperature values of 39.7±0.290C and 39.3±0.360C were observed in the ST infected rabbits at 6 and 15 days post infection, respectively. However, the highest mean temperature observed in the

uninfected group of rabbits was 38.7±0.230C and was recorded at days 5 and 10 of the experiment (Figure 2). Although the observed fever in both the infected groups coincides with the period of parasitaemia, there was however no statistically significant correlation (KT, r = 0.1755, p>0.05; ST, r = 0.06297, p>0.05) between parasitaemia and rectal temperature in both KT and ST groups (Table 4).

Clinical signs displayed by both infected groups of rabbits were dullness, depression, weakness, rough hair coat, epiphora and areas of alopecia around the rump and tail regions. Pale ocular mucous membrane and mortality rate of 60% (3 out of the 5 rabbits) were observed in the KT group. Two out of the 5 (40%) rabbits infected with the ST isolate also died one each at 18 and 21 days of infection. Clinical signs were not observed in the control rabbits.

The mean PCV of the KT infected rabbits decreases progressively during the course of the infection from the initial mean value of 32.0±5.24% to the lowest value of 22.3 ±3.51% at day 15 post infection. The mean PCV of this group however later appreciated to 29.0 ±2.83% 6 days latter (21 dpi). Mean PCV values of the KT group were significantly ($p < 0.05$) lower at days 8, 11, 15 and 18 post infection (Table 1).

However, a gradual decrease in mean PCV was observed in the ST group from the initial value of 39.0 ±5.24% to a minimum mean value of 26.3 ±2.63% at day 15 post infection. The PCV value of this group later improved to 33.3 ±6.42 at 21 days post infection. The decrease in the PCV of the ST infected rabbits was only statistically significant at day 15 post infection compared with control. The PCV of the uninfected rabbits remained relatively unchanged from the initial mean of 33.5 ±4.65% during the course of the experiment, with the minimum and maximum values of 32.5 ±3.7% and 35.5 ±3.79%, respectively, observed at days 11 and 21 post infection (Table 1). There was an inverse statistically significant correlation ($r = -0.7262$, $P < 0.05$) between parasitaemia and PCV in the KT infected group. The correlation between parasitaemia and PCV was however not significant ($r = -0.6070$, $P > 0.05$) in the ST group (Table 4).

Infection with ST isolate of the T. evansi was characterized by progressive leukocytosis from the initial mean WBC count of 4.3 ± 1.33 x109/L to a statistically significant ($p < 0.05$) mean counts of 11.1 ±4.43 x109/L and 17.6 ±2.85 x109/L on days 11 and 18 post infection, respectively when compared with KT and control. Meanwhile the WBC counts of the KT infected rabbits remained relatively within the range of the initial mean count of 4.8 ±1.45 x109/L during the course of the experimental infection with mild statistically insignificant ($p > 0.05$) leukocytosis of 7.4 ± 4.9 x109/L and 6.2 ±1.56 x109/L at 8 and 21 days post infection, respectively. Leukocyte counts of the uninfected rabbits was also relatively steady within the range of the initial mean count of 4.9 ± 0.21 x109/L with the minimum and maximum counts of 3.9 ± 1.11 x109/L and 5.5 ± 0.70 x109/L observed at days 2 and 8 post infection (Table 2). The leukocytosis observed in the ST infected rabbits significantly correlated ($r = 0.7958$, $p<0.05$) with parasitaemia (Table 4).

The mean total plasma protein values of the control, ST and KT groups were relatively maintained within their initial mean values of 5.9 ±0.26 g/dL, 5.9 ± 0.85 g/dL and 6.1± 0.74 g/dL, respectively, throughout the course of this experiment. However, a sudden significant ($p <0.05$) rise in mean total protein value (7.1 ± 0.61 g/dL) of the KT infected rabbits was observed at day 2 post infection (Table 3). There was no statistically significant correlation between parasitaemia and changes in total plasma protein concentration in the KT ($r = -0.1026$, $p >0.05$) and ST ($r = -0.1626$, $p >0.05$) infected rabbits (Table 4).

Observation of the organ impression smears revealed few degenerated and some complete trypanosomes in the adrenal gland, liver, heart, lung and kidney of the KT group. In the ST group degenerated trypanosomes were only seen in the lung (Table 5.0).

Discussion

Despite inoculation of the rabbits with approximately same number of trypanosomes, shorter pre-patent period and higher level of parasitaemia were observed in the KT isolate infected rabbits. This may suggests that the KT

Table 1: Mean PCV (%) ±SD of uninfected control (UC) rabbits and those experimentally infected with Sokoto (ST) and Kano (KT) isolates of Trypanosoma evansi.

Groups	Days post infection (dpi)								
	0	2	5	8	11	15	18	21	Remarks
UC (n = 5)	33.5 ±4.65[a]	(27.0 - 40.0)	33.0 ±4.08[a]	32.8 ±3.59[b]	32.5 ±3.70[b]	34.0 ±3.56[a,b]	34.3 ±3.86[b]	35.5 ±3.79[a]	No mortality
	(29.0 - 40.0)	(28.0 - 39.0)	(30.0 - 39.0)	(30.0 - 38.0)	(29.0 – 37.0)	(30.0 - 37.0)	(30.0 - 38.0)	(30.0 - 38.0)	
ST (n = 5)	39.0 ±5.24[b]	36.6 ±3.58[b]	32.0 ±4.30	28.6 ±3.13[a]	29.0 ±2.92[a]	26.3 ±2.63[a]	27.0 ±2.00[a]	33.3 ±6.42[b]	n = reduced to 4 & 3 at 18 and 21 dpi respectively
	39.0 ±5.24[b]	(33.0 - 42.0)	(26.0 - 38.0)	(26.0 - 32.0)	(25.0 - 32.0)	(24.0 - 30.0)	(25.0 - 29.0)	(26.0 - 38.0)	
KT (n = 5)	32.0±5.24[c]	33.0±5.24[c]	27.6±4.51[c]	23.6 ±2.61[b]	23.6 ±4.62[b]	22.3 ±3.51[b]	23.7 ±3.79[b]	29.0 ±2.83[c]	n = reduced to 4, 3 & 2 at 15,18 and 21 dpi respectively
	(27.0 - 40.0)	33.0±5.24[c]	(37.0 - 42.0)	(20.0 - 26.0)	(20.0 - 30.0)	(19.0 - 26.0)	(21.0 - 28.0)	(27.0 - 31.0)	

Table 2: Total WBC counts (x109/L) ±SD of uninfected control (UC) rabbits and those experimentally infected with Sokoto (ST) and Kano (KT) isolates of Trypanosoma evansi.

Groups	Days post infection (dpi)								
	0	2	5	8	11	15	18	21	Remarks
UC (n = 5)	4.9 ± 0.21[a]	3.9 ± 1.11[a]	4.1 ± 0.79[a]	5.5 ± 0.70[a]	4.6 ± 0.59[a]	4.8 ± 0.98[a]	5.3 ± 0.89[a]	4.3 ±0.31[a]	No mortality
	(4.6 - 5.1)	(2.8 - 5.4)	(3.1 – 5.0)	(4.8 - 6.1)	(4.0 – 5.2)	(3.6 - 6.0)	(4.3 – 6.2)	(3.9 – 4.6)	
ST (n = 5)	4.3 ± 1.33[b]	8.6 ± 6.88[b]	8.0 ± 4.65[b]	9.4 ± 3.99[b]	11.1 ±4.43[a,b]	10.8 ± 6.34[b]	17.6 ±2.85[a,b]	10.2 ±7.25[b]	n = reduced to 4 & 3 at 18 and 21 dpi respectively
	(2.6 – 6.0)	(5.0 - 20.9)	(4.6 - 16.1)	(6.1 - 15.8)	(7.2 - 18.2)	(5.6 - 20.0)	(15.1 - 20.7)	(7.9 - 12.4)	
KT (n = 5)	4.8 ±1.45[c]	4.1 ± 1.81[c]	4.2 ±1.94[c]	7.4 ± 4.91[c]	4.5 ± 1.54[b]	4.4 ±1.20[c]	5.0 ± 0.91[b]	6.2 ±1.56[c]	n = reduced to 4, 3 & 2 at 15,18 and 21 dpi respectively
	(3.1 - 7.1)	(2.1 - 6.6)	(2.1 - 6.0)	(2.8 - 14.1)	(3.0 - 6.5)	(3.2 - 5.6)	(4.0 – 5.8)	(6.0 - 7.0)	

Table 3: Total plasma protein (g/dL) ±SD of uninfected control (UC) rabbits and those experimentally infected with Sokoto (ST) and Kano (KT) isolates of Trypanosoma evansi.

Groups	Days post infection (dpi)								
	0	2	5	8	11	15	18	21	Remarks
UC (n = 5)	5.9 ±0.26[a]	5.9 ±0.12[a]	6.0 ±0.34[a]	6.2 ± 0.43[a]	6.2 ± 0.43[a]	6.2 ± 0.54[a]	6.0 ± 0.76[a]	6.5 ± 0.26[a]	No mortality
	(5.6 - 6.2)	(5.8 - 6.0)	(5.6 - 6.4)	(5.8 - 6.8)	(5.8 – 6.8)	(5.8 - 7.0)	(5.0 – 6.8)	(6.2 – 6.8)	
ST (n = 5)	5.9 ± 0.85[b]	6.8 ± 0.86[b]	6.0 ±1.03[b]	5.4 ± 1.15[b]	5.4 ± 1.08[b]	5.9 ± 1.25[b]	6.3 ± 0.58[b]	6.5 ± 0.50[b]	n = reduced to 4 & 3 at 18 and 21 dpi respectively
	(5.0 – 7.0)	(5.8 - 7.6)	(4.8 - 6.8)	(4.0 - 6.4)	(4.0 - 6.8)	(4.2 - 7.0)	(6.0 - 7.0)	(6.0 - 7.0)	
KT (n = 5)	6.1± 0.74[c]	7.1 ± 0.61[a]	6.4 ±0.56[c]	5.5 ± 0.66[c]	5.9 ± 0.33[c]	6.3 ± 0.6[c]	5.7 ± 0.50[c]	6.0 ±0.0[c]	n = reduced to 4, 3 & 2 at 15,18 and 21 dpi respectively
	(5.1 - 7.1)	(6.6 - 7.9)	(5.8 - 7.0)	(4.4 - 6.0)	(5.6 - 6.4)	(5.8 - 7.0)	(5.2 – 6.2)	(6.0 - 6.0)	

Mean values with same superscripts within column are statistically significant: $p<0.05$.
UC= Uninfected control, ST= Sokoto isolate infected group, KT= Kano isolate infected group n= number of rats in the group, ( ) = range

Table 4: Relationship between parasitaemia, rectal temperature, PCV, total WBC count and total plasma protein in rabbits infected with Kano (KT) and Sokoto (ST) isolates of *T. evansi*

Correlated Variables	Pearson's correlation, r	P –value
Parasitaemia and rectal temperature	KT - 0.1755	0.55
	ST - 0.06297	0.83
Parasitaemia and packed cell	KT - 0.7262	0.04*
	ST - 0.6070	0.11
Parasitaemia and total WBC count	KT - 0.06944	0.87
	ST - 0.7958	0.02*
Parasitaemia and total plasma protein	KT - 0.1026	0.81
	ST - 0.1626	0.70

Table 5: Organ/tissue distributions of Kano (KT) and Sokoto (ST) isolates of Trypanosoma evansi in experimentally infected rabbits.

Organs	KT group	ST group
Brain	-	-
Eye	-	-
Lung	+	+
Heart	+	-
Liver	+	-
Spleen	-	-
Adrenal gland	+	-
Kidney	+	-
Bone marrow	-	-

Key: + = trypanosomes seen, - = no trypanosome seen

isolate is more virulent than the ST isolate as the former was able to multiply and establish parasitaemia earlier than the later. Egbe-Nwiyi et al. (2003) and Jatau et al. (2012) made similar observation with virulent relapsed isolates of and *T. evansi* respectively, in experimentally infected rats. According to Trail et al. (1991) the degree of parasitaemia in *Trypanosoma* infected animal is among the indices determining the severity of the disease.

The pyrexia and other clinical signs observed in this work coincided with the patency of infection and is in agreement with findings of Orhue and Nwenze, (2004) in T. brucei infected rabbits. Pyrexia is a consistent finding in *trypanosomosis* and usually follows the pattern of parasitaemia in the infected animals (Taylor and Authie, 2004)

Significant decrease in PCV values was observed in the infected rabbits which correlate with the periods of high parasitaemia. This is in agreement with the previous records of *T. evansi* infections (Gutierrez et al. 2005; Shehu *et al.,* 2006; Jatau *et al.,* 2012) and it strongly suggests that the presence of live trypanosomes in circulation and the level of parasitaemia may be responsible for the development of anaemia (Holmes and Jennings, 1976; Trail *et al.,* 1991). Anaemia is a consistent finding in *trypanosoma*-infected animal and determines the severity of infection (Anosa, 1988; Trail *et al.,* 1991). The significant and progressive decrease in PCV of the KT infected rabbits as against the mild anaemia observed in the ST group further suggest that the KT isolate may be more pathogenic than the ST isolate in the infected rabbits.

Moreover the significant leucucocytosis observed in the ST infected rabbits is an indication that this group responded favourably to the infection than the KT infected group. This is based on the fact that tolerant animals

develop leukocytosis and non-tolerant animals develop leucopenia (Paling *et al.,* 1991).

It was observed that the *T. evansi* isolates were able to invade some of the organs examined. Previous work had also shown that *T. evansi* was capable of invading these organs (Sudarto, *et al.,* 1990; Jatau *et al.,* 2010). Similar observation has also been made by Lawal et al. (2007) in experimentally *T. brucei* infected rats. The invasive ability of the *T. evansi* isolates therefore, seems to be in line with that of the brucei group, to which *T. evansi* belongs (Losos, 1980). The fact that the KT isolate was able to invade about 56% of the organs observed as against the ST isolate which was only found in 11% of the organs is suggestive that the former is more tissue invasive than the later.

Conclusion

From the findings of this study, it can be concluded that rabbits are suitable model for the pathogenicity studies of *T. evansi* infection, since all the infected group of the animals (KT, and ST groups) showed similar signs but with varying degree of severity in which Kano isolate of *T. evansi* appears to be more virulent than the Sokoto isolate. More so the result of the study may also suggests that rabbits especially those in the wild could play a role in the enzootiology of *Trypanosomosis* due to *T. evansi* in endemic areas.

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IMMUNOGENICITY AND PATHOLOGY OF FORMALIN-KILLED-SEPA *SALMONELLA ENTERICA PARATYPHI A* ANTIGEN IN PULLET.

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Abstract

Intensive poultry production in Nigeria is currently on the increase with associated health challenges. This work investigated the immunogenicity of a documented *Salmonella enterica Paratyphi A* (SEPA) of poultry. A formalin-killed-SEPA containing 1x107cfu/ml was used as antigen. Seventy-five pullets were immunized orally with the antigen at 4 weeks of age and another 25 pullets (UC) served as unimmunized control. First and second booster doses were given at 10th and 29th day post-immunization (p.i). Three groups of immunized pullets (IA, IB, and IC) each of the 25 pullets were challenged with 0.5ml broth containing: 1x108cfu/ml SEPA (IA), 1x108cfu/ml Salmonella Gallinarum-(SG) (IB), while the immunized control group (IC) was fed with sterile broth. The unimmunized control group (UC) was infected with 1x108cfu /ml *Salmonella enterica Paratyphi A*. The formalin-killed vaccine of SEPA was immunogenic in poultry as indicated by the presence of O and H antibodies in the sera of pullets, producing protection only in pullets challenged with homologous strain, whereas morbidities and mortalities were recorded in unimmunized UC pullets challenged with homologous SEPA strain and IB challenged with heterologous strain (SG) during a 21-day experimentation. Also the inactivated-SEPA vaccine was able to protect birds against homologous challenge. These findings show that the inactivated-SEPA as a potential candidate vaccine offers protection of pullets against the emerging SEPA infection in pullets to avert severe economic losses in growing poultry industry and make the much needed animal protein available to feed the nation's population.

Keywords: *Salmonella enterica Paratyphi A*, immunogenicity, vaccine, poultry.

IMMUNOGENICITE ET PATHOLOGIE DE L'ANTIGENE DE *SALMONELLA ENTERICA PARATYPHI A* (SEPA) TUEE AVEC DU FORMOL CHEZ LA POULETTE

Résumé

La production intensive de volaille au Nigeria augmente actuellement, et engendre des problèmes de santé connexes. Ce travail a étudié l'immunogénicité de *Salmonella enterica Paratyphi A* (SEPA) documentée de la volaille. Une SEPA tuée avec du formol contenant 1x107cfu/ml a été utilisée comme antigène. Soixante-quinze poulettes âgées de 4 semaines ont été immunisées par voie orale avec cet antigène, et 25 autres poulettes (UC) ont servi de groupe témoin non vacciné. Les première et deuxième doses de rappel ont été données aux 10ème et 29ème jours post-immunisation (p.i). Trois groupes de poulettes immunisées (IA, IB, et IC), chacun comprenant 25 poulettes, ont reçu 0,5 ml de bouillon contenant 1x108cfu/ml SEPA (IA), 1x108cfu/ml Salmonella Gallinarum-(SG) (IB), tandis que le groupe témoin immunisé (IC) a reçu du bouillon stérile. Le groupe témoin non immunisé (UC) a été infecté avec 1x108cfu /ml de Salmonella enterica de sérotype Paratyphi A. Le vaccin à la SEPA tuée avec du formol était immunogène chez la volaille comme l'a montré la présence des anticorps O et H dans le sérum des poulettes, produisant une protection uniquement chez les poulettes exposées à une souche homologue, alors que des morbidités et mortalités ont été enregistrées chez les poulettes UC non immunisées exposées à la souche homologue SEPA et les poulettes IB exposées à la souche hétérologue (SG) pendant une expérimentation de 21 jours. De plus, le vaccin à base de SEPA inactivée a pu protéger les oiseaux contre la souche homologue. Ces résultats montrent que la SEPA inactivée comme vaccin potentiel offre une protection des poulettes contre l'infection SEPA émergente qui permettrait d'éviter de graves pertes économiques dans l'industrie avicole nigériane et d'offrir à la population grouillante de cette nation les protéines animales dont elle a besoin.

Mots-clés : *Salmonella enterica Paratyphi A*, immunogénicité, vaccin, vollaille.

Introduction

The poultry population in Nigeria is estimated as 65,269,582 as at 2003 (Adene and Oguntade, 2006), and more recently at 140 million according to FAO statistical database (FAO, 2009). Intensive poultry production is becoming popular in the country. However, disease outbreaks, among others, *salmonellosis* has constituted a stumbling block to profitable poultry production in terms of treatment costs, antimicrobial resistance and mortalities with resultant economic losses to farmers and unavailablity of high quality protein to the fast-growing human population. Despite serious surveillance and control measures, *salmonellosis* has not been completely eradicated even in the developed economies of the world (Antune *et al.,* 2002). Serovars of Salmonella organisms have been associated with many diseases ranging from self-limiting gastroenteritis to life threatening systemic infections in several hosts, including humans (Ellermeier and Slauch, 2007). Salmonella enterica subspecies enterica serotype Typhi is the aetiological agent of typhoid fever in humans, a disease associated with morbidity in many regions of the World, with not less than 12-33 million cases occurring annually (Janvier *et al.,* 2007). Salmonella Paratyphi A is one of the serovars of Salmonella reported to be primarily restricted to human, and has been identified as aetiological agent of paratyphoid enteric fever among other agents like Salmonella Paratyphi B and C (Shu Lin-Liu and Kenneth, 1995; Janvier *et al.,* 2007). However, for the first time, *Salmonella enterica Paratyphi A* was isolated from a disease outbreak in a commercial poultry farm in Ibadan, Oyo State, Nigeria (Ogunleye *et al.,* 2005), and the pathogenicity and haematological changes associated with the isolate in pullets were documented (Ogunleye *et al.,* 2006 ; Ogunleye *et al.,* 2009). More recently, other outbreaks in poultry with high morbidity and mortality in pullets referable to Salmonella enterica subsp enterica serovar Paratyphi A infection has occurred in the same country (personal communication).

Contaminated poultry meat and poultry-products, such as eggs play important role in the transmission of Salmonella infections, which is currently a major global health burden (WHO, 1988; Antunes, 2002). Vaccination remains an effective tool for the prevention of Salmonella infections (Cardenas and Clement, 1992; Gast *et al.,* 1993; Shlim *et al.,* 1995; Leenar *et al.,* 1999; Mittrucker and Kaufmann, 2000; Mastroeni *et al.,* 2001; Norimatsu *et al.,* 2004). Hence, the need for the development of efficacious vaccines strategy to contain Salmonella infection in animals has been acknowledged by the relevant stakeholders and World Health Organization against the backdrop of increasing antimicrobial resistance (Gast *et al.,* 1993; WHO, 1988). A developing country like Nigeria will need vaccine to avert severe economic losses being associated with *salmonellosis* so that the much needed animal protein will be available to feed the teeming population.

The current study investigated the immunogenicity of a formalin-killed Salmonella Paratyphi A vaccine in groups of pullets challenged with un-attenuated homologous strain and heterologous (Salmonella gallinarum) strain respectively.

Materials and Methods

Experimental birds

The 100 Hubbard Golden Comet pullets donated by Amo Byng Nigeria Limited®, Awe, Oyo State Nigeria were used for the protection/cross protection experiment. A group of 75/100 were immunized, while the remaining 25/100 pullets tagged UC were left as unimmunized control group. Approved Institutional Animal Care guidelines were obtained from the designated committee in the Faculty of Veterinary Medicine, University of Ibadan, Nigeria (Certificate no: ethic/07/07). The chicks were raised under strict hygienic conditions in experimental cage facilities at the Faculty of Veterinary Medicine, University of Ibadan, Nigeria. The chicks were fed ad libitum with antibiotic-free commercial chick mash donated by the feed meal unit of the Hope Farms® Ltd, Ibadan, Oyo State, Nigeria up to the 9th week of age. From the 9th week to the end of the experiment, the birds were fed with antibiotic-free commercial growers mash donated by Amo Byng Nigeria Limited®.

The birds were routinely vaccinated with the National Veterinary Research Institute (NVRI®) Vom, Plateau State, Nigeria vaccines against: Newcastle disease on the 1st and 16th days;and 6th and 10th week (using intra ocular and Lasota vaccines, Lasota and Komorov vaccine, respectively). The Infectious Bursal Disease (Gumboro, Live attenuated) vaccine (NVRI®) were administered on the 8th and 18th days and in the 4th week of age, respectively. The birds were dewormed in the 9th and 11th weeks of age, respectively using piperazine (piperazine wormer Pfizer® products Plc, Ikeja, Nigeria) at dosage of 0.7g/litre drinking water. Salmonella Paratyphi A isolate

The *Salmonella enterica Paratyphi A* (SEPA) for the vaccine was isolated in July 2002 from Salmonella outbreak in a poultry farm in Ibadan Oyo state, Nigeria. It was a non-lactose fermenter on MacConkey agar with characteristics yellowish glistening colonies and was found to be Gram-negative, it was motile unlike other pathogenic Salmonella species of poultry, short rod, oxidase, urease, citrate, indole, salicin, sucrose and H2S negative, while the organism was glucose, sorbitol and dulcitol positive. Based on biochemical and serological tests it was identified as SEPA (Ewings, 1986; Barrow and Feltham, 1993; Ogunleye *et al.,* 2005; Ogunleye *et al.,* 2006; Ogunleye *et al.,* 2009).

Preparation of Formalin-Killed SEPA Vaccine

A formalin-killed vaccine was prepared from the SEPA with slight modification as described by Bernard et al. (1990). Briefly, the isolate was inoculated into sterile trypticase Soy broth (TSB) and incubated at 37°C overnight. One millilitre (1ml) of 0.1% formalin was added to 1ml of the overnight broth culture. Excess formaldehyde was reduced by addition of sodium bisulfite (at 23mM final concentration). The mixture was then diluted with another 4ml of sterile TSB and was subsequently incubated at 37°C overnight (using this dilution ratio, more than sufficient volume required to immunize each birds with 0.5ml killed antigens were prepared each time the birds were vaccinated. Samples from the inactivated Salmonella isolate were inoculated onto blood agar and MacConkey agar and incubated at 37°C for 18 hours.

Immunization

When the chicks were 4 weeks old, the sera were screened for the presence of antibodies to Salmonella Paratyphi AH and OH by slide agglutination test using the Febrile antigen kit (Fortress Diagnostic Limited®, Unit 2CAntrum Technology Park, BT41 IQS United Kingdom), to check for reactors.

At the 4th week of age, 75 birds were immunized, tagged (I), orally with 0.5ml formalin-killed SEPA containing 1x107cfu/ml. The first and second vaccine booster doses were given after the 10th and 29th days following the first immunization to allow for build up of antibodies. Another 25 birds served as unimmunized control group / tagged (UC), each bird was fed orally with 0.5ml of sterile TSB during the vaccination of I. After the second booster doses, the I was separated into 3 groups of 25 birds per group tagged (IA, IB, and IC)

Widal test serological screening

Febrile antigen kit manufactured by Fortress Diagnostic Limited®, Unit 2C Antrum Technology Park, Antrum BT41 IQS United Kingdom, containing S. Typhi H, S. Typhi O, S. Paratyphi AO, S. Paratyphi AH, S. Paratyphi BO, S. Paratyphi BH, S. Paratyphi CO, S. Paratyphi CH with positive and negative control were used for the slide agglutination test screening of 10 birds from each immunized groups and 10 from the unimmunized group; 12 days following the third immunizing dose. Twenty-nine days post third immunization; all the experimental birds were screened in slide agglutination test using the aforementioned febrile antigen kit. Subsequently a cross sectional screening of the antibody titres for 45 pullets (15 per group) of the immunized birds which were earlier positive by slide agglutination method, were determined by Widal tube agglutination test as described by Cruickshank *et al.,* (1975), with slight modifications. Briefly, each positive serum sample was serially diluted with normal Saline (0.85% NaCl), in two series of sterile tubes starting from 1:20 to 1:2560 while the 9th tube contained only saline. S. Paratyphi AH and AO antigens were then added. For H agglutination, the tubes were incubated at 37°C for 2 hours

followed by room temperature for 30 minutes, while in the case of O agglutination, the tubes were incubated at 37°C for 4hours and kept at 4oC overnight before reading.

Protection experiment/Cross protection Experiment

At the 16th week of age, cloaca swabs of the experimental pullets were examined bacteriologically for Salmonella by Standard methods (Ewings, 1986; Barrow and Feltham, 1993), to check for shedders.

By 16th week of age, IA was challenged orally with 0.5ml of the 8 hours TSB culture containing 1x108cfu/ml SEPA, while the group IB was challenged with 0.5ml of the 8 hours TSB culture containing 1x108cfu/ml S. gallinarum. The IC (served as immunized control: it was immunized but fed with sterile broth culture and not with any infectious agent), while the UC (the group that was not vaccinated at all) was challenged with 1x108 cfu/ml of SEPA to serve as unimmunized control. The pullets were subsequently fed antibiotics-free growers mash and water ad libitum. The birds were observed daily for clinical signs, and dead birds were examined for pathological lesions.

Results

Results of the immunization experiment

Birds and vaccine control before experiment

The validated formalin-inactivated broth culture of SEPA showed no growth on blood agar and MacConkey agar after 18 hours incubation at 37°C .We showed that the cloaca samples of the 100 pullets used for the experiments yielded no growth of Salmonella species to eliminate pre-experimental exposure. None of the experimental pullets screened for the presence of antibodies to S. Paratyphi AO and S. Paratyphi AH by slide agglutination method prior to vaccination at the 4th week of rearing was positive.

Assessment of the formalin-killed SEPA vaccine immunogenicity

The assessment, the ability of the SEPA vaccine candidate for antibody production on the immunized birds prior to challenge with homologous and heterologous Salmonella enterica, in a cross sectional study of 10 pullets from each group of experimental birds twelve days after the second vaccine booster dosage (3rd immunization), through slide agglutination test showed that at least 8 of 10 pullets from the immunized groups showed positive antigen-antibody reaction to S. Paratyphi AO and AH antigen. By contrast none of the 10 serum samples from the unvaccinated control (UC) was positive for S. Paratyphi AO and AH antigens in slide agglutination test. Twenty-nine days later (prior to challenge of the birds in each groups with relevant Salmonella enterica according to the experimental design), all the 75 birds in the three vaccinated groups (IA, IB, IC) showed positive results for the slide agglutination reaction, while the 25 pullets in unvaccinated group (UC) showed a negative slide agglutination reaction. Table 1 shows the antigen/ antibodies responses of the experimental birds following immunization with formalin killed SEPA vaccine candidate.

Clinical findings/pathology of protection experiment

Group IA birds vaccinated and orally challenged with SEPA

The pullets in group IA did not develop any clinical disease following oral challenge with SEPA, and no death was recorded throughout 21 days of the investigation. No histopathological lesion was observed in spleen (Figure 1b), proventiculus (Figure 1d) and other organs of pullets euthanized with chloroform.

Unvaccinated control group UC but orally challenged with SEPA

In contrast clinical manifestations of the disease and notable pathological lesions were observed in the group UC. Three days post-innoculation p-i., all the 25 birds became apparently inactive. By the 6th day post infection, they all showed signs of dullness, somnolence, decreased feed and water intake followed by yellowish watery diarrhoea. The first mortality was recorded on the 7th day p-i and the mortality rose to 24% (6/25) by 21 days p-i when the experiment was terminated. Pathological lesions were observed in the dead birds from group UC. The intestines showed severe necrosis of the intestinal epithelium, moderate

Table 1: Antigen/ antibody response of the experimental birds to formalin- killed vaccine candidate of *Salmonella enterica Paratyphi A.*

Antigen/antibody Response	Group IA (n= 25)	Group IB (n= 25)	Group IC(n=25)	Group UC(n=25)
Slide agglutination SPAO positive	25	25	25	
Slide agglutination SPAH positive	25	25	25	
Average Ab/titre for SPAO(n=15)	320	320	320	
Average Ab/titre for SPAH(n=15)	160	160	160	

Key: AB/titre= antibody titre, SPAH= Salmonella Paratyphi A – H Febrile antigen, SPAO= Salmonella Paratyphi A – O Febrile antigen

infiltration of lamina propia with mononuclear cells. There was moderate pulmonary oedema of the lung. The liver showed multifocal hepatic degeneration and necrosis (Figure 1a), severe lymphoid depletion of the spleen (Figure 1c) and severe glandular necrosis of proventiculus (Figure 1e). The birds in this group produced typical gross lesion of *salmonellosis* in poultry in form of marked bronze coloration of the liver and engorgement of the gall bladder with bile (Figure 1f).

Group IB birds vaccinated and orally challenged with S. Gallinarum

The birds in group IB did not show any apparent clinical sign until 9 days p-i when the first mortality was recorded. All the 25 birds were gradually inactive with mortality increasing progressively. It reached 28% (7/25) by 15 days p-i and up to the end of the experiment.

Notable histopathological lesions were observed namely: moderate diffuse hepatic necrosis with widespread vascular thrombosis, distension of sinusoids and vascular congestion, and diffuse fatty changes of hepatocytes with marked vascular congestion of the liver, severe diffuse necrosis of renal tubular epithelia of the kidneys, marked congestion and thickening of lamina propia with mononuclear infiltration of intestine, diffuse severe hyperaemia and oedema of lungs (Figures not shown).

Vaccinated control group IC but not challenged

For the group IC, there was no clinical sign, no mortality, no pathological lesion in the birds euthanized with chloroform.

Discussion

Salmonella Paratyphi A is one of the serovars of Salmonella documented to be restricted to human, and has been identified as aetiological agent of paratyphoid enteric fever among other agents like Salmonella Paratyphi B and C (Shlim *et al.,* 1995). This work investigated the immunogenicity of a candidate SEPA vaccine prepared from the strain described from poultry (Ogunleye *et al.,* 2005; Ogunleye *et al.,* 2006). Due to increasing implication of Salmonella Paratyphi A with human typhoid fever in some parts of the world such as, Nepal, India subcontinent, Israel, Indonesia to mention a few, particularly among people otherwise vaccinated with existing vaccines like Ty2a and V1 Typhoid vaccines, the need to develop an effective vaccine against S. Paratyphi A has been acknowledged (Schwartz *et al.,* 1990; Pang *et al.,* 1995; Shu Lin-Liu and Kenneth, 1995; Leenar *et al.,* 1999; Meltzer *et al.,* 2005).

The inactivated-SEPA vaccine in the current investigation produced 100% protection in immunized pullets challenged with homologous strain. This is in agreement with (Roesler *et al.,* 2006; Janvier *et al.,* 2007). However, the immunity produced by the SEPA vaccine was not cross protective because the immunized pullets challenged with the Salmonella gallinarum, showed a short period of protection followed by clinical signs and mortalities due to Salmonella gallinarum. Hence, candidate SEPA vaccine appears species-specific, similarly to the observation of Roesler *et al.,* (2006) who found that a homologous

inactivated Salmonella vaccine produced decreases the prevalence of Salmonella typhimurium infection in the offspring following immunization of sows in an integrated pig-breeding herd.

The development of stable, efficacious and safe vaccines against *salmonellosis* in poultry is generally accepted as the most practical measure to eradicate the pandemic of Salmonella food-borne illness (Pang *et al.,* 1991; Cardenas and Clement, 1992; Mastroeni *et al.,* 2001; Braden, 2006; Roesler *et al.,* 2006). Orally delivered vaccines elicit mucosal and systemic immune responses to antigens as compared with vaccines delivered via the parenteral routes (Cardenas and Clement, 1992). Therefore, in order to control new outbreaks by SEPA in poultry, we developed a formalin-killed SEPA vaccine. In the present study, oral vaccination

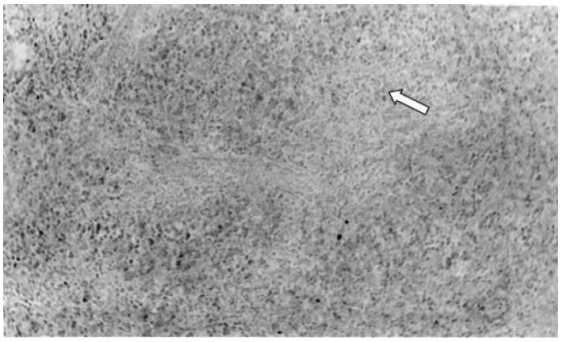


Figure 1a: Liver from group UC challenged with SEPA showing multifocal hepatic degeneration and necrosis (Arrow) H&E X100

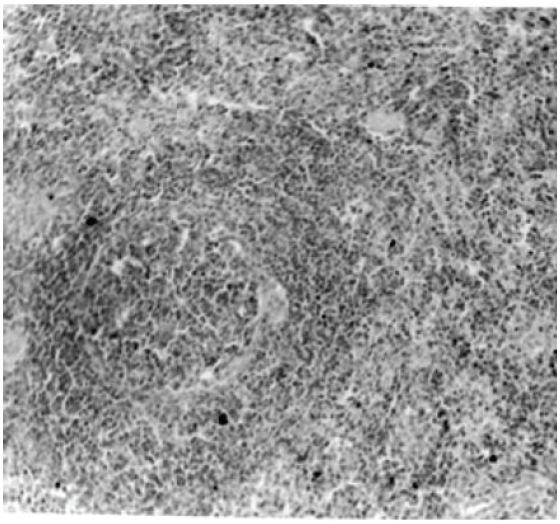


Figure 1b: Spleen from group IA showing no lesion H&E X 100

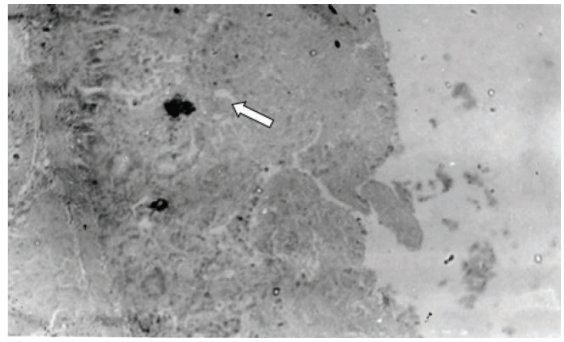


Figure 1c: Spleen from group UC challenged with SEPA showing severe lymphoid depletion (Arrow) throughout the parenchyma H&E x100.

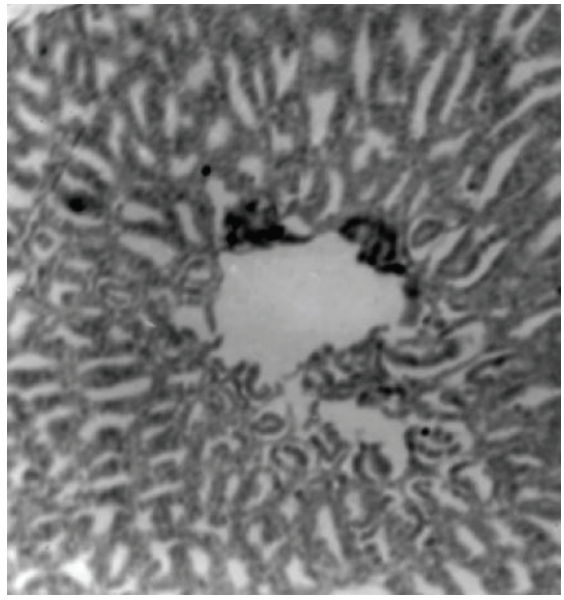


Figure 1d: Proventiculus from group IA showing no significant lesion H&E X100.

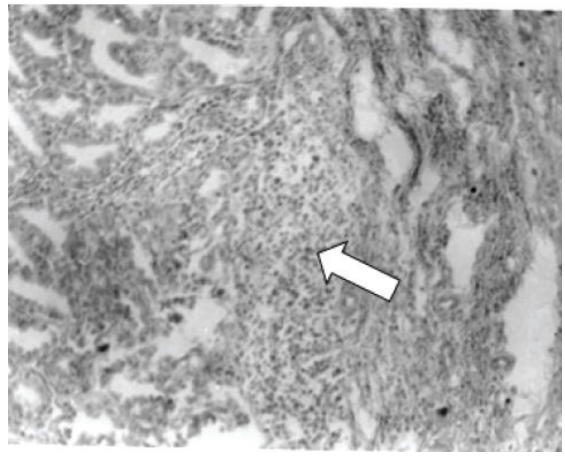


Figure 1e: Proventiculus from group UC challenged with SEPA showing severe glandular necrosis (Arrow) H&E X100.



Figure 1f: Avian visceral at post-mortem from group UC challenged with SEPA showing marked bronze- coloration of the liver with engorgement of the gall bladder with bile.

was used since oral vaccine offers a potential solution to problem of *salmonellosis* of poultry. It was shown that the immunogenicity of SEPA vaccine for poultry, as indicated by the presence of O and H antibodies in the sera of pullets. It was shown that this inactivated SEPA vaccine was able to protect birds against homologous challenge. This formalin-killed SEPA vaccine is thus a potential candidate vaccine to protect poultry against infection by Salmonellla enterica Paratyphi A.

Conclusion

The above inactivated candidate vaccine offers a good prophylactic protection against this emerging SEPA infection in poultry. It will protect the farmers and the nation against severe economic losses and the much needed animal protein will be available to feed the teeming population. Indirectly, it will safeguard the human population against possible infection by SEPA through poultry and poultry products. *Salmonella enterica Paratyphi A* has been established to be a major cause of typhoid fever among others in some regions of the world (Schwartz *et al.,* 1990; Pang *et al.,* 1995; Shu Lin- Liu and Kenneth, 1995; Leenar *et al.,* 1999).

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SEROLOGICAL AND VIROLOGICAL STUDY OF NEWCASTLE DISEASE AND AVIAN INFLUENZA IN CHICKENS IN RURAL AREAS IN SENEGAL

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Abstract

Serological survey on the prevalence of Newcastle disease (NCD) virus antibodies using haemagglutination inhibition test (HI) and virological detection by RT-PCR of highly pathogenic avian influenza (HPAI) H5N1, were carried out in 6 regions of Senegal from June to November 2008. Rural chickens were raised in free ranging traditional and backyard systems. A total of 668 birds were sampled and 150 cloacae swabs from wild birds were used for the study. Data on medical care (treatments) and hygiene were collected using a questionnaire. The overall seroprevalence of NCD virus antibodies was 54.4% in these rural chickens but the prevalence of protective Newcastle disease virus antibodies in vaccinated chickens was 70.6% and in unvaccinated chickens 43.8%. These results suggest that for the "vaccinated chickens" group, the vaccination was not well carried out, furthermore the results also imply the possibility of circulation of a wild strain of NCD virus. There was a significant difference in prevalence between regions and the use of antibiotics for vaccinated and unvaccinated chickens. Multiple correspondence analysis classified regions in four sectors in which emphasis has to be laid on vaccination or training. To further understand the epidemiology of NCD in rural chickens in Senegal, characterisation of isolates and risk factor studies should be conducted. No HPAI H5N1 virus was detected in the present study. This confirms the negative status of Senegal for HPAI H5N1. Guinea fowls, Ducks, Turkeys and chickens were positive to the HI test.

Keywords: Prevalence, Newcastle disease, Avian Influenza, Rural chickens, Senegal.

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Introduction

Indigenous chicken make up 80% of total chicken population in Senegal (Traoré, 2006). Indigenous rural chickens are either raised under free ranging or backyard extensive production. In fact, the latter and the former (traditional and backyard) should be considered as two separate systems of production in rural areas. The traditional system is characterised by free ranging animals with rudimentary or no housing at all and without medical care, whereas in the backyard system the farmers (women) are better organised (trained as village vaccinators) there is vaccination against Newcastle, there is medical care and diet supplements and above all, the existence of more modern hen housing. These units also possess rapid growth mixed breed cocks. The industrial system is mainly focused around the Dakar region and is estimated at 12 million chicken population. Ninety seven (97%) percent of these are imported from Brazil and the remaining 3% from parent breeders in Dakar. Eighty percent of this intensive and modern system is practiced around the Dakar region. Indigenous village chickens play important roles in the rural economy. Poultry constitutes an important source of income and high quality animal protein in the rural socio-economy with little capital investment. However, the emergence and transmission of infectious diseases such as avian influenza and Newcastle disease pose a huge threat to indigenous chicken production.

Newcastle disease (NCD) is caused by an avian paramyxovirus (APMV-1 serotype) that belongs to the genus Avulavirus. NCD is endemic and was found to be the most important disease of village poultry in Senegal (Gueye, 1998). It is associated with a high mortality leading to heavy economic losses for the rural population especially in the months of February and May (Arbelot et al., 1997).

Avian Influenza virus is classified as a member of the Orthomyxoviridae family. To date, all outbreaks of the highly pathogenic form have been caused by influenza A virus of the H5 and H7 subtypes. Before 2003, outbreaks of this virus in poultry seldom occurred (Werner & Harder, 2006) and were reported only in Europe and America (Kalthoff *et al.,* 2010).

The first confirmed outbreak of H5N1 HPAI on the African continent occurred in January, 2006, at a commercial farm in Kaduna State, Nigeria (Saidu *et al.,* 2008). H5N1 HPAI then spread to 6 countries of the West African sub-region; Niger and Cameroon in February, 2006, Burkina Faso and Ivory Coast in March, 2006, Ghana in April, 2007 and Togo in June, 2007. The last outbreaks ended in July and September, 2008 in Nigeria and Togo, respectively.

A strong suspicion of commercial farms, a weakness in biosafety and biosecurity both in the rural and\or urban poultry management and in the distribution chain, and the porous nature of the borders seem to be the main factors which contribute to the spread of this disease in this part of the continent.

This study was designed to determine the serological status of NCD virus infections among village chickens in Senegal. In addition, the presence of H5N1 HPAI virus in village chickens and wild birds was detected in order to assess their role in the epidemiology of both diseases in village poultry.

Materials and Methods

Study area

The study was carried out in 6 regions of Senegal (Dakar, Saint Louis, Kaolack, Thies, Kolda, Louga) from June to November 2008. Fresh faecal swab samples were also collected from wild birds in the Djoudj national park.

In each region, the villages and the poultry owners were randomly selected.

Two different types of samples were collected from each chicken, namely blood samples and tracheal swabs.

Animal management

The traditional system characterised by free range where birds scavenge in the backyard, was used in all the regions. Certain investments in chicken housing were made in the Dakar region or Thies (backyard system). Many avian and non-avian species live in these backyards, with no hygiene and a handful of grains are generally distributed periodically.

In some villages of Dakar (region), Thies and Saint-Louis, there was more veterinary care and vaccination against NCD than in other areas (Gueye, 1998).

Questionnaire survey

A questionnaire was used to obtain information from poultry owners about frequent clinical signs observed, mortality, backyard cleaning, treatment and vaccination against NCD during sample collection.

Collection of serum, swabs and laboratory analysis

Blood samples and tracheal swabs were collected in 668 avian specimens with 638 chickens (vaccinated and unvaccinated against NCD), 15 ducks, 3 pigeons, 3 turkeys and 9 guinea fowls. All the birds were bled via the wing vein into 5 ml sterile tubes. Sera were separated after centrifugation at 3000 t/min for 15 min, then transferred into eppendorf tubes, which were labelled and stored at -20°C until they were used for testing.

Faecal swabs were collected from fresh faeces in 150 wild birds (Spur-winged goose: Plectropterus gambiensis; Caspian tern: Hydroprogne caspia; Gull-billed Tern: Gelochelidon nilotica; Collared pratincole: Glareola pratincola) in the Djoudj park.

Newcastle Haemagglutination inhibition (HI) assay: HI was done according to CNEVA (Centre National d'Etudes Vétérinaires et Alimentaires, France) technique (PICAUT *et al.,* 1993) in U bottomed-shaped microtitre plates. Newcastle disease vaccine HB1 strain obtained from CEVAC vaccine (CEVAC UNI L®: live freeze-dried HB1vaccine; batch 01025D1J exp: 02-2010; CEVA Santé Animale) was used as antigen after reconstitution. After a two fold serial dilution of test serum, 4 haemagglutinating units (HA) of Newcastle disease virus were added to each well and kept at room temperature for 25-30 minutes. 1% chicken RBCs (red blood cells) was added to each well and gently mixed and left to settle for about 45 minutes at room temperature. The titre was considered as the highest dilution of serum that caused complete inhibition of 4 HA of antigen. A titre greater than or equal to 1.16 was considered as positive. Furthermore, titres of value 1024 and above could denote a double contact between wild and vaccine viruses.

PCR analysis was performed to detect avian influenza virus H5N1 HPAI (M gene) in 668 tracheal swabs of domestic birds and 150 faecal swabs of wild birds.

RNA isolation

RNA was isolated from Tracheal and cloacae swabs using commercial RNeasy Mini Kit (Qiagen®, USA) as recommended by the supplier. Total nucleic acid was quantified using a spectrometer at 260 nm wave length. RNA extract were kept at 4°C until used for PCR amplification.

Reverse transcription (RT)

One step RT-PCR kit (Qiagen®, USA) was synthesized using the One Step RT-PCR enzyme mix containing Qiagen omniscript, Reverse transcriptase, sensiscript reverse transcriptase and Hotstar Taq DNA polymerase in a total volume of 50µl for 30 min at 50°C.

Primers: A set of primers:

IA/M1 (5' - AGC GTA GAC GCT TTG TC – 3') and IA/M2 (5' – GAC GAT CAA GAA TCC AC– 3') that flanks the region encompassing the cleavage site of the fusion protein gene (M) was used in the study. The expected size of PCR product was 601 bp.

Polymerase chain reaction (PCR):

PCR was carried out in a total volume of 50µl containing 4µl of the total RNA, 10µl of 1x PCR buffer Qiagen, 2µl of 0.1 M DTT, 2µl of 10mM dNTP, 2µl One step Qiagen enzyme mix, 0.6µM of primers IA/M1 and IA/M2, 29µl of RNase free water. The thermocycler conditions were as follows: 15 min at 95°C (initial denaturation), followed by 40 cycles of 1 mn at 94°C (denaturation), 1 mn at 53°C (annealing), 1 mn at 72°C (elongation). The PCR ended with a final elongation for 7 min at 72°C.

Detection of PCR products:

PCR products were separated in 1.5% agarose gel in 1x TAE buffer stained with ethidium bromide, compared with molecular mass marker and visualized by ultraviolet (UV) transillumination.

Statistical analysis

Data collected were statistically analyzed using Chi-square test ($p< 0.05$) to determine the association between explanatory variables, seropositivity and vaccination status respectively. Statistical analyses were performed using R.2.9.2. A Multiple Correspondence Analysis (MCA) was used to describe the relative position of regions and some important variables such as vaccination, mortality and treatment using the questionnaire data (FactomineR®, Husson *et al.,* 2009).

Results

Newcastle disease

Prevalence and HI titre

Prevalence of antibodies to Newcastle disease by species

The Chi-square test for antibody seropositivity by species indicated that there was a significant difference between species ($p=0.0026$). All the samples from turkeys (n= 3) and guinea fowls (n= 9) were positive as shown in table 1. Chickens (n=638) and domestic duck (n= 15) had 54.38% and 33.33% of positives respectively. No pigeon tested positive. Only chickens were vaccinated.

With the low number of samples for other species, all analyses that follow will be based only on chickens.

Prevalence Newcastle disease antibodies virus by region

The overall seropositivity of NCD virus antibodies was 54.4% (CI: 49.6- 57.4). Comparisons between Dakar and other regions indicated that Dakar's positivity is significantly higher than that of Kolda, Louga ($p< 0.001$), and Kaolack, Saint-Louis ($p<0.01$). No statistically significant difference was observed between Dakar and Thies regions (Table 2).

Prevalence of Newcastle disease virus antibodies in vaccinated and unvaccinated chickens

The antibody seroprevalence with vaccination effect is shown in table 3. Overall, the seroprevalence was 70.6% (CI 95%: 0.6452- 0.7609) in vaccinated birds and 43.8% (CI 95%: 0.3879- 0.4889) in unvaccinated chickens. There was a significant difference between vaccinated and unvaccinated chickens ($p< 0.001$). Almost 30% of vaccinated chickens tested negative to HI.

The percentage of vaccinated chickens was between 0 % (Saint Louis) and 79.5% (Dakar) as shown in Table 3. The difference was highly significant ($p<0.001$). No vaccination was used in selected backyard farms in Saint Louis. For unvaccinated chickens, the percentage ranged from 22% (Kolda) to 71% (Thies). For both vaccinated and unvaccinated groups, the percentage of chickens which have a positive serology is statistically lower in farms which use antibiotics than those that do not use them. No statistical differences were observed between the presence of other birds, the mortality, the backyard cleaning, the clinical signs (respiratory, diarrhoea, nervous) for vaccinated or unvaccinated chickens. Altogether, in both the vaccinated and unvaccinated groups, farmers cleaned up their hen houses.

Distribution of Newcastle antibodies HI titre classes by region

The distribution of NCD virus antibody titre according to region is shown in Table 4. There were 220 chickens out of 638 (34.5%) with no NCD antibodies in these regions and a quarter of these chickens come from Kolda region.

Higher antibody titre have been observed in 12% (78/638) of the chickens and half come from the region of Thies.

Distribution of Newcastle antibodies titre by vaccination status

The distribution of Newcastle virus antibodies titre for vaccinated and unvaccinated chickens is shown in Figure 1. The number of unvaccinated subjects is greater than that of the vaccinated subjects, with HI negative antibody titre ≤ 16 and HI positive antibody titre ≥ 512.

Multiple Correspondance Analysis (MCA)

The first two factorial axes explain 77% of the total variability of the data. Figure 2 shows that with respect to vaccination, treatment and mortality, regions can be classified into four different groups:

Table 1: Newcastle disease prevalence by species, Senegal, 2008

Species	Number of animals	Number of positive/total	Positives (%)
Ducks	15	5/15	33.33
Turkeys	3	3/3	100
Pigeons	3	0/3	0
Chickens	638	347/638	54.38
Guinea fowls	9	9/9	100

Table 2: Overall prevalence of Newcastle disease virus antibodies, Senegal, 2008

Regions	Number of chickens	Positivity (%)	Confidence interval (95%)
Dakar	104	74	64.35-81.91
Kaolack	106	51.9	42.01-61.61
Kolda	121	29.8	21.96-38.84
Louga	110	50.9	41.25-60.49
Saint-Louis	93	51.6	41.07-62.01
Thies	104	72.1	62.32-80.24
TOTAL	**638**	**54.4**	**49.6 – 57.4**

Avian Influenza

All the 668 tracheal swabs from domestic poultry and 150 faecal swabs from wild birds tested negative to gene M of avian influenza virus H5N1 HPAI by RT-PCR.

Discussion

The main objective of this study was to determine the seropositivity of the Newcastle disease in traditional poultry and wild birds, which is the main cause of death of these birds and to detect the presence of influenza virus as well.

Serological prevalence of Newcastle in traditional chickens, in this study, was 54.4% and was lower than that obtained by Arbelot et al. (1997), in Dakar and Thies regions (with prevalence 84% in the rainy season and 98% in dry season). However, NCD seroprevalence estimate of traditional poultry in this study is higher than that recorded in Niger (14%, Courtecuisse *et al.,* 1990), Mauritania (5%, Bell *et al.,* 1990), Zambia (36.9%, Alders *et al.,* 1994), Mali (32.9%, Sylla *et al.,* 2003), and Madagascar (21%, Maminiaina *et al.,* 2007).

The prevalence of Newcastle disease virus antibody titre (≥ 16) was 70.6% in vaccinated chickens and 43.8% in unvaccinated chickens. This means that about a quarter of the vaccinated chickens and half of unvaccinated chickens were likely to be susceptible to the disease.

This high prevalence of vaccinated chickens (70% have protective antibodies) is quite important, but some antibody titre higher than 1024 were observed in Saint Louis and Thies, suggesting the possible circulation of wild strains of Newcastle disease in chickens as indicated by Arbelot et al. (1997).

A suggested hypothesis is that this high titre could be due to chickens which might have survived former Newcastle outbreaks, where there is often a constant transmission of the virus in the backyard via other avian species such as pigeons, guinea fowls and ducks. This study, the sampled turkeys, guinea fowls, and ducks, tested positive. These species can serve as a reservoir and could play a role in spreading the disease (Weingartl *et al.,* 2003). However, these results indicated that the presence of other avian species, do not appear to influence the seroprevalence of Newcastle disease antibodies. Similarly, clinical signs (diarrhoea, respiratory and nervous) observed by farmers could denote the circulation of mesogenic and velogenic viral strains in this production system (Beard and Hansson, 1981). It will be necessary to study Newcastle viral strains circulating in Senegal, and also observe the likely causes of

Table 3: Newcastle disease virus HI antibodies in chickens in Senegal, 2008

		Vaccinated (n=252)		Unvaccinated (n=386)	
		HI negative (%)	HI positive (%)	HI negative (%)	HI positive (%)
Region	Dakar	18/88 (20.5)	70/88 (79.5)	9/16 (56.2)	7/16 (43.8)
	Kaolack	20/62 (32.3)	42/62 (67.7)	31/44 (70.5)	13/44 (29.5)
	Kolda	17/34 (50.0)	17/34 (50.0)	68/87 (78.2)	19/87 (21.8)
	Louga	14/46 (30.4)	32/46 (69.6)	40/64 (62.5)	24/64 (37.5)
	Saint-Louis	0/0	0/0	45/93 (48.4)	48/93(51.6)
	Thies	5/22 (22.7)	17/22 (77.3)	24/82 (29.3)	58/82 (70.7)
Respiratory signs		21/73 (28.8)	52/73 (71.2)	60/97 (61.9)	37/97 (38.1)
		53/179 (29.6)	126/179 (70.4)	157/289 (54.3)	132/289 (45.7)
Diarrhoea		20/68 (29.4)	48/68 (70.6)	19/50 (38.0)	31/50 (62.0)
		54/184 (29.3)	130/184 (70.7)	198/336 (58.9)	138/336 (41.1)
Nervous signs		31/115 (27.0)	84/115 (73.0)	54/124 (43.5)	70/124 (56.5)
		43/137 (31.4)	94/137 (68.6)	163/262 (62.2)	99/262 (37.8)
Mortality		13/47 (27.7)	34/47 (72.3)	48/78 (61.5)	30/78 (38.5)
		61/205 (29.8)	144/205 (70.2)	169/308 (54.9)	139/308 (45.1)
Backyard cleaning		4/21 (19.0)	17/21 (81.0)	25/41 (61.0)	16/41 (39.0)
		70/231 (30.3)	161/231 (69.7)	192/345 (55.7)	153/345 (44.3)
Antibiotics treatment		56/166 (33.7)	110/166 (66.3)	136/263(51.7)	127/263 (48.3)
		18/86 (20.9)	68/86 (79.1)	81/123 (65.9)	42/123 (34.1)
Other bird species		60/186 (32.3)	126/186 (67.3)	132/261 (50.6)	129/261 (49.4)
		14/66 (21.2)	52/66 (78.8)	85/125 (68.0)	40/125 (32.0)
TOTAL		74/252 (29.4)	178/252 (70.6)	217/386 (56.2)	169/386 (43.8)

Table 4: Distribution of Newcastle antibody HI titres by region, Senegal, 2008

Regions	**Antibodies HI titre**		
	0-8	**16-512**	**≥ 1024**
	Negative	**Positive**	**Strongly positive**
Dakar	27	69	8
Kaolack	51	47	8
Kolda	85	29	7
Louga	54	55	1
Saint-Louis	45	26	22
Thies	29	43	32

vaccination failures.

The present data suggest that the proximity to large urban areas such as Dakar and Thies offers easier access to the supply of veterinary drugs. This would therefore be important factor reckon with, given of their peri-urban situation of the farms around these areas (more frequent urban markets, proximity to large villages with birds and genetic improvement by the introduction of cocks). In these two regions, the traditional system was replaced by backyard system where women participate as compared with Kolda, Saint Louis, Louga regions. The income generated by this speculation helps provide for children's education and other personal needs (Gueye, 1998).

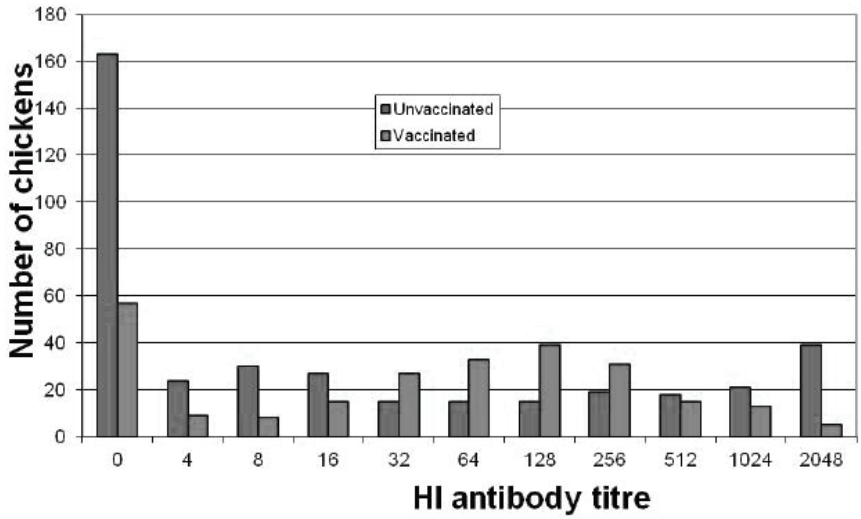


Figure 1: The distribution of Newcastle virus antibodies titre for vaccinated and unvaccinated chickens

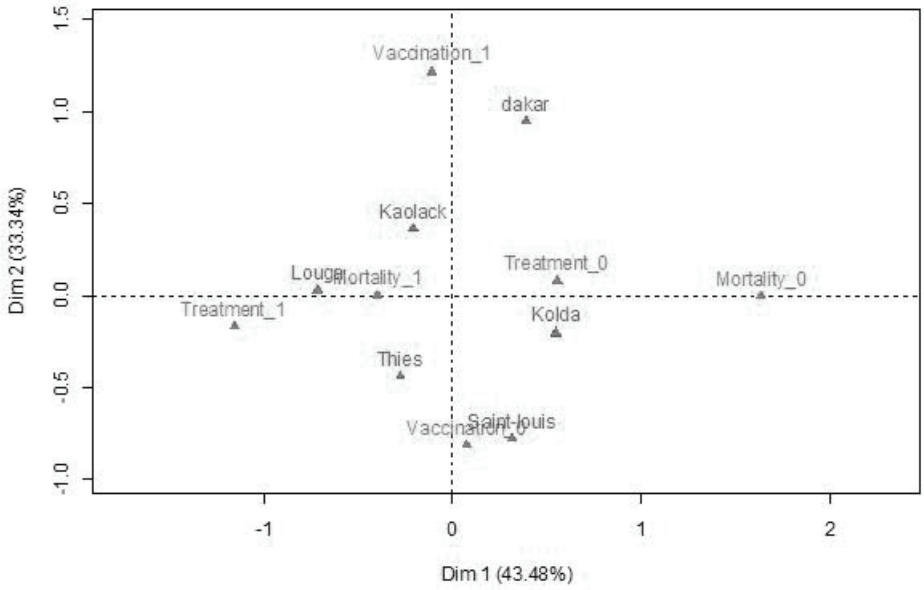


Figure 2: Classification of regions according to vaccination, treatment and mortality

Multiple correspondence analysis summarizes current results by classifying the regions into four sectors, with major factors being vaccination, mortality and antibiotic treatment used. According to these sectors, emphasis should be made on vaccination and/or training of farmers. Vaccination and primary hygiene measures should be emphasized upon in other regions through sensitisation of the population, namely in Saint Louis.

In the sampled regions, there was no trace of the H5N1 HP virus in village birds, as well as in wild birds from the Djoudj Park. These results confirm the negative status of Senegal, to the presence of H5N1 HP virus.

Conclusions

The study contains some bias but since birds were randomly sampled, the questionnaire was short and precise, and the laboratory analyses were based on standard methods, we therefore consider the results to be valid.

Backyard cleaning seemed to be more frequent with farmers who vaccinated, unlike

those with unvaccinated chickens and the difference was statistically significant. This can be easily understood because farmers who vaccinate their animals have a greater tendency to implement good hygiene and veterinary care (prevention and treatment).

Further investigative studies might be done on the phylogenetic strains, which circulated in Senegal and the risk factors associated with Newcastle disease annual outbreaks in rural chickens, in order to build a prediction model for the spread of the disease.

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ENQUÊTES ENTOMOLOGIQUE ET PARASITOLOGIQUE SUR LES TRYPANOSOMOSES BOVINES À LA STATION D'AVÉTONOU/ITRA, AU TOGO

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Abstract

Cross-sectional entomological and parasitological surveys were conducted between mid-August and September 2011 on 180 cattle at the Agronomic Research Institute of Togo (ITRA) station of Avetonou located in the Prefecture of Agou in Togo. These surveys were intended to update epidemiological data on animal *trypanosomosis* in this area. On parasitological point of view, two trypanosome species (Trypanosoma vivax and Trypanosoma congolense) were identified. The average prevalence value of trypanosome infections was 22.78% with a predominance of T. vivax infections (80.49%), followed by T. congolense infections (12.19%), with the possibility of mixed infections caused by these two trypanosomes species (7.32%). The mean Packed Cell Volume (PCV) of animals is 27.34 ± 0.88. On the entomological side, Glossina tachinoides was captured in all surrounding area to the station with an apparent fly density of 2 per trap per day. Contrary to G. tachinoides species, Glossina palpalis palpalis was only captured along the Zio River located approximately 0.8 km in the north-east of the station with an apparent fly density of 0.2 per trap per day.

Keys-Words: Animal *Trypanosomosis*, Trypanosoma, Glossina, Prefecture of Agou, Togo.

Résumé

Des enquêtes transversales entomologique et parasitologique ont été conduites entre mi-août et septembre 2011 sur 180 bovins à la station de recherche d'Avétonou/Institut Togolais de Recherches Agronomiques (ITRA) située dans la Préfecture d'Agou au Togo. Ces enquêtes avaient pour objectif d'actualiser des données épidémiologiques sur les trypanosomoses animales dans cette zone. Du point de vue parasitologique deux espèces de trypanosomes (Trypanosoma vivax et Trypanosoma congolense) ont été identifiées. La prévalence moyenne des infections trypanosomiennes est de 22,78 % avec une nette prédominance des infections à T. vivax (80,49 %), suivi des infections T. congolense (12,19%) avec possibilité d'infections mixtes causées par ces deux espèces de trypanosomes (7,32%). L'hématocrite moyen des animaux est de 27,34 ± 0,88. Sur le plan entomologique, Glossina tachinoides a été capturé dans tous les milieux environnant la zone d'étude avec une densité apparente par piège par jour égale à 2. Contrairement à l'espèce G. tachinoides, Glossina palpalis palpalis n'a été capturée que sur le long du fleuve Zio, situé à environ 0,8 km au Nord-Est de la station, avec une densité apparente par piège par jour égale à 0,2.

Mots clés: Trypanosomoses animales, Trypanosoma, Glossina, Préfecture d'Agou, Togo.

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Introduction

En Afrique subsaharienne, la productivité des animaux reste faible, en partie, à cause des trypanosomoses animales qui constituent l'une des plus importantes contraintes au niveau pathologique (Peregrine, 1994 ; Swallow, 2000). Les glossines ou mouches tsétsé, qui en sont les principaux vecteurs, occupent généralement les meilleures terres de l'Afrique de l'Ouest, notamment les zones humides (Diall, 2001). Le Togo est un pays de l'Afrique de l'Ouest entièrement logé dans la zone de distribution des glossines (Dao, 1998) avec plus de 85 % de sa superficie infestée de glossines et 95 % du cheptel bovin sous la menace des trypanosomoses animales. Annuellement, plusieurs cas cliniques de trypanosomoses sont rapportés au Togo, occasionnant ainsi des dépenses supplémentaires et un manque à gagner aux éleveurs. Par ailleurs, des données actualisées sur la répartition spatiale des trypanosomoses et de leurs vecteurs ne sont pas disponibles, pourtant indispensables dans des stratégies de lutte contre ces maladies. C'est dans cette logique que nous avons entrepris sur le plan national, une série d'études dont la présente effectuée à la station de recherche d'Avétonou/ITRA afin d'actualiser la situation épidémiologique des trypanosomoses animales et la distribution des glossines dans la zone.

Matériel Et Méthodes

Zone d'étude

L'étude a été réalisée à la Station de recherche d'Avétonou située dans la préfecture d'Agou (région des plateaux). Elle est localisée entre les parallèles 6° 40' et 6° 55' de latitude Nord, et 0° 40' et 0° 55' de longitude Est. D'une superficie totale de 650 ha, la végétation de la station est constituée d'un ensemble de savane arborée traversée par endroits de forêts galeries. Cette végétation est dominée par une strate herbacée, constituée d'Andropogon sp. et de Panicum sp. Le fleuve Zio, situé environ à 0,8 km au Nord- Est de la Station, constitue avec ses affluents, le réseau hydrographique le plus important. Le climat est de type soudano-guinéen avec deux saisons pluvieuses de mars à juin et de septembre à octobre et deux saisons sèches de juillet à août et de novembre à février. La préfecture d'Agou présente, en outre, un microclimat dû au relief dont l'altitude adoucit les températures et provoque des précipitations plus abondantes dans les plaines. La pluviométrie moyenne annuelle varie entre 1000 et 2000 mm (avec des plus grandes précipitations en juin) mais elle présente des variations interannuelles importantes. Le relief de la préfecture d'Agou est essentiellement marqué par le mont Agou, le plus haut sommet du Togo avec 986 m de hauteur qui se dresse à la pointe Sud de la chaîne d'Atakora. L'agriculture et l'élevage sont les principales activités économiques du milieu.

Méthode

Enquête entomologique

L'enquête entomologique a été conduite en Août 2011. Elle avait pour objectif de déterminer les espèces de glossines présentes, leur densité apparente par piège (DAP). Les pièges biconiques standards Challier -Laveissière, 1973 et les pièges mono-coniques de type Vavoua (Laveissière et Grebaut, 1990) ont été déployés à intervalle de 100 à 200 m pendant 24 heures. Chaque piège a été identifié par un numéro. Les mouches capturées ont été dénombrées suivant l'espèce et le sexe. La densité apparente par piège (DAP) a été définie comme le nombre moyen de glossines (mouches tsétsé) capturées par piège et par jour. La portion du fleuve Zio du milieu, la Station et ses villages limitrophes ont été prospectés avec les deux types de pièges (biconiques et monoconiques posés alternativement). Déployés le long de la végétation riveraine du fleuve Zio, les pièges ont été posés autour des parcs à bétail, des points d'eau, dans les pâturages, à la porcherie de la station, puis autour des bergeries et des porcheries villageoises. En pratique 3, 10 et 12 pièges ont été posés respectivement le long du fleuve Zio, à la Station et dans les villages environnants, soit un total de 25 pièges.

Enquête parasitologique

Elle a été conduite en septembre 2011 et a porté sur trois troupeaux de 180 bovins répartis comme suit: (i) 68 bovins de race

N'dama, (ii) 52 bovins métis (Goudali x N'dama ; Goudali x race locale Somba) et (iii) 60 bovins de race locale Somba. Le prélèvement de sang a été fait à la veine jugulaire dans un tube « vacutainer » avec anticoagulant et conservé sous glace avant examen microscopique. La mesure de l'hématocrite (Packed Cell Volume : PCV) et la recherche microscopique des trypanosomes vivants, ont été réalisées par la méthode de buffy coat (Murray *et al.,* 1977) dans un délai maximum de 4 heures après le prélèvement. En effet, après une centrifugation différentielle du sang dans des tubes capillaires à une vitesse de 12 000 tours/min pendant 5 minutes, la lecture de l'hématocrite a été effectuée sur un lecteur standard. Ensuite, le buffy coat a été examiné au microscope après son étalement entre lame et lamelle. Les différentes espèces de trypanosomes ont été identifiées sur la base de leurs mobilités et de leurs morphologies. Par ailleurs, des informations sur l'âge et la race des animaux ainsi que la date du dernier traitement trypanocide ont été relevées sur une fiche d'enquête.

Analyse de données

Les données ont été saisies et analysées à l'aide du tableur Excel.

Résultats

Résultats entomologiques

Les résultats entomologiques sont consignés dans le tableau I. Au total, deux espèces de glossines riveraines, Glossina palpalis palpalis et Glossina tachinoides, ont été capturées. L'espèce G. palpalis palpalis n'a été capturée que sur le long du fleuve Zio. Quant à l'espèce G. tachinoides, elle a été capturée sur tous les sites de piégeages (fleuve Zio, station, villages environnants) et constitue l'unique espèce capturée à la Station et dans les villages environnants. La densité apparente par piège et par jour de glossines à l'échelle de la zone a été de 2,2 dont 2 pour G. tachinoides et 0,2 pour G. palpalis palpalis. Elle est de : (i) 2 sur le Zio dont 0,33 pour G. tachinoides et 1,67 pour G. palpalis palpalis; (ii) 2,5 à la Station et (iii) 2 dans les villages (tableau I).

Résultats parasitologiques

Les résultats parasitologiques sont consignés dans le tableau II. Deux espèces de trypanosomes ont été identifiées. Il s'agit de Trypanosoma vivax et Trypanosoma congolense. La prévalence moyenne était de 22,78 % dont 33 cas de T. vivax (18,33%), 05 cas de T. congolense (2,78%) et 03 infections mixtes T. vivax-T. congolense (1,67 %). Cette prévalence se répartit dans les troupeaux comme suit: (i) troupeau de race N'dama 16,18 % (11/68), (ii) troupeau de race locale Somba 23,33 % (14/60) et (iii) troupeau métis (Goudali x N'dama ; Goudali x race locale Somba) 30,77 % (16/52). Cette différence observée des valeurs de prévalence entre ces différents groupes de bovins n'est pas significative au seuil 5% (comparaison basée sur le test de Khi2). Ces résultats parasitologiques ont montré une nette prédominance de T. vivax sur T. congolense soit 80,49 % des infections totales (figure 1). Outre les trypanosomoses, des cas de microfilaires ont été diagnostiqués.

Hématocrite des animaux

Pour tous les animaux confondus, l'hématocrite moyen des animaux a été de 27,34 ± 0,88. L'hématocrite moyenne la plus élevée par troupeau a été de 29,67 ± 1,32 dans le troupeau de race locale Somba, suivie de celle du troupeau de race N'dama 27, 28 ± 1,48 et enfin celle du troupeau métis (Goudali x N'dama ; Goudali x race locale Somba) 24,73 ± 1,44 (tableau III). Cette différence de

Tableau I: Densités de glossines capturées par site et par espèce à Avétonou

Site de piégeage	Nbre de pièges	Glossina tachinoides				Glossina p. palpalis				DAP globale
		Mâles	Femelles	Total	DAP	Mâles	Femelles	Total	DAP	
Zio	3	00	1	1	0,33	1	4	5	1,67	2
Station	10	4	21	25	2,5	00	00	00	00	2,5
Villages	12	9	15	24	2	00	00	00	00	2
DAP	**-**	**0,52**	**1,48**	**-**	**2**	**0,04**	**0,16**	**-**	**0,2**	**2,2**

Tableau II: Prévalences trypanosomiennes

Troupeau	Taille	T.c	T.v	T.c+T.v	Prévalence (%)
N'dama	68	02	08	01	16,18
Race locale Somba	60	02	01	02	23,33
Métis (Goudali x N'dama ; Goudali x race locale Somba)	52	01	15	00	30,77
Total	**180**	**05**	**33**	**03**	**22,78**

T.c: Trypanosoma congolense **T.v:** Trypanosoma vivax

Tableau III: Hématocrite des bovins

Troupeau	Taille	Hct(%)	Ecart type	Intervalle de confiance (α=0,05)	Effectif Hct < 25%
N'dama	68	27,28	6,33	27,28 ± 1,48	21
Race locale Somba	60	29,67	5,28	29,67 ± 1,32	07
Métis (Goudali x N'dama ; Goudali x race locale Somba)	52	24,73	5,40	24,73 ± 1,44	24
Total	**180**	**27,34**	**6,03**	**27,34 ± 0,88**	**52**

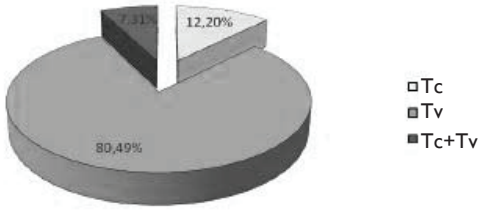


Figure 1: Pourcentage des différentes infections

moyennes de l'hématocrite observée entre les différents troupeaux est significative au seuil 5% (comparaison basée sur le test d'écart réduit). Le pourcentage de bovins dont l'hématocrite a été inférieur à 25 (seuil classiquement considéré comme révélateur d'un état anémique) a été de 28,89 % soit 52/180 (tableau III). Ce pourcentage, par troupeau, a été de 11,67% chez les bovins de race locale Somba (7/60), de 30,88% chez les N'dama (21/68) et de 46,15% dans le troupeau métis (24/52).

Discussion

D'après les résultats entomologiques, de façon générale, seules deux espèces de glossines riveraines (G. tachinoides et G. palpalis palpalis) ont été capturées dans la zone d'étude à Avétonou. Dans cette même zone, Mawuéna et Yacnambé (1988a) ont rapporté qu'en 1977 la densité de G. palpalis palpalis était de 1 à la station et 4 sur le Zio et celle de G. tachinoides était de 0,6 à la station et de 2 sur le Zio. En 1986, ces densités (résultats d'une année d'enquête) avaient considérablement diminué (G. palpalis palpalis : 0,1 à la station et 0,8 sur le Zio ; G. tachinoides : 0,05 à la station et de 0,2 sur le Zio), cependant G. palpalis palpalis demeurait l'espèce glossinaire la plus importante. Les résultats obtenus sur les mêmes sites, 25 ans après, montrent une quasi absence de G. palpalis palpalis à la station. Par contre sa densité sur le Zio est passée de 0,8 en 1986 à 1,67 en 2011. G. tachinoides est concentrée à la station où sa densité est de 2,5 alors qu'elle était de 0,05 en 1986. La non capture de G. palpalis palpalis à la station pourrait s'expliquer par la réduction de sa densité et aussi par la courte durée de la pose de pièges (24 heures). L'augmentation des densités de G. tachinoides à la station et de G. palpalis palpalis sur le Zio serait due à un relâchement dans la lutte contre les mouches tsétsé. En effet, depuis la fin du projet Germano-togolais en 1988, aucune lutte de grande envergure n'a été entreprise. Les actions de lutte se sont limitées aux applications épicutanées d'insecticides sur les animaux.

A la station d'Avétonou, bien que les bovins du centre soient composés en majorité d'animaux trypanotolérants, ces derniers ont été fortement infestés par les trypanosomes.

Les prévalences enregistrées au niveau

de chaque troupeau sont : (i) 30, 77 % dans le troupeau métis (Goudali x N'dama ; Goudali x race locale Somba), (ii) 23,33 % dans le troupeau de race locale Somba et (iii) 16,18 % dans le troupeau N'dama. La moyenne de la prévalence obtenue à la station d'Avétonou est de 22,78 %. Cette dernière valeur est nettement supérieure à celle obtenue par Mawuéna et Yacnambé (1988b) et qui était de 2,8%. Les espèces trypanosomiennes identifiées dans les infections sont T. vivax et T. congolense, et ces résultats sont en accord avec ceux obtenus par les deux précédents auteurs (Mawuéna et Yacnambé (1988b)). Tous les animaux de la station bénéficient d'une chimioprophylaxie à base du Chlorure d'Isométamidium à la dose de 0,5 mg/kg de poids vif tous les trois mois. Malgré ce calendrier prophylactique, une prévalence relativement élevée présagerait qu'aux côtés de la forte pression glossinaire, les traitements trypanocides appliqués seraient inefficaces ; d'où une suspicion de la résistance aux trypanocides. Une étude complémentaire sur la chimiorésistance aux trypanocides permettrait de mieux clarifier la situation. Il est aussi intéressant de signaler que des analyses complémentaires par la PCR avec les amorces spécifiques des parasites permettraient d'indiquer des prévalences plus élevées que celles décelées par la technique parasitologique classique par l'examen du buffy coat (Desquesnes *et al.,* 1999). En effet, nos résultats parasitologiques ont été obtenus par cette dernière technique classique de buffy coat qui est une technique reconnue être sensible pour un diagnostic de terrain et permettant d'identifier de façon rapide les animaux dont les parasitémies sont de l'ordre de 300 à 700 trypanosomes par ml de sang (Very *et al.,* 1990 ; Uilenberg, 1998). Cependant, de faibles parasitémies peuvent échapper à cet examen direct (Desquesnes et De La Rocque, 1995).

L'hématocrite moyen des bovins a été supérieur à 25 (27,34 ± 0,88) pour l'ensemble des animaux avec des valeurs moyennes par troupeau allant de 24,73 ± 1,44 pour le troupeau métis à 29,67 ± 1,32 pour le troupeau de race locale Somba, avec une différence significative entre différents troupeaux. Etant donné que, le degré d'anémie chez un animal dépend aussi bien d'autres facteurs tels que l'état nutritionnel, pathologique (helminthoses, hémoparsitoses, infestation par les tiques, etc…) et de l'âge de l'animal (Agyemang *et al.,* 1990; Agyemang *et al.,* 1991 ; Kaufmann *et al.,* 1992), la différence entre les différents troupeaux serait difficile à expliquer. Toutefois, le degré d'anémie établi par la méthode de micro-hématocrite est une mesure fiable de l'état général d'un animal atteint de trypanosomoses (Murray et Dexter, 1988).

Conclusion

Les trypanosomoses animales sont bien présentes à la station d'Avétonou de l'Institut Togolais de Recherche Agronomique du Togo. Les glossines qui en sont les principaux vecteurs ont été capturées (Glossina palpalis palpalis et Glossina tachinoides) sur le fleuve Zio, à la Station d'Avétonou et dans les villages environnants de la station. Les infections parasitaires sont essentiellement dues à Trypanosoma vivax. Bien qu'un calendrier prophylactique à base du Chlorure d'Isométamidium tous les trois mois soit adopté, il a été noté des prévalences parasitologiques relativement élevées. Une étude complémentaire sur la chimiorésistance aux trypanocides dans cette zone permettrait de mieux clarifier la situation.

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QUANTITATIVE ANALYSIS OF OXYTETRACYCLINE RESIDUE IN BEEF AND CHICKEN MEAT FROM CITIES OF SOUTHWEST NIGERIA

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Summary

This study evaluated the levels of oxytetracycline residue in chicken and beef from major abattoirs and chicken marketing outlets in two metropolitan cities of southwest Nigeria. Samples of cattle (kidney, liver and muscle) and chicken (liver and muscle) from each city were obtained between January 2010 and December 2011. Oxytetracycline residue was determined by high performance liquid chromatography (HPLC). The mean residue concentrations of 1324.7±148.0, 856.6±118.0 and 651.7±101.3µg/kg were obtained in bovine kidney, liver and beef respectively with 8.7%, 8.6% and 11.2% above MRLs (kidney 1200µg/kg, liver 600µg/kg and muscle 400µg/kg). The levels in chicken liver and muscle were 1042.0±122.8 and 615.0±91.8µg/kg, respectively, of which 17.4% and 18.4% were above MRLs. The levels were significantly higher ($P<0.05$) in chicken than in beef and also in wet than dry season in beef. This study showed that a high proportion of beef and chicken consumed in the cities contained violative levels of oxytetracycline residue arising possibly from indiscriminate antibiotics usage in Nigeria livestock industry. These could predispose meat consumers to the associated health risks of antibiotic residue. Regulatory control of antibiotics usage, residue monitoring in Nigerian meat will ensure food safety and participation in international meat trade.

Keywords: Oxytetracycline, Residue, Beef, Chicken, HPLC, Southwest Nigeria

ANALYSE QUANTITATIVE DE RESIDUS D'OXYTETRACYCLINE DANS LA VIANDE DE BOEUF ET DE POULET DES CITES DU SUD-OUEST DU NIGERIA

Résumé

Cette étude a évalué les niveaux de résidus d'oxytétracycline chez les poulets et boeufs de grands abattoirs et points de vente de poulets dans deux villes métropolitaines du sud-ouest du Nigeria. Des échantillons de viandes de bovins (rein, foie et muscle) et de poulets (foie et muscle) de chaque ville ont été prelevés entre janvier 2010 et décembre 2011. Les résidus d'oxytétracycline ont été déterminés par chromatographie liquide à haute performance (CLHP). Les concentrations moyennes de résidus de 1324,7 ± 148,0 ; 856,6 ± 118,0 ; et 651,7 ± 101.3µg/kg ont été observées dans les reins, le foie et la viande de bovins respectivement avec 8,7%, 8,6% et 11,2% au-dessus des LMR (reins 1200µg/kg, foie 600µg/kg et muscle 400µg/kg). Les concentrations dans le foie et les muscles de poulet étaient respectivement de 1042,0 ± 122,8 et 615,0 ± 91.8µg/kg, dont 17,4% et 18,4% étaient au dessus des LMR. Les concentrations étaient significativement plus élevées ($P <0,05$) pour le poulet par rapport au boeuf, et également en saison humide par rapport à la saison sèche pour le boeuf. Cette étude a montré qu'une proportion élevée de viande de boeuf et de poulet consommée dans les villes contenait des concentrations de résidus d'oxytétracycline excessives résultant éventuellement de l'usage anarchique d'antibiotiques dans l'industrie avicole du Nigeria. La présence de ces résidus pourrait prédisposer les consommateurs de viande aux risques sanitaires associés aux résidus d'antibiotiques. Le contrôle réglementaire de l'usage des antibiotiques, le contrôle des résidus dans la viande nigériane permettra d'assurer la sécurité sanitaire de ces aliments et la participation du pays au commerce international de la viande.

Mots-clés : Oxytetracycline, Résidu, Boeuf, Poulet, HPLC, Sud-Ouest du Nigeria

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Introduction

Antibiotics are used in livestock for prophylaxis and therapeutic purposes against bacterial infections. They are also used in livestock to improve productivity and as growth promoter antibiotics (GPA). Since the first discovery of penicillin by Alexander Fleming in 1928 (Fleming, 1929) and the subsequent demonstration of its clinical importance in the 1940s (Abraham *et al.,* 1941), antimicrobial agents have played revolutionary role in human and animal health. The benefits of drug use in livestock result from the maintenance of animal health thereby reducing the chance that pathogenic agents will spread to humans from animals.

Oxytetracycline, streptomycin, penicillin and sulphamethazine have been reported as the most frequently used and detected antibiotics in tissues of food animals (Phillips *et al.,* 2004; Posyniak *et al.,* 2005; Salehzadeh *et al.,* 2006). Tetracyclines are broad-spectrum antibiotics that show activity against Gram-positive and Gram-negative bacteria, including mycoplasmas, chlamydiae, rickettsias, spirochetes, actinomycetes, and some protozoa (Chopra and Roberts, 2001). About 65% of the antibiotics prescribed for veterinary therapeutic use within the European Community are tetracyclines (Sarmah *et al.,* 2006). Also, in the United States, tetracyclines are the most commonly used antibiotics in a variety of animal species (disease treatment and prevention; growth promotion), plants, and humans (Mellon *et al.,* 2001). Consequently, their residues were often reported in meat, milk and eggs (Rivere and Spoon, 1995; Phillips *et al.,* 2004; Posyniak *et al.,* 2005)

The potential health hazards of drug residues in food can be categorised into three namely; toxicological, microbiological and immunological effects (Goldfrank *et al.,* 2002). Also, other public health hazards associated with the ingestion of violative levels of antibiotics residues in food chain include; allergic or hypersensitivity reactions, carcinogenic, mutagenic and toxic effects in man (Dewdney *et al.,* 1991; Berends *et al.,* 2001; Nisha, 2008). Economic loses also arise from meat, milk and eggs condemnations at national and international trade levels when residues are detected at violative levels. Furthermore, milk contaminated with even low concentrations of antimicrobial drug residues also create technological difficulties in the production of fermented milk products by inhibiting the growth of the starter cultures leading to economic loss in dairy industry (Heeschen and Blüthgen, 1991). Tetracyclines and their residues have been reported to also cause permanent discolouration of teeth, chronic nephrotoxicity, hepatotoxicity and skin hyperpigmentation (Robert, 1996; Goldfrank *et al.,* 2002). Therefore, ethical, hygienic, technological and ecological effects of veterinary drug residues in foodstuffs are of public health and socioeconomic reasons for monitoring and foods of animal origin are due importance (Honkanen-Buzalski and Suhren, 1999, Nonga *et al.,* 2009).

In developing countries, the use of antibiotics is characterised by lack of adequate regulatory control (Al-Mustafa and Al-Ghamdi, 2002; Okeke *et al.,* 2005). The quantities being used are difficult to obtain due to indiscriminate use (Mitema *et al.,* 2001). In Nigeria, antibiotics as well as other veterinary drugs are available over the counter and easily accessible by livestock farmers (Dina and Arowolo 1991; Kabir *et al.,* 2004). A greater proportion of cattle in Nigeria are reared by the nomadic herdsmen who administer chemotherapeutic agents without veterinary prescription, while poultry farmers also routinely use antibiotics for their flocks (Dipeolu and Alonge, 2002; Kabir *et al.,* 2004).

Withdrawal periods are established for veterinary drugs residue of toxicological concern to ensure safe concentration before slaughtering of treated animals or harvesting milk and eggs for human consumption (Goetting *et al.,* 2011). The withdrawal periods for tetracyclines vary between 7 to 21 days in different edible tissues from food animals (Posyniak *et al.,* 1998). Veterinarians and livestock producers have ethical responsibility for prudent use of drugs in livestock and are expected to observe the withdrawal periods. When drug manufacturers and national legislative directions are followed by livestock producers and the veterinarians, drug residue

levels in food of animal origin are expected to be within safe limits. However, when levels are exceeded they are usually caused by improper use and as such food should legally not be allowed for human consumption (Ivona and Mate, 2002).

International efforts by FAO/ WHO Codex Alimentarius Commission (CAC) and other national agencies are involved in setting residues safety standards: maximum residue limits (MRLs) and acceptable daily intake (ADI) for the various drugs and chemicals used in livestock production are ongoing and are major requirements in food exports. The MRL set by the EU legislation for tetracycline, oxytetracycline as well as chlortetracycline in raw cow milk is set to 0.1mg/kg (100ng/g) (Council Regulation 2377/90/EEC), while CAC (2009) recommended maximum residue limit (MRL) is 200µg/kg in muscle, 600µg/kg in liver and 1200µg/kg in kidney.

The widespread use of antimicrobials in livestock management and possible violations of MRLs require routine monitoring and surveillance of the use of veterinary drugs and drug residues. However, in Nigeria there are no residues inspections and monitoring programmes in place, coupled with the unregulated access to veterinary drugs by livestock producers the risks of residues in meat eggs or milk are very high.

Several studies conducted in Nigeria on drug residues deposit in food animal products (Oboegbulem and Fidelis, 1996; Dipeolu and Alonge 2002; Kabir *et al.,* 2004) have demonstrated the presence of antibiotic residues in meat and animal products. Most of these studies were based on microbiological inhibition techniques that did not specifically classify and quantify the antibiotics involved. Also, there are no national MRLs or residues inspection and monitoring programmes in place, coupled with the unregulated access and use of veterinary drug (including antibiotics) by livestock producers in Nigeria. This study of residue risk assessment was aimed at detecting and quantifying the levels of oxytetracycline residue in chicken and beef from major abattoirs and chicken marketing outlets two metropolitan cities of southwest Nigeria.

Materials And Methods

Study location

Lagos and Ibadan metropolis are the two largest and fast growing commercial cities in the southwest Nigeria with human population of 9.1 million and 3.6 million respectively (National Bureau of Statistics, 2006). The two municipal central abattoirs in Lagos and Ibadan metropolis namely are Oko-Oba and Bodija abattoirs respectively. Approximately 300 and 1000 heads of cattle are slaughtered daily respectively for distributions to retail meat markets across the cities. The cities are supplied with cattle mainly from northern Nigeria and peri-urban agro-pastoralist and nomadic cattle herds. Also, large and small scales of commercial poultry farms exist within the cities and at the peri-urban areas. Live and slaughtered chickens are sold at the farm gates and in Oko-Oba (Lagos), and Mokola (Ibadan) markets.

Sampling procedure

Cattle and table size chicken carcasses were randomly obtained from the two city abattoirs, retail chicken markets and poultry farms during the periods of August 2009 and July 2010 with the assistance of State meat inspectors. Ten commercial poultry farms within and around the cities producing commercial chicken broilers were randomly selected. Sixty samples of each meat portion were randomly obtained simultaneously from the sample locations..Approximately 50 to 100 grams of liver, kidney or muscle samples were obtained from each cattle carcass and also from broiler chicken livers, and breast/thigh muscles. The samples were well labelled and wrapped in polythene bags and transported in cool boxes with dry ice or freezer packs at 4oC. Five broiler chickens reared at the University of Ibadan free of tetracycline antibiotic were also obtained as blank samples for recovery experiment. The samples were subsequently transported to the food and meat hygiene laboratory, University of Ibadan and stored at -20^{o}C until time of analysis.

Calibration curve and recovery experiment

Analytical grade oxytetracycline

chloride was purchased from Sigma Chemical Co., St. Louis MO. USA. Serial dilutions of the standard oxytetracycline solutions (1 : 100 (10 ppm), 1 : 200 (5 ppm), 1 : 400 (2.5 ppm), 1 : 500 (1.25 ppm), 1 : 1000 (0.1 ppm), 1 : 10000 (0.01 ppm) were injected into the HPLC machine.

The different concentrations and the corresponding peak areas were plotted to obtain the calibration curve (Figure 1). For the recovery experiment to determine the sensitivity of the extraction and elution procedures, 25 grams of edible portions of tetracycline free broiler chickens were spiked in triplicate with 4 ppm and 8ppm of the analytical standard oxytetracycline solutions. The tissues were allowed to equilibrate for about 30mins before proceeding with extraction and for reverse phase HPLC analysis.

The analysis and quantification of oxytetracycline residues in the analytes were performed at Chemical Analysis Laboratory of the Nigeria Institute of Science Laboratory Technology (NISLT), Ibadan. A high-performance liquid chromatography machine HPLC (Agilent, 1200 series, Germany) with UV detector equipped with a constant flow pump and a variable wavelength UV detector set at 360nm and flow rate of 1.5mls/min was used. Elution of oxytetracycline from the analyte was done on a nucleosil C- 18 (10µ, 250 x 4.0mm ID) column with mobile phase; Methanol-Acetonitrile-0.01M aqueous Oxalic acid solution, pH 2.0 (1: 1.5: 2.5) as described by Muriuki *et al.,* (2001). The mobile phase was filtered through 0.22µm membrane filter using vacuum pump and regularly degassed by sonication. The HPLC machine was also flushed at interval with blank methanol and mobile phase was allowed to run through the machine for equilibration and conditioning during which stable baseline was obtained on the recorder monitor. The column and tubing were regularly checked to ensure leak proof.

The coefficient of correlation (R2) values of 0.993 and the linear equation y=20.7x + 8.611 where y is the peak area and x is the concentration in ppm were obtained. The mean retention time for the oxytetracycline ranged from 2.8 to 3.5 minutes while the detection limit was 0.01ppm. The mean recovery of oxytetracycline from the spiked tissues was between 80.0% and 92.5%. Figures 2 showed the typical HPLC chromatographs with peak areas from oxytetracycline standard (a) and residue in samples (b) obtained from detector.

Sample preparation:

The extraction and clean-up procedures involve solvent extraction and liquid-liquid partitioning procedures to obtain the analyte were performed at the Food and Meat Hygiene Laboratory, University of Ibadan. The extraction was done by cutting approximately 25g of each sample which was thoroughly homogenized with 75ml of 1N hydrochloric acid, followed by protein precipitation of 8ml of the homogenate

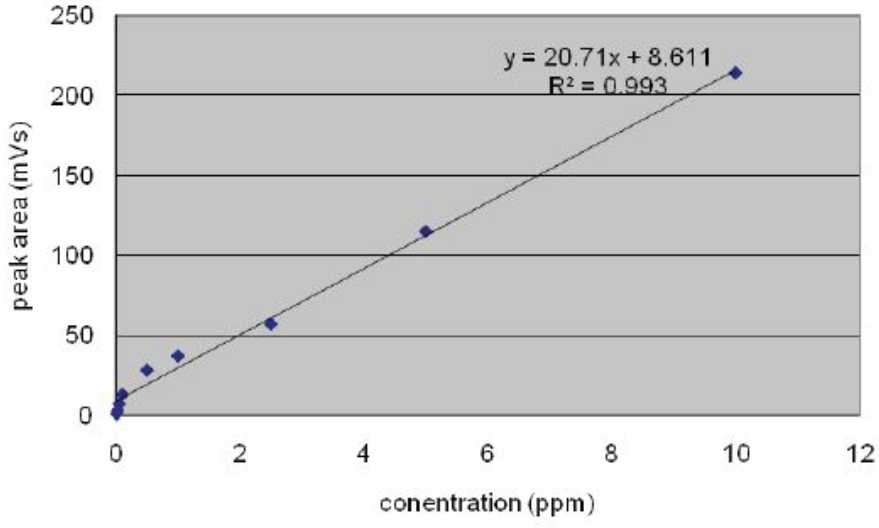


Figure. 1: Calibration curve of oxytetracycline analytical standard

by thoroughly swirling with 32mls acetonitrile to obtain filtrate of the supernatant. The sample clean-up was achieved by adding 20 mls of the filtrate to 20mls hexane and 20mls methylene chloride in a separatory funnel and vigorously shaken resulting in separation into aqueous and organic layers.

The aqueous layer containing the analyte was collected into scintillation vials for HPLC analysis. HPLC analysis of oxytetracycline in samples

The injection volume of 20µl analyte from each sample (in duplicate) was injected through the column. Separation and resolution occurred in the column and detector resulting in peaks (chromatographs) shown on the monitor with the peak areas and retention times recorded by the computer recorder. The mean peak areas of the samples corresponding to the retention time between 2.8 to 3.5 minutes obtained from the reference standard were recorded as positive for oxytetracycline. Quantification of oxytetracycline residues in the samples were obtained from calculation by substituting the mean peak areas in the linear equation obtained from the calibration curve of the standard.

Statistical Analysis

Descriptive statistics of the prevalence of oxytetracycline residue in samples from different locations were obtained. The prevalence of the residue were compared by Chi-square test while Statistical significance of different residue levels were obtained by repeated measure ANOVA followed by Tukey multiple comparisons and student t-test (at 95% confidence level $p<0.05$) using GraphPad Prism statistical software (GrapPad Software Inc, USA).

Results

Oxytetracycline residue in cattle from Lagos and Ibadan abattoirs

The prevalence of oxytetracycline residue detected from cattle kidney, liver and muscle samples from Oko-Oba (Lagos) abattoir during the wet season were 56.7%, 50.0% and 48.3%; and 60.0%, 60.0%, and 50.8% during the dry season respectively. Oxytetracycline residue prevalence in meat obtained from Bodija (Ibadan) abattoir during the wet season was 73.3% in kidney, 61.7% in liver and 56.7%; while during the dry season it was 58.3%, 53.3% and 46.7% in kidney, liver and muscle, respectively. The overall mean oxytetracycline residue levels were 1324.7±148.0, 856.6±118.0 and 651.7±101.3µg/kg in bovine kidney, liver and muscle, respectively. The residue levels in the different meat types were of variable mean concentrations in all the abattoirs and sources shown in Table 1.

Oxytetracycline residues in broiler chicken from farms and markets

The results obtained from the chicken samples indicated oxytetracycline residue prevalence of 71.7 and 75.0% in liver and muscle respectively from Ibadan poultry farms. While 63.3% and 60.0% prevalence were obtained in liver and muscle respectively from Ibadan market. The prevalence from Lagos poultry farms were 53.3% in liver and 60.0% in muscle and also liver 41.7% and kidney 56.7% from Lagos chicken market. The overall mean concentration of the residue in chicken muscle and liver samples in this study was 1042.0±122.8 in liver and 615.0±91.8µg/kg in muscle. The distribution of the mean residue levels in the chicken across the market and farm locations are shown in Table 2 with a range of 41.7 to 60.0% containing residue above the CAC MRLs. Chicken samples from Ibadan farms had the highest prevalence of the residue. The overall mean residue concentrations in liver were significantly higher ($p<0.05$) than in the chicken muscle. However, there was no significant difference ($p> 0.05$) between the mean residue in chicken from farms and the markets within and across the cities.

Comparison of oxytetracycline residue in beef and chicken within and across the sources

The overall prevalence of 55.4% and 67.4% were obtained from beef and chicken respectively. The mean oxytetracycline residue levels of 1324.7±148.0µg/kg, 856.6±118.0µg/kg and 651.7±101.3µg/kg were obtained in bovine kidney, liver and beef, respectively. While in chicken, the overall concentrations were 1042.0±122.8µg/kg in liver and 615.0±91.8µg/

Table 1: Mean (µg/kg), range and proportion of positive samples for oxytetracycline residue in cattle meat types from Lagos and Ibadan municipal abattoirs

Location (abattoir)	*Season*	*Parameters*	*Tissue types*		
			Kidney	*Liver*	*Muscle*
Oko-Oba (Lagos)	Wet season	Positive	34/60	35/60	38/60
		Mean ±SD	1267.0 ±476.4[a]	904.9±221.4[b]	729.6±274.3[c]
		Range	76.7-2409.0	163.6-1708.0	178.1-1926.0
		% above MRL*	31.7	40.0	46.7
	Dry season	Positive	36/60	32/60	35/60
		Mean ±SD	1436.0±154.1[a]	620.7±175.6[b]	346.2 ±103.8[c]
		Range	308.4-2819.0	240.8-1824.0	67.1-1709.0
		% above MRL*	40.0	41.7	45.0
Bodija (Ibadan)	Wet season	Positive	38/60	40/60	41/60
		Mean ±SD	1544±470.8[d]	917.5 ±315.2[e]	766.2 ±313.9[f]
		Range	598.1-5059.0	163.6-2544.0	211.9-2790.0
		% above MRL*	53.3	46.7	56.7
	Dry season	Positive	36/60	34/60	39/60
		Mean ±SD	1054 ±276.5[d]	618.5 ±105.6[e]	355.8 ±107.8[f]
		Range	163.6-3736.0	110-2191.0	67.1-1245.0
		% above MRL*	30.0	40.0	45.0

superscripted values a, b, c, d,e, f in columns are significantly different ($p < 0.05$)

*MRL (Codex Alimentarius commission) kidney 1200µg/kg, liver 600µg/kg and muscle 400µg/kg (CAC, 2009).

Table 2: Mean (µg/kg), range and proportion of positive samples for oxytetracycline residue in broiler chickens from Lagos and Ibadan

City Chicken source	**Parameters**	**Tissue types**	
		Liver	**Muscle**
Lagos poultry farms	Positive	42/60	35/60
	Mean ±SD	1130 ±614.4[g]	752.4 ±393.7[h]
	Range	164-2674	231-1853
	% above MRL*	56.7	58.3
Lagos chicken market	Positive	43/60	45/60
	Mean ±SD	895.7±473.8[g]	583.8 ±396.0h
	Range	56.7	58.3
	% above MRL*	51.7	60.0
Ibadan poultry farms	Positive	43/60	45/60
	Mean ±SD	895.7 ±473.8	583.8 ±396d
	Range	299-2481	164-1129
	% above MRL*	53.3	60.0
Ibadan chicken markets	Positive	38/60	36/60
	Mean ±SD	986.1 ±449.1	559.4 ±349
	Range	270-1911	183-1525
	% above MRL*	41.7	56.7

superscripted values g, h, i, j, in columns are significantly different ($p < 0.05$)

kg in muscle. The overall residue levels obtained in this study were significantly higher ($p<0.05$) than CAC MRLs (1200, 600 and 200µg/kg in kidney, liver and muscle respectively). Bovine kidney had the highest residue levels followed by liver and least in the muscle. The residue concentrations in beef during the wet season were significantly higher than in dry season. Residue levels in the chicken liver were significantly higher ($p<0.05$) than in the chicken muscle. The overall prevalence and mean concentrations of oxytetracycline residue in chicken meat were significantly higher ($p<0.05$) than in beef. There was no statistical significant difference in mean residue levels of OTC across the metropolitan abattoirs; also between the mean residue in chicken from farms and the markets within and across the cities.

Discussion

The mean residue concentrations in the different meat types ranged between 766.2 and 1544.0µg/kg during the wet season and between 555.8 and 1354.0µg/kg in dry season. These results also showed variable proportions of the meat containing the residues above codex MRLs. Therefore mean residue concentrations in muscle liver and kidney were significantly different ($p< 0.05$) with kidney having the highest followed by liver and least in the muscle. The tissues residue levels obtained in the wet season were higher compared with the dry season. Residues of oxytetracycline were detected at 56.2% and 64.2% prevalence in cattle and chicken meat samples respectively in this study indicated that more than 50% meat being consumed in the study area contained antibiotics. This could be a result of the common practice of indiscriminate use of antibiotics in livestock production and non adherence to withdrawal periods before slaughtering.

The results of this study showed a higher prevalence than 45.6% obtained by Muriuki *et al.,* (2001) in a survey of beef samples from Kenya slaughterhouses who also used HPLC. In Nigeria, previous studies by Dipeolu and Alonge, (2002) reported tetracycline residue between 13.3 to 16.6% prevalence in cattle from Ogun and Lagos States. While Ibrahim *et al.,* (2010) obtained 26% prevalence tetracycline residue in cattle from Sokoto abattoir and 0.1% by Fagbamila *et al.,* (2010) in commercial eggs from northern Nigeria. These authors employed microbial inhibition method which cannot detect protein bounded residues in tissues that require physicochemical extraction. Therefore, the higher prevalence obtained in this study could be due to the better specificity and sensitivity of HPLC analysis employed in this study than the screening tests.

The mean residue levels obtained from this study varied with the meat types and they were mostly higher than CAC MRLs for each meat type (200, 600 and 1200µg/kg for muscle, liver, and kidney). The results also agree with the work of Muriuki *et al.,* (2001) who also reported mean oxytetracycline residues higher than the MRLs in cattle from Kenya slaughter houses. Also, Al-Ghamdi *et al.,* (2000) in Saudi Arabia showed that antibiotic residue were present in 69.7% of broiler and 60% layer chicken samples. While Salehzadeh *et al.,* (2006) also obtained oxytetracycline residues 95.55% of farms survey in Iran with 27.77%, 95.55% and 18.88% of muscle, liver and kidney samples containing residues of oxytetracycline above MRLs.

The results also showed that the mean oxytetracycline levels were higher in liver and kidney than muscle, but the level in cattle muscle was higher than in chicken muscle. This could be due to the fact that cattle were administered with intramuscular injectable brands of the drugs while chicken were dosed through feed and water. Nisha (2008), reported that tetracyclines are usually concentrated in the kidney and liver, such that residue concentrations in edible tissues can be predicted from kidney cortex concentrations. There was no significance difference in the mean residue concentrations across the different locations but higher during the wet season than in the dry season. These results could indicate different husbandry practices in poultry and cattle production as well as varying disease prevalence for which oxytetracycline was commonly used along with other antibiotics throughout the year.

The extensive use of veterinary drugs

in animal production occasioned by the growth and intensification of animal production in order to meet the increasing population demand for animal protein have become a major global public health concern (Botsoglou and Fletouris, 2001; Jafari *et al.,* 2007). The high prevalence of residues obtained could be due to indiscriminate and misuse of antibiotics by farmers as well as lack of strict regulation and control of antimicrobial use in Nigerian livestock production. Therefore there are several national and international regulatory control and monitoring efforts to ensure safety of livestock products meant for human consumption. Antibiotics are the most important and frequently used group of veterinary drugs in therapeutic quantities against animal diseases and also in sub-therapeutic quantities for growth promotion and disease prophylaxis (Aarestrup *et al.,* 2008). The presence of their residues in food of animal origin has been frequently associated with misuse by livestock producers. Oxytetracycline, one of the most commonly used antimicrobial in different classes of livestock, was detected and quantified in this study.

These results indicate that a large proportion of cattle and chicken meats being consumed in Nigeria contain varying detectable levels of oxytetracycline residue above MRLs with resultant public health implications. Also, this trend will hamper prospect of international trade in meat products and huge loss of potential foreign exchange in most developing countries through WTO, because such products cannot meet the standards.

Conclusion

This study provided the first major quantitative survey of oxytetracycline residue in cattle and chicken meat that were being consumed in cities of southwest Nigeria. The prevalence of oxytetracycline residue obtained was significantly higher in chicken than in beef and during wet than dry season in beef. Our present study indicated that greater proportion of the meat being consumed in Nigerian cities contained oxytetracycline residues as well as other antibiotics above international food safety standards. This is of great public health concern that requires appropriate regulatory actions. Therefore, there is need for establishment of national pharmaco-epidemiological surveillance program on antibiotic usage, residues and resistant food-borne pathogens along the food chain to protect meat consumers in Nigeria. National monitoring of distribution, prescription and usage of antibiotic as well as application of HACCP whereby chemical and microbial hazards from meat are strictly monitored and controlled from farm to fork is hereby recommended. This should involve proper structuring of livestock production in the country, regular extension education for livestock producers, marketers and processors on good animal husbandry practice to ensure safe animal protein supply. Veterinary supervision of livestock production for proper diseases diagnosis, prescription of antibiotics and observance of withdrawal period in food animal production in Nigeria are equally recommended. Good management practices, the use of probiotics and vaccines as alternatives to antibiotic prophylaxis are also recommended.

There is also the need to review and amend the enabling laws on animal disease control, handling of veterinary drugs and meat inspection in Nigeria to ensure safety of meat consumers. The Veterinary Directorate of National Directorate for Food and Drug Administration and Control (NAFDAC) should be properly structured to function like the Centre for Veterinary Medicine of Food and Drug Administration of US and European Union Centre Veterinary Medicine.

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EFFECT OF AQUEOUS EXTRACT OF EUPHORBIA HIRTA ON HAEMATOLOGICAL AND BIOCHEMICAL PARAMETERS OF SPRAGUE DAWLEY RATS

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Abstract

An oral toxicity assessment of the aqueous extract of the whole plant of Euphorbia hirta selected for use in animal healthcare in Ghana was carried out in Sprague Dawley (SD) rats. The extract was administered at a dose rate of 500 - 2000mg/kg per os for 14 days. At the end of the period, blood samples were collected by jugular veno-puncture for haematological and biochemical analysis to assess liver and kidney function. Significant ($P < 0.01$) decreases occurred in the values of red blood cell (RBC) counts of the groups receiving 1000mg/kg and 2000mg/kg of the extract. Consequently haemoglobin concentrations also decreased significantly ($P < 0.001$) compared with the control. The haematocrit (HCT) value of these groups also decreased significantly ($P < 0.05$) below that of the control. Biochemical studies did not suggest any malfunction or abnormality of the renal or hepato-biliary systems. The present results indicate that aqueous extract of the whole plant of E. hirta up to a dose of 500mg/kg orally is generally safe. This finding perhaps justifies the widespread use of the plant in the treatment of several diseases all over the world and may also be used for the treatment of diseases in animals. However, caution should be taken with doses > 500mg/kg as these may induce anaemia.

Key words: Aqueous extract, toxicity, rats, Euphorbia hirta.

EFFET DE L'EXTRAIT AQUEUX D'EUPHORBIA HIRTA SUR LES PARAMÈTRES HAEMATOLOGIQUES ET BIOCHIMIQUES DES RATS SPRAGUE DAWLEY

Resume

Une évaluation de la toxicité orale de l'extrait aqueux de la plante entière d'Euphorbia hirta sélectionné pour pour son utilisation dans les soins de santé animale au Ghana a été réalisée chez des rats Sprague Dawley (SD). L'extrait a été administré à une dose de 500 - 2000mg / kg per os pendant 14 jours. A la fin de cette période, des échantillons de sang ont été prélevés par ponction dans la veine jugulaire pour analyse hématologique et biochimique afin d'évaluer les fonctions hépatique et rénale. On a observé des diminutions significatives ($P < 0,01$) des numérations érythrocytaires (RBC) des groupes recevant 1000mg/kg et 2000mg/kg de l'extrait aqueux. Par conséquent, les concentrations d'hémoglobine ont également diminué de façon significative ($P < 0,001$) par rapport au groupe témoin. La valeur hématocrite (HCT) de ces groupes a également diminué de façon significative ($P < 0,05$), en dessous de celle du groupe témoin. Les études biochimiques n'ont suggéré aucun dysfonctionnement ou anomalie du système rénal ou du système hépato-biliaire. Les présents résultats indiquent que l'extrait aqueux de la plante entière d'E. hirta jusqu'à une dose de 500mg / kg par voie orale est généralement sain. Cette constatation justifie peut-être l'utilisation généralisée de la plante, partout dans le monde, dans le traitement de plusieurs maladies, et elle peut aussi être utilisée pour le traitement de maladies animales. Cependant, des précautions doivent être prises avec les doses > 500mg/kg car celles-ci sont susceptibles de provoquer une anémie.

Mots-clés : Extrait aqueux, toxicité, rats, Euphorbia hirta.

Introduction

Poor health is one of the most important constraints to livestock production under the traditional smallholder production systems. Each year large numbers of farm animals are lost to diseases. It is estimated, for example, that control of diarrhoea and parasitic disease in these animals could increase their production by about 60% (Aryee *et al.,* 1991). Use of modern chemotherapeutic medicines is currently seen as the most effective means of controlling livestock diseases. In rural Ghana, however, especially in the main animal production zones of the country, orthodox veterinary medicines are not available in many places or are expensive and out of reach of many livestock farmers (Otchere *et al.,* 1997). The supply of these medicines is also erratic and not sustainable. This is further complicated by the non-availability of timely expert veterinary services, which easily leads to drug residues in food animals as a result of misuse of medicines. Many smallholder farmers therefore rely on much cheaper and available herbal medicines for keeping their animals healthy and productive (Otchere *et al.,* 1997; Kolawole *et al.,* 2007; Toyang *et al.,* 2007).

Recently, there has been an increased awareness on the use of herbs in animal healthcare practices (MacCorkle, 1986). In Ghana, currently, human healthcare is receiving phenomenal inputs from the use of herbal medicines as alternative medicine for the management of many ailments. In contrast, animal healthcare has not benefited from such advancement (Mzamo *et al.,* 1998). The comparatively low cost of herbal medicines, their relative safety and their availability can impact positively on animal healthcare for increased production.

Euphorbia hirta, Linn (Euphorbiceace) known locally as Animakoa (Twi) occurs widely throughout west tropical Africa and most parts the world (Lind and Tallntire, 1971; Sofowora, 1982; Kumar, *et al.,* 2010). Extracts or decoction of the plant collected in the flowering and fruiting stages is used for treating asthma and respiratory problems such as bronchitis and other respiration complaints in many countries (Sofowora, 1982; Muhammad and Muhammad, 2005; Bala, 2006; Igoli *et al.,* 2007; Ogueke *et al.,* 2007; Sonibare and Gbile, 2008; El-Mahmood, 2009; Huang *et al.,* 2012). In Ghana, Nigeria and parts East Africa, the plant in addition to treating respiratory problems is fed to cattle, sheep and goats to induce or increase lactation (Kokwaro, 1993; Mzamo *et al.,* 1998).

In spite of the massive indigenous knowledge on the ethnoveterinary uses of this plant, pharmacological, particularly toxicological studies that will enhance scientifically formulated preparations for animal use are scanty. Since herbal medicine is affordable, available and acceptable, there is a need to research into the use of herbal medicine for the treatment of disease in animals. In contributing to the research to integrate scientifically formulated herbal medicines into animal healthcare in Ghana, this paper reports result of toxicological evaluation of the whole plant aqueous extract of E. hirta for use in animals.

MATERIALS AND METHODS

Extraction of plant material

The mature whole plant materials of E hirta were collected from Mampong-Akwapim and surrounding areas in the Eastern Region of Ghana by scientists of the Centre for Scientific Research into Plant Medicine (CSRPM). The harvested plants were sun dried for 3 - 5 days after which they were pulverized. One kilogram of the dried pulverized parts was boiled under reflux condensation in 2 litres of water for about 1 hour. The extract was filtered hot in a tincture press and allowed to cool. The filtrate was freeze-dried for use.

Animals and experimental design

Healthy adult Sprague Dawley (SD) rats (150 – 250g) of both sexes obtained from Noguchi Memorial Institute of Medical Research (NMIMR) and maintained at the animal house of the Department of Pharmacology, College of Health Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana were used. They were kept in rat cages and fed a balanced pelleted diet obtained from Ghana Agricultural Foods Company Ltd (GAFCO) Tema, with free access to water and exposure

to a 12h light-dark cycle. The study was conducted in accordance with internationally accepted principles for laboratory animal use and care (EEC directive of 1986: 86/609 EEC).

Toxicity Studies

The animals were divided into four groups (n=6). One group served as control and was administered 0.5ml of 2% Tragacanth (Sigma-Aldrich Co. LLC, USA) for 14 days. The other three groups received daily doses of the aqueous extract of E. hirta (500, 1000 and 2000 mg/kg p.o), respectively for 14 consecutive days. All animals in the various groups were observed continuously over the study period, for changes in various autonomic effects such as frequent urination, convulsion, salivation, respiratory distress, diarrhoea and vomiting among others. Behavioural responses, mortality and other signs of toxicity were also monitored by visual observation. Blood samples were obtained from 3 animals in each group, the control as well as the treated ones at the end of 14 days and analyzed for haematological and biochemical effects.

Haematological effects

Samples of blood were collected from the jugular vein into tubes containing 0.1 ml 4% aqueous solution of di-potassium salt of ethylenediaminetetraacetic acid (EDTA). Total erythrocytes and leucocytes counts, haemoglobin concentrations and packed cell volume (PCV) were performed on both treated and control animals. Blood parameters were obtained with an autoanalyzer (CELL – DYN 1700 version 1.08, USA).

Biochemical assays

For biochemical assays, blood samples were collected into tubes without an anticoagulant. The samples were allowed to clot on the bench for 2 hours and the centrifuged at 80 rpm for 10 minutes; the serum was harvested and stored at -20oC. This was used for the determination of Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST), Gamma-glutamyl transpeptidase (GGT) values and alkaline phosphatase (ALP) to assess the effects of E. hirta on hepatic cells. Blood urea nitrogen (BUN) and creatinine assays were also carried out to assess renal toxicity in the rats. The auto-analyzer, Random Access Chemistry System (Elan Diagnostics, Smithfield, RI, USA) was used for this analysis.

Statistical Analysis

Statistical evaluation was performed using GraphPad Prism Ver. 5.0, for Windows XP. All results are presented as mean ± S.E.M. Data were analyzed using one-way analysis of variance (ANOVA) and, when appropriate, by a Bonferroni post hoc test. Results were considered significant at $p < 0.05$.

Results

Effect of oral administration of Euphorbia hirta on rats

The administration of Euphorbia hirta (500 – 2000mg/kg) orally to rats over a period of 14 days produced no clinical signs, adverse effects or deaths in the animals. The rats were alert with no observable behavioural, motor or neurological changes. No gastrointestinal tract disorder, respiratory distress or locomotor activity was observed in any of the animals in both the treated or control groups.

Effect on haematological parameters

Significant ($P < 0.01$) decreases occurred in the values of red blood cell counts of the groups receiving 1000 and 2000mg/kg of the extract. Consequently haemoglobin concentrations of this group also showed significant ($P< 0.001$) decreases, while the mean haemoglobin concentrations also decreased significantly compare to the control. The HCT value of the group also increased significantly ($P < 0.05$) above that of the control (Table 1).

Effect on serum biochemistry

The 14-day oral administration of Euphorbia hirta (500 – 2000mg/kg) induced insignificant effects in serum enzymes, bilirubin, total protein, albumin and globulin levels (Table 2). Also the oral administration of Euphorbia hirta extract (500 – 2000mg/kg) did not show any significant effect on the blood urea nitrogen (BUN) and creatinine levels (Table 3).

Table 1: Effect of the 14-day toxicity test of Euphorbia hirta extract (500 – 2000mg/kg) on haematological parameters in rats.

Parameters	Control	500 mg/kg	1000 mg/kg	2000 mg/kg
Red blood cells (M/µL)	6.27 ± 0.14[a]	6.37 ± 0.18[a]	4.37 ± 0.4[b]	4.11 ± 0.10[b]
Haemoglobin (g/dL)	16.00 ± 0.55[a]	14.80 ± 0.36[a]	10.30 ± 0.33[b]	10.50 ± 0.46[b]
Haematocrit (%)	38.77 ± 0.80[a]	39.67 ± 2.16[a]	31.47 ± 1.17[b]	32.50 ± 0.76[b]
Mean corpuscular volume (fL)	59.00 ± 0.40[a]	57.97 ± 0.14[a]	73.90 ± 8.41[a]	64.50 ± 0.76[a]
Mean corpuscular haemoglobin (pg)	24.23 ± 0.12[a]	23.40 ± 0.20[a]	24.07 ± 1.60[a]	23.73 ± 1.04[a]
Mean corpuscular haemoglobin concentration (g/dL)	40.57 ± 0.17[a]	40.40 ± 0.37[a]	32.90 ± 1.97[b]	33.50 ± 0.85[b]
Platelets (K/µl)	937.30± 62.67[a]	995.30 ± 4.66[a]	378.0 ±71.30[a]	614.00 ± 211.80[a]
White blood cells (Kµ/L)	11.60 ± 2.71[a]	9.07 ± 0.69[a]	15.17 ± 4.42[a]	6.30 ± 0.65[a]
Lymphocytes (%)	8.67 ± 2.11[a]	7.00 ± 0.71[a]	13.20 ± 7.30[a]	5.50 ± 0.00[a]
[a] Mid cells (%)	2.03 ± 0.43[a]	1.57 ± 0.15[a]	1.20 ± 0.20[a]	1.00 ± 0.00[a]
Granulocytes (%)	0.95 ± 0.65[a]	0.50 ± 0.06[a]	0.37 ± 0.06[a]	0.45 ± 0.15[a]

[a] MID cells include less frequently occurring cells and rare cells correlating to monocytes, eosinophils, basophils, blast and other precursor white cells.

Values are mean ± S.E.M, n = 3, a,b… Value with same superscripts across rows are not significantly different (P≤ 0.05).

Effect of Aqueous Extract of Euphorbia Hirta on Haematological and Biochemical Parameters of Sprague Dawley Rats

Table 2: Effect of the 14-day toxicity of Euphorbia hirta extract (500 – 2000mg/kg) on serum biochemistry of rats.

Parameters	Control	500mg/kg	1000mg/kg	2000mg/kg
Aspartate aminotransferase (UI/L)	38.33 ± 21.71[a]	55.33 ±12.44[a]	52.33 ±5.20[a]	58.33 ± 5.21[a]
Alanine aminotransferase (UI/L)	65.67 ± 9.38[a]	75.33 ± 5.04[a]	97.00 ± 9.00[a]	95 ±1.15[a]
Alkaline phosphatase (UI/L)	152.00 ± 18.18[a]	190.30±13.30[a]	294 .00±44.74[b]	245.30 ± 7.67[a]
Gamma-glutamyl transpeptidase (UI/L)	7.33 ± 0.67[a]	5.67±1.66[a]	9.00 ± 1.00[a]	5.67± 0.67[a]
Bilirubin Total (mg/dL)	0.47 ± 0.12[a]	0.37 ± 0.12[a]	0.57 ± 0.15[a]	0.30 ± 0.07[a]
Bilirubin Direct (mg/dL)	0.37 ± 0.08[a]	0.33 ± 0.08[a]	0.23 ± 0.03[a]	0.17 ± 0.03[a]
Bilirubin Indirect (mg/dL)	0.23 ± 0.08[a]	0.23 ± 0.09[a]	0.23 ± 0.03[a]	0.13 ± 0.03[a]
Total protein (g/dL)	8.30 ± 0.70[a]	8.30 ± 0.36[a]	8.07± 0.24[a]	8.00 ± 0.35[a]
Albumin (g/dL)	3.67 ± 0.08[a]	3.93 ± 0.08[a]	3.53 ± 0.17[a]	3.80 ± 0.20[a]
Globulin (g/dL)	4.63 ± 0.77[a]	4.37 ± 0.45[a]	47.33 ± 7.13[a]	39.00 ± 6.25[a]

Values are mean ± S.E.M, n = 3, a,b..Values with same superscript across rows are not significantly different (P≤ 0.05).

Table 3: Effect of the 14-day oral administration of Euphorbia hirta extract (500 – 2000mg/kg) on serum BUN and Creatinine content in rats.

Parameters	Control	500mg/kg	1000mg/kg	2000mg/kg
Blood urea nitrogen (mg/dL)	20.47 ± 0.20	29.23 ± 2.75	23.43 ± 1.43	15.77 ± 6.70
Creatinine(mg/dL)	0.73 ± 0.07	0.66 ± 0.04	0.51 ± 0.04	0.64 ± 0.05
BUN/Creatinie	29.00 ± 3.06	45.00 ± 6.25	47.33 ± 7.16	39.00 ± 6.25

Values are mean ± S.E.M, n = 3, P was not significant at 95% level.

Discussion

During the 14-day toxicity test of E. hirta extracts, food consumption and body weights were comparable to the control group. For the haematological parameters evaluated, the aqueous extract of E. hirta caused significant decreases in erythrocyte count, haemoglobin and haematocrit at the 1000 mg/kg and 2000mg/kg dose levels. This result is similar with those of Adedapo *et al.,* (2004) who observed significant reduction in the RBC counts and haemoglobin levels after administering alcohol E. hirta extracts to Wister rats for 14 days in Nigeria. These results indicate that extracts of the plant could induce anaemia. Therefore one must take care in the use of extracts of this plant at higher doses.

Hepatic function was monitored by the evaluation of the serum level of transaminases ALT, AST and alkaline phosphatase (ALP). Only the alkaline phosphatase level significantly increased in the groups receiving 1000 g/kg and 2000mg/kg of the E hirta extract. ALP is reported to be present in large numbers of cell but only in a few cells is its activity sufficient to be of clinical importance. According to Wingard et al. (2002) moderate or acute hepatic disease is observed only in patients showing higher than a five-fold increase in ALP level. Therefore, the variation in ALP observed in rats treated with E. hirta at 1000mg/kg is unlikely to have any toxicological significance. ALT and AST are particularly useful in measuring hepatic necrosis, especially in small animals (Cornelius, 1989; Duncan *et al.,* 1994) their elevation is associated with liver damage. In this study, however, no significant increases in AST, ALT or GGT were observed in any of the animals dosed with E. hirta. This suggests that the plant did not cause any liver damage. In contrast to the present findings however, Adedapo *et al.,* (2004) reported significant increases in AST and ALT in rats administered with 10,000mg/kg bwt of E. hirta and concluded that the elevated levels may indicate damage caused by the plant. However, the dose of 10,000mg/kg bwt used by Adedapo and co-workers was very high and unlikely to be the dose required in clinical use.

Bilirubin (total, unconjugated and conjugated) and serum proteins were also assessed as a measure of liver function. However, no elevations in bilirubin levels were observed.

The extract of E. hirta did not cause any significant changes in the levels of serum total proteins, albumin or globulin compared with the control animals. This result suggests that the extract did not affect protein synthesis at the dose levels studied.

Renal function was evaluated by means of serum urea and creatinine levels. It should be noted that blood urea nitrogen (BUN) and creatinine are compounds in the blood derived from proteins, which are eliminated by the kidneys and most often used to assess renal function (Iyayi and Tewe, 1998). High blood levels may be accompanied by nitrogen retention that may provoke renal diseases. The crude extract of E. hirta in this study did not cause significant changes in the serum levels of these substances compared with the control values. Indeed, extracts of this plant has been found to contain polyuronides such as tannins that possess antioxidant properties and consequently protect renal tissues (Satyanarayana *et al.,* 2001). The administration of 500-2000mg/kg body weight of E. hirta over 14 days did not show any alterations in morphology of the major organs in the animals used in this study. These suggest that the plant extract may not be toxic at the dose levels used. This correlates well with the haematological and biochemical data.

Conclusion

The aqueous extract of E hirta was generally found to be nontoxic when toxicity studies in rats were carried out. The present results in addition to the several reported medicinal properties corroborate the use of the plant in folk medicine. It may be concluded from the foregoing that, E hirta when used for medicinal purposes in animal healthcare up to the dose level of 500mg/kg body weight will be safe. However, more work will have to be carried out to determine the potency of the plant on animal diseases at these concentrations.

Impact

Though, massive indigenous knowledge on the ethnoveterinary uses of plant medicines exist, issues of safety and potency has prevented their wide use in modern veterinary practice. This work reveals that E. hirta can be safely used in animal healthcare as an alternative to orthodox medicines for increased production especially in rural areas of Africa if its potency against animal diseases can be established.

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EFFET DE LA FERTILISATION AU MOLYBDÈNE SUR LES BIOMASSES ET LE NOMBRE DES NODULES D'ARACHIS GLABRATA À DIFFÉRENTES PÉRIODES DE FAUCHE DANS L'OUEST CAMEROUN

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Resume

L'Effet de la fertilisation au molybdène sur les biomasses et le nombre des nodules d'Arachis glabrata a été évalué à différentes périodes de fauche à la Ferme d'Application et de Recherche de l'Université de Dschang en 2011. Un dispositif factoriel comparant quatre doses de Molybdène sous forme de Molybdate d'ammonium (0 ; 0,75 ; 1,5 et 2,25 kgMo/ha) et six (6) périodes de fauches sur des pots de 0,0346 m2 de surface en trois répétitions, soit un total de 72 pots expérimentaux, a été utilisé. A chaque période de fauche, les plantes étaient coupées en deux : la partie aérienne et les racines nodulées. Ensuite, les nodules étaient séparés des racines par dépôt des pots dans un bassin contenant de l'eau. L'eau par la suite faisait dissoudre lentement la terre qui se dégageait des racines. Les nodules étaient alors collectés et comptées. Les nodules des trois pots par niveau de fertilisation et les parties aériennes étaient pesées puis placées dans une étuve ventilée à 60°C jusqu'à poids constant pour la détermination des biomasses, en kg MS/ha et t MS/ha respectivement pour les nodules et les parties aériennes. Les résultats obtenus ont montré que la fertilisation au molybdène a peu influencé la biomasse foliaire d'Arachis glabrata. La valeur la plus élevée (9,21 tMS/ha) a été obtenue au 180e jour de fauche avec le niveau de fertilisation 0 kgMo/ha. Alors que la plus faible (1,07 tMS/ha) a été obtenue au 30e jour de fauche avec le niveau de fertilisation 1,5 kgMo/ha. Une relation linéaire (R2= 0,88) hautement significative ($p<0,01$) a été observée entre la biomasse foliaire et les périodes de fauche. Les données sur la biomasse nodulaire et le nombre des nodules ont évolué de manière variable en fonction des différents niveaux de fertilisation au molybdène. Les valeurs les plus élevées 91,62 kgMS/ha et 322,33 ont été observées au 180e jour de fauche avec la dose de 0 kgMo/ha respectivement pour la biomasse nodulaire et le nombre. Les plus faibles 1,25 kgMS/ha et 22,00 ont été obtenues au 30e jour de fauche avec les doses de 0,75 et 1,50 kgMo/ha respectivement. Dans cette étude, des relations Logarithmiques (R2 = 0,8 et R2 = 0,64) hautement significatives ($p<0,01$) respectivement entre la biomasse nodulaire et la période de fauche d'une part et entre la biomasse nodulaire et la biomasse foliaire d'autre part ont été observées. Cependant, aucune relation n'a été observée entre le nombre des nodules et la période de fauche. Les résultats obtenus montrent donc que dans les conditions de cet essai, les paramètres étudiés ont plus été influencés par l'âge de la plante que par la fertilisation au molybdène. Toutefois, les doses de 0,75 ; 1,5 et 0 kgMo/ha ont permis d'obtenir la biomasse aérienne, la biomasse nodulaire et le nombre des nodules les plus élevés d'Arachis glabrata respectivement.

Mots clés: Arachis glabrata, Molybdène, période de fauche, nodules et biomasse

THE EFFECT OF MOLYBDENUM FERTILIZATION ON ARACHIS GLABRATA BIOMASS AND NODULE NUMBER IN DIFFERENT MOWING PERIODS IN WESTERN CAMEROON

Abstract

The effect of molybdenum fertilization on biomass and the number of nodules of Arachis glabrata was assessed at the Teaching and Research Farm of the University of Dschang in 2011 at different periods of mowing. A factorial design comparing four doses of molybdenum as ammonium molybdate (0, 0.75, 1.5 and 2.25 kgMo / ha) and six (6) periods of mowing in tubs of 0.0346 sq.m each repeated three times,

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representing a total of 72 experimental tubs used. At each mowing period, the plants were cut into two: the visible part and nodulated roots. The nodules are then separated from roots by placing the tubs in a water basin. The water then slowly disolves the earth coming off the roots. The nodules were then collected and counted. The nodules in the three tubs per level of fertilization and visible parts were weighed and then dried in a 60 ° C ventilated oven to constant weight to determine biomass in kg MS/ha and tMS/ha for nodules and visible parts respectively. The results showed that molybdenum fertilization had little influence on leaf biomass from Arachis glabrata. The highest value (9.21 tMS / ha) was obtained at 180 days from mowing, with a fertilization level of 0 kgMo/ha, while the lowest value (1.07 tDM/ha) was obtained at 30 days from mowing, with a level of fertilization of 1.5 kgMo/ha. A highly significant ($p<0.01$) linear relationship ($R2 = 0.88$) was observed between leaf biomass and mowing periods. The data on nodular biomass and number of nodules variably changed according to the different levels of molybdenum fertilization. The highest values 91.62 kgMS/ha and 322.33 were noted at 180 days from mowing at a dose of 0 kgMo/ha for nodular biomass and nodule number respectively. The lowest values 1.25 kgMS/ha and 22.00 were obtained at 30 days from mowing at doses of 0.75 and 1.50 kgMo/ha respectively. In this study, highly significant ($p <0.01$) logarithmic relations ($R2 = 0.8$ and $R2 = 0.64$) between nodular biomass and mowing period on the one hand and between nodule biomass and leaf biomass on the other hand were observed. However, no relationship was observed between the number of nodules and the mowing period. Thus, the results show that under the conditions of this experiment, the parameters studied were more influenced by the age of the plant rather than by molybdenum fertilization. However, doses of 0.75; 1.5 and 0 kgMo/ha achieved the highest above-ground biomass, nodular biomass and number of nodules of Arachis glabrata respectively.

Key words: Arachis glabrata, Molybdenum, mowing period, nodules and biomasse.

Introduction

Les résultats des effets bénéfiques de la fertilisation azotée pour accroître les rendements des cultures sont connus et disponibles (Pamo, 1991; Lhoste *et al.,* 1993; Morot-Gaudry, 1997; Lawlor *et al.,* 2001 ; Tendonkeng *et al.,* 2009 ; 2010 ; 2011a et 2011b). Mais l'adoption de cette pratique à base d'éléments chimiques, par les producteurs reste limitée à cause du coût élevé des engrais, de l'irrégularité dans sa disponibilité et des problèmes environnementaux de pollution du sol (Rippy *et al.,* 2004).

Cette situation difficile exige la recherche d'autres méthodes de fertilisation peu onéreuses adaptées et durables. Les légumineuses offrent un potentiel et des atouts souvent ignorés (Kone et Tapondjou, 2010). La plante Arachis glabrata, originaire de l'Amérique latine est une plante pérenne de petite taille, poussant à partir de souches centrales ramifiées. Comme beaucoup de légumineuses, elle a la capacité de fixer l'azote atmosphérique et le transformer en une forme assimilable par les plantes via la symbiose qu'elle réalise avec les bactéries du genre Rhizobium. Elles pourraient ainsi être une opportunité pour améliorer la nutrition azotée des plantes et augmenter les rendements des cultures (Bado, 2002). En effet, les légumineuses de couvertures comme Mucuna pruriens, Pueraria phaseoloïdes, Lablab purpureus, permettent une accumulation assez importante d'azote allant de 61 à 650 kg/ha dans les jachères améliorées (Segda *et al.,* 1998).

Cependant, la déficience en éléments comme le molybdène, ou le bore et le zinc peut compromettre la croissance et la production des légumineuses (Roberge et Toutain, 1999). Le molybdène est un constituant de l'enzyme impliquée dans la fixation de l'azote gazeux par les Rhizobiums des légumineuses et de celle impliquée dans la réduction des nitrates. Le NH4+ provenant de cette réduction est incorporé dans les squelettes carbonés pour former les acides aminés par le processus d'assimilation de l'azote (Morot-Gaudry, 1997).

Si plusieurs travaux effectués sur les processus symbiotiques ont concerné surtout les légumineuses des zones tempérées, et le rôle important joué par ceux-ci dans l'amélioration de la nutrition minérale des plantes a été prouvé (Diouf *et al.,* 2003), par contre peu d'information existent sur celles des zones tropicales (IRD, 2007), encore moins sur les facteurs pouvant influencer le processus de nodulation des légumineuses dans l'espace et dans le temps. Le présent travail a donc pour objectif de contribuer à l'amélioration des connaissances

sur la nodulation et la production d'Arachis glabrata en présence du molybdène.

Materiel et Methodes

Zone d'étude

L'étude a été conduite à la Ferme d'Application et de Recherche (FAR) de l'Université de Dschang entre Juin et Novembre 2011. La FAR est située en région Soudano-guinéenne du Cameroun à 05°20' latitude Nord et 10°03' longitude Est et à une altitude moyenne de 1410 m. Le climat de la région est équatorial de type Camerounien, modifié par l'altitude. Les températures oscillent entre 10°C (juillet – août) et 25°C (février) avec une insolation annuelle de 1800 heures et une humidité relative variant entre 40 – 97%. Les précipitations varient entre 1500 et 2000 mm par an. La saison sèche va de mi-novembre à mi-mars et la saison des pluies de mi-mars à mi-novembre correspondant à la période de culture dans cette région.

Conduite de l'essai

Les éclats de souche d'Arachis glabrata ont été plantés au centre de chaque pot de superficie 0.0346 m² pour l'étude des biomasses et du nombre de nodules.

Un dispositif factoriel (4 × 6) comparant quatre (4) doses de Molybdène sous forme de Molybdate d'ammonium (0 ; 0,75 ; 1,5 et 2,25 kg/ha) et six (6) périodes de fauches, en trois répétitions, soit un total de 72 tubs ont été utilisé dans cet essai. Les pots remplis de terre ont été placés côte à côte par lot de 18 et par niveau de fertilisation, dans les mêmes conditions de température, d'humidité et d'éclairage.

Mise en place des éclats de souches

La préparation des pots expérimentaux a consisté au remplissage de ceux-ci avec de la terre prélevée dans le site expérimental. Des touffes d'Arachis glabrata ont été prélevées dans les parcours de la FAR. Ces touffes ont été habillés (ont subi une réduction de taille des racines et des feuilles) à l'aide de ciseaux. Des éclats de souche de deux (2) rejets obtenus par division manuelle ont été repiqués au centre de chaque pot, à une profondeur de 4 cm.

Fertilisation et entretien des plantes

Un engrais phosphaté sous forme de superphosphate simple (100kg par hectares, soit 0,346g par unité expérimentale) à 21 % et un engrais potassique sous forme de chlorure de potassium (35kg par ha soit 0,121g par unité expérimentale), a été appliqué sur l'ensemble des 72 tubs comme engrais de fond. Un mois après la mise en place d'éclats de souches de la légumineuse et après la première levée, un désherbage manuel des pots a été pratiqué. Ensuite, les pots ont été fertilisés au molybdène en une seule fois selon le plan expérimental. Afin d'assurer une homogénéité d'application et compte tenu des quantités très faibles de molybdène, chaque dose à apporter par niveau de fertilisation au molybdène a été dissous dans 180ml d'eau de façon à apporter exactement 10ml de solution au pied de chaque plante à l'aide d'une seringue de 10cc. L'entretien a consisté au désherbage des pots et au nettoyage des espaces entre les pots et des allées entre les blocs de pots.

Collectes des données

Un mois après la fertilisation au molybdène et deux mois après la mise en place des éclats de souche d'Arachis glabrata trois (3) pots par niveau de fertilisation pour un total de 12 pots étaient prélevés au hasard et ceci se répétait tous les 30 jours (correspondant à la période de fauche) pour l'évaluation des biomasses et du nombre des nodules. A chacune des 6 périodes de collecte des données, les plantes étaient séparées en deux parties : la partie aérienne et les racines nodulées. Ensuite, les nodules étaient séparés des racines par dépôt des pots dans un bassin contenant de l'eau. L'eau par la suite faisait dissoudre lentement la terre qui se dégageait des racines. Les nodules étaient alors collectés et comptés. Les nodules des trois pots par niveau de fertilisation et les parties aériennes étaient pesés puis séchés dans une étuve ventilée de marque Gallemkamp à 60°C jusqu'à poids constant pour la détermination des biomasses en kg MS/ha et t MS/ha respectivement pour les nodules et la partie aérienne.

Analyses statistiques

Les données sur les biomasses (feuillue et nodulaire) et les nombres des nodules ont subi une transformation logarithmique, Log10 (x+1) (Gomez & Gomez, 1984), à cause des très grandes variations entre les écarts types des moyennes, avant d'être soumises à une analyse de variance suivant le Model Linéaire General (MLG). Lorsque les différences existaient entre les traitements, les moyennes étaient séparées par le test de Duncan au seuil de signification 5% (Steel et Torrie, 1980).

Resultats

Effet de la fertilisation au molybdène sur la biomasse foliaire d'Arachis glabrata en fonction de la période de fauche

Les variations de la biomasse aérienne d'Arachis glabrata en fonction des doses de molybdène à différentes périodes de fauche montrent que la fertilisation au molybdène a peu influencé la biomasse foliaire d'Arachis glabrata (Tableau 1). La valeur la plus élevée (9,21tMS/ha) a été obtenue au 180e jour de fauche avec le niveau de fertilisation 0kgMo/ha, alors que la plus faible (1,07tMS/ha) a été obtenue au 30e jour de fauche avec le niveau de fertilisation 1,5kgMo/ha. Cette valeur est statistiquement comparable à celles obtenues avec les doses de 0 et 2,25kgMo/ha. Mais elle est significativement ($p>0,05$) plus faible que celle obtenue avec le niveau de fertilisation 0,75kgMo/ha à cette période de fauche. Au 60e, 120e et 150e jour de fauche, la biomasse foliaire a baissé avec la fertilisation jusqu'à la dose de 1,5kgMo/ha avant de croître avec la dose maximale de fertilisation. Cependant, aucune différence significative n'a été observée entre les biomasses en fonction des doses de fertilisation à ces périodes de fauche. Par contre, c'est l'effet contraire qui a été observé au 90e jour de fauche. A cette période de fauche, les valeurs de biomasse obtenues avec les doses de 0 (1,30tMS/ha) et 0,75kgMo/ha (1,32tMS/ha) ont été comparables et significativement inférieures à celles obtenues avec les doses de 1,5kgMo/ha (2,98tMS/ha) et 2,25kgMo/ha (2,80tMS/ha) qui ont été par ailleurs comparables.

Biomasse foliaire (t/ha) = 0,037x jours + 0,298, ($R2 = 0,88$)

Effet de la fertilisation au molybdène sur la biomasse nodulaire (kgMS/ha) d'Arachis glabrata en fonction de la période de fauche

La fertilisation au molybdène a influencé de manière variable la biomasse nodulaire d'Arachis glabrata aux différentes périodes de fauche (Tableau 2). La valeur de la biomasse nodulaire la plus élevée (91,62kgMS/ha) a été obtenue au 180e jour de fauche avec la dose de 1,5kgMo/ha. Alors que la plus faible (1,25kgMS/ha) a été observée avec la dose de 0,75kgMo/ha au 30e jour de fauche. A cette première période de fauche, la fertilisation a permis une augmentation statistiquement comparable ($p>0,05$) de la biomasse nodulaire mais tout en restant significativement ($p<0,05$) inferieur à celle obtenue des pots non fertilisés. La valeur de la biomasse nodulaire observée au 90e jour de fauche avec la dose de 1,5kgMo/ha (64,64 gMS/ha) a été significativement ($p<0,05$)

Tableau 1 : Evolution de la biomasse foliaire d'Arachis glabrata aux différentes périodes de fauche en fonction des doses croissantes de Molybdène.

Traitements	Périodes de fauche (jours)					
(kgMo/ha)	30	60	90	120	150	180
0	1,52^{ab}	2,57	1,30^{b}	5,22	6,21	9,21
0,75	1,68^{a}	1,95	1,32^{b}	4,67	4,67	3,32
1,5	1,07^{b}	1,68	2,98^{a}	3,11	3,41	6,87
2,25	1,15^{ab}	1,91	2,80^{a}	4,49	7,79	2,42
SEM	0,0197	0,0423	0,0455	0,077	0,0636	0,0794
Prob.	0,093	0,958	0,041	0,837	0,462	0,187

a,b : les moyennes portant les mêmes lettres dans la même colonne sont significativement comparables au seuil de 5%.

Tableau 2: Evolution de la biomasse nodulaire (kgMS/ha) d'Arachis glabrata en fonction des doses de molybdène à différentes périodes de fauche.

Traitements	Périodes de fauche (jours)					
(kgMo/ha)	30	60	90	120	150	180
0	3,66[a]	89,88	23,80[bc]	72,64[a]	52,02	74,37
0,75	1,25[b]	16,28	17,15[c]	41,71[ab]	32,47	33,43
1,5	1,54[b]	20,42	64,64[a]	27,94[b]	42,10	91,62
2,25	2,12[b]	18,11	46,72[ab]	58,38[ab]	76,78	32,37
SEM	0,0413	0,129	0,0848	0,0838	0,0777	0,0178
Prob.	0,09	0,684	0,025	0,142	0,521	0,293

a,b,c : les moyennes portant les mêmes lettres dans la même colonne sont significativement comparables au seuil de 5%.

supérieure à celles obtenues avec les doses de 0 (23,80kgMS/ha) et 0,75kgMo/ha (17,15kgMS/ha) qui ont été par ailleurs comparables entre elles. Il en est de même de celles observées avec les doses de 1,5 et 2,25kgMo/ha. La biomasse nodulaire obtenue avec la dose de 0kgMo/ha (72,64kgMS/ha) au 120e jour a été significativement ($p<0,05$) plus élevée que celle observée avec la dose de 1,5kgMo/ha (27,94kgMS/ha). Cependant, elle a été statistiquement comparable à celles observées avec les doses de 0,75 et 2,25kgMo/ha.

Au 150e jour de fauche, la fertilisation au molybdène a permis une augmentation des biomasses nodulaires de la plante. Cependant, aucune différence significative ($p<0,05$) n'a été observée entre les valeurs de biomasses.

Aux périodes de fauche de 60 et 180 jours, les valeurs de biomasse nodulaire de la légumineuse ont évolué en dents de scie avec la fertilisation. Toutefois aucune différence significative ($p<0,05$) n'a été observée entre les différentes valeurs de biomasse en fonction des différents niveaux de fertilisation au molybdène.

Des relations logarithmiques significatives ($p<0,01$) entre les biomasses nodulaires et les périodes de fauches d'une part et entre les biomasses nodulaire et foliaire d'autre part, ont été observées comme l'indique les coefficients de détermination (R2) des équations ci-après :

- Biomasse nodulaire (kg/ha) = 22,046 x Ln (jours) - 54,79 (R2 = 0,80)
- Biomasse nodulaire (kg/ha) = 25,924xLn (Biomasse foliaire) + 9,91 (R2 = 0,64)

Effet de la fertilisation au molybdène sur le nombre des nodules d'Arachis glabrata en fonction de la période de fauche.

La fertilisation au molybdène à différentes périodes de fauche a peu influencé les données sur le nombre des nodules de la légumineuse (Tableau 3). Le nombre le plus élevé (322,33) a été observé à la dernière période de fauche avec le niveau de fertilisation 0kgMo/ha et le plus faible (22,00) à la première

Tableau 3 : Evolution du nombre des nodules d'Arachis glabrata en fonction des doses de molybdène à différentes périodes de fauche

Traitements	Périodes de fauche (jours)					
(kgMo/ha)	30	60	90	120	150	180
0	40,00	125,33	103,67[b]	313,67[a]	215,00	322,33
0,75	32,67	61,67	101,00[b]	118,33[b]	132,33	152,33
1,5	22,00	97,67	299,00[a]	111,33[b]	192,00	290,33
2,25	22,33	82,00	181,00[ab]	237,00[a]	301,00	144,67
SEM	0,0628	0,0653	0,0683	0,0719	0,0849	0,0852
Prob.	0,402	0,758	0,013	0,09	0,686	0,390

a,b : les moyennes portant les mêmes lettres dans la même colonne sont significativement comparables au seuil de 5%.

période de fauche avec la dose de 1,5kgMo/ha.

Au 30e et 120e jour de fauche, la fertilisation au molybdène a permis une baisse du nombre de nodules avec les doses de 0,75 et 1,5kgMo/ha avant de croître sans être significativement différente au 30e et significative ($p<0,50$) au 120e jour avec l'apport de la dose maximale de fertilisation.

Au 150e jour de fauche, la fertilisation a augmenté le nombre des nodules. Cependant, aucune différence significative ($p<0,05$) n'a été observée entre les valeurs obtenues quelque soit le niveau de fertilisation.

La valeur obtenue avec la dose de 0,75kgMo/ha au 90e jour de fauche a été comparable ($p>0,05$) à celles obtenues des plantes fertilisées avec les doses de 0 et 2,25kgMo/ha. Elle est cependant significativement ($p<0,05$) inférieure à celle obtenue avec la dose de 1,5kgMo/ha, qui est elle-même comparable à celle obtenue avec la dose de 2,25kgMo/ha.

Les nombres obtenus aux périodes de fauche de 60 et 180 jours ont évolué en dents de scie avec les niveaux croissants de molybdène. Les valeurs les plus élevées (125,33 et 322,33) ont été obtenues avec la dose de 0kgMo/ha et les valeurs les plus faibles (61,67 et 144,67) avec les doses de 0,75 et 2,25kgMo/ha respectivement. Cependant, aucune différence significative ($p>0,05$) n'a été observée entre les nombres de nodules en fonction des doses de fertilisation à ces deux périodes de fauche. De même, aucun lien n'a été établie entre le nombre des nodules et la période de fauche.

Discussion

La fertilisation au molybdène a peu influencé la biomasse foliaire d'Arachis glabrata. Ce résultat est contraire à ceux de Berkić et al. (2004) qui ont constaté une augmentation de la biomasse aérienne avec la fertilisation au molybdène en présence d'azote sur le pois chiche (cicer arietinum). Cette différence pourrait donc s'expliquer par la présence de l'azote. La valeur la plus élevée (9,21tMS/ha) obtenue au 180e jour de fauche avec le niveau de fertilisation 0kgMo/ha et la plus faible (1,07tMS/ha) au 30e jour de fauche avec le niveau de fertilisation 1,5kgMo/ha, montre plutôt que la biomasse a augmenté avec l'âge de la plante. En effet la hausse de rendement en biomasse est significativement affectée par la durée de la période de croissance (Enoh *et al.,* 2004). La relation hautement significative ($p<0,01$) établie entre les biomasses foliaires et les périodes de fauche montrerait que la biomasse foliaire pourrait être prédite dans 88% des cas par de la période de fauche.

Les biomasses nodulaires d'Arachis glabrata obtenues aux différentes périodes de fauche ont été influencées de manière variable par la fertilisation au molybdène. Toutefois, des différences significatives ($p<0,05$) ont été observées entre les valeurs de biomasses en fonction des doses de molybdène aux 30e, 90e et 120e jours de fauche.

A la première période de fauche, les valeurs de biomasses nodulaires obtenues avec les différents niveaux de fertilisation au molybdène ont été significativement ($p<0,05$) inférieures à celles observées sur les plantes non fertilisées. Cette situation s'expliquerait par la richesse du sol en azote. En effet, en début de croissance, les légumineuses utilisent préférentiellement l'azote du sol au détriment de l'azote atmosphérique via la symbiose (légumineuses-Rhizobium). Ce résultat concorde avec les observations faites par L'taief et al. (2009) selon lesquelles la présence de l'azote dans le sol baisse la biomasse nodulaire. Le molybdène étant le constituant de l'enzyme impliquée dans la fixation de l'azote gazeux par le Rhizobium des légumineuses et de celle impliquée dans la réduction des nitrates, laisserait penser qu'à ce stade de développement de la légumineuse, le molybdène ne joue pas encore son rôle et aurait donc un effet plutôt inhibiteur sur la formation de nodules.

Au 90e jour de fauche, la fertilisation a augmenté la biomasse nodulaire jusqu'à la dose de 1,5kgMo/ha avant de baisser avec l'apport de la dose maximale. Cette observation suggérerait qu'au-delà de 1,5kgMo/ha, le molybdène devient phytotoxique dans nos conditions comme l'ont affirmé Odet et al. (1987) qui ont fixé le seuil au tour de 2kgMo/ha. A moins que le sol contienne déjà une quantité importante de Molybdène á la quelle vient s'ajouter la dose de fertilisation pour la

porter au seuil de phytotoxicité.

La baisse observée au 120e jour de fauche (période de forte pluviométrie dans la zone d'étude) est en accord avec les résultats des travaux de Purcell et al. (1997) qui ont remarqué que les fortes précipitations peuvent être à l' origine de la baisse de la biomasse nodulaire. Alors que la sécheresse aurait plutôt un effet positif sur la croissance des nodules à cause d'une plus grande allocation des photosynthétas aux nodules.

Aux autres périodes de fauche, aucune différence significative ($p>0,05$) n'a été observée entre les valeurs de biomasses nodulaires en fonction des différents niveaux de fertilisation au molybdène. Ce résultat corrobore ceux de Jongruaysup et al. (1993) qui ont remarqué que la présence du molybdène n'a pas d'effet significatif sur l'initiation et le développement des nodules de Vigna mungo L. Toutefois, la valeur la plus élevée (91,62 kgMS/ha) obtenues au 180e jour de fauche avec la dose de 1,5 kgMo/ha est supérieure à celle observée (86,71 kgMS/ha) par L'taief et al. (2009) sur le pois chiche en saison pluvieuse. Les relations logarithmiques établies entre la biomasse nodulaire et la période de fauche d'une part et entre la biomasse nodulaire et la biomasse foliaire d'autre part, qui se traduisent par des coefficients de détermination relativement élevée ($R2 = 0,80$ et $R2 = 0,64$ respectivement) montrerait que dans 80 et 64% des cas, les proportions de variation de la biomasse nodulaire pourraient être expliquées par la période de fauche et par la biomasse foliaire respectivement.

Le nombre de nodules d'Arachis glabrata observé à différentes périodes de prélèvement a évolué de manière variable en fonction des différents niveaux de fertilisation au molybdène. Les nombres ($p<0,05$) les plus élevés observés au 90e (299,00) et au 120e (313,67) jour de fauche avec les doses de 1,5 et 0 kgMo/ha respectivement serait dû au stade phénologique de la plante. En effet à ces périodes, la plante avait atteint le stade floraison, période pendant laquelle la croissance des racines est limitée au profit de celles des nodules (Bot *et al.,* 2003). Mais d'une manière générale, la fertilisation au molybdène n'a pas significativement ($p>0,05$) influencée le nombre de nodules aux autres périodes de fauche. Aussi, les valeurs les plus élevées dans l'ensemble de l'essai ont été observées sur les plantes non fertilisées. Ceci est en accord avec les résultats de Berkić et al. (2004) sur le pois, qui ont remarqué que la fertilisation au molybdène n'a pas d'effet sur le nombre de nodules et que les valeurs les plus élevées ont été réalisées dans des variantes sans molybdène. L'évolution en dents de scie du nombre des nodules observée à la dernière période de fauche avec les niveaux croissants de molybdène ne corrobore pas les investigations de Jongruaysup *et al.,*(1993) qui ont déclaré que le nombre des nodules baisse avec l'application du molybdène.

Indépendamment des différents niveaux de fertilisation au Molybdène, les variations du nombre de nodules avec les périodes de fauche pourraient aussi s'expliquer par la durée d'activité relativement brève des nodules, de l'ordre de quelques semaines chez les légumineuses herbacées. Cette courte période d'activité est compensée par l'apparition de générations successives de nodosités (Trinchant *et al.,* 1997). Cela pourrait également expliquer l'absence de relation entre le nombre des nodules et les périodes de fauche observée dans cette étude.

Conclusion

De l'étude de l'effet de la fertilisation au molybdène sur les biomasses et le nombre des nodules d'Arachis glabrata à différentes périodes de fauche, il ressort que :

- la fertilisation au molybdène a peu influencé la biomasse foliaire d'Arachis glabrata.
- Il semble plutôt que la biomasse a augmenté avec l'âge de la plante que sous l'effet du molybdène ;
- la fertilisation au molybdène a influencé de manière variable la biomasse nodulaire d'Arachis glabrata aux différentes périodes de fauche ;
- aux différentes périodes de fauche, la fertilisation au molybdène a également peu influencé les données sur le nombre de nodules de la légumineuse.

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EFFECT OF SORGHUM -BARLEY BREWER'S SPENT GRAIN AS A FEED INGREDIENT ON BROILER PERFORMANCE AND CARCASS CHARACTERISTICS

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Summary

The objective of the study was to determine if the inclusion of Sorghum-Barley Brewer's Spent Grain (SBBSG) in broiler diets will affect growth performance and carcass characteristics. A total of 380 Ross broiler chicks were brooded on a common diet without SBBSG for 28 days after which they were randomly assigned to five experimental diets, T1, T2, T3, T4, and T5 representing 0, 4, 8, 12 and 16% SBBSG inclusion respectively in a completely randomized design. There were 4 replicates per treatment and each replicate had 19 birds. The test period was 28 days and consisted of a grower phase (14 days) and a finisher phase (14 days). Water and feed were provided ad libitum. Production parameters measured included average daily gain, feed intake and feed conversion efficiency. At the end of the trial 3 birds from each replicate were sacrificed and used for carcass analysis. Parameters measured included dressing percentage, intestinal weight, liver and gizzard weight and abdominal fat. There were no significant differences (P>0.05) among all diets for any of the production parameters measured. Similarly, except for abdominal fat where birds on T5 had significantly higher (P < 0.05) values (37.99g) compared with T1, T2, T3 and T4 (18.47, 17.41, 21.07 and 29.4g) respectively, all other carcass parameters measured were similar (P > 0.05). There was no significant reduction in cost of feed per unit gain with increasing levels of SBBSG. However, the combined effect of an increasing trend in final body weight, similar feed intake and FCE could sum up to make SBBSG an attractive alternative, cost-wise to more conventional feed ingredients. The present study shows that beyond the brooding stage, SBBSG can be included in broiler diets up to 16% without adverse effects on performance, and up to 12% without a significant increase in abdominal fat.

Keywords: Sorghum-Barley Brewer's Spent Grain (SBBSG), Broilers, Weight gain, Feed Conversion efficiency, Carcass characteristics.

EFFET DE LA DRECHE DE SORGHO/ORGE COMME INGREDIENT ALIMENTAIRE SUR LA PERFORMANCE ET LES CARACTERISTIQUES DE CARCASSE DES POULETS DE CHAIR

Résumé

L'objectif de l'étude était de déterminer l'ellet de l'inclusion de la drêche de sorgho/orge (SBBSG) dans l'alimentation des poulets de chair aura une incidence sur le performances de croissance et les caractéristiques de la carcasse. Au total, 380 poussins de chair de race Ross ont été couvés sur un régime commun sans SBBSG pendant 28 jours, et par la suite ils ont été assignés de manière aléatoire à cinq régimes expérimentaux, T1, T2, T3, T4 et T5 représentant respectivement une inclusion de SBBSG de 0 ; 4 ; 8 ; 12 et 16% dans un dispositif complètement aléatoire. Il y a eu 4 répétitions par traitement et chaque répétition avait 19 poussins. La période d'essai était de 28 jours et consistait en une phase de croissance (14 jours) et une phase de finition (14 jours). L'eau et de la nourriture ont été fournies ad libitum. Les paramètres de production mesurés comprenaient le gain moyen quotidien, la prise alimentaire et l'efficacité de la conversion des aliments. A la fin de l'essai, 3 poussins de chaque répétition ont été sacrifiés et utilisées pour analyse des carcasses. Les paramètres mesurés comprenaient le rendement de la carcasse, le poids intestinal, le poids du foie et du gésier et la graisse abdominale. Il n'y avait pas de différences significatives (P> 0,05) entre les régimes pour tous les paramètres de production mesurés. De même, à l'exception de la graisse abdominale où les oiseaux sur traitement T5 avaient des valeurs significativement (p <0,05) plus élevées (37,99g) par rapport aux traitements T1, T2, T3 et T4 (respectivement 18,47 ; 17,41 ; 21,07 et 29,4g),

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tous les autres paramètres de carcasse mesurés étaient similaires (P> 0,05). Il n'y a pas eu de réduction significative du coût des aliments par unité de gain avec des niveaux croissants de SBBSG. Cependant, l'effet combiné d'une tendance à l'augmentation du poids final corporel, une prise alimentaire similaire et de l'efficacite de la comension des alimets pourraient se combiner pour rendre le régime SBBSG come une alternative intéressante, économique par rapport aux ingrédients classiques. La présente étude montre qu'après l'étape de couvaison, le régime SBBSG peut être administré aux poulets de chair jusqu'à 16% sans effets négatifs sur la performance, et jusqu'à 12% sans augmentation significative de la graisse abdominale.

Mots-clés: Drêches de sorgho /orge (SBBSG), poulets de chair, gain pondéral, efficacité de conversion des aliments, caractéristiques de carcasse.

Introduction

The broiler industry in Ghana has faced severe challenges over the years, impeding its capacity to increase production to meet the growing national demand for poultry meat. High input costs such as day-old chicks, feed, medication, labour, water and electricity have all played a role in driving the cost of production up, with feed alone contributing about 70% of total production costs (USDA, 2008; Aning, 2006). The resultant high cost of locally produced chicken meat has contributed to the high patronage of the cheaper imported frozen chicken even though consumers prefer the taste and freshness of the former (Cato et al., 2011). The major feed ingredients in poultry diets in Ghana are maize, soybean meal and wheat bran. However maize forms a large part of human diets and as a result there is competition for this grain resulting in high market prices.

The supply of wheat bran, which represents approximately 16 and 20% respectively of broiler and layer diets, depends on production activities of existing flour mills in the country. However wheat, the raw material from which wheat bran is obtained is imported, and together with soybean meal, lend themselves to wide price fluctuations which in turn depend on the comparative cost of the dollar relative to the local currency, the cedi.

Competition between man and livestock for maize, and over dependence on wheat bran whose supply and price depend entirely on the world market price of imported wheat, are strong reasons why local producers and feed mills need to look elsewhere for energy sources for poultry diets. Brewers' spent grains offer such an option and several studies have been carried out on their use in poultry diets (Adama *et al.,* 2007; Husaini *et al.,* 2010).

In recent years some beer breweries in Ghana, particularly in the Ashanti Region, have incorporated malted sorghum as an ingredient in their production of beer. Although the use of brewers' spent grain is not new, this particular product is quite unique and unlike any that is produced by any other brewery in Ghana. This unique brewers' spent grain is known as Sorghum-Barley Brewers' Spent Grain (SBBSG). It is made up both of sorghum and barley but the proportion of these grains is not known. Little to no information exists on the nutrient composition of this relatively new product or its suitability as a feed ingredient for birds. However small to medium scale swine producers (particularly in the Ashanti region of Ghana) feed it in the wet form to pigs with mixed results. Grain sorghum and barley contain digestible nutrients for poultry (NRC, 1994) and it can be inferred that some of these nutrients still remain in the by-products after the malting and brewing process; hence the popularity of brewers spent grain in monogastric feeding. There is every indication that the brewery industry in Ghana is expanding, meaning that the supply of this raw material will increase. There is therefore the need to investigate it's suitability for use in poultry diets if it is to be well managed.

The hypothesis of this study was therefore that SBBSG can be included in broiler diets without negatively affecting broiler performance. The objectives were to investigate the efficacy, growth related responses and carcass characteristics of broilers fed diets with increasing levels of SBBSG.

Materials and Methods

The trial was carried out at the Livestock and Poultry Research Centre (LIPREC), Institute of Agricultural Research (IAR), College of Agriculture and Consumer Sciences (CACS) of the University of Ghana (UG).

Processing of Sorghum-Barley Brewer's Spent Grain

The fresh SBBSG with a moisture content of 95% was obtained from Guinness Ghana Brewery Ltd (GGBL), Kumasi and transported to the Department of Animal Science, Kwame Nkrumah University of Science and Technology (KNUST) Kumasi. It was spread thinly on a clean concrete floor and sun-dried for 14 days with daily turning to obtain a moisture content of about 7-8%. The SBBSG was bagged in nylon sacks (25kg) and transported to LIPREC where it was stored in a cool, dry and airy environment for subsequent incorporation into feed.

Experimental Diets

Five diets were formulated to contain 0%, 4%, 8%, 12% and 16% of SBBSG representing T1, T2, T3, T4, and T5 respectively for the grower phase (from 28 -41 d) and the finisher phase (42-56 d). These levels of gradual inclusion of SBBSG came at the expense of wheat bran which dropped progressively from 16 to 0% in T1 to T5 respectively. Diets for the grower and finisher phases contained 21 and 20% protein respectively, and an estimated ME of 12MJ/kg. In order to achieve an iso-nitrogenous and iso-caloric diet for the different treatments for both the grower and finisher phases, it was necessary to adjust slightly the levels of corn and soybean meal in the diets.

Management of Experimental Birds

Three hundred and eighty 28-day-old broiler chicks were randomly assigned to the five (5) experimental diets in a Completely Randomised Design (CRD) with four (4) replicates per treatment. There were 76 birds in each treatment and 19 birds per replicate. Since this is the first time of feeding this peculiar brewery waste product to broilers, day-old chicks were avoided as a precaution. So, the experimental birds had already been brooded for 28 days on a standard commercial broiler starter diet. Each replicate group of broilers was housed in deep litter pens measuring 2m x 2m x 2m (Length X Breadth X Height). The birds were individually weighed at the start of the experiment and subsequently on a weekly basis. Birds in each replicate were given a known amount of feed and water on a daily basis. Feed leftover was weighed the next day prior to feeding to determine feed disappearance. The leftover feed was then discarded and fresh feed provided. Standard routine vaccination protocols were strictly adhered to. The birds were fed with the experimental grower diet for 14d after which they were switched onto the finisher diet. The whole trial lasted 28d; performance and feeding records were recorded and summarized on a weekly basis.

Carcass Analysis

At 18:00 hrs on day 27 of the trial, three birds from each replicate were randomly selected and tagged for carcass analysis. The birds were starved for 12hrs and at 06:00 on day 28, they were weighed. Subsequently they were slaughtered by jugular venipuncture (Osei *et al.,* 2010). They were defeathered after scalding in boiling water and immediately transferred into a cold room where they were stored overnight at -400C. The next day, the whole frozen carcasses were weighed. Individual weights of the head, neck, shanks and intestines, and also some internal organs (including liver and gizzard) were taken. Abdominal fat which had solidified following the overnight freezing was carefully separated and the weight recorded.

Chemical Analysis

The proximate chemical composition of both the ingredients and experimental diets was analyzed using methods outlined by AOAC, (1995). Calcium and Phosphorus were determined according to the methods outlined by James (1996) and AOAC (1995). Amino acid analysis of the feed ingredients was carried out using the procedure described by Andrews and Balder (1985). Methionine and cystine were determined using performic acid oxidation according to the method of Moore (1963). Total NSP and sugar fractions were analysed

using Gas Liquid Chromatography (Englyst and Hudson, 1987).

Statistical Analysis

All data gathered were subjected to statistical analysis using the Generalised Linear Model procedure of the Statistical Analysis Systems Institute (SAS, 1999). Significant differences among means were separated using the Student Newman-Kuels (SNK) Test. The model used was as follows;

$$Yij = \mu + Di + Eij$$

Where; Yij is the observation, μ is the mean, Di is the effect of diet and Eij is the error term.

Results

Table I shows the chemical composition of SBBDG. The composition and calculated values of the broiler grower and finisher diets are shown in Tables 2 and 3 respectively. The levels of the major ingredients in both grower and finisher diets were determined based on existing commercial broiler diets in Ghana.

Production parameters

`There were no significant differences (P>0.05) among the experimental diets for the various performance parameters measured (final body weight, daily feed intake, daily weight gain and feed conversion efficiency; Table 4). Final body weights of broilers on T1, T2, T3, T4 and T5 were 2106.3, 2053, 2121, 2148 and 2152 g respectively. Similarly average daily feed intake and feed conversion efficiency were not different for the various treatments, averaging 129g and 0.4 respectively. Although differences among treatments were not significant (P > 0.05), slight increases in average daily gain were observed as the level of SBBSG increased in diets. Birds on T1 gained 50.2g day whilst those on T5 gained 52.62g per day.

Carcass characteristics

Carcass weight, dressing percentage, liver, gizzard and intestinal weights showed no significant differences (P>0.05) among the various treatments, (Table 5). Increasing the amount of SBBSG in diets however resulted in an increase in abdominal fat weight. Abdominal fat weight of chickens on T5 was 37.99g. This was significantly higher (P<0.05) than abdominal fat weights of birds on T1, T2, T3 and T4 (18.47, 17.41, 21.07 and 29.4g) respectively. Again the abdominal fat weight of birds on T4, although lower than that of birds on T5, was significantly higher (P<0.05) than that obtained for birds on T1 to T3.

Table 1: Chemical Composition of Sorghum Barley Brewer's Spent Grain

Parameter	SBBSG
Dry Matter (%)	95.8
Crude Protein (%)	27.58
Crude Fibre (%)	14.75
Total Ash (%)	11.81
Ether Extract (%)	10.22
Calcium (mg/100g)	252.9
Phosphorus (mg/100g)	715.2

Cost of feed

The cost of feed required to produce a kilogram live weight was similar (P>0.05) among the various treatments (Table 5). There was however a trend towards a reduction in cost as the level of SBBSG in the diet increased. It cost 2.26 Ghana Cedis (Gh. C) to produce a kg of broiler meat for birds on T1, and 2.14, 2.01, 2.11 and 2.04 Gh. C to produce a kg of broiler meat for birds on T2, T3, T4 and T5 respectively.

Discussion

Performance parameters measured in this study included final body weight, body weight gain, feed intake and feed conversion efficiency (Table 4). The similarities observed among birds fed the different treatment diets imply that SBBSG inclusion in broiler diets in this particular trial had no adverse effects on performance.

The possible negative effects of NSP on nutrient bioavailability (Slominski *et al.,* 2004) were not observed in the present study. It can be speculated that the negative effects were within tolerance levels for broiler growers and finishers. Additionally the brewing processes (malting, grinding and brewing) and drying of

Table 2: Composition of the broiler grower diets

Ingredients (%)	0% SBBSG*	4% SBBSG	8% SBBSG	12% SBBSG	16% SBBSG
Maize	51.4	53	54.3	56.6	57.5
Soybean meal	27	25.5	24.8	23	22.1
Wheat bran	16	12	8	4	0
SBBSG	0	4	8	12	16
Salt	0.5	0.5	0.5	0.5	0.5
Shell grits	2	2	2	2	2
Lysine	0.25	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25	0.25
Dicalcium phosphate	0.5	0.8	0.8	0.8	0.8
Soybean oil	1.5	1.1	0.5	0	0
Broiler Premix	0.25	0.25	0.25	0.25	0.25
RE31	0.15	0.15	0.15	0.15	0.15
Toxin Binder[2]	0.2	0.2	0.2	0.2	0.2
Total	**100**	**100**	**100**	**100**	**100**
Calculated Analysis					
**ME, MJ/kg	11.62	11.65	11.64	11.69	11.80
CP, %	20.00	21.51	21.70	21.50	21.60
Total NSP, mg/g	81.09	91.59	102.97	113.59	124.39
Water Insoluble NSP mg/g	10.66	21.94	33.32	44.60	55.92
Water Soluble NSP mg/g	70.43	69.65	69.65	68.99	68.47
Ca, %	0.94	1.00	1.00	1.00	1.00
Total P, %	0.63	0.66	0.64	0.61	0.59
Available P, %	0.25	0.30	0.30	0.30	0.30
Total Lys, %	1.31	1.27	1.26	1.23	1.21

**Sorghum-Barley Brewer's Spent Grain*

[1]:Lactobacillus sp, Bacillus sp, Saccharomyces sp and Fermentation products.

[2]: Mycofix® Select 3.0 by Biomin

the wet SBBSG may have helped reduce the negative effects of the NSP in the experimental diets (Mitaru *et al.,* 1984; Nortey, 2001; Nortey *et al.,* 2007; Selle *et al.,* 2009).

Feed intake influences final weight and feed conversion efficiency (Austic and Nesheim, 1990; Ferket and Gernat, 2006). It can be implied therefore that the lack of significant differences in final body weight and feed efficiency among birds on the different treatments may be due to the similar amounts of feed and nutrients consumed among experimental birds. The SBBSG used in this trial did not depress appetite. Adama *et al.,* (2007) in a similar trial fed 20% of sorghum dried brewer's grain to broilers and observed no influence on broiler performance. Husaini *et al.,* (2010) fed 7.5 and 15% of conventional brewer's spent grain to broiler starters and finishers respectively and also observed no influence on performance. However, Denstadli *et al.,* (2010) who fed 40% of brewer's dried grain to broiler chickens, realized that though it did not affect feed consumption, there was a significant reduction in weight gain and loss in feed efficiency.

This was attributed to the dilution of the energy value of the feed at the 40% inclusion level. For this particular trial feeding up to 16% of SBBSG in broiler finisher diets

Table 2: Composition of the broiler grower diets

Ingredients (%)	0% SBBSG*	4% SBBSG	8% SBBSG	12% SBBSG	16% SBBSG
Maize	54.45	56.05	57.35	59.65	60.55
Soybean meal	24	22.5	21.8	20	19.1
Wheat bran	16	12	8	4	0
SBBSG	0	4	8	12	16
Salt	0.5	0.5	0.5	0.5	0.5
Shell grits	2	2	2	2	2
Lysine	0.2	0.2	0.2	0.2	0.2
Methionine	0.25	0.25	0.25	0.25	0.25
Dicalcium phosphate	0.5	0.8	0.8	0.8	0.8
Soybean oil	1.5	1.1	0.5	0	0
Broiler Premix	0.25	0.25	0.25	0.25	0.25
RE31	0.15	0.15	0.15	0.15	0.15
Toxin Binder[2]	0.2	0.2	0.2	0.2	0.2
Total	**100**	**100**	**100**	**100**	**100**
Calculated Analysis					
**ME, MJ/kg	11.74	11.76	11.75	11.81	11.93
CP, %	19.00	19.00	20.60	20.40	20.50
Total NSP, mg/g	79.32	93.92	101.21	111.82	122.61
Water Insoluble NSP mg/g	10.45	22.14	33.11	44.39	55.72
Water Soluble NSP mg/g	68.87	71.79	68.10	67.43	66.89
Ca, %	1.00	0.99	0.99	0.99	0.99
Total P, %	0.62	0.65	0.63	0.60	0.60
Available P, %	0.25	0.30	0.30	0.30	0.30
Total Lys, %	1.18	1.15	1.14	1.10	1.10

**Sorghum-Barley Brewer's Spent Grain*

[1]:Lactobacillus sp, Bacillus sp, Saccharomyces sp and Fermentation products.

[2]: Mycofix® Select 3.0 by Biomin

proved sufficient for adequate growth for growing, finishing broilers. The present study implies that SBBSG can serve as a good replacement for wheat bran and some soybean meal without compromising the performance of broiler chickens.

Carcass weight, dressing percentage, gizzard, liver, intestinal and abdominal fat weights were the carcass parameters measured (Table 5). The lack of significant differences in carcass weight and dressing percentage are likely due to similar feed intake, FCE and weight gain values. These factors are known to influence carcass traits in broilers (Austic and Nesheim, 1990; Ferket and Gernat, 2006; Chiba, 2009). Similar findings have been reported in other studies using other forms of brewer's spent grain in broiler diets. This was due to the high nutritional value of these ingredients (Chukwudi *et al.,* 2010; Denstadli *et al.,* 2010; Husaini *et al.,* 2010). The results observed for carcass characteristics in the current study therefore indicate that feeding SBBSG did not compromise the final saleable product. The size of liver was similar for birds fed on the different treatments. Significant differences could have served as an indication for further work into possible physiological or metabolic imbalances in the birds as the level of SBBSG increased in the diets.

Table 4: Effect of SBBSG on broiler performance

Parameter	0% SBBSG*	5% SBBSG	8% SBBSG	12% SBBSG	16% SBBSG	SEM ±	Probability
Initial weight (g)	706.30	677.50	692.20	701.50	707.20	11.97	NS
Final weight (g)	2106.00	2053.00	2121.00	2148.00	2152.00	37.00	NS
Feed intake (g/b)	128.55	125.05	127.85	129.50	128.60	7.61	NS
Weight gain (g/b	50.20	50.59	54.75	52.62	52.62	52.41	NS
Feed Conversion							
Efficiency	0.40	0.41	0.43	0.41	0.40	0.02	NS

** Sorghum Barley Brewer's Spent Grain*
SEM: Standard error of means
NS: Not Significant

Table 5: Effect of SBBSG on carcass characteristics

Parameter	0% SBBSG*	5% SBBSG	8% SBBSG	12% SBBSG	16% SBBSG	SEM ±	Probability
Carcass weight(g)	1526.70	1575.80	1553.30	1637.50	1644.20	50.46	NS
Dressing %	68.07	70.43	68.34	68.36	69.19	0.46	NS
Liver (g)	33.34	34.30	31.28	35.16	31.46	1.03	NS
Gizzard (g)	59.86	58.84	56.65	61.64	59.03	3.41	NS
Intestines (g)	105.18	98.30	107.07	108.13	115.76	5.56	
Fat (g)	18.47^{a}	17.41^{a}	21.07^{a}	29.40^{b}	37.99^{c}	2.76	0.05
**Feed cost/kg							
Gain	2.26	2.14	2.01	2.11	2.04	0.11	NS

** Sorghum Barley Brewer's Spent Grain*
*** Unit: GH¢/kg*
SEM: Standard error of means.
NS: Not Significant
^{abc} Means with same superscripts within the same row are not significantly different (P > 0.05)

Results from the present study on weight of intestines showed slight increasing trends with increase in SBBSG levels in the diets. An increase in total fibre content of diets is associated with increased weight of intestines and gizzard (Banfield *et al.,* 2002; Hetland *et al.,* 2003; Gabriel *et al.,* 2008). In the present study, the total NSP content in the grower diets increased progressively from 81.09 mg/g in T1 to 124.39mg/g in T5, while in the finisher diets this value ranged from 79.32mg/g in T1, to 122.61 mg/g in T5 respectively. This is a direct result of higher levels of SBBSG. However it is clear from the Tables 2 and 3 that, while soluble NSP remains fairly constant with increasing levels of SBBSG, the non soluble NSP is increasing. Hence, the greater contributor to the increase in total NSP with increasing SBBSG levels is the water insoluble portion of NSP. This is of significance because according to Jørgensen *et al.,* (1996) water soluble NSP's have a greater impact on increasing the size of the digestive tract than water insoluble NSP. While water insoluble NSP reduces transit time in the GIT, water soluble NSP tends to gelatinize by absorbing water and thereby delaying transit time. An adaptation of the GIT in such a situation will be to increase in size and weight. Since the soluble NSP tended to stay constant with increases in SBBSG levels, this may be the reason for the observed lack of significant increases in GIT size with increasing levels of SBBSG in the diet.

Weight of abdominal fat increased as the level of SBBSG increased in the diets. Birds on T4 and T5 accumulated abdominal fats

which were significantly different (P<0.05) from each other and higher than fat accumulated by birds on the other diets. It has been shown that increases in dietary NSPs lead to a reduction of abdominal fat in broilers (Saki, et. al., 2011; Shirzadi et. al., 2009; Moharrery, 2006). A reason for this may be that soluble NSP in the GIT causes a reduction in nutrient, particularly fat, absorption. For this trial no such increases in soluble NSPs were observed with increases in dietary SBBSG. The expected reductions in abdominal fat were therefore not observed. A precise ratio of dietary ME to amino acid is required to control fat accumulation in broiler finishers (Jackson *et al.,* 1982). There is little to no information on the degree of availability of amino acids and the ME content of this particular SBBSG to broilers. In formulating these diets, assumed amino acid availability and ME values for brewers spent grain (Donkor and Attoh-Kotoku, 2009), were used. These values could be wrong. If that were so, then the negative effects of these deviations will be magnified as the inclusion level of SBBGS goes up in the diets. It is also possible that some essential amino acid(s) was (were) lacking and hence the others would be catabolised and stored as fat, especially as their amounts would increase with increasing levels of SBBSG. These may therefore be some of the reasons for the increase in abdominal fat weights of broilers on T4 and T5.

The cost of feed per kilogram weight gain was similar (P>0.05) among the experimental diets. However, there was a trend towards decreasing cost of feed as dietary SBBSG levels increased (data not shown) and the relatively cheap cost of the SBBSG may be responsible for this. This has also been reported by several other researchers working on different forms of brewery spent grains (Adama et. al., 2007; Madubuike *et al.,* 2007; Salissou, 2009; Chukwudi et. al., 2010; Denstadli et. al., 2010 and Husaini et. al., 2010). The accumulated effect of increasing and decreasing trends in average daily gain and cost of feed per kg live weight gain respectively could sum up in overall cost savings with use of SBBSG.

Conclusions

The results of this preliminary study on the use of SBBSG in poultry diets suggest that:

1. SBBSG is a potential feed ingredient for use in broiler diets. There were no negative effects when SBBSG was included up to 16% in broiler diets.
2. Though this product is cheap at the source of production at the breweries, additional labour is required to quickly reduce its moisture content to below 10% if quality is to be maintained.
3. The ME content and nutrient availability to broilers of this new by-product needs to be determined in further studies if precise diet formulations are to be attained.
4. Finally a precise knowledge of the nutrient availability could make this product an extremely valuable feed ingredient for reducing the cost of broiler feed and result in cheaper cost of broiler meat to the average Ghanaian.

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GROWTH RESPONSE AND CARCASS TRAITS OF BIRDS FED DIETS CONTAINING THREE DIFFERENTLY PROCESSED DISCARDED VEGETABLE-BOVINE BLOOD-RUMEN CONTENT MIXTURES.

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Abstract

A total of 1,080 day-old Marshal broilers were fed diets containing discarded vegetable-fresh bovine blood-fresh rumen digesta (P1), discarded vegetable-ensiled bovine blood-fresh rumen digesta (P2) and discarded vegetable-fresh bovine blood-ensiled rumen digesta (P3) at three levels of inclusion (0, 3 and 6 %) at the starter phase while 540 of the birds were transferred and redistributed in the finisher phase. Data on growth response, blood profile and carcass characteristics were taken. At the 8th week of the study, 3 broiler chickens were randomly selected from each replicate for carcass evaluation. Data were subjected to Completely Randomized Design in a 3 x 3 factorial experimental layout. At the starter phase, results showed that birds fed P1 and P3 had significantly ($P<0.05$) higher final weight but birds fed P3 had the highest ($P<0.05$) survivability percentage compared with P1. The interaction of processing methods and levels of inclusion significantly ($P<0.05$) influenced the performance characteristics measured. In the interaction between processing methods and levels of inclusion showed a decrease ($P<0.05$) in the final weight as the levels of inclusion of P1 and P2 increased at the starter phase. At the finisher phase, the feed intake increased with increased levels of inclusion. The interaction between processing methods and levels of inclusion significantly ($P<0.05$) affected the performance characteristics measured Results of the carcass characteristics showed that birds fed 3% inclusion of P2 had the least ($P<0.05$) thigh weight while those fed 6% inclusion of P1 had the least value for abdominal fat. This study revealed that for enhanced growth performance; broiler chickens could be fed diets containing discarded vegetable-fresh bovine blood-ensiled rumen digesta (P3) up to 6% level of inclusion.

Key words: Growth response; carcass traits; processed mixtures; discarded vegetable; bovine blood; rumen content.

CROISSANCE ET CARACTÉRISTIQUES DE CARCASSE DES OISEAUX RECEVANT DES RÉGIMES CONTENANT TROIS MÉLANGES TRAITÉS DIFFÉREMMENT À BASE DE LÉGUMES REBUTÉS/SANG BOVIN/CONTENU RUMINAL

Résumé

Au total, 1080 poulets de chair de une Marshal âgés de 1 (un) jour ont été nourris avec des aliments contenant un mélanges de légumes rebutés/sang bovin frais / digesta ruminal frais (P1), de légumes rebutés/sang bovin ensilé/digesta ruminal frais (P2), et de légumes rebutés/sang bovin frais/digesta ruminal ensilé (P3), à trois niveaux d'inclusion (0, 3 et 6%) lors de la phase de démarrage tandis que 540 de ces oiseaux ont été transférés et répartis de nouveau pendant la phase de finition. Les données sur la réaction de croissance, le profil sanguin et les caractéristiques de carcasse ont été collectées. A la 8ème semaine de l'étude, 3 poulets de chair ont été choisis de manière aléatoire dans chaque répétition pour évaluation de la carcasse. Les données ont été soumises à un dispositif complètement aléatoire dans un schéma expérimental factoriel 3x3. Pendant la phase de démarrage, les résultats ont montré que les oiseaux nourris aux P1 et P3 avaient un poids final significativement ($P <0,05$) plus élevé, tandis que les oiseaux nourris au P3 avaient le pourcentage de survie le plus élevé ($P <0,05$) par rapport à ceux nourris

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au P1. L'interaction des méthodes de traitement et des niveaux d'inclusion a significativement influencé (P <0,05) les caractéristiques de performance mesurées. L'interaction entre les méthodes de traitement et les niveaux d'inclusion a révélé une diminution (P <0,05) du poids final car les niveaux d'inclusion de D1 et D2 avaient augmenté lors de la phase de démarrage. Lors de la phase de finition, la prise alimentaire a augmenté avec l'augmentation des niveaux d'inclusion. L'interaction entre les méthodes de traitement et les niveaux d'inclusion a affecté de façon significative (P <0,05) les caractéristiques des performances mesurées. Les résultats des caractéristiques de carcasse ont montré que les oiseaux recevant une inclusion à 3% de P2 avaient le poids de cuisse minimal (P <0,05) tandis que ceux recevant une inclusion à 6% de P1 avaient la valeur minimale pour la graisse abdominale. Cette étude a révélé que pour améliorer la performance de croissance, les poulets de chair peuvent être alimentés avec des régimes contenant des légumes rebutés/sang bovin frais/digesta ruminal ensilé (P3) jusqu'au niveau d'inclusion à 6%.

Mots-clés : réaction de croissance ; caractéristiques de carcasse ; mélanges traités ; légumes rebutés ; sang bovin ; contenu ruminal.

Introduction

The discipline of poultry nutrition has undergone remarkable changes during the past 50 years. Nutritional requirements have changed substantially due to dramatic changes in the genetics of the birds used (Havestein et. al., 2003), and due to improved nutritional knowledge. The unprecedented increase in feed prices during the past years are by now an unfortunate matter of record. The costs of virtually all feed ingredients, especially corn, soybean meal, phosphate, fat and even vitamins have gone far beyond any reasonable expectation. The ingredient, soybean meal occupies an important place in the poultry industry worldwide (Ekunseitan, 2012). Its importance lies in the fact of being the reference standard for all protein source-ingredients.A number of challenges confronting soybean utilization in animal diets have been cited and discussed by Kerley and Allee (2003) and their respective magnitudes debated. The authors further reported an increased demand for vegetable oil for biodiesel production as well as increased demand for animal protein in developing countries, scarcity and attendant increase in price.

In Nigeria, soybean meal accounts for approximately 92% of the total oilseed meals fed to livestock and poultry. A shift in its availability will result in partially collapse feedstuff production and a need in finding a suitable alternative. In recent years efforts are being geared towards the use of locally available alternative feedstuffs such as agro industrial by-products and farm wastes that may bring about the expected reduction in feed cost and consequently poultry products. The various investigations on the use of cheaper industrial by-products and farm wastes in poultry nutrition are being intensified with the aim of determining their efficiency of utilization for growth and production. Blood and rumen contents are major pollutants in abattoirs and slaughter slabs in developing countries especially in Nigeria. But with a critical view at the present situation in Nigeria where the by-products from thousands of animals slaughtered in the abattoir are left to waste, then less can be spoken of those slaughtered at the unapproved premises because there is no full implementation of the animals' slaughter and abattoir regulations (Aganga and Belino, 1983).

The potential of processing these materials as mixture has been unexplored and fewer reports are available for its inclusion in poultry diets. Evaluating the nutritional and potentials of wholesome mixtures of the three waste materials tend to justify the need to search for least–cost alternative protein and energy sources for livestock feeds in periods of scarcity of soybean meal and comparative effectiveness when used as substitute. Limited information is available on mixture of vegetable with rumen content and bovine blood and optimum level of replacement for major plant and animal protein sources. This study was thereby aimed at evaluating the nutritional potential of bovine blood, amaranth and rumen digesta when processed and mixed at equal proportions and its application in animal nutrition.

Materials and Methods

Area depiction

The study was carried out from July – November 2011 at Alao Farms, Tanke-Akata, Ilorin, Kwara state, Nigeria located at an altitude of 1412 ft, longitude 4o 35'N and latitude 8o 30'E (Google earth, 2013).The study area is located in the Southern Guinea Savannah ecological zone of Nigeria.

Experimental birds and management

A total of 1080, day old Hubbard strain broiler were used for the study. The birds were sourced from Obasanjo Farms, Igboora, Ogun state. The experiment was a 3 x 3 factorial experimental layout with factors due to processing methods and dietary levels of inclusion of mixtures in the diets. The inclusion of the three differently processed mixtures in the diet meal was at 3 levels: 0, 3 and 6 % giving rise to 9 dietary treatment groups of 120 birds each and 3 replicates of 40 birds each. At the finisher phase, a total of 540 birds from the broiler starter population were re-allotted in the same factorial experimental layout to obtain 9 dietary treatment groups of 60 birds each and 3 replicates of 20 birds each.

Collection and Preparation of Processed mixtures

Discarded vegetable (Amaranthus spp.) which has wilted and undergone decomposition was obtained from two commercial markets namely; Ipata and Oja-Oba in Ilorin, Kwara state, Nigeria between July and November, 2011. Fresh blood and rumen content were collected from several cattle that have been physically examined and ascertained fit for slaughtering at Ipata market slaughter slab in Ilorin, Kwara state, Nigeria. Blood was collected directly from the neck of the slaughtered cattle with the aid of wide-mouthed but shallow plastic bowls, used one after the other to minimize blood loss and contamination. Fresh rumen content was collected immediately the visceral was split from freshly slaughtered cattle. The dietary mixtures were then processed as follows:

Preparation of Mixtures

Discarded vegetable, fresh bovine blood and fresh rumen digesta (P1)

The chopped Amaranthus, fresh bovine blood and bovine rumen digesta were weighed out (weight for weight) at a ratio of 1:1:1 and mixed. The mixture was cooked and stirred intermittently for duration of 2 hours until the mixture was semi-dried. Stirring was done to prevent burning. After cooking, the mixture was sundried to constant moisture content.

Discarded vegetable, ensiled bovine blood and fresh rumen digesta (P2)

Freshly collected blood was covered up in an hermetic container for 3 days. It was then mixed with chopped Amaranthus and fresh bovine rumen digesta at a ratio of 1:1:1 in a drum. The mixture was subjected to similar cooking procedure as P1 above.

Discarded vegetable, fresh bovine blood and ensiled rumen digesta (P3)

Freshly collected bovine rumen was obtained from bowels of cattle. Rumen content was left for few hours in open air to reduce moisture and then covered up in an hermetic container and subjected to a fermentative process (ensiling) for three days. Ensiled rumen content was then mixed with chopped Amaranthus and fresh bovine blood at ratio 1:1:1 and subjected to similar cooking method as P1 above.

Drying of Processed Mixtures

All mixtures were dried using solar radiation. Black polythene nylons were purchased and spread on an open concrete floor. Semi-dried mixture which was properly drained of liquid using perforated aluminum containers was spread evenly on the nylon sheets. Mixtures were weighed intermittently until constant moisture content was obtained.

Experimental diets and management of birds

Nine experimental diets were formulated and used throughout the duration of the study. The processed mixtures were used to replace soybean meal in diet to meet the nutrient requirements of birds at the starter and finisher phases. The crude protein and metabolizable energy (Kcal/Kg) contents of the feed was within the recommended range (NRC, 1994). Brooding was done in groups of 40 birds each

Table 1: Composition (%) of Broiler starter diets

Ingredient	Replacement levels of Soybean meal								
	1	2	3	4	5	6	7	8	9
	0%	3%	6%	0%	3%	6%	0%	3%	6%
Maize	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00
Wheat offal	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Groundnut Cake	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	17.50
Soybean meal	20.00	17.00	14.00	20.00	17.00	14.00	20.00	17.00	14.00
P1	-	3.00	6.00	-	-	-	-	-	-
P2	-	-	-	-	3.00	6.00	-	-	-
P3	-	-	-	-	-	-	-	3.00	6.00
Fish meal (72%)	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Fish meal (72%)	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Oyster shell	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
*Vit./Min. premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Palm-Oil	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Analysis									
Crude protein (%)	21.09	20.97	21.16	21.09	20.71	20.59	21.09	20.70	20.69
Crude fibre (%)	4.42	4.48	4.53	4.42	4.63	4.82	4.42	4.60	4.77
Ether extract (%)	6.78	4.23	6.03	6.78	4.43	4.71	6.78	4.81	7.41
**ME(MJ/kg)	12.60	11.39	11.74	12.60	11.54	11.43	12.60	11.65	12.07

*Premix composition per kg diet: Vit A :400000IU, Vit D:80000IU, Vit E:40000ng, Vit k3:800mg, Vit B1:1000MG, Vit B2:6000mg, Vit B6:500mg, VitB12:25mg, Niacin:6000mg, Panthothenic acid:2000mg, Folic acid: 200mg, Biotin:8mg, Manganese:300000g, Iron:8000mg, Zinc:20000g, Cobalt:80mg, Iodine:400mg, Selenium:40mg, Choline:800000g

**Estimated using the formula by Pauzenga (1985) i.e: ME(kcal/kg) = (37.7 x CP%) + (81.8 x EE%) + (35.5 x NFE%). ME(MJ/kg) = ME(kcal/kg)* (4.18/1000).

for three weeks using electrical bulbs. The birds were fed ad libitum and managed intensively throughout the duration of the experiment with necessary routine medications provided accordingly. Housing protocols, handling the animals as well as study design were pre-approved by the faculty ethics committee of the Federal University of Agriculture, Abeokuta. Diet composition is shown in Tables 1 and 2.

Chemical analysis

After sun-drying and subjection to particle size reduction, triplicate chemical analyses were was carried out on each processed mixture AOAC (2005) standards. Dried meals were milled through a 1 mm screen. Dry matter content was determined by drying the samples at 105oC for 24 h, cooled in desiccators and weighed until constant weight was attained. Ash was determined after ignition in a muffl e furnace at 500 ± 15oC for 4 h. Crude protein (CP) was estimated from N determined using a Kjeltec 2300 Analyser Unit (Kjeltec auto distillation unit, model: Kjeltec 8200) after samples were digested in concentrated sulphuric acid. Ether extract (EE) was determined by using petroleum ether (bp 60–80oC) extraction in a Soxhlet extracting system (Phillip Harris, Birmingham, England: Model: 2050). Crude fibre (CF) was determined using fibertec cold extraction unit (Model: 1021).

Data Collection

Records of feed intake, weight gain and mortality was taken weekly. Protein efficiency ratio (PER), feed conversion ratio (FCR) and protein intake were calculated.

Carcass Evaluation

At the end of the 8th week, 3 birds which were of average weight for each replicate were used for carcass evaluation. They were slaughtered, bled, plucked and eviscerated. The live weight, plucked weight and dressed weight were taken. Also, cut-up parts (Head, neck, wings, breast, thighs, drumstick, back and shank), organs and offals (Gizzard, liver, lungs, heart and kidney) were weighed as criteria for the measurement of carcass performance. All weights were taken using Mettler® top-loading sensitive scale and expressed as percentages of the live weight.

Data Analysis

The data generated were arranged in a 3 x 3 factorial experimental layout and subjected to a completely randomized design (CRD). Significant differences among treatment means were resolved by Duncan's Multiple Range Test (Duncan, 1955) as contained in of the SAS statistical package (2011). Statistical signifi cance was established when probability was 95 %.

Experimental Model:

$$\gamma ijk = \mu + \tau i + \beta j + (\tau\beta)ij + \varepsilon ijk$$

Table 2: Composition (%) of Broiler finisher diets

Ingredient	**Replacement levels of Soybean meal**								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
	0%	**3%**	**6%**	**0%**	**3%**	**6%**	**0%**	**3%**	**6%**
Maize	44.00	44.00	44.00	44.00	44.00	44.00	44.00	44.00	44.00
Wheat offal	12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00
Groundnut Cake	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Soybean meal	15.00	12.75	10.50	15.00	12.75	10.50	15.00	12.75	10.50
P1	-	2.25	4.50	-	-	-	-	-	-
P2	-	-	-	-	2.25	4.50	-	-	-
P3	-	-	-	-	-	-	-	2.25	4.50
Fish meal (72%)	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Bone meal	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Oyster shell	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
*Vit./Min. premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Palm-Oil	2.50	2.50	-	-	-	-	-	-	-
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Analysis									
Crude protein (%)	22.57	21.28	21.43	22.57	21.07	21.00	22.57	21.08	21.03
Crude fibre (%)	3.98	4.02	4.05	3.98	3.43	4.28	3.98	4.11	4.23
Ether extract (%)	4.05	5.85	6.40	4.05	4.65	4.92	4.05	5.71	5.71
**ME(MJ/kg)	12.95	10.76	9.51	12.95	11.65	11.61	12.95	10.70	10.51

*Premix composition per kg diet: Vit A :400000IU, Vit D:80000IU, Vit E:40000ng, Vit k3:800mg, Vit B1:1000MG, Vit B2:6000mg, Vit B6:500mg, VitB12:25mg, Niacin:6000mg, Panthothenic acid:2000mg, Folic acid: 200mg, Biotin:8mg, Manganese:300000g, Iron:8000mg, Zinc:20000g, Cobalt:80mg, Iodine:400mg, Selenium:40mg, Choline:800000g

Estimated using the formula by Pauzenga (1985) i.e: ME(kcal/kg) = (37.7 x CP%) + (81.8 x EE%) + (35.5 x NFE%). ME(MJ/kg) = ME(kcal/kg) (4.18/1000).

Where:

γijk =	Observed value of the dependent variable (output)
μ =	Population mean (Overall mean)
τi =	Effect of Processing methods (i = P1, P2, P3)
βj =	Effect of levels of inclusion (j = 0, 3 and 6%)
$(\tau\beta)ij$ =	Interaction Processing methods and levels of inclusion
εijk =	Random error

Results

Chemical composition (% air-dry basis) of processed mixtures and Soybean meal

Table 3 shows chemical composition (% air-dry basis) of the three processed mixtures and soybean meal. Crude protein was highest in P1. The crude protein contents of P2 and P3 were 40.79 and 41.92%, respectively and less than the value obtained in P1. Crude fibre values obtained for P2 and P3 were 60.12 and 48.85% higher than the crude fibre of P1. The fibre value was least in soybean meal. Similar observation was made for the ash content of the mixtures with the lowest value in soybean meal. Energy content was considerably lower for P2, P3 and soybean meal being 8.12, 4.19 and 3.23 % less than the gross energy value obtained for P1.

Main effect of mixtures from the different processing methods and levels of inclusion on performance characteristics of broiler chickens at starter phase

The performance characteristics of broiler chickens at starter phase is shown in Table 4. During the starter phase of the experiment, processed mixtures significantly ($P<0.05$) influenced survivability and final weight. However, processed mixtures had no significant ($P>0.05$) effect on feed intake, weight gain, protein intake, PER and FCR. Birds fed P1 and P3 had similar final weight which were significantly ($P<0.05$) higher than value obtained on P2. Survivability was however least in birds on P1. Levels of Inclusion had no significant ($P>0.05$) effect on final weight, feed intake, daily weight gain, FCR and survivability.

Details of interaction between mixtures from the different processing methods and levels of inclusion on performance characteristics of broiler chickens at starter phase

The results of the effect of interaction between mixtures from the different processing methods and levels of inclusion on performance characteristics shown in Table 5 revealed significant ($P<0.05$) differences in all parameters measured except weight gain, PER and FCR. There was a decrease in the final weight as the levels of inclusion of P1 and P2 increased. This was not in tandem with that observed in birds fed P3. Feed intake was statistically similar across treatment except at 3 % inclusion of P1 and P2 where highest and least values, respectively were obtained. Survivability was statistically higher at 3 % inclusion of P3 but within comparable range to that obtained on birds at 0 % inclusion of P1.

Main effect of mixtures from the different processing methods and levels of inclusion on performance characteristics of broiler chickens at finisher phase

The performance characteristics of broiler chickens at finisher phase is shown in Table 6. The results showed that PER was significantly ($P<0.05$) affected by processed mixtures. PER values was highest in birds fed diet containing P3 and least for birds on diets containing P1 and P2, respectively. The levels of inclusion had a significant effect on feed intake, protein intake, PER and FCR. Values for feed intake were significantly similar at 3 and 6 % level of inclusion and higher than birds fed at 0 %. The PER and FCR did not follow any particular trend with increasing levels of inclusion, highest value for both parameters was obtained at 3 and 6% levels of inclusion.

Details of interaction between mixtures from the different processing methods and levels of inclusion on performance characteristics of broiler chickens at finisher phase

The effect of interaction between mixtures from the different processing methods and levels of inclusion on performance

Table 3: Chemical composition (% Air-dry Basis) of processed mixtures and soybean meal

Parameter	P1	P2	P3	SBM
Moisture (%)	14.90	11.40	13.00	N/A
Protein (%)	50.36	40.88	41.48	45.00
Ether Extract (%)	1.03	1.57	1.74	3.50
Crude Fibre (%)	8.25	13.21	12.28	6.50
Ash (%)	9.99	14.44	11.25	6.0
Nitrogen-free extract (%)	15.47	18.50	20.25	N/A
Metabolizable energy (Kcal/kg)	2500.85	2297.74	2395.97	2420.00

SBM: Soybean meal

Table 4: Main effect of mixtures from the different processing methods and levels of inclusion on performance characteristics of broiler chickens at starter phase

	Processed Mixtures				*Levels of Inclusion*				
Parameter	**P1**	**P2**	**P3**	**SBM**	***0***	***3***	***6***	**SBM**	***P x LI***
Initial weight(g)	40.67	40.56	40.25	0.18	40.66	40.40	40.40	0.19	NS
Final weight(g)	669.33^{a}	642.10^{b}	682.68^{a}	9.98	675.61	666.19	652.26	11.18	*
Feed intake(g/ bird/day)	50.26	49.02	49.41	0.68	50.19	49.63	48.87	0.66	*
Weight gain(g/ bird/day)	21.75	21.44	22.31	0.47	22.38	21.82	21.31	0.46	NS
PER	2.45	2.52	2.53	0.06	2.44	2.50	2.57	0.06	NS
FCR	2.18	2.18	2.10	0.03	2.13	2.17	2.16	0.04	NS
Survivability (%)	89.16^{a}	94.85^{ab}	97.02^{a}	2.05	95.93	91.87	93.24	2.40	*

a, b: Means in the same row by factor with different superscripts differ significantly(P<0.05)
SEM: Standard Error of Mean.; PER: Protein Efficiency Ratio; FCR: Feed conversion Ratio; *: Significant; NS: Not significant

characteristics of broiler chickens at finisher phase is shown in Table 7. There were significant (P< 0.05) effects on feed intake, protein intake, PER and FCR. An upward trend was observed in feed intake for each processed mixture, as the level of inclusion of mixture increased from 0 to 30% an increase in feed intake was evident in P1 and P2 but not so pronounced in birds fed diet containing P3. PER values were within the same range at 0 and 3% inclusion levels of the P2 and P3. In addition, FCR value was best at 0% level of inclusion of each mixture. However, no significant effect was observed for final weight, daily weight gain and survivability.

Main effect of mixtures from the different processing methods and levels of inclusion on carcass quality of broiler chickens at finisher phase

Table 8 shows the effect of processed mixtures on carcass yield of the experimental birds. Processed mixtures had no significant effect (P>0.05) on most parameters measured except live weight, shank and lungs. An increase in live weight was observed across the row of mixtures in feed with the least and highest value obtained in birds fed on P1 and P3, respectively. In addition, processed mixtures had significant effect on the shank and lungs with highest values obtained in birds fed P1. On the other hand, the levels of inclusion had no significant (P>0.05) effect on all the parameters considered. However, higher live weight was recorded for birds fed at 30% level of inclusion while those on 15% level of inclusion had the highest dressing percentage. Also the abdominal fat (P>0.05) reduced with increasing levels of inclusion of the diets.

Table 5: Details of interaction between mixtures from the different processing methods and levels of inclusion on performance characteristics of broiler chickens at starter phase

		Processed Mixtures			
Parameter	**Levels of inclusion**	**P1**	**P2**	**P3**	**SEM**
Final Weight (g)	0	702.79^{a}	656.07^{ab}	668.16^{ab}	14.82
	3	675.59^{ab}	635.81^{b}	687.16^{a}	
	6	629.63^{b}	634.43^{b}	692.71^{a}	
Feed Intake (g/bird/day)	0	50.42^{ab}	50.93^{ab}	49.23^{ab}	11.06
	3	51.90^{a}	47.80^{b}	49.17^{ab}	
	6	48.46a^{b}	48.32^{ab}	49.82^{ab}	
Survivability (%)	0	96.75^{a}	95.12^{ab}	95.93^{ab}	4.285
	3	82.11^{b}	94.31^{ab}	99.19^{a}	
	6	88.62^{ab}	95.12^{ab}	95.93^{ab}	

$^{a, b, c, d, e:}$ Means in the same row by factor with different superscripts differ significantly (P<0.05)
SEM: Standard Error of Mean; P1: Discarded vegetable, fresh bovine blood and rumen digesta

Table 6: Main effect of mixtures from the different processing methods and levels of inclusion on performance characteristics of broiler chickens at finisher phase

	Processed Mixtures				***Levels of Inclusion***				
Parameter	**P1**	**P2**	**P3**	**SBM**	***0***	***3***	***6***	**SBM**	***P x LI***
Initial weight(g)	637.78	632.78	647.22	6.45	638.33	633.33	646.11	6.40	NS
Final weight(g)	1983.50	2012.80	2025.20	23.55	2019.4	1979.10	2022.10	23.76	NS
Feed intake(g/bird/day)	115.61	117.63	118.33	1.53	113.57^{b}	118.28^{a}	119.72^{a}	1.31	*
Weight gain(g/bird/day)	48.02	49.29	49.07	0.86	49.12	48.07	49.20	0.87	NS
PER	2.09^{b}	2.02^{b}	2.20^{a}	0.07	2.04^{b}	2.02^{b}	2.24^{a}	0.06	*
FCR	2.43	2.41	2.44	0.04	2.33^{b}	2.50^{a}	2.46^{a}	0.03	*
Survivability (%)	96.11	96.11	95.00	1.89	97.78	95.00	94.44	1.83	NS

$^{a, b}$: Means in the same row by factor with different superscripts differ significantly(P<0.05)
SEM: Standard Error of Mean.; PER: Protein Efficiency Ratio; FCR: Feed conversion Ratio; *: Significant; NS: Not significant

Table 7: Details of interaction between mixtures from the different processing methods and level of inclusion on performance characteristics of broiler chickens at finisher phase

		Processed Mixtures			
Parameter	**Levels of inclusion**	**P1**	**P2**	**P3**	**SEM**
Final Weight (g)	0	110.69^{b}	114.87^{ab}	115.14^{ab}	2.262
	3	115.20^{ab}	118.61^{a}	121.03^{a}	
	6	120.93^{a}	119.42^{a}	118.81^{a}	
PER	0	2.64^{a}	2.06^{c}	2.04^{c}	0.063
	3	1.90^{c}	2.10^{c}	2.04^{c}	
	6	2.32^{b}	1.90^{c}	2.52^{a}	
FCR	0	2.32^{b}	2.32^{b}	2.34^{ab}	0.055
	3	2.52^{a}	2.47^{ab}	2.50^{ab}	
	6	2.45^{ab}	2.44^{ab}	2.49	

Table 8: Main effect of mixtures from the different processing methods and inclusion level on carcass quality of broiler chickens at finisher phase

Table 4: Main effect of mixtures from the different processing methods and levels of inclusion on performance characteristics of broiler chickens at starter phase

	Processed Mixtures				*Levels of Inclusion*				
Parameter	**P1**	**P2**	**P3**	**SBM**	***0***	***3***	***6***	**SBM**	***P x LI***
Live weight (g)	2166.67^{b}	2288.89^{ab}	2366.67^{a}	157.61	2211.11	2277.78	2333.33	50.92	NS
Plucked Weight (g)	1956.29^{b}	2038.03^{ab}	2065.63^{a}	48.02	1966.78	2027.68	2068.26	51.7	NS
Plucked Weight (%)	90.29	89.04	87.28	2.65	88.95	89.02	88.64	0.98	NS
Dressing Percentage	65.81	65.57	65.22	0.88	66.14	65.17	65.32	2.63	NS
Cut-up part1									
Back	14.42	14.26	14.64	1.03	14.12	14.40	14.49	0.39	NS
Breast	17.78	18.36	18.30	1.38	18.66	17.54	18.24	0.48	NS
Thigh	11.00	10.28	11.04	0.74	10.82	10.58	10.92	0.25	*
Drumstick	10.12	10.34	9.77	0.71	10.00	10.18	10.04	0.26	NS
Shank	4.31^{a}	4.08^{ab}	3.80^{b}	0.33	4.16	4.14	3.89	0.11	*
Wings	8.11	8.15	7.74	0.43	8.13	8.12	7.75	0.16	NS
Head	2.73	2.59	2.58	0.28	2.77	2.58	2.56	0.10	NS
Neck	3.97	3.79	3.85	0.42	3.99	3.87	3.81	0.15	NS
Organ2									
Liver	2.33	2.20	2.33	0.39	2.09	2.40	2.41	0.12	*
Kidney	0.44	0.39	0.48	0.12	0.46	0.48	0.38	0.39	*
Gizzard	2.26	2.04	2.02	0.09	2.11	2.25	1.97	0.10	*
Heart	0.52	0.45	0.46	0.10	0.44	0.50	0.49	0.04	NS
Abdominal fat	0.52	0.45	0.46	0.15	1.16	1.34	0.96	0.04	*
Lungs	0.70^{a}	0.53^{b}	0.55^{ab}	0.13	0.60	0.61	0.57	0.05	NS

^{a,b}: Means in the same row by factor with different superscripts differ significantly($P<0.05$). ^{1,2}: Expressed as percentages of the live weight; SEM: Standard Error of Mean; *: Significant; NS: Not significant.

Details of interaction between mixtures from the different processing methods and levels of inclusion on carcass quality of broiler chickens at finisher phase

The results of the effect of interaction between mixtures from the different processing methods and levels of inclusion on carcass quality shown in Table 9 revealed significant ($p<0.05$) differences in thigh, shank, liver, kidney, gizzard and abdominal fat. Birds fed 3% inclusion of P1 had the highest gizzard and thigh values. In addition, there was a similar trend of decrease in shank value as the levels of inclusion of P2 and P3 were increased. Birds fed 0% inclusion level of P3 had the least and highest value for liver and kidney weight respectively. However, it was observed that 3% P2 inclusion in the diets gave the highest value of 1.67% in abdominal fat with the least value obtained at 6% inclusion of P1.

Discussion

The determined chemical composition revealed that the processed mixtures had crude protein (40.88, 41.48 and 50.36%) and metabolizable energy which can adequately replace soybean meal in broiler diets and within comparable range as that obtained by

Table 9: Details of interaction between mixtures from the different processing methods and levels of inclusion on Carcass Quality of broiler chickens at finisher phase

		Processed Mixtures			
Parameter	**Levels of inclusion**	**P1**	**P2**	**P3**	**SEM**
Cut part[1]					
Thigh	0	10.99^{ab}	10.49^{ab}	10.98^{ab}	0.43
	3	11.41^{a}	9.84^{b}	10.58^{ab}	
	6	10.61^{abc}	10.52^{ab}	10.92^{ab}	
Shank	0	4.4^{ab}	4.23^{abc}	3.86^{bc}	0.19
	3	4.59^{a}	4.02^{abc}	3.82^{bc}	
	6	3.95^{bc}	3.99^{bc}	3.73^{c}	
Liver	0	2.07^{b}	2.30^{ab}	1.89^{b}	0.2
	3	2.48^{ab}	1.96^{b}	2.75^{a}	
	6	2.43^{ab}	2.33^{ab}	2.47^{ab}	
Kidney	0	0.43^{ab}	0.39^{ab}	0.57^{a}	0.07
	3	0.51^{ab}	0.45^{ab}	0.47^{ab}	
	6	0.39^{ab}	0.33^{b}	0.41^{ab}	
Gizzard	0	2.13^{ab}	2.14^{ab}	2.05^{ab}	0.17
	3	2.51^{a}	2.15^{ab}	2.08^{ab}	
	6	2.15^{ab}	1.84^{b}	1.94^{ab}	
Abdominal fat	0	1.03^{ab}	1.40^{ab}	1.04^{ab}	0.26
	3	1.26^{ab}	1.67^{a}	1.11^{ab}	
	6	0.57^{b}	1.07^{ab}	1.25^{ab}	

$^{a, b}$: Means in the same row by factor with different superscripts differ significantly($P<0.05$)
SEM: Standard Error of Mean.; PER: Protein Efficiency Ratio; FCR: Feed conversion Ratio; *: Significant; NS: Not significant

Aduku (1993). The ingredients thereby rank alongside other conventional protein sources especially of plant origin (Schmidt, 1971). Animal by-products should be carefully used, as they are not easily standardized as a result of the differences in production processes and origins of the residues included in animal meals. This may have been one of the reasons for the varied nutrient composition observed when included in experimental feeds. The crude protein values obtained for the three mixtures were higher than those obtained when bovine blood and rumen content were combined at ratio 1:3 as reported by Adeniji (1996).

The processed mixtures though higher in some chemical constituents (fibre and ash), however contains negligible or no antinutritional factors (ANFs) (Adeniji, 1996; Fasuyi, 2007) when compared with soybean meal. The ANFs in soybean meal are known to cause depressed growth performance in poultry and swine (Cromwell, 1999). The popularity of soybean meal in swine and poultry feeds is largely due to its high concentration of protein (44 to 48%) and its excellent profile of highly digestible amino acids (Cromwell, 1999). Although not a perfect blend of amino acids (it tends to be low in methionine) while the materials used in this novel mixture are blends of various amino-acids profile.

The percentage survivability obtained in the experiment is an indication that the processed mixtures are non-toxic. This could be associated with probably low level of toxins and pathogenic organisms in the diets as a result of cooking the discarded vegetable, bovine blood and rumen digesta and dried properly before incorporation into experimental diets. Cooking and drying of the abattoir wastes not only serve to kill many pathogenic organisms but also to improve the palatability, nutrient digestibility and also to prolong the shelf life in

terms of length of storage of the ingredients. It also confirms that the processing method utilized was adequate even as the level of inclusion of processed mixtures in the diet increased.

Processed mixtures significantly affected final weight gain which was statistically similar in birds fed on P1 and P3 at the starter phase. The observed lower weight in P2 may be attributed to the high content of fibre in P2 which reflected in the feed intake though statistically similar with others. These findings agree with earlier reports (McDonald et al; 1995). Levels of inclusion had no significant effect on all parameters measured except in protein intake. The gradual decrease in feed and protein intakes with increase in the level of mixtures in the diet may be due to the fact that the increased fibre content depresses birds' consumption. Fibre creates bulkiness of the feed in the gut and this reduces the feed consumption of the birds, hence they ate less and their nutrient requirement was met with less feed consumed.

Feed intake was statistically similar across treatment except at 3% inclusion of P1 and P2 where the highest and least values were obtained. Bray et al. (1971) stated that feed consumed by chicks is governed by the energy and protein level in the feed and this was reflected in the results obtained from the birds fed the three processed mixtures at varying levels of inclusion. The varied intake of feed in birds may be due to the energy content of the feed, this is attributable to the fact that chicks eat to satisfy their energy requirement (Maynard et al., 1979) and also low energy and low protein diets influence performance. Ogundipe et al. (2003) also stated that when controlling protein level, energy levels of the ration must be considered because energy is the main factor controlling feed intake.

The levels of inclusion had significant effect on feed intake, protein intake, PER and FCR at the finisher phase of growth. An increase in level of inclusion resulted in a concomitant increase in feed intake of birds. Values for 3 and 6% inclusion were significantly similar. The gradual increase in feed consumption may be due to the fact that the birds consumed more feed to meet their energy and daily requirement. (Atteh, 1990; Odeniya, 2002). This was evident in the protein intake which influenced the PER values obtained.

The dressing percentages were within the range reported for broilers by Longe (1986) and Sogunle et al. (2009). The drumstick which is part of the most economically important portion of the carcass composition and also provide the greatest portion of edible meat in broilers (Smith and Teeter, 1987 ; Fanimo *et al.,* 1996) was greatly favoured by P2 having a better value though comparable to P1 and P3. An increase in live weight value was observed across the row of processed mixtures with the least and highest values obtained in P1 and P3, respectively.

The details of interaction between mixtures from the different processing methods and level of inclusion on carcass quality revealed significant differences in thigh, shank, kidney, gizzard and abdominal fat. Birds fed 3% inclusion in P1 had the highest gizzard value compared with inclusion levels in P2 and P3. Gizzard is known to respond particularly rapidly to changes in the diet (Svihus, 2011). This present study was in contrast with the result that high fibre is reported to cause gizzard enlargement to cope with fibre handling (Fasina *et al.,* 2004). This deviation may be as a result of the composition of the processed mixtures (plant and animal origin) being different from fibre component of most conventional feedstuffs, combination ration and replacement level in feed and the processing method employed in the preparation of the mixture which could be adequate in reducing the fibre effect since the physical properties of these components are different as well as having gizzard stimulating properties. The non-significant effect on gizzard in this study lies also in the lack of differences in particle size distribution after milling. This agrees with Amerah et al. (2009b) who opined that very fine fibre particles do not stimulate gizzard development and enlargement. Therefore the increase in size of the gizzard is a logical consequence of an increased need for particle size reduction, due to the stimulative effect of the increased grinding activity of the two pairs of gizzard muscle.

Conclusion

This present study showed that there was no detrimental effect of processed mixtures in diets fed to birds. The percentage survivability obtained in the experiment is an indication that the mixture is not toxic. It also confirms that the processing method utilized was adequate even as the level of inclusion of mixtures in diet increased. In addition, the values for feed intake were similar at 3% and 6% level of inclusion at the finisher phase. In the interaction between mixtures from the different processing methods and levels of inclusion on performance characteristics, a decrease in the final weight was observed as the levels of inclusion of P1 and P2 increased at the starter phase. Increasing levels of inclusion of the three processed mixtures increased digestibility of ether extract of the birds except at P3. Birds could be fed diet containing P3 (discarded vegetable-fresh bovine blood-ensiled rumen content) at up to 6% inclusion levels for enhanced performance in terms of the final weight and feed conversion ratio. The mixtures can serve as suitable replacements for soybean in terms of chemical constituent.

Impact

The development of an animal is consequent on the nutritional status of the animal. The increase in price of feed ingredient in developing countries has greatly reduced the rate of expansion of the poultry industry. In order to halt this drift, emphasis has been focused towards the use of economical and efficient feeds stuffs such as abattoir wastes. This will allow for saving in the feed cost without reducing the nutritive value of the ration and also in converting waste into feedstuffs capable of serving as replacement for soybean and other protein ingredients in the poultry industry. This innovation has been presented to farmers in Ilorin and environs with appreciable number adopting and reporting positive results.

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THE EFFECT OF FEEDING SORGHUM-BARLEY BREWER'S SPENT GRAIN (SBBSG) ON PERFORMANCE AND EGG QUALITY OF LAYING HENS

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Abstract

The objective of the study was to establish if inclusion of Sorghum-Barley Brewer's Spent Grain (SBBSG) in laying hen diets will affect laying performance and egg quality. A total of 300 Bovan Brown laying hens (37 weeks old) were randomly allocated in a completely randomized design, to five dietary treatments, T1, T2, T3, T4 and T5, representing 0, 5, 10, 15 and 20% SBBSG inclusion in the diets, respectively. Each treatment was replicated 4 times and the trial lasted 112 days. Feed and water were provided ad-lib. Eggs laid were collected twice daily and laying performance and egg quality characteristics determined. There were no significant differences (P>0.05) among treatment diets for hen day and hen housed egg production, daily feed intake and egg weight. FCE was similar for T4 and T5 (0.54 and 0.54 respectively) and significantly better (P<0.05) than for T1, T2 and T3 (0.53, 0.53 and 0.53 respectively). Birds on T5 had eggs with significantly thicker shells (0.44mm) than that of birds on T1 to T4 (0.42, 0.42, 0.43 and 0.42 mm respectively). Birds that had no SBBSG in their diet (T1) had egg yolks with the highest cholesterol concentration (414.9 mg/dl). This was significantly higher (P<0.05) than cholesterol concentration of eggs from T2, T3, T4 and T5 (266.66, 279.62, 243.52 and 246.61 mg/d), respectively. Feed cost per kg egg produced for birds on T5 was 1.33 GH¢. This was significantly lower (P<0.05) than feed cost for birds on T1 (1.41 GH¢) and similar (P>0.05) to birds fed T2 to T4 (1.38, 1.37 and 1.35 GH¢), respectively. The present study shows that SBBSG can effectively be included in laying hen diets up to 20% without any adverse effect on laying performance and egg quality while at the same time reducing egg cholesterol content and being cheaper.

Keywords: Sorghum-Barley Brewer's Spent Grain (SBBSG), Layers, Egg Quality

L'EFFET DE LA DRECHE DE SORGHO/ORGE (SBBSG) COMME INGREDIENT ALIMENTAIRE SUR LA PERFORMANCE ET LA QUALITE DES OEUFS DES POULES PONDEUSES

Résumé

L'objectif de l'étude était de déterminer si l'inclusion de la drêche de sorgho/orge (SBBSG) dans les régimes de poules pondeuses aurait une incidence sur la performance de ponte et la qualité des oeufs. Au total, 300 pondeuses Bovans Brown (âgées de 37 semaines) ont été réparties de manière aléatoire dans un schéma complètement randomisé à cinq traitements diététiques, T1, T2, T3, T4 et T5, représentant respectivement des taux d'inclusion de SBBSG de 0,5% ; 10% ; 15% et 20% dans les régimes. Chaque traitement a été répété 4 fois, et le processus a duré 112 jours. La nourriture et l'eau ont été servies à volonté. Les oeufs pondus étaient recueillis deux fois par jour ; et la performance de ponte et la qualité des oeufs ont été déterminées. On n'a pas relevé de différences significatives (P> 0,05) entre les régimes de traitement pour la production d'oeufs (hen day and hen housed egg ?), la prise alimentaire quotidienne et le poids de l'oeuf. Le taux de conversion alimentaire était similaire pour les traitements T4 et T5 (respectivement 0,54 et 0,54), et significativement meilleur (P <0,05) par rapport aux traitements T1, T2 et T3 (respectivement 0,53, 0,53 et 0,53). Les coquilles des oeufs des oiseaux au T5 étaient significativement plus épaisses (0,44mm) que celles des oiseaux aux traitements T1 à T4 (respectivement 0,42, 0,42, 0,43 et 0,42 mm). Les oiseaux qui n'ont pas reçu de SBBSG dans leur alimentation (T1) avaient des jaunes d'oeufs avec un taux de cholestérol élevé (414,9 mg / dl). Ce taux était significativement plus élevé (P <0,05) que le taux de cholestérol des oeufs des poules aux traitements T2, T3, T4 et T5 (respectivement

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266,66 ; 279,62 ; 243,52 et 246,61mg / d). Le coût des aliments par kg d'oeufs produits pour les oiseaux au traitement T5 était de 1,33 GH ¢. Ce coût était significativement plus faible (P <0,05) que le coût des aliments des oiseaux au traitement T1 (1,41 GH ¢) et similaire (P> 0,05) à celui des oiseaux aux traitements T2 à T4 (respectivement 1,38 ; 1,37 et 1,35 GH ¢). Le régime alimentaire avec inclusion à 20% de SBBSG (T5) était moins cher (P <.05) par kg d'oeufs produits par rapport au régime avec 0% inclusion de SBBSG (T1). La présente étude montre que la SBBSG peut effectivement être incorporée dans les régimes des poules pondeuses jusqu'à 20% sans aucun effet négatif sur la performance de ponte et la qualité des oeufs et réduire en même temps le taux de cholestérol des oeufs, et est moins chère.

Mots-clés : Drêches de sorgho-orge, poules pondeuses, qualité de l'oeuf

Introduction

High cost of production has been a major challenge of the domestic poultry industry in Ghana in the last 10 years. The main factors responsible for this are the high cost of day old chicks, feed, medication, water, electricity and lack of access to credit facilities. Prime among these factors, is feed which alone makes up to about 70% of the cost of production (Aning, 2006). In egg production, high production costs translate directly into an increase in the cost of eggs on the local market. The high cost of feed is a result of the high cost of feed ingredients. Major feed ingredients in layer feed manufacturing are maize, soybean meal and wheat bran. Major feed mills in Ghana use about 225,000 mt of maize annually for poultry feed. In 2009, about 1.62 million metric tons (mt) of maize, was produced in Ghana. This is below what is needed for human consumption alone, according to the Statistics, Research and Information Directorate, (SRID), of the Ministry of Food and Agriculture, Ghana (SRID, 2010). Therefore, there is competition for maize among consumers, leading to an increase in the price of locally produced chicken and eggs.

Wheat bran is a by-product of wheat processing for wheat flour. All the wheat used by flour mills in Ghana is imported. About 9% of the total weight of the wheat grain is bran (AAFCO, 1988). Since the price of wheat bran is dependent largely on the world price of wheat, there is a wide fluctuation in the local cost of wheat bran. Most of the soybean meal intended for feed manufacturing in Ghana is imported, and so this commodity has wide price fluctuations, mostly determined by the strength of the local currency, the cedi, against the US dollar. Hence fluctuations in the price of these major inputs as well as competition for maize makes feed for poultry expensive.

Since cost of feed is the highest expenditure item, it also represents the biggest opportunity for reducing the cost of layer, and hence egg production (Aning, 2006). In this regard, most farmers and feed mills in Ghana are constantly searching for new and cheaper alternatives to conventional feed ingredients. One such alternative is brewers spent grain. The use in animal feed is not new (Adama *et al.,* 2007; Husaini *et al.,* 2010). However, in recent years breweries in Ghana have began to include sorghum in their brewing formulae creating in the process, a unique brand of brewers spent grain called sorghum-barley brewers spent grain (SBBSG). SBBSG is currently being produced in appreciable quantities by a large international brewery and local farmers in the Ashanti Region of Ghana use this by-product in the wet form to feed pigs as part of their daily ration.

No information exists in Ghana on the nutrient availability of this ingredient to monogastrics. Again no information exists on its appropriateness as an ingredient in layer rations. It can be speculated however that since both barley and sorghum grain contain appreciable levels of digestible nutrients for poultry (NRC, 1994) some of these nutrients, especially the non-fermentable nutrients, will still remain after the grain has been used for brewing purposes.

The hypothesis of this study was that SBBSG can be effectively included in layer diets without negatively affecting laying performance. The objective was therefore to investigate the efficacy, laying performance, and egg quality of laying hens fed diets with included SBBSG.

Materials and Methods

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

Processing of SBBSG

Fresh SBBSG was obtained from Guinness Ghana Brewery Ltd (GGBL), Kumasi and transported to the Department of Animal Science, Kwame Nkrumah University of Science and Technology (KNUST) Kumasi. The fresh by-product with a moisture content of 95%, was spread thinly on a clean concrete floor and sun-dried for 14 days with daily turning till the moisture content dropped to approximately 7-8%. The dry storable product was subsequently bagged in nylon sacks (25kg) and transported to LIPREC where it was stored in a cool, dry and airy environment for subsequent incorporation into feed.

Experimental diets

Five diets were formulated to contain 0%,5%,10%,15% and 20% of SBBSG representing T1, T2, T3, T4, and T5, respectively (Table 1). These incremental levels of SBBSG came at the expense of wheat bran which dropped from 20% to 0% in T1 and T5, respectively. A maximum inclusion of 20% SBBSG was chosen to reflect the average inclusion level of wheat bran in most layer diets in Ghana. In order to maintain an isonitrogenous and isocaloric level for all the treatments, slight adjustments in maize and soybean meal inclusion levels were made.

Management of Experimental Birds

A total of 300 Bovan Brown laying hens (37-weeks old) were assigned, in a Completely Randomised Design (CRD), to the five experimental diets with four (4) replicates per treatment. Birds were housed in groups of 15 in communal cages, each cage measuring 2m * 2m * 1m (L *B* H). After relocation, the hens were allowed nine (9) days to acclimatize to their new environment and thereafter fed on the experimental diet for another seven (7) days for conditioning after which the trial commenced. Birds on a particular treatment were fed the same diet throughout the period of study. A known amount of fresh feed was provided each morning at 06:00hrs. Prior to feeding, any feed left over in the troughs was collected and weighed, to determine feed intake. Fresh water was provided ad lib. Eggs were collected twice a day at 08:00 and 16:00 and grouped according to treatment and replicate. Records of egg weight, hen-day and hen-housed egg production performance were kept daily and summarized on a weekly basis. The trial lasted for 112 days.

On days 56, 84 and 112 of the trial, four eggs were randomly selected from each replicate. They were selected from eggs that had been laid within a two hour period between 10:00 and 12:00hr. Their external and internal qualities including egg weight, shell thickness, Haugh Unit and albumin height were determined within a six hour period after collection. On day 112, three eggs were randomly selected from each replicate, cracked open and the contents carefully separated into yolk and albumin. A small amount of yolk was collected into a sampling tube and preserved in a refrigerator at 40oC for cholesterol analysis which was conducted within 48 hours.

Chemical Analysis

The proximate chemical composition of both the ingredients and experimental diets was analyzed using methods outlined by AOAC, (1995). Calcium and Phosphorus were

determined according to the methods outlined by James (1996) and AOAC (1995). Amino acid analysis of the feed ingredients was carried out using the procedure described by Andrews and Balder (1985). Methionine and cystine were determined using performic acid oxidation according to the method of Moore (1963). Total NSP and sugar fractions were analysed using Gas Liquid Chromatography (Englyst and Hudson, 1987).

Egg quality analysis

Internal and external egg quality measurements were determined on day 56, 84 and 112. Four eggs laid within a two hour period and selected from each replicate were used. Parameters measured included egg weight, shell thickness, Haugh Unit and albumin height. The Shell Thickness Gauge™ (Orka Food Technology) was used to measure shell thickness. The Haugh Unit was calculated according to the method described by Haugh (1937);

$$HU = 100 * \log \left[H - \frac{\sqrt{G\,(30\,W0.37 + 100)} + 1.9}{100} \right]$$

Where:

- HU = Haugh unit
- H= observed height of the albumen in millimeters
- W = weight of egg in grams
- G = the gravitational constant, 32.2

Since the birds were from the same strain and were of the same age and managed similarly, the concerns of Silversides (1994) about the use of the Haugh unit for comparisons are not relevant in the current study.

Cholesterol determination was based on the enzymatic method as described by Allain *et al.,* (1974). The underlying principle behind this method is that Cholesterol is present in animal tissues and fluids as cholesterol esters and free cholesterol. Cholesterol esters are hydrolysed by cholesterol esterase and the cholesterol is then measured by oxidizing with cholesterol oxidase to form hydrogen peroxide. The hydrogen peroxide then reacts with phenol and 4-aminoantipyrine present to form a red quinoneimine dye. The intensity of the resultant dye is directly proportional to the level of cholesterol present in the sample. About 10µl of the egg yolk was pipetted into a test tube and 100µl of the test reagent added. The mixture was allowed to stand for about 10 minutes for colour development, after which time the absorbance was read from a spectrophotometer at a wavelength of 546nm.

Cholesterol was then calculated as;

$$\text{Cholesterol Concentration} = \frac{\Delta \text{Absorbance of Standard} \times \Delta \text{ Concentration of Standard}}{\Delta \text{Absorbance of Sample}}$$

Δ = Change

Statistical Analysis

All data gathered were subjected to statistical analysis using the Generalised Linear Model procedure of the Statistical Analysis Systems Institute (SAS, 1999). Significant differences among means were separated using the Student Newman-Kuels (SNK) Test. The model used was as follows;

$$Yij = \mu + Di + Eij$$

Where;
Yij is the observation, µ is the mean, Di is the effect of diet and Eij is the error term.

Results

Table 1 shows the chemical composition of SBBDG. The composition and calculated values of the layer diet is shown in Table 2. The levels of the major ingredients are based on existing commercial layer diets in Ghana.

Performance

There were no significant differences ($P>0.05$) among the experimental diets for average daily feed intake (ADFI), hen housed, hen day egg production and average egg weight (Table 3). Feed conversion efficiency of hens on T4 and T5 (0.54 and 0.54, respectively) was significantly better ($P<0.05$) than for hens on T1, T2 and T3 (0.53, 0.53 and 0.53, respectively).

With the exception of egg weight where there were no differences (P>0.05), all the other egg quality parameters studied showed significant differences among treatment diets. Albumen height values were significantly higher (P<0.05) for hens on T2, T4 and T5 than that obtained for hens on T1 and T3. A similar pattern was observed in Haugh units, where birds on T2, T4 and T5 had values of 88.07, 88.34 and 88.12, respectively and these were significantly higher (P<0.05) than values obtained for hens on T1 and T3, 85.29 and 85.26, respectively. The shell thickness of hens on T5 was significantly better (P<0.05; 0.44mm) than hens on T1, T2, T3 and T4: 0.42, 0.42, 0.43 and 0.42mm, respectively). Birds on T1 had egg yolks with the highest cholesterol concentration (414.9 mg/dl) and this was significantly different (P<0.05) from cholesterol concentration of eggs from T2, T3, T4 and T5, 266.66, 279.62, 243.52 and 246.61mg/dl respectively.

Feed cost per kg of eggs produced for hens on T5 was 1.33 Ghanaian Cedis (Gh¢). This was significantly lower (P<0.05) than that for hens on T1 (1.41 Gh¢). However for hens on T2, T3 and T4, feed cost per kg egg produced (1.38, 1.37 and 1.35 Gh¢ respectively) were similar (P>0.05) to each other and also to T1 and T5.

Discussion

Most cereal grains and their byproducts contain anti-nutritive factors. The major ANFs in wheat bran and barley are arabinoxylans, cellulose and beta-glucans (Aalto, *et al.,* 1988; Andersson *et al.,* 1999; Bacic and Stone, 1991; Zijlstra *et al.,* 1999), while in sorghum, tannins, phytic acid, trypsin and amylase inhibitors are common (Mohammed *et al.,* 2010). High amounts of dietary fibre limit the bioavailability of nutrients to swine and poultry (Schulze *et al.,* 1994; Lenis *et al.,* 1996; Slominski *et al.,* 2004). Arabinoxylans and beta glucans reduce nutrient digestion, absorption and utilization resulting in reduced laying performance (Bedford, 2000; Sivhus and Gullord, 2002; Ravindran *et al.,* 2007; Taylor, 2008; Woyengo *et al.,* 2008). Considering the large differences in NSP contents of the diets (Table 4) it would be expected that some differences in performance among hens on the different treatments may be observed. However, in this study addition of SBBSG did not affect hen day, hen housed egg production and ADFI suggesting that nutrient intake and availability were not affected by inclusion of SBBSG in the diets. This may be explained by the relative concentrations of soluble and insoluble components of the total NSP in each diet (Table 4). Generally soluble NSP's exert a greater impact on nutrient availability, and hence performance, than insoluble NSP (Antoniou *et al.,* 1981; Classen *et al.,* 1985; Campbell *et al.,* 1986; Hesselman and Aman, 1986). In this trial although total and insoluble NSP increased with increasing levels of SBBSG, soluble NSP levels did not follow any particular trend and remained quite low. This might explain the absence of any significant negative effect on overall performance. The similarity in the hen day and hen housed measures of egg production implies that mortality was minimal. This is another confirmation that SBSSG inclusion in the diets did not have any negative effect.

The levels of ANF's particularly tannins, trypsin inhibitors and amylase inhibitors were not determined in this trial. However, it is known that physical/mechanical, chemical or biological processing can be used to partly reduce the negative effects of anti-nutritional factors in cereal by-products (Mitaru *et al.,* 1984; Nortey, 2001; Nortey *et al.,* 2007; Selle *et al.,* 2009 and Kumar *et al.,* 2010). Since no loss of performance was observed with increasing levels of fibre in this trial, it can be speculated that the ANF's present were either within tolerable limits, or were rendered harmless by the various stages of production from barley/sorghum to beer and the by-product, SBBSG.

Hens fed the 15% and 20% SBBSG diets had better (P<0.05) feed conversion efficiency (0.54) than those on the other treatments (0.53). Factors such as metabolisable energy, rate of egg production, nutrient content of diet and feed intake are all known to affect feed utilization in poultry (Austic and Nesheim, 1990). In formulating these diets, assumed amino acid availability and ME values for brewers spent grain (Donkor and Attoh-Kotoku, 2009), were used. Increasing the level of SBBSG resulted in

better FCE, implying that the assumed nutrient values used in formulating diets for this trial were not far from right.

The levels of intrinsic enzyme activity in the cereal by-products used for this study were not determined.

With the exception of egg weight where there were no differences (P>0.05), all the other egg quality parameters studied showed significant differences among treatment diets. Albumen height values were significantly higher (P<0.05) for hens on T2, T4 and T5 than that obtained for hens on T1 and T3. A similar pattern was observed in Haugh units, where birds on T2, T4 and T5 had values of 88.07, 88.34 and 88.12, respectively and these were significantly higher (P<0.05) than values obtained for hens on T1 and T3, 85.29 and 85.26, respectively. The shell thickness of hens on T5 was significantly better (P<0.05; 0.44mm) than hens on T1, T2, T3 and T4: 0.42, 0.42, 0.43 and 0.42mm, respectively). Birds on T1 had egg yolks with the highest cholesterol concentration (414.9 mg/dl) and this was significantly different (P<0.05) from cholesterol concentration of eggs from T2, T3, T4 and T5, 266.66, 279.62, 243.52 and 246.61mg/dl respectively.

Feed cost per kg of eggs produced for hens on T5 was 1.33 Ghanaian Cedis (Gh¢). This was significantly lower (P<0.05) than that for hens on T1 (1.41 Gh¢). However for hens on T2, T3 and T4, feed cost per kg egg produced (1.38, 1.37 and 1.35 Gh¢ respectively) were similar (P>0.05) to each other and also to T1 and T5.

Discussion

Most cereal grains and their byproducts contain anti-nutritive factors. The major ANFs in wheat bran and barley are arabinoxylans, cellulose and beta-glucans (Aalto, *et al.,* 1988; Andersson *et al.,* 1999; Bacic and Stone, 1991; Zijlstra *et al.,* 1999), while in sorghum, tannins, phytic acid, trypsin and amylase inhibitors are common (Mohammed *et al.,* 2010). High amounts of dietary fibre limit the bioavailability of nutrients to swine and poultry (Schulze *et al.,* 1994; Lenis *et al.,* 1996; Slominski *et al.,* 2004). Arabinoxylans and beta glucans reduce nutrient digestion, absorption and utilization resulting in reduced laying performance (Bedford, 2000; Sivhus and Gullord, 2002; Ravindran *et al.,* 2007; Taylor, 2008; Woyengo *et al.,* 2008). Considering the large differences in NSP contents of the diets (Table 4) it would be expected that some differences in performance among hens on the different treatments may be observed. However, in this study addition of SBBSG did not affect hen day, hen housed egg production and ADFI suggesting that nutrient intake and availability were not affected by inclusion of SBBSG in the diets. This may be explained by the relative concentrations of soluble and insoluble components of the total NSP in each diet (Table 4). Generally soluble NSP's exert a greater impact on nutrient availability, and hence performance, than insoluble NSP (Antoniou *et al.,* 1981; Classen *et al.,* 1985; Campbell *et al.,* 1986; Hesselman and Aman, 1986). In this trial although total and insoluble NSP increased with increasing levels of SBBSG, soluble NSP levels did not follow any particular trend and remained quite low. This might explain the absence of any significant negative effect on overall performance. The similarity in the hen day and hen housed measures of egg production implies that mortality was minimal. This is another confirmation that SBSSG inclusion in the diets did not have any negative effect.

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Table 1: Chemical Composition of Sorghum Barley Brewer's Spent Grain

Parameter	SBBSG
Dry Matter (%)	95.8
Crude Protein (%)	27.58
Crude Fibre (%)	14.75
Total Ash (%)	11.81
Ether Extract (%)	10.22
Calcium (mg/100g)	252.9
Phosphorus (mg/100g)	715.2

Table 2: Composition of the layer diets (%)

Ingredient	0% SBBSG*	5% SBBSG	10% SBBSG	15% SBBSG	20% SBBSG
Maize	52.1	49.7	48.15	45.6	44.55
Wheat bran	20	15	10	5	0
Soybean meal	18	20	21.5	24	25
SBBSG	0	5	10	15	20
Lysine	0.15	0.15	0.15	0.15	0.15
Methionine	0.15	0.15	0.15	0.15	0.15
Shell grits	8	8	8	8	8
Dicalcium phosphate	0.5	0.55	0.6	0.65	0.7
Salt	0.5	0.5	0.5	0.5	0.5
Layer premix	0.25	0.25	0.25	0.25	0.25
RE 31	0.15	0.5	0.5	0.5	0.5
Toxin Binder2	0.2	0.2	0.2	0.2	0.2
Total	**100**	**100**	**100**	**100**	**100**
Calculated Analysis					
ME, Mcal/kg	3.00	3.00	3.00	3.00	3.00
CP, %	17.00	17.00	17.00	17.00	17.00
Ca, %	3.37	3.38	3.38	3.39	3.40
Total P, %	0.62	0.57	0.53	0.49	0.44
Available P, %	0.25	0.25	0.25	0.25	0.25
Total Lys, %	**0.95**	**0.96**	**0.97**	**0.99**	**0.98**

*: Sorghum-Barley Brewer's Spent Grainyer Premix.
[1](Lactobacillus sp, Bacillus sp, Saccharomyces sp and Fermentation products)
[2] Mycofix Select 3.0 by Biomin

1984; Nortey, 2001; Nortey *et al.,* 2007; Selle *et al.,* 2009 and Kumar *et al.,* 2010). Since no loss of performance was observed with increasing levels of fibre in this trial, it can be speculated that the ANF's present were either within tolerable limits, or were rendered harmless by the various stages of production from barley/ sorghum to beer and the by-product, SBBSG.

Hens fed the 15% and 20% SBBSG diets had better ($P<0.05$) feed conversion efficiency (0.54) than those on the other treatments (0.53). Factors such as metabolisable energy, rate of egg production, nutrient content of diet and feed intake are all known to affect feed utilization in poultry (Austic and Nesheim, 1990). In formulating these diets, assumed amino acid availability and ME values for brewers spent grain (Donkor and Attoh-Kotoku, 2009), were used. Increasing the level of SBBSG resulted in better FCE, implying that the assumed nutrient values used in formulating diets for this trial were not far from right.

The levels of intrinsic enzyme activity in the cereal by-products used for this study were not determined. However, there is evidence that there are intrinsic enzymes in cereal grains which promote the breakdown and subsequent release of stored nutrients during germination (Duodu *et al.,* 2003). Therefore, if this by product contained appreciable levels of intrinsic enzymes it could be speculated that these enzymes may have played a role in alleviating some of the negative effects of increased levels of SBBSG.

Table 3: Effect of SBBSG on performance and egg quality of laying hens

Parameter	0% SBBSG*	5% SBBSG	10% SBBSG	15% SBBSG	20% SBBSG	SEM±	P value
Daily Feed Intake (g)	109.03	108.89	108.51	108.53	104.66	1.37	0.1393
Hen Day (%)	86.58	89.64	86.31	84.21	87.97	1.43	0.1487
Hen Housed (%)	83.28	87.97	85.58	83.58	87.38	1.70	0.2133
FCE1	0.53[a]	0.53[a]	0.53[a]	0.54[b]	0.54[b]	0.004	0.036
Egg Weight (g)	58.85	59.59	59.40	59.65	60.02	0.30	0.1076
Haugh Unit	85.29[a]	88.07[b]	85.26[a]	88.34[b]	88.12[b]	0.65	0.0006
Shell Thickness (mm)	0.42[a]	0.42[a]	0.43[a]	0.42[a]	0.44[b]	0.01	0.0024
Albumen Height (mm)	7.28[a]	7.83b	7.41[a]	7.75[b]	7.76[b]	0.10	0.0004
Egg Weight (g)	61.36	63.49	62.43	7.75[b]	7.76[b]	0.10	0.0004
Yolk Cholesterol (mg/dl)	414.90[a]	266.66[b]	279.62[b]	243.52[b]	246.61[b]	5.48	0.0013
Mortality (%)	6.90	5.00	3.33	6.67	3.33		
Feed Cost/kg Egg(GH¢/kg)	1.41[a]	1.38[ab]	1.37[ab]	1.35[ab]	1.33[b]	0.02	0.0328

* Sorghum-Barley Brewer's Spent Grain
SEM: Standard error of means
abMeans with same superscripts in the same row are not significantly different
[1]Calculated on per egg basis

Table 4: NSP concentrations of the experimental diets

NSP* (mg/g)	0% SBBSG*		5% SBBSG		10% SBBSG		15% SBBSG		20% SBBSG	
	Total NSP**	Water Insoluble	Total NSP	Water Insoluble	Total NSP	Water Insoluble	Total NSP	Water Insoluble	Total NSP	Water Insoluble
Arabinose	26.61	23.94	26.23	24.37	25.73	23.17	24.58	23.17	26.15	22.92
Xylose	38.32	35.27	39.79	37.67	40.52	34.78	37.71	35.96	42.76	37.39
Mannnose	2.58	1.52	3.11	1.99	3.34	2.09	3.88	2.78	4.96	2.90
Galactose	11.53	9.49	11.34	9.81	11.23	9.97	12.39	11.47	12.70	11.24
Glucose	35.50	35.74	41.91	37.82	40.32	41.02	41.77	44.50	44.56	48.19
Uronic acid	11.92	11.38	13.50	11.32	12.19	12.05	12.75	12.40	13.62	10.07
Total	**126.43**	**117.32**	**135.87**	**122.96**	**133.32**	**12.05**	**133.08**	**130.26**	**144.76**	**132.69**

* Sorghum-Barley Brewer's Spent Grain
**Non starch polysaccharide

Egg quality characteristics

The USDA (2000) recommends that Haugh Unit values of <60, 70-80, and >90 are not acceptable, acceptable and excellent respectively. In this trial birds on the various treatments had eggs with Haugh Units falling within the acceptable range. However, unexplained significant differences ($P<0.05$) existed among experimental diets, with birds on T1 and T3 having eggs with the least Haugh Unit values (85.29 and 85.26 respectively) compared with the rest. Some factors reported to influence Haugh Unit either individually or in combination include age, strain and health status of the bird, diet, storage and environmental temperature at which eggs are stored (Tweneboah, 2000; FAO, 2003; Keener *et al.,* 2006). For this trial, it is speculated that a

major contributor to differences in Haugh Unit was diet, since it was the only major factor that varied.

Diets high in fibre have been demonstrated to reduce the cholesterol content of eggs. Using chicory fructans, Chen *et al.,* (2005) demonstrated that both oligofructose and inulin reduced egg yolk cholesterol by 18.64 and 16.44%, respectively when fed to laying hens. These fibres also increased cholesterol concentrations in both the jejunum and faeces. In another study to investigate the effects of different dietary levels of yucca powder on the performance, blood parameters and egg yolk concentration of laying quails, Kaya *et al.,* (2003) determined that addition of 200ppm of yucca powder to layer diets resulted in slight reductions in egg yolk cholesterol concentration. When layer diets were supplemented with β-Cyclodextrin, Park et al., (2005) observed a reduction in the cholesterol contents of egg yolk. In this trial, egg yolk cholesterol was significantly reduced from 414.9mg/dl in T1 to 246.61mg/dl in T5 with corresponding NSP levels of 126.43mg/g and 144.76mg/g, respectively. Based on the available literature it can be speculated that this reduction in total yolk cholesterol was a direct result of increasing dietary NSP levels.

Conclusions

Results of this preliminary study on the use of SBBSG in layer diets suggest that:

- SBBSG is a potential feed ingredient for use in layer diets and can be included up to 20% in layer diets without any negative effects on production performance or egg quality characteristics.This level of SBBSG resulted in better internal egg quality, thicker shells as well as reduced cholesterol levels.
- Feed cost per egg produced tended to be cheaper.
- There is the need for additional labour to quickly reduce the moisture content of this product to below 10% if quality is to be maintained.
- The ME content and nutrient availability to layers of this new by-product needs to be determined in further studies if precise diet formulations are to be attained with the ultimate aim of reducing the cost of eggs to the average Ghanaian.
- Finally, precise knowledge of the nutrient availability could make this product a valuable feed ingredient for reducing the cost of layer feed and result in cheaper cost of eggs to the average Ghanaian.

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DIGESTIBILITÉ IN VIVO DES FEUILLES FRAÎCHES DE BANANIER (MUSA SP.) TRAITÉES À LA MÉLASSE CHEZ LES BREBIS DJALLONKÉ (OVIS ARIES)

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Résumé

Une étude portant sur l'ingestion et la digestibilité in vivo des feuilles fraîches de bananier non traitées et traitées à 10% de mélasse chez les brebis Djallonké a été menée à la Ferme d'Application et de Recherche et au Laboratoire de Nutrition Animale de l'Université de Dschang entre Août et Septembre 2009. Pour cela, six brebis ont été utilisées et reparties en deux lots de trois animaux chacun. Le lot témoin (lot 1) a reçu les feuilles fraîches de bananier non traitées et le lot 2 les feuilles fraîches de bananier traitées à 10% de mélasse. Des échantillons des feuilles fraîches de bananier non traitées et traitées à 10% de mélasse ont été prélevés et conduit au laboratoire pour l'analyse de leur composition chimique. Les brebis ont été maintenues dans des cages de digestibilités individuelles. Tous les matins, avant de fournir une nouvelle ration, les refus de chaque ration étaient pesés. De même, les quantités de fèces et d'urines de chaque brebis étaient évaluées et des échantillons étaient prélevés et conduits au laboratoire pour les analyses. Les résultats de cette étude ont montré que le traitement à la mélasse n'a pas influencé la teneur en protéines brute ; que la teneur en cellulose brute des feuilles non traitées a été supérieur ($p<0,05$) à celle des feuilles traitées à 10% de mélasse. L'ingestion de la matière sèche a été significativement améliorée par le traitement à la mélasse. De plus le traitement des feuilles fraîches de bananier à 10% de mélasse n'a pas permit d'améliorer la digestibilité apparente de la matière sèche et de la cellulose brute. Par contre, la digestibilité de l'azote des feuilles fraîches de bananier traitées à 10% de mélasse (75,54%) a été significativement plus élevée ($p<0,05$) que celle des feuilles fraîches de bananier non traitées (69,55%). Le traitement des feuilles fraiches de bananier à la mélasse permet donc d'améliorer l'ingestion de la MS et la digestibilité de l'azote chez la brebis Djallonké.

Mots clés : brebis Djallonké, mélasse, feuilles fraîches de bananier, ingestion, digestibilité.

IN VIVO DIGESTIBILITY OF MOLASSES-TREATED FRESH BANANA LEAVES (MUSA SP.) IN WEST AFRICAN DWARF SHEEP (OVIS ARIES)

Abstract

A study on the intake and in vivo digestibility of untreated and 10% molasses-treated fresh banana leaves in West African Dwarf sheep was conducted at the Teaching and Research Farm and Animal Nutrition Laboratory of the University of Dschang between August and September 2009. For this, six sheep were used and assigned to two groups of three animals each. The control (group 1) received untreated fresh leaves and Group 2 received fresh leaves treated with 10% molasses. Samples of untreated fresh banana leaves and those treated with 10% molasses were collected and taken to the laboratory for analysis of their chemical composition. The sheep were kept in individual digestibility cages. Every morning, before providing a new ration, refusals of each ration were weighed. Similarly, the amounts of feces and urine of each sheep were evaluated and samples were collected and taken to the laboratory for analysis. The results of this study showed that molasses treatment did not affect the crude protein content, and that crude fiber content of untreated leaves was higher ($p <0.05$) than that of leaves treated with 10% molasses. Dry matter intake was significantly improved by molasses treatment. In addition, the treatment of fresh banana leaves with 10% molasses did not improve the apparent digestibility of dry matter and crude fiber. However, digestibility of

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nitrogen in fresh banana leaves treated with 10% molasses (75.54%) was significantly higher ($p < 0.05$) than that of untreated fresh banana leaves (69, 55%). Molasses-treated fresh banana leaves therefore improve MS intake and nitrogen digestibility in West African dwarf sheep.

Key Words: West African dwarf sheep, molasses, fresh banana leaves, intake, digestibility.

Introduction

En Afrique tropicale, les petits ruminants jouent un rôle socio-économique très important dans la vie des populations (Pamo *et al.,* 2002). En effet, ils interviennent de manière prépondérante dans les rites culturels, traditionnels et religieux des différents peuples (dots funérailles, sacrifices…). Par ailleurs, ils constituent une caisse d'épargne facilement mobilisable en cas de besoin (rentrée scolaire, maladies) et représentent une source de protéines d'origines animales (Pamo et Pieper, 1995).

Malgré ces multiples avantages, la productivité des ovins et caprins demeure faible dans cette région à cause de nombreuses contraintes entre autre sanitaires et surtout alimentaires (Pamo *et al.,* 2004). La région de l'Ouest Cameroun a connu une importante croissance démographique ces dernières décennies, elle a conduit à l'accroissement des surfaces cultivées au détriment des terres pâturables. Dans ce contexte, la quantité et la qualité des fourrages disponibles pour les animaux ne suffisent pas à couvrir leurs besoins. L'insuffisance et la mauvaise valeur nutritive des fourrages se trouvent parmi les principaux facteurs limitant la productivité des ruminants à l'ouest Cameroun et dans toute la zone tropicale (Morhan-Fer, 1989). Ainsi, la supplémentation de la ration de base apparait donc comme une stratégie inévitable pour pallier ces contraintes.

Pour assurer cette supplémentation, il est souvent conseillé d'utiliser des sous produits de l'agro-industrie tels que les tourteaux, généralement trop couteux pour les élevages modestes. Aussi, cette stratégie n'a-t-elle jamais permis de résoudre le problème de manière efficace et surtout durable (Tendonkeng, 2006).

Les résidus de récoltes peu couteux et disponibles peuvent constituer des ressources potentielles, capables d'assurer cette supplémentation (Chermiti, 1994). Parmi ces résidus de récolte, on retrouve les feuilles de bananier. Avec des taux de protéines brutes pouvant atteindre les 15% de la matière sèche (Ffoulkes et Preston, 1977) les feuilles de bananier peuvent être d'excellents fourrages. Son ingestion pourrait être améliorée par l'ajout des glucides facilement fermentescible dans le rumen. De nombreuses études ont montrées que la mélasse peut rehausser la valeur nutritive de nombreux fourrages de qualité moyenne (Chenost et Kayouli, 1997). Cependant, aucune investigation n'a été conduite sur l'ingestion et la digestibilité in vivo des feuilles fraîches de bananier traitées à la mélasse chez le mouton Djallonké. C'est donc pour combler ce vide que cette étude a été menée. Elle a pour objectif de contribuer à l'amélioration de l'alimentation des ruminants tropicaux à travers la valorisation d'une ressource peu coûteuse et disponible : les feuilles fraîches de bananier.

Matériel et Méthodes

Zone d'étude

L'essai a été conduit d'Août à Septembre 2009 à la Ferme d'Application et de Recherche (FAR) de l'Université de Dschang, situé à 5°26 de Latitude Nord, à 10°26 de Longitude Est et à une altitude d'environ 1420m dans l'Ouest Cameroun. Le climat de la région est équatorial de type Camerounien d'altitude. Les précipitations varient entre 1500 et 2000 mm et les températures oscillent entre 20 et 28°C. La saison sèche va de mi-novembre à la mi-mars et la saison des pluies de mi-mars à mi-novembre et correspond à la principale période des cultures (Pamo *et al.,* 2004).

Matériel animal

Six brebis Djallonké achetées dans les marchés à la périphérie de Dschang ont servi dans cette étude. Leur âge déterminé à partir de leur dentition était situé entre 12 et 24 mois (Corcy, 1991). Ces animaux ont été repartis en

deux lots de trois animaux chacun et mis dans des cages métaboliques (ou de digestibilité) individuelles.

Matériel végétal

Le matériel végétal était constitué essentiellement de feuilles de bananier récoltées chaque matin dans les plantations de la Ferme d'Application et de Recherche de l'Université de Dschang. Deux traitements ont été expérimentés : les feuilles fraîches de bananier hachées non traitées d'une part et celle traitées à 10% de mélasse. Pour obtenir ce dernier traitement, la quantité de mélasse à utiliser a été estimée en prenant 10% de la quantité des feuilles de la ration (2700 g). Cette quantité de mélasse a été ensuite dissoute dans 1125ml d'eau. La solution obtenue a été ensuite mélangé aux feuilles jusqu'à leur imprégnation complète par la mélasse. Le mélange était pesé de nouveau pour n'avoir que la quantité à donner aux animaux.

Conduite de l'essai

Une période d'adaptation à la nouvelle ration et aux cages métaboliques de huit jours a été observée avant le début de l'essai. Le premier jour de la période d'adaptation, toutes les brebis ont reçu 2400 g de Brachiaria ruziziensis haché. En plus, les brebis du lot 1 (témoin) ont reçu 300 g de feuilles fraîches de bananier non traitées et celles du lot 2, 300 g de feuilles fraîches de bananier traitées à 10% de mélasse. Par la suite, chaque jour, la quantité de Brachiaria ruziziensis était réduite de 400 g et la quantité de feuilles fraîches de bananier non traitées et traitées à 10% augmentait de 400 g dans chaque lot jusqu'au septième et huitième jour. Les sept jours suivants ont été consacrés à la collecte des données. Pendant cette période, chaque brebis recevait quotidiennement 2700g de feuilles fraîches de bananier non traitées pour le lot 1 et traitées à 10% de mélasse pour le lot 2.

Collecte des données

Un échantillon de 500 g de feuilles fraîches de bananier non traitées et 500 g de feuilles fraîches de bananier traitées à 10% de mélasse ont été prélevés et séchés au laboratoire de nutrition animale pour la détermination de la composition chimique. Chaque matin, les fèces et les urines de chaque brebis ont été récoltées et pesées. Un échantillon de 100 g de fèces était prélevé, emballé dans un sachet plastique et conduit au laboratoire de nutrition animale pour l'analyse bromatologique. Les urines étaient collectées dans une éprouvette graduée, 100 ml étaient prélevé et conservé au réfrigérateur à 4°C pour la détermination de l'azote.

Analyses statistiques

Les données ont étés analysés au moyen du logiciel SPSS 17.0, les paramètres de l'ingestion et de la digestibilité in vivo ont été soumis à l'analyse de la variance à une voie de classification. Lorsque les différences existaient entre les moyennes, elles étaient séparées par le test de Student- Newman- Keuls au seuil de 5%.

Résultats

Composition chimique des feuilles fraîches de bananier non traitées et traitées à 10% de mélasse

Le tableau 1 présente la composition chimique des feuilles fraîches de bananier traitées à 0 et 10% de mélasse. Il ressort de ce tableau que les teneurs en matières sèches, cendres matières organiques et lipides ont peu varié avec les niveaux de traitement à la mélasse. La teneur en cellulose brute a diminué avec le traitement à la mélasse. Elle a été de 27,03% dans les feuilles fraîches de bananier non traitées contre 21,29%MS après le traitement à 10% de mélasse. La teneur en glucide quant à elle a été plus élevée dans les feuilles traitées à la mélasse. La teneur en protéines brute la plus élevée a été obtenue avec les feuilles fraîches de bananier non traitées (19,57%MS).

Ingestion des feuilles fraîches de bananier non traitées et traitées à 10% de mélasse

Le tableau 2 présente l'ingestion de la matière sèche, de la cellulose brute et l'utilisation digestive de l'azote par les brebis Djallonké. L'ingestion de la matière sèche et de l'azote des brebis nourries aux feuilles fraîches de bananier traitées à 10% de mélasse (lot 2) respectivement de 278,21 et de 80,12 g/

Tableau 1: Composition chimique des feuilles de bananier traitées à 0 et 10% de mélasse

Eléments	Niveaux de traitement (%)	
	0	10
Matière sèche (%)	95,81	95,90
Cendres (%MS)	13,66	12,90
Matière organique (%MS)	82,15	83,00
Cellulose brute (%MS)	27,03	21,29
Glucide (%MS)	57,38	58,89
Protéines brute (%MS)	19,57	19,02
Lipides (%MS)	5,20	5,09

MS : matière sèche.

Tableau 2: Ingestion de la matière sèche, de la cellulose brute et utilisation digestive de l'azote par les brebis Djallonké

	Lot 1	Lot 2
Ingestion de la MS (g/brebis/j)	1598,2±288,10[a]	1818,9±242,23[b]
Ingestion de la MS (g/brebis/j/kg^{0,75})	252,23±34,56[a]	278,21±28,02[b]
Ingestion de la CB (g/brebis/j)	431,99±77,87[b]	387,2±51,57[a]
Ingestion de la CB (g/brebis/j/kg^{0,75})	94,55± 12,95[b]	87,20±8,77[a]
Azote ingérée (g/brebis/j)	310,10±53,26[a]	345,9±46,07[b]
Azote ingérée (g/brebis/j/kg^{0,75})	74,21±10,17[a]	80,12±8,07[b]
Azote fécal (g/brebis/j)	54,74±10,86[a]	61,76±10,22[b]
Azote fécal (g/brebis/j)kg^{0,75})	20,07±3,01[a]	21,99±2,72[b]
Azote urinaire (g/brebis/j)	38,88±1,46[b]	23,01±4,62[a]
Azote urinaire (g/brebis/j)kg^{0,75}	15,57±0,43[b]	10,48±1,59[a]

MS : matière sèche, CB : cellulose brute.

[a, b]: les moyennes portant la même lettre sur la même ligne ne sont pas significativement différente (p>0,05).

Tableau 3: Digestibilité apparentes de la matière sèche, de la cellulose brute et de l'azote des feuilles fraiches de bananier traitées à 0 et 10% mélasse

	Niveau de traitement (%)	
	0	10
Digestibilité apparente de la MS (%)	76,20±2,62[a]	77,17±1,93[a]
Digestibilité apparente de la CB (%)	70,00±3,08[a]	73,39±2,61[a]
Digestibilité apparente de l'azote (%)	69,55±3,03[a]	75,54±0,56[b]

a, b: les moyennes portant la même lettre sur la même ligne ne sont pas significativement différente (p>0,05).

brebis/kg0,75 est significativement plus élevée (p<0,05) que celles des brebis nourries aux feuilles fraîches de bananier non traitées (lot 1). Par contre, l'ingestion de la cellulose brute par les brebis du lot 1 (94,55 g/brebis/kg0,75) est significativement plus élevée (p<0,05) que celle des brebis du lot 2 (87,20 g/brebis/kg0,75). Concernant l'azote, les fèces des brebis du lot 2 contiennent significativement plus d'azote (61,76 g/brebis/kg0,75) que ceux du lot 1 (54,74 g/brebis/kg0,75) (p<0,05). En revanche, la teneur en azote urinaire des animaux du lot 1 (15,57 g/brebis/kg0,75) est significativement supérieur (p<0,05) à celle des brebis du lot 2 (10,48 g/brebis/kg0,75).

Digestibilité apparente

Les digestibilités apparentes de la matière sèche, de la cellulose brute et de l'azote des feuilles fraiches de bananier traitées à 0 et 10% de mélasse sont présentées dans le tableau 3. Il apparaît de ce tableau que les digestibilités apparentes de la matière sèche et de la cellulose brute des feuilles fraiches

de bananier non traitées et traitées à 10% de mélasse ont été comparables ($p>0,05$). Par contre, le traitement à 10% de mélasse a amélioré significativement ($p<0,05$) la digestibilité de l'azote dans les feuilles fraîches de bananier.

Discussion

La teneurs en matières minérales obtenues dans cette étude sont supérieures à celles trouvées par Göhl (1975) et par la FAO (2000) sur des feuilles fraîches de bananiers. En effet, ils ont indiqué que la teneur en cendres des feuilles de bananier est comprise entre 8,8 et 9% de matière sèche. La teneur en cellulose brute des feuilles fraîches de bananier traitées à 10% de mélasse (21,29%MS) a été inférieure aux 24%MS observées par Göhl en 1975 et par la FAO en 2000. Cependant, cette valeur est inférieure à la teneur obtenue dans les feuilles fraîches de bananier non traitées (27,03%MS).
Le teneur en protéines brutes des deux niveaux de traitement a peu varié. La valeur la plus élevée a été obtenue dans les feuilles fraîches de bananier non traitées (19,57%MS), tandis que celle des feuilles fraîches traitées à 10% de mélasse a été de 19,02%MS. Ces valeurs sont largement supérieures à celles obtenues par Göhl (1975) et par Belewe et Belewe (1999) qui ont respectivement trouvé ¬6,81 et 9,9%MS. Mais elles sont assez proches de celles obtenues par Ffoulkes et Preston (1977) et par la FAO (2000) qui ont indiqué que les feuilles de bananier contiennent 15%MS de protéines brutes. Ces taux élevés de PB pourraient s'expliquer par un facteur tel que la saison, le sol et ou le stade végétatif du bananier au moment de la récolte des feuilles.

L'ingestion de la MS a été significativement plus élevée chez les brebis ayant consommé les feuilles fraîches de bananier traitées à 10% de mélasse (278,21g/j/kg0,75) par rapport aux brebis nourries aux feuilles fraîches de bananier non traitées à la mélasse (260,46g/j/kg0,75). Ce résultat qui montre que l'ingestion d'un aliment dont les teneurs en azote et fibres sont élevées est stimulée par l'apport de mélasse est conforme aux résultats obtenus par Ffoulkes et Preston (1977), Chesworth (1996) et Boukila et al. (2009). Ces auteurs ont montré que l'apport d'un supplément énergétique fait que les microorganismes du réseau rumen l'utilisent pour dégrader les aliments et donc accélérer le transit intestinal. Ce qui améliore considérablement l'ingestion du fourrage. De plus, la mélasse fournit la matière première (chaîne carbonée) nécessaire à la synthèse des protéines microbiennes, la prolifération de ces microorganismes aurait permit de mieux dégrader les feuilles fraîches de bananier (Gadoud *et al.,* 1992 ; Chesworth, 1996).

L'ingestion de la CB a été significativement plus élevé chez les brebis nourries aux feuilles fraîches de bananier non traitées (97,64g/j/kg0,75) que chez les brebis nourries aux feuilles fraîches de bananier traitées à 10% de mélasse (89,98g/j/kg0,75). Ce résultat pourrait être directement lié à la teneur en CB des feuilles fraîches de bananier non traitées. En effet, on retrouve plus de CB dans les feuilles fraîches de bananier non traitées, les brebis qui les ont consommé en ont donc ingéré plus que celles qui ont été nourries aux feuilles fraîches de bananier traitées à 10% de mélasse.

L'ingestion de l'azote est significativement plus élevée ($p<0,05$) chez les brebis nourries aux feuilles fraîches de bananier traitées à 10% de mélasse (94,55g/j/kg0,75) que chez celles nourries aux feuilles fraîches de bananier non traitées (87,20g/j/kg0,75). Ces résultats sont conformes avec ceux de Merkel et al. (1999) et Boukila et al. (2009) qui ont montré que l'apport d'énergie facilement assimilable améliore l'ingestion de l'azote.

Par ailleurs, le traitement à 10% de mélasse a amélioré significativement ($p<0,05$) la digestibilité de l'azote des feuilles fraîches de bananier. Ce résultat est conforme à ceux obtenus par Demarquily et al. (1998) et Chenost et Kayouli (1998) qui ont montré que l'apport de mélasse accroît significativement la digestibilité apparente de l'azote

Conclusion

De l'étude sur l'ingestion et la digestibilité in vivo des feuilles fraîches de bananier non traitées et traitées 10% de mélasse, il ressort que :

Les teneurs en MS, cendres, PB et lipides n'ont pas été différents entre les deux niveaux de traitement des feuilles fraîches de bananier. Par contre, la teneur en CB a diminué avec le traitement à la mélasse.

Le traitement à la mélasse a amélioré l'ingestion de l'azote des feuilles fraîches de bananier chez la brebis Djallonké, induisant ainsi une meilleure digestibilité apparente de l'azote. Cependant, l'ajout de mélasse n'a pas influencé de manière significative, les digestibilités apparentes de MS et de CB.

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FACTORS AFFECTING VIRULENCE OF E. COLI ISOLATES FROM DIARRHEIC SHEEP AND GOATS.

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Abstract

This study was carried out to investigate factors affecting virulence of E. coli in sheep and goats; parameters mesearued were, incidence of E. coli, effect of age, haemolytic activity serotyping of the pathogenic E. coli, detection of enterotoxin, astA and verotoxin genes of E. coli isolates using Polymerase chain reaction And antibiogram to the E. coli isolates.

E. coli was isolated at highest percentage from diarrheic sheep at age ranged from 1 to 6 month, highest percentage from diarrheic goats aged from 7 to 12 month.

E. coli isolated from diarrheic sheep gave alpha, beta and gamma hemolysis at percentage of 66.7 %, 18.2 % and 15.1%, respectively. Moreover, E. coli isolated from diarrheic goats gave alpha, beta and gamma hemolysis at percentage of 55%, 30% and 15 %, respectively.

The results revealed that serotype O26 was the most E. coli serotype (31.4%) that gave a positive Congo red followed by E. coli serotype O78 (22.9%) and E. coli serotype O86 (17.1%). Results of detection of verotoxin 2 gene of E. coli isolated from diarrheic sheep and goats revealed that the gene could not be detected in all examined E. coli isolates (7 isolates). On the other hand, results of detection STa gene in E. coli isolated from diarrheic sheep and goats revealed that 5 out of 7 E. coli isolates (71.4 %) were detected. astA gene of E. coli isolated from diarrhoeic sheep and goats was detected in all the examined isolates (7 isolates). It could be concluded that E. coli isolated from diarrhoeic sheep and goats was pathogenic and producer for enterotoxin and astA. Moreover, PCR is a simple and rapid method for detection of STa , astA and VT2 genes of E. coli

FACTEURS AFFECTANT LA VIRULENCE DES ISOLATS E. COLI CHEZ DES MOUTONS ET CHÈVRES DIARRHÉIQUES

Resume

La présente étude a été réalisée pour étudier les facteurs qui affectent la virulence de la bactérie E. coli chez les moutons et les chèvres. Les paramètres mesurés étaient l'incidence de E. coli, l'effet de l'âge, le sérotypage de l'activité hémolytique de l'E. coli pathogène, la détection de l'entérotoxine, les gènes astA et vérotoxine des isolats de l'E.coli en utilisant la réaction en chaîne à la polymérase (PCR) et un antibiogramme des isolats de l'E. coli.

La bactérie E. coli a été isolée au plus haut pourcentage chez les moutons diarrhéiques âgés de 1 à 6 mois et au plus haut pourcentage chez les chèvres diarrhéiques âgés de 7 à 12 mois.

La bactérie E. coli isolée chez les moutons diarrhéiques a donné une hémolyse alpha, bêta et gamma respectivement au pourcentage de 66,7%, 18,2% et 15,1%. De plus, la bactérie E. coli isolée chez les chèvres diarrhéiques a donné une hémolyse alpha, bêta et gamma respectivement au pourcentage de 55%, 30% et 15%.

Les résultats ont révélé que le sérotype O26 était le sérotype le plus important de l'E. coli (31,4%) qui a donné un rouge Congo positif, suivi du sérotype O78 (22,9%) et du sérotype O86 (17,1%). Les résultats de la détection du gène vérotoxine 2 de l'E. coli isolée chez les moutons et chèvres diarrhéiques ont révélé que le gène n'a pas pu être détecté dans tous les isolats d'E. coli examinés (7 isolats). D'autre part, les résultats de la détection du gène STa chez les bactéries E. coli isolées chez les moutons et chèvres diarrhéiques a révélé que 5 des 7 isolats d'E. coli (71,4%) ont été détectés. Le gène astA de la bactérie E. coli isolée chez les moutons et chèvres diarrhéiques a été détecté dans tous les isolats examinés (7 isolats). On

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pourrait conclure que la bactérie E. coli isolée chez les moutons et chèvres diarrhéiques est pathogène et producteur d'entérotoxine et d'astA. En outre, la PCR est une méthode simple et rapide de détection des gènes STa, astA et VT2 des bactéries E. coli.

Mots-clés : isolats d'E coli, virulence, moutons, chèvres, PCR

Introduction

Verotoxin-producing Escherichia coli (VTEC) produce two families of verotoxins,VT1 and VT2 (Mainil, 1999). VTEC can cause severe diseases in humans such as haemorrhagic colitis (HC) and haemolytic uraemic syndrome (HUS). Those VTEC strains that are able to induce HC and HUS are called enterohaemorrhagic E. coli (EHEC) (Mainil, 1999). VTEC and EPEC have been associated with diarrhoea in goat kids (Orden *et al.,* 2003).

Strains of E. coli produce protein toxins, many of which appear to play a role in disease process. These include alpha haemolysin, enterohaemolysin, verocytotoxins (Shiga-like toxins), cytotoxic necrotizing factor (CNF) and enterotoxins (Gyles ,1992).

EnteroAggregative heat-StableToxin1 (EAST1) is encoded by the astA gene, a 117 bp long DNA sequence (Savarino et al. 1991). EAST1 is thought to play a role in EAEC pathogenicity even if not all EAEC strains harbour the astA gene (Huang et al. 1994). Epidemiological studies have associated this gene with E. coli pathotypes other than EAEC like ETEC and EHEC and other bacteria genus including Salmonella (Savarino et al. 1996 and PaivadeSousa et al. 2001). The astA gene has also been detected in E. coli strains isolated from animal hosts including pigs, cattle, and sheep (Veilleux et al. 2006).

Quinn et al. (2002) revealed that two types of enterotoxins, heat labile (LT) and heat stable (ST) have been identified each type of enterotoxin has two subgroups. Many strains of ETEC from pigs produce LT1. A second heat-labile toxin, LT2, has been demonstrated in some ETEC strains isolated from cattle. One of the heat-stable enterotoxin subgroups, Sta, has been identified in strains of ETEC isolated from porcine, bovine, ovine and human specimens.

PCR was a valuable and sensitive method for determining the virulence factors of E. coli strains and seemed to give good results in epidemiological investigation of diarrheogenic E. coli (Osek *et al.,* 1999).

Material and Methods

Material

A total of 163 rectal swabs were collected from sheep and goats. 112 rectal swabs from diarrheic sheep and 51 rectal swabs from diarrhetic goats. The samples were collected, labeled and transported in ice box to the laboratory. Media used for primary isolation and identification of E.coli were prepared according to Cruickshank *et al.,* 1975):

Antisera intended for the serological identification of E. coli were used.

The primers were selected to amplify E. coli were:

1. Stable toxin STa gene (Ojeniyi et al., 1994). downstream primer (STa2) with a sequence of 5' ATA ACA TCC AGC ACA GGC AG 3' and 5' TCC GTG AAA CAA CAT GAC GG 3'.
2. EAST-1 downstream primer with a sequence of 5' TAG GAT CCT CAG GTC GCG AGT GAC GGC 3' and 5' TGC CAT CAA CAC AGT ATA TCC 3' .
3. STX2 (VT2) downstream primer with a sequence of 5' GGA TGC ATC TCT GGT CAT TG 3' and 5' CTT CGG XXX CCT ATT CCG GC3. These primers were synthesized and supplied by Gibco BRL life Technologies Inc.

Ready –To-GOTM PCR Beads (Amersham pharmacia Biotech. Inc.):

These beads contain the following ingredients:Taq DNA polymerase, 10 x reaction Buffer (Kc 1500 mM and Tris-HCl 100 mM pH 9.0), 25 mM MgCl2 and dNTP mix (10 mM of each dNTP).

Antibiotic discs:

The antibiotic discs and their

concentration were presented in the following Table (1).

Methods:

Collection of samples:

Rectal swabs were taken from diarrhoeic sheep and goats by means of sterile cotton swabs (Boyd *et al.,* 1974). The collected samples were transferred in ice bags to the laboratory where the samples were subjected to bacteriological examination as soon as possible.

Bacteriological examination:

Bacteria was identified according to Edwards and Ewing (1972); Finegold and Martin (1982); Koneman et al. (1993) and Quinn et al. (2002

Serotyping of E. coli:

Slide agglutination test was carried-out for serotyping of E. coli according to Ewing (1986). Antimicrobial sensitivity test: Antimicrobial resistance testing was performed by the disk diffusion method according to guidelines established by the National Committee for Clinical Laboratory Standards (Nccls, 2006).

Detection the gene coding enterotoxin and verotoxin of E.coli isolates using polymerase chain reaction

Bacterial template DNA: are you referring to the extraction?

DNA template was prepared from E. coli cells according to Nishikawa et al. (1988).

Oligonucleotide primers set STa:

Primers were dissolved in nuclease-free water to obtain 50 – 100 p mol concentration (Sambrook *et al.,* 1989 and Baumforth *et al.,* 1999)

Oligonucleitide primers set SLT-II:

Primers were dissolved in nuclease-free water to obtain 50 – 100 p mole concentration. 5 µl of two primers were used in PCR mixture (Fratemico *et al.,* 2000).

Programming the thermal cycler:

1. Programming the thermal cycler for detection pf STa gene: (Sambrook *et al.,* 1989; Baumforth *et al.,* 1999 and samer, 2001):

The thermal cycler (Biometra, personal cycler) was programmed and presened in Table (2).

2. Programming the thermal cycler for detection of SLT-II gene: (Pina *et al.,* 2001):

The thermal cycler was programmed as presented in table (3).

Results

Incidence of E. coli isolated from diarrheic sheep and goats at different ages

Results presented in table (4) revealed the incidence of E. coli isolated from diarrheic

Table 1: The antibiotic discs and their concentration

Antibiotic	Concentration
Colistin (CT)	10 ug
Neomycin (N)	30 ug
Marbofloxacin (MAR)	30 ug
Tulathromycin (TUL)	30 ug
Levofloxacin (LEV)	5 IU
Norfloxacin (NOR)	10 IU
Ofloxacin (OFX)	5 IU
Trimethoprim/Sulfamethoxazole (SXT)	25 ug
Gentamicin (CN)	10 ug
Tobramycin (TOB)	10 IU

Table 2: Setting up the thermal cycler for STa gene:

No. of cycles	°C	Time / min	Target
1 cycle	94	5	DNA template denaturation
30 cycles of	94	1	Denaturation
			Primer annealing
	57	1	Extension
1 cycle	72	2	Final extension
1 cycle	72	10	preservation
	4	∞	

Table 3: Setting up the thermal cycler for SLT-II gene:

No. of cycles	°C	Time	Target
1 cycle	94	5min	DNA template denaturation
35 cycles of	94	20sec	Denaturation
			Primer annealing
	57	1min	Extension
1 cycle	72	1min	Final extension
1 cycle	72	10min	preservation
	4	∞	

Table 4: Results of E. coli isolated from diarrheic sheep at different ages.

Age of sheep	No. of examined samples	No. of E. coli isolates	%
From (1) to (6) month	72	23	31.94
From (7) to (12) month	29	7	24.14
Over one year	11	3	27.27
Total	**112**	**33**	**29.5**

sheep. E.coli was isolated at highest percentage (31.94%) from diarrheic sheep at age ranged from 1 to 6 month.

Results presented in table (5) revealed the incidence of E. coli isolated from diarrheic goats. E. coli was isolated at highest percentage (78.6%) from diarrheic goats at age ranged from 7 to 12 month.

Results of E. coli isolated from sheep and goats were presented in table (6). E. coli was isolated at the highest percentage (41.9) from sheep and goats at 7-12 month old.

Results of haemolysis produced by E. coli isolated from Diarrheic sheep and goats

Results presented in table (7) show that E. coli isolated from diarrheic sheep gave alpha, beta and gamma hemolysis at percentage of 66.7 %, 18.2 % and 15.1%, respectively. More over, E. coli isolated from diarrheic goats gave alpha, beta and gamma hemolysis at percentage of 55%, 30% and 15 %, respectively.

Results of Congo red test of E. coli isolated from Diarrheic sheep and goats.

Results of Congo red test revealed that 90.9% of E .coli isolated from diarrheic sheep and 90% E. coli isolated from goats gave positive results (Tabe, 8).

Relationship between E. coli serotypes and its Congo red test.

The results presented in table (10) revealed that E. coli serotype O26 was the most E. coli serotype (31.4%) that gave a positive Congo red followed by E. coli serotype O78 (22.9%) and E. coli serotype O86 (17.1%).

The percentage was calculated according to total No. of serotyped E. coli (35 isolates).

Results antibiotic sensitivity test of E.

Table 5: Results of E. coli isolated from diarrheic goats at different ages.

Age of sheep	No. of examined samples	No. of E. coli isolates	%
From (1) to (6) month	72	23	31.94
From (7) to (12) month	29	7	24.14
Over one year	11	3	27.27
Total	**112**	**33**	**29.5**

Table 6: Results of E. coli isolated from diarrheic sheep and goats at different ages.

Age of sheep	No. of examined samples	No. of E. coli isolates	%
From (1) to (6) month	95	28	29.5
From (7) to (12) month	43	18	41.9
Over one year	25	7	28
Total	**163**	**53**	**32.5**

coli isolated from diarrheic sheep and goats.

Results of antibiotic sensitivity test of E.coli isolated from diarrheic sheep and goats indicated that levofloxacin, norfloxacin and ofloxacin were the most effective antibiotics (table, 1)

Results of detection of verotoxin 2 gene of E. coli isolated from diarrheic sheep and goats.

Results of detection of verotoxin 2 gene of E. coli isolated from diarrheic sheep and goats revealed that the gene could not be detected in all examined E. coli isolates (7isolates) (Fig.1).

Results of detection of STa gene of E. coli isolated from diarrheic sheep and goats.

Results of detection STa gene of E. coli isolated from diarrheic sheep and goats revealed that 5 out of 7 (71.4%) were positive (Fig.2).

Results of detection of EAST1 gene of E. coli isolated from diarrheic sheep and goats.

Results of detection EAST-1 gene of E. coli isolated from diarrheic sheep and goats revealed that all the examined isolates (7 isolates) were positive (Fig. 3).

Discussion

Results presented in table (4, 5 and 6) revealed the incidence of E. coli isolated from diarrheic sheep and goat. E.coli was isolated at highest percentage (31.94%, 21.7 % and 29.5%) from diarrheic sheep at age ranged from 1 to 6 month. The age factor played a role in the incidance of E. coli where the recovery among cattle calves and buffalo calves was relatively lower to 11.9% and 21.4% respectively at age of 1 – 3 months, 8.8% and 15.7% respectively at age of 3 – 6 months. Similar observation was recorded by (Asma *et al.,* 1996 and Abd El Kader *et al.,* 2001).

Results presented in table (7) show that E. coli isolated from diarrheic sheep gave alpha, beta and gamma hemolysis at percentage of 66.7 %, 18.2 % and 15.1%, respectively. Moreover, E. coli isolated from diarrheic goats gave alpha, beta and gamma hemolysis at percentage of 55%, 30% and 15 %, respectively. These results are coincident with those described by Samer (2001) reported that only 4.91% (9/140) of the E. coli isolates from diarrhoeic animals were α-Hly producing E. coli. and he added that, there is no association between α-Hly and VT production. The haemolytic E. coli isolates used as a phenotypic marker or virulence factor of E. coli isolates associated with diarrhoea. The 2 E. coli O157 isolates and the 6 haemolytic E. coli isolates (one of 7 haemolytic E. coli isolates was E. coli O157) and randomly 6 non-haemolytic E. coli isolates considered three groups (Gaber, 2002).

Results of Congo red test revealed that 90.9% of E .coli isolated from diarrheic sheep and 90% E. coli isolated from goats gave positive results (Table, 8). Alskan (1995) observed that Congo red positive E. coli cultures bind enough Congo red dye to obtain a red colony after 24 hours of incubation and that Congo red test may be used as a screening test for detection

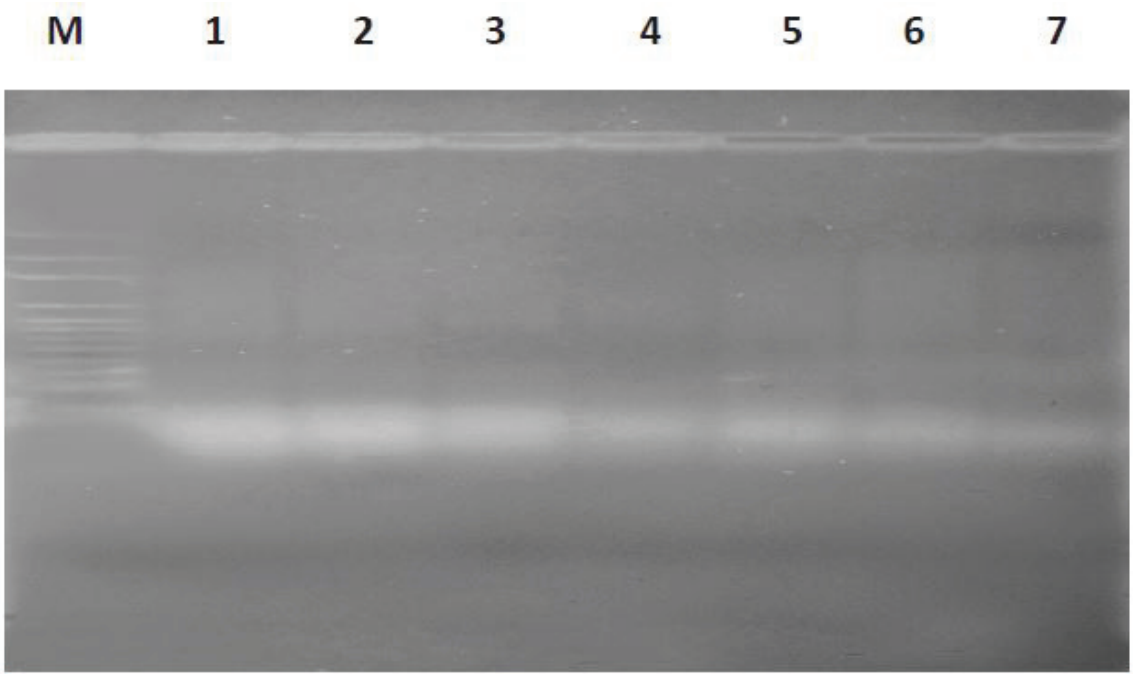


Figure 1: Results of amplification verotoxin 2 gene by PCR. Lane M: DNA molecular weight marker. Lanes 1, 2, 3, 4, 5, 6 and 7 were negative E. coli isolates for VT2 gene.

Factors Affecting Virulence Of E. Coli Isolates From Diarrheic Sheep And Goats

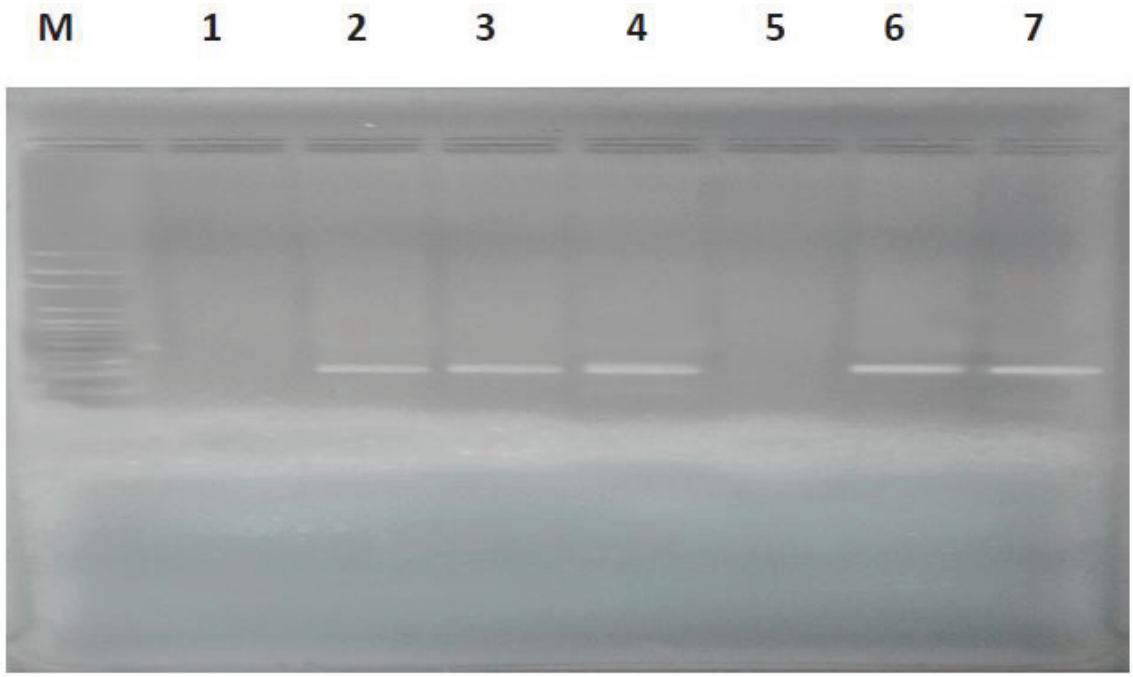


Figure 2: Results of amplification STa gene by PCR. Lane M: DNA molecular weight marker. Lanes 2, 3, 4, 6 and 7 were positive E. coli isolates for STa gene while lanes 2, 1 and 5 were negative.

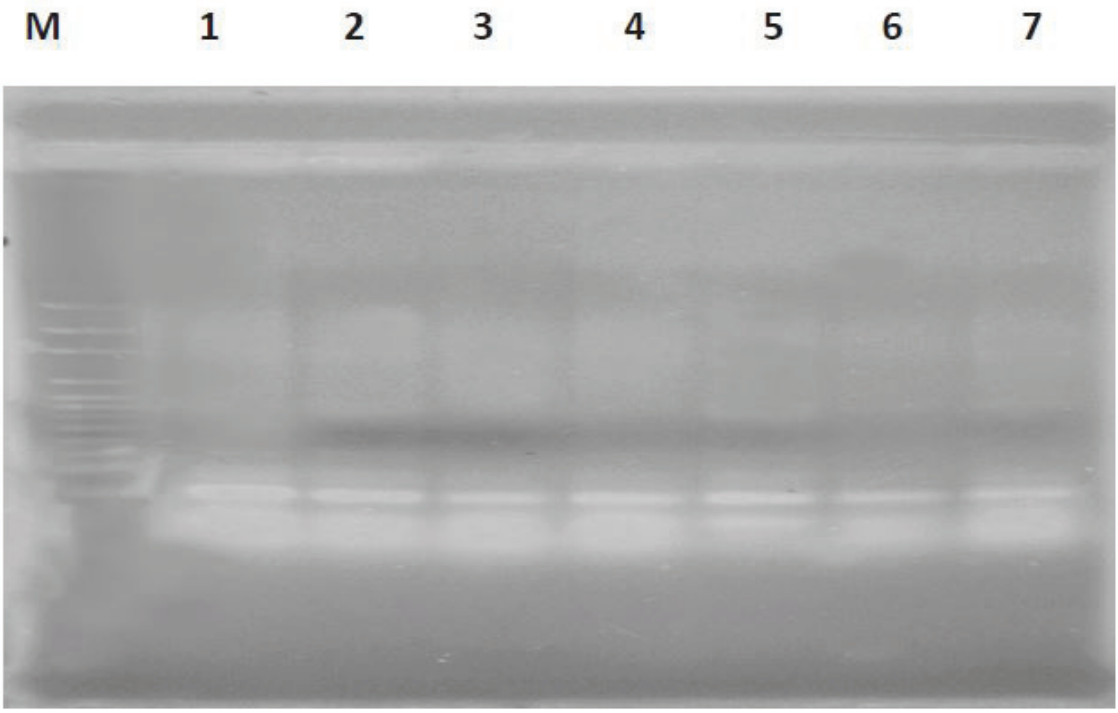


Figure 3: Results of amplification astA gene by PCR. Lane M: DNA molecular weight marker. Lanes 1, 2, 3, 4, 5, 6 and 7 were positive E. coli isolates for astA.

Table 7: Results of haemolysis produced by E. coli isolated from Diarrheic sheep and goats.

Animal species	No. of *E. coli* isolates	*Alpha hemolysis*		*Beta hemolysis*		*Gamma hemolysis*	
		No.	%	No.	%	No.	%
Sheep	33	22	66.7	6	18.2	5	15.1
Goats	20	11	55	6	30	3	15
Total	**53**	**33**	**62.3**	**12**	**22.6**	**8**	**15**

Table 7: Results of haemolysis produced by E. coli isolated from Diarrheic sheep and goats.

Animal species	No. of E. coli isolates	Positive Congo red	
		No. of isolates	%
Sheep	33	30	90.9
Goats	20	18	90
Total	**53**	**48**	**90.6**

of virulent E. coli. Congo red dye agar test can be used for primary screening of non invasive E. coli from potentially invasive E. coli (Moussa et al. 2006 and Sharma et al. 2006).

The results presented in table (10) revealed that E. coli serotype O26 was the most E. coli serotype (31.4%) that gave a positive Congo red followed by E. coli serotype O78 (22.9%) and E. coli serotype O86 (17.1%).

The results of antibiotic sensitivity of E.coli to chemotherapy revealed that E.coli was sensitive to levofloxacin, norfloxacin, ofloxacin and trimethoprim/sulfamethoxazole. On the other hand E.coli was resistant to colistin and neomycin (Table, 11).Our results agree with those obtained by Aisha and Youseif (1999) who found that all E. coli isolates were sensitive to gentamycin, enrofloxacin and in sulphate. Glisson *et al.,* (2004) recorded that chickens that received enrofloxacin had significantly less mortality, lower average gross pathology scores, and better feed conversion ratios than did

Table 9: Relationship between E. coli serotypes and its hemolytic activities.

Serotypes of E. coli	Alpha hemolysis E. coli		Betahemolysis E. coli		Gamma hemolysis E. coli	
	No.	%	No.	%	No.	%
O 114	1	2.9	0.0	0.0	0.0	0.0
O 55	2	5.7	0.0	0.0	0.0	0.0
O 78	4	11.4	2	5.7	3	8.6
O 25	0.0	0.0	1	2.9	0.0	0.0
O 158	1	2.9	0.0	0.0	0.0	0.0
O 86	3	8.6	3	8.6	0.0	0.0
O 26	8	22.6	1	2.9	2	5.7
O 128	4	11.4	0.0	0.0	0.0	0.0
Total	**23**	**65.7**	**7**	**20**	**5**	**14.3**

Table 10: Relationship between E. coli serotypes and Congo red.

Serotypes of E. coli	Results of Congo red test			
	Positive		negative	
	No	%	No	%
O114	1	2.9	0.0	0.0
O55	2	5.7	0.0	0.0
O78	8	22.9	1	2.9
O25	1	2.9	0.0	0.0
O158	1	2.9	0.0	0.0
O86	6	17.1	0.0	0.0
O26	11	31.4	0.0	0.0
O128	4	11.4	0.0	0.0
Total	**34**	**97.1**	**1**	**2.9**

The percentage was calculated according to total No. of serotyped E. coli (35 isolates).

chickens that received either oxytetracycline or no medication. Also they found that chickens which received enrofloxacin had significantly less mortality, lower pathology scores than those that received sulfadimethoxine and numerically feed conversion than the sulfadimethoxine group. Moreover, E. coli isolates from diseased chickens diagnosed with colibacillosis in Henan province, China were resistance to trimethoprim-sulfamethoxazole (100%), oxytetracycline (100%), ampecillin (83%), enrofloxacin (83%) and ciprofloxacin (81%).

Results of detection of verotoxin 2 gene of E. coli isolated from diarrheic sheep and goats revealed that the gene could not be detected in 7 examined E. coli isolates. These results match with those descried by Heuvelink et al. (1998) who found that verocytotoxin producing E. coli (VTEC) produces either or both of two phage-encoded toxins VT1 and VT2. Verotoxins are though to cause vascular endothelial damage which observed by haemorrhagic colitis and haemolytic uraemic syndrome. An additional virulence factor contributing to the pathogenicity of VTEC is the formation of attaching and effacing lesions in the intestine of the host. Verocytotoxin (VT)-producing E. coli (VTEC) of different serotypes have become a major concern in human and animals diseases in different countries in the few years (Mohamed *et al.,* 1985; Ryan *et al.,* 1986; Karamali, 1989; Kleanthous *et al.,* 1990; Gyles, 1992; Wieler *et al.,* 1992). (Begum *et al.,* 1993) assessed PCR technique for the identification of Shiga-like toxin (SLT) producing E. coli.

The role of enterohaemolysin in the pathogenesis of VTEC infection is not known

(Chart *et al.,* 1998). There is no conversing data for a role in human infections and the toxin may be more important in assisting infection of animals (Law, 2000).

Results of detection STa gene of E. coli isolated from diarrheic sheep and goats revealed that 5 out of 7 (71.4 %) were positive. Pathogenic E. coli strains may produce a variety of toxins with different activities: heat-labile (LT) and heat-stable (ST) enterotoxins, shiga toxins and cytotoxic necrotizing factors 1 and 2 (CNF1 and CNF2). Shiga toxins are produced by EHEC strains (Riley *et al.,* 1983 and Quinn et al. 2002). Enterotoxigenic strains usually produce two enterotoxins, LT and ST, which have distinct roles in the pathogenesis. E. coli isolates producing CNF2 are common in cattle (Pohl *et al.,* 1993). Many of these strains also produce F17b fimbriae and aerobactin and are resistant to the killing action of serum.

Results of detection EAST-1 gene of E. coli isolated from diarrheic sheep and goats revealed that all the examined isolates 8 were positive. Our result agree with those mentioned by Law (2000) who cited that the high prevalence of the astA genotype in O157 isolates is striking, indeed the incidence is considerably higher than that found in EAggEC. He added that in E. coli O157 infection, it is possible that EASTI contributes to the initial phase of watery diarrhoea seen in patients with HC.

The virulence of E. coli is multifactorial and contain properities are associated primarily with virulent strains. One of these is the ability to produce a haemolysin (Cavalieri *et al.,* 1984). The α-haemolysin is belived to be a virulence factor (Bohach and Snyder, 1985). The production of enterohaemolysin by some serogroups of E. coli was closely associated with (VT) production (Beutin and Montengro, 1990).

The study aimed to detect the gene coding for the production of heat stable enterotoxin (STa) and Shiga-like toxin type 2 (SLT-II) by three groups of E. coli isolates using PCR. Also, to detect the gene expression by infant mouse assay and vero cell assay.

SLT production by non-O157 SLTEC is similar to that for serotype O157, namely that the SLTS are phage – encoded and strains may produce either one or both SLT. I and SLT. II (Marguerite, 1996).

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AFRICAN UNION - INTERAFRICAN BUREAU FOR ANIMAL RESOURCES (AU-IBAR)

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

The Editor in Chief
January 2013

Aims and scope

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

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

The BAHPA encourages submission of papers on all major themes of animal health and production, wildlife management and conservation, including:

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- Marketing, economics
- Infectious and non infectious disease
- Parasitology
- Genetic improvement and biotechnology
- Animal production, nutrition and welfare
- Science and policy in animal health and production
- Beekeeping and honey bees
- Ecology and climate change impacts on animal resources in Africa
- wildlife management
- Fisheries and aquaculture development
- Food safety and food hygiene
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- Biosecurity
- Animal resources trade and value chain
- Socio economics and economics of animal resources development

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The language of submission should be either in U.K. English or Standard French. The abstract is translated to the other three languages of the African Union (Arabic, English, French and Portuguese), by the editors, after acceptance. Full articles submitted in French will also be published in English.

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Full papers of original research

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9. Acknowledgements. Where necessary acknowledgements of grants and technical assistance should be included under this heading. . Please also include any potential conflict of interests if appropriate. Suppliers of materials should be named and their location (town, state/county, country) included.
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- *Conference Proceedings:* Stock A, 2004. Signal Transduction in Bacteria. In the Proceedings of the 2004 Markey Scholars Conference, pp: 80-89.
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