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Abstract not exceeding 200 words giving a synopsis of the findings presented and the conclusion(s) reached.

Introduction stating the purpose of the work.

Materials and Methods used.

Results presented concisely

Discussion of significance.

Acknowledgements.

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BULLETIN

March 1996 VOL 44 No. 1

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MICROBIAL ISOLATES FROM MILK SAMPLES OF ANIMALS WITH CLINICAL MASTITIS IN A LARGE DAIRY FARM IN TANZANIA

D.M. KAMBARAGE, M.M.A. MTAMBO, S.I. KIMERA AND A.P. MUHAIRWA
Department of Veterinary Medicine and Public Health, Faculty of Veterinary Medicine, Sokoine University of Agriculture, Box 3021, Morogoro, Tanzania.

SOUCHES MICROBIENNES ISOLEES DES ECHANTILLONS DE LAIT D’ANIMAUX SOUFFRANT DE MAMMITE CLINIQUE DANS UNE GRANDE FERME LAITIERE EN TANZANIE

Résumé
L’examen clinique visant à détecter la mammitre a montré que sur 800 vaches teintes et en lactation, 6% avaient moins de 4 quartiers de la mamelle qui fonctionnaient. 78% des vaches ayant moins de 4 quartiers qui fonctionnaient en avaient 3 en bon état. 17% en avaient 2 et un faible pourcentage n'avait qu'une têtine qui soit intacte. Les souches bactériennes les plus répandues sur les quartiers atteints de mammitre étaient les coliformes (76%). On a recueilli à partir des échantillons de lait, l'espèce Streptococcus hémolytique α (54%), les bacilles à gram négatif (49%), l'espèce Corynebacterium (13%) et d'autres microbes (non-classifiés) 30%.

Abstract
Clinical examination for mastitis showed that out of 800 dry and milking cows, 6% had less than four functional quarters. Of the cows with defective quarters, 78% had three functional ones, whereas 17% had two and only a small percentage had one functional teat. Common Bacterial isolates from mastitic quarters were coliforms (76%), a haemolytic Streptococcus species α (54%), gram negative bacilli (49%). Corynebacterium species (13%) and other (unclassified) microbes (30%) were recovered from milk samples.

Introduction
The dairy industry, still in infancy in Tanzania, comprises large and small-scale holder units. The backyard small-scale dairy farming is sprouting in many urban and periurban areas in the country. Animals vary from pure breeds (Friesian, Jersey, Aryshire and Mwapwa breeds) and their crosses with indigenous Zebu cattle (Bos indicus). The dairy animals form only 2% of the total population of 12.8 million cattle in Tanzania.

Feeds and diseases are the major constraints of the dairy industry. Although a variety of diseases are likely to affect dairy animals, mastitis appears to be one of the most devastating cause of considerable loss of milk production and income to the dairy industry1,2,3,4. The loss in milk production is caused by both clinical and subclinical mastitis. Subclinical mastitis has been shown to account for 70% of the reduced milk production caused by mastitis1,5,5.

Infected quarters produce 30% less milk than non-infected ones throughout the lactation period and this has been attributed to lactogenic tissue damage7.

A number of causative agents and predisposing factors have been implicated in causing mastitis. These vary according to the type of animals, management, feeding and geographical location8. Studies conducted in some large dairy farms and small-scale holder units in Tanzania showed the average annual incidences of clinical and sub-clinical mastitis to range between 2.2–2.8% and 40–71.6% respectively9,10. In Kenya, 2.3–3% and 48% of the animals had clinical and subclinical mastitis respectively10. The level of subclinical mastitis in Norway was found to be 31%11.

The largest dairy farm in Tanzania is located at Kitulo plateau in the southern highlands, at an altitude of 300 m above sea level and 60 km from the town of Mbeya. Milk is transported
daily to the Mbeya processing plant in lorries with no cooling facilities. At the plant, the milk is tested for quality (based on bacterial count) and prices vary according to the grades. Thus, the maximisation of profits of this dairy farm depends on the level of milk production and the bacteriological quality of the milk delivered to the processing plant. The bacteriological quality of milk depends on the mastitis status in the herd, hygiene during milking and hygienic handling of milk and storage facilities. Although studies have been carried out in small holder units and some large dairy farms in the coastal zone and elsewhere in Tanzania\(^4\), no similar studies have been conducted in this large and potentially productive farm. Therefore, the aim of this study was to carry out preliminary studies of mastitis in this herd with emphasis on the number of functional quarters per cow and the determination of etiological agents of clinical mastitis.

Materials and Methods

All cows (dry and milking animals) in the five operational units were thoroughly examined for the number of functional quarters and for clinical evidence of mastitis. Cardinal signs of mastitis (i.e. evidence of elevated temperature, pain, swelling, symmetry, consistency and gross changes of the milk) were used as criteria for diagnosis of the disease. Milk swabs (in Stuart’s transport medium, Oxoid, UK) were aseptically taken from quarters with clinical evidence of the disease and immediately transported to the laboratory for culture. Samples for bacteriology were also taken from 12 randomly selected cows with no evidence of clinical mastitis.

Milk samples were streaked on blood or MacConkey agar media and incubated at \(37^\circ\)C for 24–48 hours. At 24 hours all plates were examined for growth and colony morphology, haemolysis, pigmentation, lactose fermentation and inhibition of growth. Those without significant growth were incubated for another 24 hours. Significant bacterial colonies at either 24 or 48 hours were selected, subcultured for 24 hours, gram-stained and evaluated further on the basis of fermentation of a variety of sugars.

Results

The results show that out of 800 adult cows examined (dry and milking animals), 46 animals had less than four functional quarters (Table 1) thus, representing 5.8% of the animals. Out of these 46 cows, 36 (78.3%) had three functional quarters whereas, eight (17.4%) and two animals (4.3%) had two and one functional quarters respectively. The cause of this reduced number of functional quarters was likely to be previous attacks of mastitis as revealed by the records of some of the animals. Records of some animals showed that they suffered the disease 2–4 years back suggesting that mastitis may have been a problem in this farm for quite some time. Although some animals did not have records of dates on which they had mastitis, the implication of this is that there has been a great loss of milk production over the years. The loss of a total of 56 quarters of the 46 animals may result in a loss of approximately 31.5% of the total milk production of the affected animals, resulting in an immense loss of revenue to the farm. The loss of quarters is also likely to be a cause of early culling of low milk-producing animals.

<table>
<thead>
<tr>
<th>Number of functional quarters</th>
<th>Number of animals</th>
<th>Percent of animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>36</td>
<td>78.3</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>17.4</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>4.3</td>
</tr>
<tr>
<td>0</td>
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</tbody>
</table>

Table 1: Cows with less than four functional quarters

Forty-six cows out of 800 animals which were found to have less than four functional quarters as detected by thorough clinical examination of the udders.

Coliforms were the most common bacterial isolates from samples taken from quarters with clinical evidence of mastitis (Table 2). Seventy-six percent of the animals with clinical mastitis were infected with coliforms. \(\alpha\) haemolytic *Streptococcus* species (other than *Streptococcus agalactiae*) were isolated from 54% of the clini-
Table 2: Bacterial isolates from animals with clinical evidence of mastitis and those with no disease

<table>
<thead>
<tr>
<th>Bacterial isolates cows)</th>
<th>Animals with clinical mastitis (37 cows)</th>
<th>Animals with no clinical disease (12)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No of positive animals</td>
<td>Percent of positive animals</td>
</tr>
<tr>
<td>Coliforms</td>
<td>28</td>
<td>75.7</td>
</tr>
<tr>
<td>a haemolytic</td>
<td>20</td>
<td>54.1</td>
</tr>
<tr>
<td>Streptococcus species</td>
<td>18</td>
<td>48.6</td>
</tr>
<tr>
<td>Gram negative bacilli</td>
<td>5</td>
<td>13.3</td>
</tr>
<tr>
<td>Corynebacterium species</td>
<td>*11</td>
<td>*29.7</td>
</tr>
<tr>
<td>Other isolates</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Types of bacteria isolated from 37 animals with clinical evidence of mastitis and 12 cows which did not show any evidence of the clinical disease. Others with one asterisk include Proteus, Staphylococcus, Micrococcus and b haemolytic Streptococcus species as well as Listeria monocytophages and Pseudomonas euruginosa. Other isolates (**) in animals with no evidence of the disease were b haemolytic Streptococcus and Staphylococcus species.

cases of mastitis. This was followed by gram negative bacilli (45.9%); of which six were \[\beta\] haemolytic, whereas 12 were non-haemolytic. Corynebacterium species were demonstrated in 14% of the cases. Other bacterial isolates were Proteus, Staphylococcus, Micrococcus species; Listeria monocytophages, Pseudomonas euruginosa, \[\beta\] haemolytic Streptococcus species and small non-haemolytic gram positive bacilli. Coliforms, \[\alpha\] haemolytic Streptococcus species and gram negative bacilli were also the main bacterial isolates from cows with no evidence of clinical mastitis. Minor isolates from these animals with no evidence of the disease were \[\beta\] haemolytic Strepotococcus and Staphylococcus species.

Discussion

The finding that coliforms were the major bacterial isolates in this farm contradicts the observation made in small holder dairy units and in other large dairy farms in Tanzania. They found Staphylococcal mastitis to be the most common form. Mahlau and Hyera also reported that the common causes of mastitis were Staphylococcal and Streptococcus species. These species have also been observed to be the most common cause of the disease in other countries. However, the extent of Streptococcus species isolation in this herd was comparable with the results in other studies in Tanzania. It was further evident that \[\beta\] haemolytic Streptococcus species (other than Streptococcus agalactiae) were responsible for a high proportion of streptococcal isolates as also reported by Shekimweri. As in this study, Shekimweri also observed that haemolytic gram negative bacilli were responsible for a significant number of culture positive milk samples.

Although other tests such as California mastitis test and somatic cell counting were not carried out to establish the level of subclinical mastitis in the farm, the isolation of the same bacteria as isolated from clinically affected animals may suggest that these 12 cows had subclinical mastitis. The number of sampled animals with no clinical evidence of mastitis was small; nevertheless, the results suggest that subclinical mastitis may be a great problem in the farm. This is supported by the fact that down-grading and discarding of milk delivered to the milk processing plant is common and this causes considerable loss of income to the farm (personal observation). However the presence of microorganisms could be due to gross contamination during and after milking.

The level of clinical and subclinical mastitis is due to a number of factors which include the level of hygiene during and after milking, presence of mastitis control programs as well as
nutritional status of the animals. For instance, cows with low vitamin A have been reported to have increased mastitis\(^5\) and also that vitamin E supplementation is associated with a decrease in the incidence of mastitis\(^6\).

Thus, a more detailed study is required to ascertain the status of mastitis in the farm and evaluate the existing and possible mastitis control programmes for purposes of devising cost-effective methods of control of the disease.

**Acknowledgement**

The authors wish to thank the Project Manager of Kitulo Dairy Farm for allowing the study to be carried out on his farm. We are also indebted to all staff of the farm for their cooperation during the course of this study.

**References**


*Received for publication on 5th September, 1994*
SALMONELLA ISOLATION FROM ANIMALS IN THE REPUBLIC OF ZAMBIA

R.N. SHARMA¹, M.M. MUSONDA¹, H.M. MUNANG'ANDU², P. MUYOYETA² AND P.G. SINYANGWE³

¹School of Veterinary Medicine, University of Zambia, Lusaka. ²Central Veterinary Research Institute, Lusaka. ³Directorate of Animal Health and Production, Lusaka.

ISOLEMENT DE SALMONELLA DES ANIMAUX EN REPUBLIQUE DE ZAMBIE

Résumé


Summary

The occurrence of Salmonella in domestic and laboratory animals in the Republic of Zambia during 1976 – 94 is reported. Sixty-nine Salmonella cultures consisting of 13 serovars were isolated. Isolation of 7 Salmonella serovars for the first time in Zambia is being recorded. These are S. Amager, S. Bareilly, S. Bonn, S. Heidelberg, S. Konondomi, S. Kisarawe and S. Welterverden.

Introduction

Animal salmonellosis has been reported from all over the world including countries in Africa. In Zambia, the first record of animal salmonellosis is connected with an outbreak of disease in calves in 1927¹; organism of Gartner – paratyphoid group were isolated. Thereafter S. Enteritidis, S. Dublin, and S. Typhimurium were reported from time to time in almost all species of domestic animals¹. Recently in a survey, Gasper and Hrabeta² added four more serovars viz. S. Kiel, S. Bardo, S. Sundswall and S. Bovismorbificans to the list of animal salmonellosis in Zambia. Falade et. al.³ reported six more serotypes from animals in Zambia. These were S. Agona, S. Livingston, S. Newington, S. Makoma, S. Schwarzengrund and S. Anatum. This paper presents isolations of salmonella from domestic and laboratory animals between 1976 and 1994 from Zambia.

Materials and Methods

Source and type of specimen: Most of the samples originated from the specimens sub-

mitted from all over the country for routine diagnosis at Central Veterinary Research Station, Mazabuka from 1976 to 1980 and subsequently at the Central Veterinary Research Institute, Lusaka between 1981 and 1994. Some samples of bovine and porcine origin were collected from Mazabuka abattoir. Cattle, pigs, sheep, goats, rabbits and guinea pigs were included for the study. The specimens from the rabbits and guinea pigs were from the laboratory animal colony of the Research Institute. Pieces of liver, gall bladder with bile, mesenteric lymph nodes and small intestines with its contents were collected for bacteriological culture.

Bacteriological examination: Specimens were cultured separately on blood agar, MacConkey agar and selenite enrichment broth and incubated for 24 hours at 37°C. The cultures were examined. Subcultures from selenite broth were made on MacConkey agar, Deoxycholate agar and/or brilliant green agar and incubated for 1 – 2 days at 37°C and examined. The examination of cultures was done as described by Edwards and Ewing⁴. Many isolates and particularly those isolated for the first time from
Zambia were sent either to Salmonella Reference Laboratory, Colindale, London or Reference Laboratory, Hamberg, Germany for typing and confirmation.

Results

Out of 1785 specimens, Salmonella was isolated from 69 cases, giving an incidence rate of 3.86%. Amongst domestic animals, cattle had the highest incidence rate (4.86%) followed by pig (4.16%), goat (2.32%) and sheep (0.98%), whereas in laboratory animals, rabbit had higher incidence (4.90%) compared to guinea pig (3.07%).

The result of isolation of Salmonella serovars is presented in Table 1. A total of 69 Salmonella serovars were identified.

<table>
<thead>
<tr>
<th>SEROVARS</th>
<th>CATTLE</th>
<th>PIGS</th>
<th>GOATS</th>
<th>SHEEP</th>
<th>RABBITS</th>
<th>GUINEA PIG</th>
<th>TOTAL</th>
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<tr>
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<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>S. Agona 4,12:f,g,s</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>1</td>
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<td>5</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>6</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2</td>
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<td>6</td>
<td>1</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>8</td>
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<tr>
<td>S. Dublin 9, 2:g,p</td>
<td>10</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>11</td>
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<td>-</td>
<td>-</td>
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<td>-</td>
<td>-</td>
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<td>-</td>
<td>-</td>
<td>-</td>
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<td>S. Kinondomi 17:a:e,n,x</td>
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<td>2</td>
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<td>1</td>
<td>-</td>
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<td>S. Typhimurium 1,4,5,12:i:1,2</td>
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<td>-</td>
<td>4</td>
<td>3</td>
<td>-</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>1</td>
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<td>S. Choleraesuis 6, 7:c:1,5</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>6</td>
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<tr>
<td>Total</td>
<td>46</td>
<td>9</td>
<td>2</td>
<td>3</td>
<td>5</td>
<td>4</td>
<td>69</td>
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<td>13.6</td>
<td>2.8</td>
<td>4.34</td>
<td>7.24</td>
<td>5.30</td>
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cultures consisting of 13 serovars were isolated. Cattle had the highest isolations (66.6%) followed by pig (13.0%), sheep (4.34%) and goat (2.8%). Rabbit and guinea pig had 7.24% and 5.79% isolations respectively.

The survey revealed isolation of 7 Salmonella serovars from the domestic and laboratory animals for the first time in Zambia. These include S. Amager, S. Bonn, Heidelberg, S. Konandomi, S. Kisarawe, S. Bareilly and S. Welteverden.

Discussion

S. Typhimurium is the most frequently isolated serovar in the present study from cattle, rabbits and guinea pigs. S. Typhimurium is a common serovar isolated from all species of animals and birds, and has been shown to cause severe losses in cattle6,12,14 and in poultry8. In Zambia, the first case of S. Typhimurium had been reported during 19571 but unfortunately no host has been indicated. Gasper and Hrabe6 isolated S. Typhimurium from one aborted foetus out of 41 examined. We did not include foeti in our study. However, diagnostic records (1976–94) of the Department of Veterinary and Tsetse Control Services1 did not reveal isolation of S. Typhimurium from cases of abortion in Zambia. Falade et al.3 did not record isolation of S. Typhimurium during their one year survey in Zambia.

S. Dublin is the second frequent serovar isolated in the present study. Its pathogenicity in calves14 and its role in cattle abortion7 is well known. In Zambia, S. Dublin was first isolated between 1955–57 from cattle farms experiencing calf mortality. Out of 10 cultures isolated during present study, 6 were from young calves having diarrhoea and mortality, confirming continued existence of this serovar in cattle population.

From the specimens of swine, 6 isolates of S. Choleraesuis, and one isolate each of S. Bareilly, S. Bovismorbificans and S. Heidelberg were isolated. The serovars most frequently implicated in swine are S. Choleraesuis and S. Typhimurium.

Except one culture of S. Dublin from rabbits, all isolates from rabbits and guinea pigs were typed S. Typhimurium (Table 1). Amongst salmonella serovars which appear occasionally in rabbits6 and guinea pigs11,13, S. Enteritidis, S. Typhimurium and S. Ochrochu have been indicated pathogenic to rabbits and guinea pigs10,13. Laboratory animal colony, from where the rabbits and guinea pigs specimens originated, occasionally, had mortality losses.

Barring a few cultures of S. Dublin from calves and S. Typhimurium from laboratory animals, most of the cultures were isolated from animals without any specific clinical syndrome. Adult animals have been shown as symptomless carriers of many salmonella serovars2.

Acknowledgement

We express our thanks to the staff of Salmonella Reference Laboratory, Colindale, London and Hamberg, Germany for typing and confirmation of some of the isolates. The active cooperation of Mr. B.K.T. Francis, FAO technical expert, during the first ten years of this study is thankfully acknowledged.

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STUDIES ON SANITARY QUALITY AND CELL COUNT OF RAW MILK FROM DAIRY FARMS SUPPLYING MILK TO DAIRY PRODUCE BOARD IN LUSAKA, ZAMBIA

PANDEY, G.S., MISRA, D.S., MULE, D. AND MUBITA, C.

School of Veterinary Medicine, University of Zambia, P.O. Box 32379, Lusaka, Zambia.

ETUDES SUR LA QUALITE HYGIENIQUE ET LE NOMBRE DE CELLULES DU LAIT CRU DES FERMES LAITIERES QUI APPROVISIONNENT EN LAIT L’OFFICE DES PRODUITS LAITIERS A LUSAKA EN ZAMBIE

Résumé
On a mené une étude sur la qualité hygiénique et le nombre de cellules du lait de 95 fermes laitières qui approvisionnent en lait l’office des produits laitiers à Lusaka en Zambie. Le nombre de cellules variait entre 0 et 122 x 10⁶/ml de lait. Le nombre d’organismes dans une plaque-standard de lait cru oscillait entre log 7,66 et 9,15/ml de lait, ce qui montre que le lait contient une forte charge bactérienne. Le nombre de coliformes était entre log 6,06 et 7,20/ml de lait, tandis que le nombre de staphylocoques variait entre log 5 et 6,38/ml de lait. Au total, 76 souches de staphylocoque étaient isolées, parmi lesquelles il y avait 17 Staphylococcus aureus. Cinq des souches Staphylococcus aureus étaient entérotoxigènes et produisant de l’entéotoxine A, B et C. Mais heureusement qu’une très forte proportion (95%) de souches de staphylocoque avait une faible chimiorésistance aux antibiotiques courants. Les enquêtes ont conclu que la qualité hygiénique du lait cru d’environ 45% des fermes laitières dans les alentours de Lusaka n’était pas bonne et provenaient de plus atteints d’inflammation. Le lait en question contient des protéines et des entéotoxines inflammatoires qui sont thermonécessantes. Il faudrait apprendre aux éleveurs et aux traiteurs les conditions d’hygiène pour la conservation de la production et l’approvisionnement en lait cru.

Summary
A study on sanitary quality and cell count of raw milk from 95 dairy farms supplying milk to the dairy produce board in Lusaka, Zambia, was done. The cell count of milk varied from 0 to 122 x 10⁶ per ml of milk. A standard plate count of raw milk ranged from log 7,66 to log 9,15 per ml of milk suggesting a high bacterial load in the milk. The coliform count ranged from log 6,06 to 7,20 per ml of milk and the staphylococcal count ranged from log 5 to 6,38 per ml of milk. A total of 76 isolates of staphylococci were isolated and 17 of them were Staphylococcus aureus. Five of the Staphylococcus aureus isolates were found to be enterotoxigeneic producing enterotoxin A, B and C. Fortunately a very high percentage (95%) of staphylococci isolates had low drug resistance to common antibiotics. The study concluded that the sanitary quality of raw milk of about 45% dairy farms around Lusaka was not good and originated from inflamed udders. Such milk has inflammatory protein and enterotoxins which are heat resistant. There is need to educate the farmers and milkers on hygienic production, storage and supply of raw milk.

Introduction
Milk is an ideal medium for the growth of various micro-organisms. Although freshly drawn milk may possess temporary ‘germicidal’ or ‘bacteriostatic’ property, growth of micro-organisms in milk is inevitable unless it is frozen. The diseases commonly spread through milk besides the common forms of food poisoning and food infection include typhoid fever, scarlet fever, septic sore throat, diphtheria, tuberculosis and brucellosis. The single development that has eliminated virtually all disease problems of microbial origin is the widespread pasteurization in the processing of milk. However, this exercise is very limited in Zambia and majority of the population still consumes unpasteurized
milk. Mogessie and Fekadu\(^{6}\) reported that the initial microbial load of raw milk and the degree of microbial proliferation during storage dictates the quality of the product and high contamination of raw milk implies a higher chance for microorganisms to survive pasteurization. Milk is sterile as it is secreted by the specialised secretory cells in the mammary gland. As the milk moves through, it becomes contaminated with bacteria that reside within the udder. The health of the cow is the first and perhaps principal factor which is related to the absence or presence of pathogen in raw milk. Mastitis, an inflammatory disease of the udder, is likely to be accompanied by the development of millions of pathogen in the infected quarter and the resultant discharge of large number of these pathogens in the milk\(^{3}\). The most common pathogens that can cause mastitis and appear in milk include \textit{S. aureus}, \textit{Streptococci}, \textit{E. coli}, \textit{P. aeruginosa}, \textit{L. monocytogenes}, \textit{Brucella}, \textit{Mycobacterium} etc. The environment of dairy cows is a second source of micro-organism in milk and these may be from unwashed teats and skin, soil, litter, feed, water, faeces, milking machine, milk handling equipment and workers who milk the cow\(^{6}\).

The Dairy Produce Board (DPB) in Lusaka is the main body which supplies pasteurised milk to the public and a good number of consumers buy unpasteurised, raw milk directly from the farmers. The board receives its raw milk from the surrounding dairy farms in Lusaka province. Therefore, it was felt necessary to investigate the sanitary quality of raw milk supplied to DPB in Lusaka as such a type of study has not been done in Zambia. With the above objective in mind, present work reports the sanitary quality and cell count of the raw milk including isolation of staphylococcus and its enterotoxigenicity.

**Materials and Methods**

**Collection of Milk Sample** — 95 raw pooled samples of milk from 95 dairy farmers were aseptically collected at different intervals from milk cans just before delivering to the DPB processing plant. These milk samples were examined and cultured within 3 hours after collection.

**Standard Plate Count** — By using sterilised water suitable dilution of \(10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}\) and \(10^{-6}\) of raw milk samples were prepared. One ml of aliquots from appropriate dilutions of milk were taken in sterilised petri dishes and mixed with 10–15 ml of melted nutrient agar and cooled at 45°C. After the agar had solidified, the plates were incubated at 37°C for 24 hours and the average count of two plates inoculated for desired dilutions were taken into account.

**Coliforms Count** — Enumeration of coliforms in the milk sample were made by employing the multiple tube technique for determination of the most probable number (MPN) using single and double strength MacConkey broth. Suitable dilutions of milk samples were inoculated in 10 ml, 1 ml, and 0.1 ml quantities in 5 tubes for each selected dilution and tubes were taken positive on showing acid and gas production after 48 hours incubation. The MPN was calculated using a statistical table\(^7\).

**Staphylococcal Count** — Suitable dilutions of milk samples were inoculated on mannitol salt agar (medium 110) for enumeration and isolation of coagulase positive staphylococcus in milk. Inoculated plates were incubated at 37°C for 18 hours and colonies surrounded by a yellow zone were counted and isolated for further characterization.

**Cell Count** — 0.01 ml of milk sample was spread over one sq. cm area on the glass slide and air-dried. The smear was treated with xylol and stained with Newman’s stain. After washing and air-drying, the smear was examined under oil immersion and leucocytic cells were counted in 10 fields and the average calculated. This average was multiplied by the microscopic factor (100,000) and thus the number of cells/ml of milk was calculated.

**Identification of Staphylococci and their enterotoxigenicity** — Typical colonies of staphylococci were picked up from Medium 110 and transferred on nutrient agar slants for further identification. Each isolate was subjected to catalase coagulase, DNAse, mannitol and glucose fermentation tests and identified upto
For testing the enterotoxigenicity, each isolate of *Staphylococcus aureus* was tested by using reverse passive latex agglutination (RPLA) kit supplied by Denka Seiskyin (Tokyo, Japan). By using the kit production of enterotoxin, A, B, C, and D was detected. Each isolate of the *Staphylococcus aureus* to be tested for enterotoxigenicity was grown in brain-heart infusion broth for 18 hours at 37°C, then centrifuged at 3000 rpm for one hour at 4°C. The supernatant was separated and tested with the latex particles sensitised with anti-enterotoxin A, B, C and D using non-sensitised latex particle as a control. The plates were incubated at room temperature for 18–24 hours and were observed for agglutination and results recorded.

**Antibiotic Sensitivity Determination** — After identifying Staphylococci at the generic level the antibiotic sensitivity pattern of each isolate was determined. Each culture was grown on brain-heart infusion broth at 37°C for 4 hours. The growth was prepared on a nutrient agar plate and after drying the plate for one hour at room temperature, the antibiotic disc of 4 antibiotics namely Ethromycin, Gentamycin, Penicillin and Cefazolin respectively were placed. After incubation of 37°C for 18 hours, the zones of inhibition were noted. The antibiotic sensitivity of each culture was recorded as resistant (R) and Sensitive (S).

**Results and Discussion**

The results of the sanitary analysis of milk samples and cell counts have been presented in Table 1. The cell count of milk supplied to DPB varied from 0–122 x 10^5 per ml of milk.

<table>
<thead>
<tr>
<th>Group of farms</th>
<th>No of dairy farms examined(95)</th>
<th>Cell count per ml of milk x 100,000 x 100,000</th>
<th>Log of count cfu/ml</th>
<th>Average standard plate count</th>
<th>Average coliform count</th>
<th>Average medium 110 count (Staphylococci)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>31/95 (29.4%)</td>
<td>0-2</td>
<td>7.66</td>
<td>6.18</td>
<td>5.06</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>34/95 (32.3%)</td>
<td>3-10</td>
<td>8.02</td>
<td>6.06</td>
<td>5.01</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>19/95 (18%)</td>
<td>11-29</td>
<td>8.52</td>
<td>6.74</td>
<td>5.35</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>8/95 (7.6%)</td>
<td>33-54</td>
<td>8.63</td>
<td>6.63</td>
<td>5.80</td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>3/95 (2.8%)</td>
<td>103-122</td>
<td>9.15</td>
<td>7.20</td>
<td>6.38</td>
<td></td>
</tr>
</tbody>
</table>
quality of such pasteurised milk and milk products will also be poor. Similar findings were reported by Gupta. Milk with such a high bacterial load may harbour many pathogens like Salmonella, Shigella, Escherichia, Staphylococci and Streptococci species of bacteria which are a known pathogen for adults and infants and may be responsible for diarrhoeal syndromes, typhoid and paratyphoid fevers. The coliforms should be absent in a 0.01 ml quantity of raw milk but the results of the present work revealed coliforms ranging from log 6.06 to 7.20 per ml of milk. This gives indication that milk supplied to DPB was produced under unhygienic conditions and may be due to faecal contamination. High coliform counts in milk may liberate endotoxins in milk which may lead to pyrexia among consumers.

On identification of staphylococci isolated from raw milk a total of 17 out of 76 isolates were Staphylococcus aureus and of the 17 isolates, five were found to be enterotoxigenic and produced enterotoxin A,B and C (Table 2). Similar results was obtained by Olson et al 1970. The Staphylococcal enterotoxins are heat resistant and may hence be responsible for food poisoning among milk consumers. The detection of the presence of the enterotoxigenic Staphylococcus aureus from raw milk has great public health significance and milk containing enterotoxigenic staphylococci should not be included in milk supply. The antibiotic sensitivity determination of these staphylococcal isolates indicated that only 5.4% isolates were resistant to gentamycin, 4% to cefazolin, 4.5% to erythromycin and 1.3% to penicillin. The result indicates that antibiotic resistance of the isolates was of a low order. (Table 3).

Therefore, based on the study, it can be

### Table 2: Biochemical characterization and enterotoxigenicity of staphylococci isolated from raw milk from dairy farms around Lusaka

<table>
<thead>
<tr>
<th>Isolates tested</th>
<th>Mor phology</th>
<th>Catalase reaction</th>
<th>Coagulase reaction</th>
<th>D’nase reaction</th>
<th>Mannitol</th>
<th>Glucose</th>
<th>Enterotoxin Production</th>
</tr>
</thead>
<tbody>
<tr>
<td>76 gram +coci</td>
<td>+ve -ve</td>
<td>+ve -ve</td>
<td>+ve -ve</td>
<td>+ve -ve</td>
<td>A B C D</td>
<td></td>
<td></td>
</tr>
<tr>
<td>%</td>
<td>100</td>
<td>92 8</td>
<td>85.5 14.5</td>
<td>46 54</td>
<td>2 2 1</td>
<td>2.5 2.5 1.5 0</td>
<td></td>
</tr>
</tbody>
</table>

### Table 3: Antibiotic sensitivity pattern of staphylococci isolated from raw milk from different dairy farms around Lusaka

<table>
<thead>
<tr>
<th>Isolates Tested</th>
<th>Antibiotic Used</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Erythromycin 15ug</td>
</tr>
<tr>
<td>74</td>
<td>S  R</td>
</tr>
<tr>
<td>%</td>
<td>95.9 4.0</td>
</tr>
</tbody>
</table>

*S =Sensitive \ R=Resistant*
concluded that the sanitary quality of the raw milk of about 45% dairy farms around Lusaka supplying milk to DPB was not of good quality. The raw milk may harbour pathogens which may lead to salmonellosis, diarrhoeal syndrome and other ailments among consumers. The high cell count from some of the farms indicated that milk from inflamed udders was being supplied and at the same time, it was produced under unhygienic conditions. Raw milk also harboured a high number of *Staphylococcus aureus* and some of them were enterotoxigenic. This may lead to food poisoning syndromes in consumers. The result further suggested that a very high % of *Staphylococcus* isolate had fortunately low drug resistance. There is need to educate the farmers on hygienic production and supply of raw milk. A further study on sanitary quality of pasteurised milk at different intervals will be useful and factors responsible for deterioration of pasteurised milk and milk products could be known.

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VAGINAL AEROBIC BACTERIAL FLORA OF APPARENTLY HEALTHY CATTLE IN VARIOUS STAGES OF THE REPRODUCTIVE CYCLE IN THE SAHEL REGION OF NIGERIA

J. D. AMIN*, L.T. ZARIA** AND REBECCA M. MALGWI*

*Department of Veterinary Surgery and Reproduction, **Department of Veterinary Microbiology and Parasitology, Faculty of Veterinary Medicine, University of Maiduguri, PMB 1069, Maiduguri, Nigeria

FLORE BACTERIENNE AEROBIE DU VAGIN DES VACHES APPAREMMENT EN BONNE SANTE AUX DIVERS STADES DU CYCLE DE REPRODUCTION DANS LA REGION DU SAHEL AU NIGERIA

Résumé
Cent trente cinq prélèvements vaginaux de vaches aux divers stades du cycle de reproduction (génisses = 22, vaches en chaleur = 30, vaches en cycle sexual = 23, vaches gestantes = 31 et vaches à l'état puerperal = 29) ont été effectués et examinés du point de vue bactériologique. Les bactéries isolées étaient les suivantes: Coynnebacterium bovis, E. coli, Bacillus spp, Staphylococcus spp négatif à la coagulase, Micrococcus spp, Streptococcus aureus, Streptococcus spp, Actinomyces pyogenes, Proteus spp et C. hofmannii. C. bovis (24,86%), E. coli (23,2%), Bacillus spp (16,29%) et Staphylococcus spp négatif à la coagulase (11,6%) étaient les souches les plus répandues. Ces souches ainsi que Streptococcus spp étaient isolées des vaches à tous les stades du cycle de reproduction. Ces organismes sont donc parmi la flore bactérienne aérobie des vaches au Sahel.

Summary
One hundred and thirty-five vaginal swabs of cattle in different phases of the reproductive cycle (heifers = 22, cows on heat = 30, cows in dioestrus = 23, pregnant cows = 31 and cows during puerperium = 29) were taken and examined bacteriologically. The bacteria isolated were: Coynnebacterium bovis, E. Coli, Bacillus spp, coagulase negative Staphylococcus spp, Micrococcus spp, Staphylococcus aureus, Streptococcus spp, Actinomyces pyogenes, Proteus spp, and C. hofmannii. C. bovis (24,86%), E. coli (23,2%), Bacillus spp (16,29%) and coagulase negative Staphylococcus spp (11,6%). These isolates as well as Streptococcus spp were isolated from cattle in all stages of the reproductive cycle. These organisms are therefore among the aerobic bacterial flora of cows in the Sahel.

Introduction
A variety of bacteria has been isolated from the vagina of cows. The commonly isolated ones include Bacillus spp, E. coli, Staphylococcus spp, Streptococcus spp, Actinomyces spp and Coynnebacterium spp. These organisms have also been associated with vaginitis and other inflammatory genital conditions¹. Bacteria found in the vagina of cows can potentially invade the uterus of cows especially at calving. These are frequently eliminated from the involuting uterus in normal fertile cows and any endometritis resolves prior to the time the cow is served². Endometritis is a common reproductive disorder in dairy cows³. As many as 67% of the post-partum cattle examined in one study had endometritis⁴. The consequences of endometritis can range from no effect on reproductive performance to sterility⁵. Endometritis increases the calving to conception interval and delays uterine and cervical involution⁶,⁷.

Resistance of cattle to non-specific infection is related to the endocrine state prevailing at the time of infection, thus resistance is thought to be highest during oestrus and parturition while uterine infection is likely to be established during the luteal phase¹,⁶,⁹. Some authors¹⁰,¹¹ documented that oestrogen enhances elimination of bacterial infection from the bovine uterus. Progesterone is said to promote con-
ditions within the genital tract which are suitable for bacterial growth. It has been reported that ovarian activity has a profound effect on the ability of the uterus to resist bacterial infection. The uterus is highly resistant to infection during oestrogenic phase but very susceptible during the progesterone phase because of low pH, favouring the growth of bacteria regularly isolated from the bovine uterus and factors associated with delayed and diminished leucocyte response to infection. At oestrus and parturition, there is a numerical change in the peripheral blood picture with a relative neutrophilia and 'shift to the left'. At oestrus, the blood supply to the genital tract is increased under the influence of oestrogen, while at parturition, there is a massive blood supply to the genitalia with a migration of white blood cells into the genital lumen for the phagocytosis of bacteria. There is also an increased genital discharge and higher concentration of secretory immunoglobulins.

This study was designed to elucidate the bacterial flora of the genitalia of apparently healthy cattle during different phases of the reproductive cycle in the Sahel.

Materials and methods

Samples were taken from a total of 135 cattle in various locations in Borno and Yobe States in the Sahel region of Nigeria. Of the 135 samples taken, 31 were from cows in the last trimester of pregnancy, 23 were from cows in dioestrous, 30 were from cows on heat, 29 were from cows during puerperium between 3–4 weeks after parturition and 22 were from (immature) heifers about one year old. These stages of reproduction were identified by rectal palpation, signs of standing heat and history of calving.

Animals to be sampled were restrained and the vulval lips were washed and disinfected and allowed to dry. The vulval lips were parted with a gloved hand and a sterile guarded swab (Sterilit, UK) was pushed about 8 cm into the vagina. The swab was withdrawn into the sheath and removed from the vagina. The swab was placed into a transport medium (nutrient broth); transported to the laboratory and streaked on 5% sheep blood agar and McConkey agar. The plates were incubated at 37°C for 24–48 hours. To determine the bacterial load of swab, colonies were scored based on growth on the first (+), second (++) third (+++) or fourth (++++) lines of streak. Where there was more than one type of colony, they were subcultured to obtain pure cultures. Further tests employed for the identification of the colonies were gram staining, catalase, coagulase and biochemical tests based on standard procedure.13,14

Results

From the 135 cattle swabbed, a total of 362 bacteria belonging to eight genera were identified. Corynebacterium spp, E. coli, Bacillus spp, Staphylococcus spp, Micrococcus spp, Streptococcus spp, Actinomyces pyogenes and Proteus spp and Streptococcus spp were the most commonly isolated organisms. They were isolated from cattle of all the reproductive stages examined (Table 1). The average bacteria isolated per swab was highest among animals in dioestrous (3.3), followed by pregnant cows (2.93), cows on heat (2.86), cows in puerperium (2.3) and heifers (1.86). However bacterial load was highest in puerperal cows followed by dioestrous, pregnant cows, cows on heat and heifers.

There was a significant difference in the isolation rate of C. bovis from animals on heat compared with heifers (P<0.01), those in puerperium (P<0.01) and dioestrous cows (P<0.05). There was also a significant difference in the isolation rate of E. Coli between those on heat and heifers (P<0.01) and dioestrous cows (P<0.05). For coagulase negative Staphylococcus, there was a significant difference in the isolation rate from those on heat and dioestrous (P<0.05). The isolate rate of Micrococcus spp between puerperal and dioestrous cows differed significantly (P<0.03) as well as for dioestrous cows compared to heifers (P<0.02). More β-haemolytic streptococci were cultured from samples taken from dioestrous cows. This was significantly different (P<0.01) from any other group. There was also a significant difference in the isolation rate for A. pyogenes isolated from
Table 1: Bacteria isolated from the vagina of apparently healthy cattle in different stages of the reproductive cycle in the Sahel

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Pregnancy (n = 31)</th>
<th>Puerperium (n = 29)</th>
<th>Reproductive stage (n = 135)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>heat (n = 30)</td>
<td>dioestrus (n = 23)</td>
<td>heifer, (n = 22)</td>
</tr>
<tr>
<td>C. bovis</td>
<td>23++</td>
<td>16++</td>
<td>27++</td>
</tr>
<tr>
<td>E. coli</td>
<td>20++</td>
<td>18++</td>
<td>25++</td>
</tr>
<tr>
<td>Bacillus spp</td>
<td>19++</td>
<td>11+++</td>
<td>14++</td>
</tr>
<tr>
<td>Staphylococcus spp*</td>
<td>8++</td>
<td>8++</td>
<td>6+</td>
</tr>
<tr>
<td>Micrococcus spp</td>
<td>5+</td>
<td>2+</td>
<td>5+</td>
</tr>
<tr>
<td>S. aureus</td>
<td>6+</td>
<td>1+++</td>
<td>3+</td>
</tr>
<tr>
<td>b Streptococci</td>
<td>2++</td>
<td>1++</td>
<td>2++</td>
</tr>
<tr>
<td>A. pyogenes</td>
<td>1+</td>
<td>3++</td>
<td>-</td>
</tr>
<tr>
<td>Proteus spp</td>
<td>12++++</td>
<td>5++++</td>
<td>4++++</td>
</tr>
<tr>
<td>a Streptococci</td>
<td>4+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C. hofmannii</td>
<td>1+</td>
<td>2++</td>
<td>-</td>
</tr>
<tr>
<td>S. faecalis</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* = Coagulase negative staphylococci
β streptococci = β-haemolytic Streptococci
α streptococci = α-haemolytic Streptococci

dioestrous cows compared with pregnant cows (P<0.01), heifers (P<0.01), puerperal cows (P<0.05) and those on heat (P<0.001).

Bacillus spp, S. aureus and Proteus spp isolation rate was not significantly different (P>0.05) when any two groups were compared.

Fewer bacteria were isolated per sample taken from heifers (an average of 1.86 bacteria per swab). Bacterial load was also low among heifers. In contrast, the average number of bacteria isolated per swab was highest among dioestrous cows (3.34) and bacterial load was highest among puerperal cows.

Discussion

C. bovis (24.86%), E. coli (23.2%), Bacillus spp (16.29%) and coagulate negative Staphylococcus spp (11.6%) were the commonest isolates. These isolates as well as Streptococcus spp were isolated from cattle in all stages of the reproductive cycle. These organisms therefore seem to be ubiquitous among cattle in the Sahel and could therefore be considered as part of the aerobic bacterial flora of cows.

The relatively lower bacterial isolation rate, load and variety among heifers suggests that sexual maturity and/or copulation is an important contributor to the bacterial flora of the genitalia. Despite their sexual inexperience however, heifers still have a resident flora in their vagina. This might have arisen as a result of ascending infection from the perineum. In spite of the anatomical closeness of the vulva to the anus, few enterobacteriaceae were isolated. This is similar to the findings in sheep15.

When bacterial load and bacteria isolated per swab were taken together, there appears to be no clear statistical association between these and the hormonal state (reproductive status) of the animals examined. This contrasts the finding of other workers9,12,16. However it is possible that oestrogen prevents active colonisation rather than mere superficial infection (by bacteria) of genitalia leading to vaginitis, cervicitis.
and endometritis. It is noteworthy however, in this study that both isolation rate and bacterial load was highest among dioestrous cows. Cows in dioestrous are in the progestosterone phase which is believed to be compatible with a higher infection rate. The higher isolation rate and bacterial load in dioestrous cows was however, not significant (P > 0.05) when compared to those on heat.

*A. pyogenes* was more commonly associated with dioestrous cows (Table 1). *A. pyogenes* has been associated with severe endometritis in cows. Others have also associated *A. pyogenes* with pyometra. It has been reported that *A. pyogenes* was more likely to cause endometritis in association with anaerobes. In this study anaerobes were not cultured and there was no evidence of endometritis in any of the cows sampled.

Many of the bacteria isolated in this study have been associated with other conditions such as vaginitis, cervicitis, endometritis and repeat breeding. However, all animals sampled in this study were apparently healthy. It therefore appears likely that the organisms only cause disease in propitious circumstances. These need to be investigated further.

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**References**


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MYCOLOGICAL EXAMINATION OF Poultry FEED USED IN NAIROBI, KENYA

GATHUMBII J.K., BEBORA L.C., MUCHIRI D.J. AND NGATIA T.A.
Department of Veterinary Pathology and Microbiology, University of Nairobi,
P.O. Box 29053, Nairobi, Kenya. "Department of Public Health, Pharmacology
and Toxicology, University of Nairobi.

EXAMEN MYCOLOGIQUE DES ALIMENTS POUR VOLAILLE UTILISES
A NAIROBI, KENYA

Résumé
L'examen mycologique de 90 échantillons de mélange d'aliments pour volaille, disponibles sur le marché, a été fait en vue d'obtenir des informations sur le niveau de contamination fongique et la fréquence de la présence de l'espèce fongique appartenant aux genres Aspergillus, Penicillium
et Fusarium. Le niveau de contamination fongique variait entre 1 x 10^4 - 1,31 x 10^6 unités constituant une colonie (UC) par gramme d'aliments. Sur la base de ces dénombrements de fongus, la qualité hygiénique de 36% de tous les échantillons examinés a été considérée inacceptable. Aspergillus spp et Penicillium spp ont été isolés de tous les échantillons à des niveaux de 1 x 10^4 - 8 x 10^5 UC/gramme d'aliments et 2 x 10^2 - 3 x 10^6 UC/gramme d'aliments respectivement. Fusarium spp a été isolé de 76,7% des échantillons à un niveau variant entre 0 - 3,1 x 10^6 UC/gramme d'aliments. Au total, 15 Aspergillus spp, 19 Penicillium spp et 3 Fusarium spp ont été identifiés.

Abstract
Mycological examination of 90 samples of commercially mixed poultry feed was done to obtain information on the level of fungal contamination and frequency of occurrence of fungal species belonging to the genera Aspergillus, Penicillium and Fusarium. The level of fungal contamination varied from 1 x 10^4 - 1,31 x 10^6 colony forming units (CFU) per gram of feed. On the basis of these fungal counts, the hygienic quality of 36% of all the samples examined was classified as unacceptable. Aspergillus spp and Penicillium spp were isolated in all samples at levels of 1.0 x 10^3 - 8.0 x 10^5 CFU/gram of feed and 2.0 x 10^2 - 3.0 x 10^6 CFU/gram of feed respectively. Fusarium spp were isolated in 76.7% of the samples at level of between 0 - 3.1 x 10^6 CFU/gram of feed. A total of 15 Aspergillus spp, Penicillium spp, and 3 Fusarium spp were identified.

Introduction
The occurrence of fungi in poultry feed lowers the hygienic quality of the feed, causes nutrient losses and may cause certain diseases such as esporgilliosis in poultry and allergy in man. Certain fungal species can also produce toxic secondary metabolites called mycotoxins that are associated with several diseases in man and animal. These mycotoxins have been demonstrated in human and animals feed in Kenya by several workers. The recognition of these mycotoxins as a widespread economic threat to profitable poultry husbandry and the demonstration of the ability of poultry to incorporate mycotoxins in poultry feed into residues in both meat and eggs has led to increased interest in the mycobiota of poultry feed. Poultry feeds used in Kenya have not received any special mycological attention to determine their hygienic quality and the distribution of mycotoxin producing fungi inspite of accumulated information on the occurrence of fungi in many of their raw materials. This investigation was carried out to obtain information on the extent of fungal contamination and distribution of fungi belonging to the genera Aspergillus, Penicillium and Fusarium in commercially mixed poultry feed used in Nairobi, Kenya.

Materials and Methods
Sampling
A total of 90 samples of commercially mixed poultry feed were randomly sampled from poultry farms in Nairobi Province and Kikuyu Division of Kiambu District.
Mycological Examination
Ten grammes of feed from each sample was diluted in 90 ml of a sterile solution of 0.9% NaCl and 0.2% Tween 80 in distilled water. This mixture was further diluted to concentration of 0.01g/ml and 0.001g/ml feed. From each of these dilutions, aliquots of 0.1ml were taken and spread onto the surface of each of the following agar media:

Agar media
Malt salt agar (MSA)\(^{(13)}\) medium containing malt extract (2%) NaCl (7.5%) and agar (2%). Dichloran rose bengal extract sucrose agar (DRYES) medium (14%) containing yeast extract (2.0%) sucrose (15%), agar (2.0), chloramphenicol (100 mg/l), botran (2.3 dichloro-4-nitroaniline) (2mg/l) and rose bengal (25 mg/l). Potato dextrose agar (PDA) (Oxoid). All plates were incubated at room temperature for 4 – 5 days.

Enumeration and identification of fungi
The estimated total number of colony forming units (CFU) per gram of feed was obtained by counting colonies, on the medium that yielded the highest number of colonies from the most diluted feed inoculum, and multiplying the count with the appropriate dilution.

Colonies of Aspergillus spp and Penicillium spp. were counted on MSA and DRYES medium and subcultured on Czapek solution agar\(^{(15)}\) for one week at room temperature. Aspergillus spp. were identified according to the classification of Raper and Thom\(^{(15)}\). Penicillium spp. were identified according to the classification of Raper and Thom\(^{(16,17)}\) and according to Pitt\(^{(18)}\). Fusarium spp. were isolated from MSA medium and subcultured on PDA (Oxoid). Single-spore isolation techniques were used to obtain cultures suitable for identification according to Booth\(^{(19)}\) and Booth\(^{(20)}\).

Results
None of the feed sampled had any macroscopic signs of impaired hygienic quality such as abnormal colour, smell or consistency. On microbiological examination, the total mould counts varied from 1.0 \times 10^4 to 1.31 \times 10^6 CFU/gram of feed. The mean was 1.115 \times 10^4 and the median 1.8 \times 10^5 CFU/gram of feed. Thirty seven (41%) of the samples had 0 – 1.0 \times 10^5 CFU/gram, 21 (23%) had between 1.0 \times 10^5 and 2.0 \times 10^5 while 32 (36%) had over 2.0 \times 10^5 CFU/gram of feed. Aspergillus spp. and Penicillium spp. were isolated in all samples examined while Fusarium spp. were isolated in 76.7% of the samples (Table 1).

<table>
<thead>
<tr>
<th>Group</th>
<th>Range</th>
<th>Mean</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus</td>
<td>2.0 \times 10^2 - 3.0 \times 10^5</td>
<td>4.19 \times 10^4</td>
<td>1.0 \times 10^4</td>
</tr>
<tr>
<td>Penicillium</td>
<td>1.0 \times 10^5 - 8.0 \times 10^5</td>
<td>6.88 \times 10^4</td>
<td>5.0 \times 10^4</td>
</tr>
<tr>
<td>Fusarium</td>
<td>0 - 3.1 \times 10^6</td>
<td>1.93 \times 10^4</td>
<td>5.5 \times 10^4</td>
</tr>
</tbody>
</table>

A total of 15 species of the genus Aspergillus (Table 2), 19 of Penicillium (Table 3) were identified. Only three species of Fusarium were identified. Those were F. moniliforme (Sheldon) in 38.8% of the samples, F. oxysporum (Schlechtendahl) in 13.3% of F. lateritium (Ness) in 1.1% of all the samples examined.

<table>
<thead>
<tr>
<th>Species</th>
<th>No. of samples</th>
<th>% (N=90)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. A. flavus Link</td>
<td>68</td>
<td>75.6</td>
</tr>
<tr>
<td>2. A. venti Wehme</td>
<td>66</td>
<td>73.3</td>
</tr>
<tr>
<td>3. A. chevalieri Thom and Church</td>
<td>33</td>
<td>36.7</td>
</tr>
<tr>
<td>4. A. candidus Link</td>
<td>25</td>
<td>27.8</td>
</tr>
<tr>
<td>5. A. ryber (Brem)</td>
<td>16</td>
<td>17.8</td>
</tr>
<tr>
<td>6. A. niger Van Tieghem</td>
<td>12</td>
<td>13.3</td>
</tr>
<tr>
<td>7. A. tamarii Kita</td>
<td>8</td>
<td>8.9</td>
</tr>
<tr>
<td>8. A. chevalieri var. intermidus Thom and Raper</td>
<td>7</td>
<td>7.8</td>
</tr>
<tr>
<td>9. A. fumigatus Fuesenius</td>
<td>7</td>
<td>7.8</td>
</tr>
<tr>
<td>10. A. versicolor (vail) Tiraboschi</td>
<td>5</td>
<td>5.6</td>
</tr>
<tr>
<td>11. A. sydowii (Bain. and sart.)</td>
<td>5</td>
<td>5.6</td>
</tr>
<tr>
<td>12. A. ochraceus Wilhelm</td>
<td>4</td>
<td>4.4</td>
</tr>
<tr>
<td>13. A. ustus (Bainier)</td>
<td>2</td>
<td>2.1</td>
</tr>
<tr>
<td>14. A. Parasititus Speare</td>
<td>1</td>
<td>1.1</td>
</tr>
<tr>
<td>15. A. terreus Thom</td>
<td>1</td>
<td>1.1</td>
</tr>
</tbody>
</table>
Table 3: Specimen of penicillium

<table>
<thead>
<tr>
<th>Species</th>
<th>No. of samples</th>
<th>% (N=90)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. P. cyclopium Westling</td>
<td>54</td>
<td>60.0</td>
</tr>
<tr>
<td>2. P. expansum (Link) Thom</td>
<td>25</td>
<td>27.8</td>
</tr>
<tr>
<td>3. P. vindicatum Westling</td>
<td>16</td>
<td>17.7</td>
</tr>
<tr>
<td>4. P. crustosum Thom</td>
<td>8</td>
<td>8.9</td>
</tr>
<tr>
<td>5. P. martensii Bourge</td>
<td>8</td>
<td>8.9</td>
</tr>
<tr>
<td>6. P. implicatum Biorge</td>
<td>6</td>
<td>6.7</td>
</tr>
<tr>
<td>7. P. purporogenum Stoll</td>
<td>6</td>
<td>6.7</td>
</tr>
<tr>
<td>8. P. oxalicum Currie + Thom</td>
<td>6</td>
<td>6.7</td>
</tr>
<tr>
<td>9. P. rugulosum Thom</td>
<td>6</td>
<td>6.7</td>
</tr>
<tr>
<td>10. P. digitatum Saccardo</td>
<td>6</td>
<td>6.7</td>
</tr>
<tr>
<td>11. P. brevicompactum Dierckx</td>
<td>6</td>
<td>6.7</td>
</tr>
<tr>
<td>12. P. granulatum Bainier</td>
<td>4</td>
<td>4.4</td>
</tr>
<tr>
<td>13. P. diversum Rapewr and Fennel</td>
<td>3</td>
<td>3.3</td>
</tr>
<tr>
<td>14. P. italicum Wehmer</td>
<td>3</td>
<td>3.3</td>
</tr>
<tr>
<td>15. P. duclauxi Delacroix</td>
<td>3</td>
<td>3.3</td>
</tr>
<tr>
<td>16. P. funiculosum Thom</td>
<td>2</td>
<td>2.2</td>
</tr>
<tr>
<td>17. P. crysosogenum Thom</td>
<td>1</td>
<td>1.1</td>
</tr>
<tr>
<td>18. P. puberulum Bainier</td>
<td>1</td>
<td>1.1</td>
</tr>
<tr>
<td>19. P. rogueforti Thom</td>
<td>1</td>
<td>1.1</td>
</tr>
<tr>
<td>20. Penicillium spp (unidentified)</td>
<td>14</td>
<td>15.6</td>
</tr>
</tbody>
</table>

Discussion

One of the most widely accepted classifications of the hygienic quality of feed on the basis of the degree of fungal contamination is that of Fletcher and Wellinger (1981)\(^1\). According to this classification, feeds of good to acceptable quality should have less than 1 x 10⁵ CFU/gram. The hygienic quality of feed with 1 x 10⁵ and that with over 2 x 10⁶ CFU/gram is considered inferior and unacceptable, respectively. On the basis of this classification, 36% of the feed samples examined in this study are considered to be of unacceptable level of fungal contamination. This proportion of unacceptable feeds is very high compared to the findings to workers in other parts of the world such as 8.5% of poultry and swine feed used in Norway and 4.5% of feed sampled in Germany\(^1\). For this reason nutrient losses due to moulding should be allowed for in calculation of poultry rations in Kenya. Methods of controlling fungal contamination such as pelleting\(^2\) should also be recommended to poultry feed manufacturers in Kenya.

Aspergillus spp, Penicillium spp and Fusarium spp were the most commonly isolated fungi, sometimes accounting for all fungi isolated (Table 1). This is agreement with similar studies done in Kenya cereals, legumes and oil seeds\(^5\,12\). The frequency of isolation of Aspergillus spp (100%), Penicillium spp. 100% and Fusarium spp (76.7%) was higher than 80.8%, 83.2% and 54.4% respectively reported for poultry feeds used in Spain\(^22\). The levels were also higher than those reported for poultry used in Norway\(^1\).

Aspergillus flavus which produces aflatoxins, was the most commonly isolated species being demonstrated in 75.6% of the samples (Table 2). This frequency of isolation is within the range of isolation of this fungus in other parts of the world. These include 76.1% in Australian animal fedes\(^23\) and 71% in Spanish mixed animal feeds\(^24\). The frequency was however higher than that reported in India (46.3%)\(^25\) but lower than that reported from poultry feed used in Norway\(^1\).

The most commonly isolated Penicillium spp, P. cyclopium, P. expansum and P. vindicatum (Table 3) are all well known mycotoxin producers\(^16\). Penicillium cyclopium has also been reported as the most commonly occurring penicillia in poultry feed used in Norway\(^1\). Penicillium cyclopium and P. vindicatum have been demonstrated to be the most commonly occurring penicillia species in stored maize\(^26\) and their high frequency of occurrence in the present study is probably because of inclusion of maize grit in poultry feed. Similarly, the high frequency of occurrence of Fusarium moniliforme is also most probably due to the previously mentioned use of maize as raw material for mixed poultry feed.

Acknowledgement

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References


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COMPARATIVE PARASITE DEVELOPMENT IN AFRICAN BUFFALO AND N’DAMA CATTLE INFECTED WITH EITHER *TRYPANOSOMA CONGOLENSE* OR *T. VIVAX*

R.O OLUBAYO†, S.K. MOLOO‡, AND J. NAESSENS‡

†Kenya Agricultural Research Institute, Veterinary Research Laboratories
P.O. Box 274, Utiru, Nairobi, Kenya;
‡International Livestock Research Institute (ILRI), P.O. Box 30709, Nairobi, Kenya

COMPARAISON DU DEVELOPPEMENT DU PARASITE CHEZ LES BUFFLES AFRICAINS ET LES N’DAMA INFECTES AVEC *TRYPANOSOMA CONGOLENSE* OU *T. VIVAX*

Résumé
Le buffle africain (*Syncerus caffer*) et le N’Dama trypanotolérant étaient comparés pour évaluer leur résistance à *Trypanosoma congoense* et *T. vivax*. Plusieurs mécanismes du système immunitaire ont été suivis pendant les infections et comparés chez les deux espèces. Selon plusieurs paramètres, les buffles étaient beaucoup plus résistants à la trypanosomiasi que les N’Dama : ils avaient une période prépatente beaucoup plus longue, leurs niveaux de parasitemie étaient plus faibles (environ 100 fois chez *T. congoense*) et l’anémie qui se manifeste par une baisse de l’hématócrite était très courte (*T. congoense*) ou inexistante (*T. vivax*) chez les buffles. Le N’Dama produisait des anticorps neutralisants avant le buffle, ce qui exclut l’hypothèse selon laquelle les anticorps constituent la cause d’une plus grande résistance du buffle à la trypanosomiasi. Les changements relatifs aux populations de lymphocytes étaient similaires chez le buffle et le N’Dama : une baisse des cellules TCD2, mais une augmentation des cellules Tγδ et des cellules B. Toutefois, les leucocytes neutrophiles ont augmenté chez le buffle et diminué chez le N’Dama, ce qui montre qu’ils peuvent jouer un rôle dans la résistance du buffle. Une population de leucocytes non-identifiés qui ne pouvait pas être reconnue d’après son phénotype de surface, apparaissait dans le sang périphérique du buffle mais pas chez le N’Dama, à peu près au moment où l’hématócrite commençait à baisser. Il est possible que ces cellules soient des cellules rougeâtres immatures produites par une érythropoïèse plus efficace chez le buffle.

Abstracts

African buffalo (*Syncerus caffer*) and trypanotolerant N’Dama cattle were compared for their resistance to overcome *Trypanosoma congoense* and *T. vivax* infections. Several compartments from the immune system were followed during the infections and compared between the two species. Several parameters suggested that the buffaloes were much more resistant to trypanosomiasis than the N’Dama cattle: they had a much longer prepatent period, their parasitemia levels were lower (about 100 fold in *T. congoense*) and anemia, measured as a drop in PCV (packed cell volume), was either very short (*T. congoense*) or not present (*T. vivax*) in buffaloes. The N’Dama produced neutralizing antibodies before the buffalo, ruling out such antibodies as the cause of the buffalo’s greater resistance to trypanosomiasis. Changes in lymphocyte populations were similar in both buffalo and N’Dama: a decrease in CD2 T cells, but an increase in γδ T cells and B cells. However, neutrophils increased in buffalo, while they dropped in N’Dama, suggesting that they may play a role in the resistance of the buffalo. An unidentified leucocyte population that could not be identified by its surface phenotype, appeared in the peripheral blood of the buffalo, but not in N’Dama, around the time that PCV started to drop. It is possible that these cells are immature erythroid cells produced by a more effective erythropoiesis in buffalo.

Introduction

The African buffalo survives successfully in trypanosomiasis endemic areas where domestic livestock succumb to trypanosomiasis. Buf-
evolved in domestic cattle which were introduced into Africa from the near East around 5,000 BC. The presence of trypanosomiasis and tsetse infestation makes it impossible to keep livestock in many of the tropical regions of Africa. At present, trypanosomiasis is mainly controlled by suppression of the tsetse fly population and by the chemotherapeutic treatment of infected animals as well as chemoprophylaxis. Due to drug resistance and toxicity, environmental pollution and chemical production costs, there is a need to find alternative means to control tsetse flies and trypanosomiasis. One solution to this problem is the use of trypanotolerant cattle. However, their trypanotolerance traits have not been adequately defined mainly due to the lack of genetic markers to be used for selection of these traits. Most of the reported studies have compared N'Dama with Zebu (Boran) and confirmed the superior capacity of N'Dama to control parasitaemia and resist the development of anaemia. Although there are some reports which have compared African buffalo with Boran cattle on their ability to control trypanosomiasis infection, there is no report which has compared N'Dama with buffalo.

The purpose of this study was to compare trypanotolerant African buffalo with N'Dama cattle for their response to Trypanosoma congolense and T. vivax infections transmitted by tsetse flies. To determine which compartment of the immune system could play a role in the resistance of the buffalo, changes in the kinetics of leukocyte sub-population, parasitaemia, packed cell volume as well as neutralizing antibody were followed and compared between the two species.

Materials and Methods

Experimental Animals

Buffaloes

Three buffaloes, two females (7181, 7448) and one male (7240) aged 21/2 years and weighing 250-280 kgs, were infected by T. congolense in the first experiment. The buffaloes were born in captivity at Kabete, Nairobi, in a tsetse and trypanosomiasis-free environment. The animals were kept in a fly proof isolation building under similar management as described before.

Two buffaloes which had been infected in the first experiment (Nos. 7181 and 7448, both females) and two naive buffalos (Nos. 7344, female and 7685, male) aged 3 years, weighing 320 – 370 kgs were infected in the second experiment by T. vivax.

N'Dama Cattle

All N'Dama's were raised at ILRAD. They were born from N'Dama cows derived from embryos transferred from dams in the Gambia to Boran recipients in Kenya. One male and two female N'Dama's, ND12, ND13 and ND14, aged 2 years and weighing 300 – 380 kgs, were used in the first experiment.

Four naive N'Dama cattle (Nos. ND15, ND16, ND17, ND21) all bulls, aged 3 years and weighing 300 – 380 kgs were used in the second experiment.

Prior to experimental infection both buffaloes and N'Dama cattle were screened for antibodies to T. congolense, T. vivax and T. brucei by immunofluorescence and found to be negative.

Parasites

Trypanosoma congolense (IL 1180) was obtained from the International Livestock Research Institute (previously ILRAD) and used in this study. This parasite was derived after one passage in mice from a clone IL 968.

The T. vivax parasites used from stock IL 2337 which was derived from GALANA/78/TRYPS 2392 after one passage in a Boran steer and was isolated from a Zebu steer in Galana, Malindi, Kenya.

Tsete Flies

Teneral Glossina morsitans centralis raised at ILRAD were allowed to feed for 21 days on the clipped flanks of a goat infected with T. congolense IL 1180 in experiment 1 or T. vivax IL 2337 in experiment 2. The tsetse were not fed for two days and were later allowed to probe
singly on slides pre-heated to 37°C. The drops of saliva were checked for the presence of metacyclic trypanosomes. The flies which had matured infections were then used to infect experimental animals by allowing five infected male flies to feed singly on each animal.

**Haematology**

Blood was collected from the jugular vein in EDTA. Determination of erythrocyte counts (RBC) and white blood cells (WBC) was done using an automatic electronic counter (Coulter Electronics, Inc. Hialeah, Florida, model P64). Differential cell counts were determined on 200 leukocytes per Giemsa stained peripheral blood smear. Packed red cell volume (PCV) percentage was measured and theuffy coat was examined for trypanosomes using phase microscopy\(^{(15)}\). The number of trypanosomes was estimated by the semi-quantitative scoring method previously described\(^{(23)}\).

**Detection of antibodies to metacyclic VSGs**

Assays for neutralizing antibodies against *T. conglolense* metacyclic forms were performed using the method described by Nantulya et al.\(^{(16)}\). Briefly, five infected tsetse in single holding tubes were allowed to probe singly into 20μl of pre- or post-infection serum in wells of a lymphocyte migratatron plate, after which the volume was made up to 1.5ml with the same serum. After incubation on ice for 30 minutes, the suspension was inoculated, in equal portions, into three BALB/c mice. Each serum was tested twice. The mice were then observed for 30 days for the development of parasitaemia. If parasitaemia did not develop in any of the six mice in a group, neutralization was considered positive for that particular serum dilution.

**Flowcytometry**

Peripheral blood mononuclear cells (PBM) were isolated by density gradient centrifugation on Ficoll-Paque (Pharmacia LKB, Biotechnology AB, Uppsala, Sweden) according to the method described previously\(^{(17)}\). A panel of monoclonal antibodies (MAbs) to bovine leukocyte antigens in cattle, sheep and goats\(^{(9)}\) was used to measure lymphocyte sub-populations and is summarized in Table 1. These MAbs cross-reacted with homologous antigens from African buffalo\(^{(18)}\).

Single and two-colour immunofluorescence of lymphocytes was performed as described previously\(^{(17)}\) on a FACStar plus (Becton Dickinson, Sunnyvale, CA). Mouse isotype-specific antibodies were obtained from Southern Biotechnology Inc., Birmingham, AL (for

| Table 1: Monoclonal antibodies* used to analyze circulating lymphocyte sub-populations in buffalo and N'Dama cattle following infection with *T. conglolense* |
|---------------------------------|---------|-----------------|
| **Monoclonal**                  | **Antigen** | **Cells detected** |
| IL-A11                          | CD4      | T-cell sub-population |
| IL-A15                          | CD1B     | Myeloid cells + some B cells |
| IL-A24                          |          | Myeloid cells |
| IL-A43                          | CD2      | Mature α/β T cells |
| IL-A58                          | Ig       | All B cells |
| CC15                            | WCI      | γδ T cell sub-population |
| CC17                            | CD5      | All T cells, some B cells |
| CC42                            | CD2      | Mature α/β T cells |
| CC58                            | CD8      | T cell sub-population |
| NAM4                            | CD11c    | Myeloid cells |

*Reviewed by Howard*

fluorescein-labelled reagents) or mersham, Bucks, U.K. (for biotinylated reagents). Phycocerythrin-conjugated streptavidin was obtained from Becton Dickinson.

**Results**

This work was accomplished in two separate experiments. In experiment 1, three buffaloes and three N.Dama cattle were infected with *T. conglolense* and observed for 140 days. Experiment 2 was carried out with *T. vivax* 12 months later using two of the buffaloes which were previously infected with *T. conglolense*, two naive buffaloes and four naive N'Dama cattle.

**Experiment 1 - *T. congoens* infection**

**Parasitaemia**

Trypanosomes were detected around the same time in the three N'Dama cattle: day 10 post-
Figure 1: Mean parasitaemia (fig 1A) and PCV (fig. 1B) in three buffaloes (triangles) and three N'Dama cattle (closed circles) following infection with *T. congolense* transmitted by *G. M. centralis*.
infection in ND12 and ND13 and day 11 in ND14. The prepatent period among individual buffaloes varied greatly, but was longer than in the cattle: day 15 post-infection in buffalo 7181, day 20 in 7448 and day 25 in 7240.

The mean parasitaemia profiles of N'Dama compared with buffalo are presented in figure 1A. There was little variation in the parasitaemia pattern between individual animals within the two species. On buffalo (7181) and one N'Dama (ND12) had occasionally higher parasitaemia counts than the rest of the group. The highest DG parasitemia index in buffalo 7181 was +4 (approximately 10^4 – 10^5 parasites per ml of blood) and was observed 21 days post-infection whereas in N'Dama it was +5 (approx. 5 x 10% parasites per ml of blood) and was observed 17 days post-infection. The mean parasitaemia index for buffalo was maintained at +2 (103 – 10^4 parasites per ml of blood) during the first 60 – 80 days of infection. From day 80 up to the end of the experimental period, both buffaloes and N'Dama maintained their parasitaemia levels below +2.

**Anaemia**

The packed cell volumes (PCV) for both animal species are summarised in figure 1B. In both species there was a drop in PCV values during the first 40 days after infection, thereafter, buffaloes maintained their PCV levels at pre-infection levels (35%), whereas N'Dama maintained their PCV at about 28% throughout the experimental of 140 days.

**Differential Cell Count**

A comparison of lymphocyte and neutrophil counts between buffalo and N'Dama is presented in Figure 2. There was a moderate increase in the number of neutrophils (neutrophilia) in buffalo 19 days post-infection.

![Figure 2: Means of percentages of differential cell counts for lymphocytes and neutrophils from 3 buffaloes (triangles) and three N'Dama cattle (closed circles) following infection with *T. congolense* transmitted by *G. m. centralis*.](image_url)
corresponding with an equal decrease in the number of lymphocytes (lymphocytopenia). This condition prevailed with moderate fluctuation until 53 days post-infection. By this time, both lymphocytes and neutrophil counts had settled at pre-infection levels. In the N'Dama the opposite occurred: a slight decrease in the number of neutrophils (neutropenia), 19 days post-infection, and a moderate increase in the number of lymphocytes (lymphocytosis) during the same period. By day 53, the lymphocyte counts were still higher than the pre-infection levels, whereas neutrophil counts were still lower. There was a slight increase in the number of monocytes (data not shown) in buffalo, but not in N'Dama.

Serology
The results of the neutralizing antibody test are summarized in Table 2. Both buffalo and N'Dama produced neutralizing antibodies to metacyclic trypanosomes. Neutralising antibodies to metacyclic trypanosomes were detected on day 14 in one N'Dama and on day 18 in the other two. In one buffalo (7448) neutralising antibodies first appeared on day 18, and in the other two on day 32.

T-cells
Flow cytometric analysis of buffalo lymphocyte sub-populations with MAbs defining T lymphocyte sub-sets revealed that there was significant decrease in the number of CD2, CD4 and CD8 positive cells (not shown). This decrease was observed on days 3 and 11 post-infection, and remained until 53 days post-infection when the lowest counts were recorded. After day 53, these sub-populations increased, but by day 103, when the last analysis was done, CD2 and CD4 T-cell numbers were still below the pre-infections levels whereas the numbers of CD8 T-cells had reached the pre-infection level. Other T-cell sub-populations, such as CD4+, CD8+ and CD2-CD8+ and others were analysed by two-colour flow cytometry, but their numbers were very low and did not change during infection.

B Cells
Flow cytometric analysis of B cells using MAbs ILA58 showed moderate variation among individual buffaloes. Analysis of the mean values during the course of infection showed a steady increase, reaching a high peak of 48% 30 days post-infection. The number of B cells expressing CD5 antigen was measured by two-colour immunofluorescence, using antibodies CC17 (CD5) and IL-A58 (Ig). There was no significant change in the number of B cells expressing CD5+ antigen in buffalo and remained below 4% in the course of this infection.

### Table 2: Detection of metacyclic neutralizing antibody in sera from buffalo and N’Dama cattle following infection with *T. congolense* by *G. m. centralis*

<table>
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<td>Buffalo</td>
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γδ T-Cells
Monoclonal antibody CC15 detects antigen WCI (9) which is present on most γδ T-cells in peripheral blood. There was a steady increase in this lymphocyte sub-population from a pre-infection level of 3.8% to a peak of 17%, 30 days post-infection. At the time of the last analysis, 103 days post-infection, the number of these cells was still high at 13%.

Unknown cell population
While measuring cell population by one or two-colour immunofluorescence, we observed in the buffalo the appearance of a population of cells which stained weakly for different lineage-restricted MAbs e.g. CD11b, CD11c, CD2 and CD4 MAbs (fig. 3), but not others like IL-A30. It was impossible to characterize this population from its antibody profile. Second step antibodies did not bind in a non-specific manner to these cells. This population made the calculation of the percentage of the different lymphocyte populations difficult. Very low numbers of this sub-population were present prior to infection and they increased from background levels, 0.5% at day 3 post-infection, to a mean of 12% on day 30, 26% on day 49 and 11% on day 103 post-infection. The number of cells fluctuated between individual buffaloes.

A comparison was made between the occurrence of this sub population in each individual animal and the parasitaemia waves. This comparison showed that this population tended to increase soon after the 1st parasitaemia peak and after subsequent peaks in each individual buffalo.

Experiment 2: T. vivax infection

Parasitaemia
The mean parasitaemia profiles of buffaloes and N'Dama cattle are presented in figure 4A. Parasites appeared in the peripheral blood 12 days post-infection of one buffalo (7181) and on day 14 in the other three. In N'dama, parasites appeared 9 days post-infection in two animals (ND15 and ND21) and on day 10 in the other two.

Buffaloes maintained their parasitaemia index between +2 and +4. The highest parasitaemia index of +5 was recorded in three N'Damas (ND15, ND17, ND21) 15 days post-infection.

A comparison between the mean parasitaemia profiles of the two groups shows that both species had high parasitaemia peak during the 2nd to 4th week post-infection. After 6 weeks of infection, parasite counts in both species declined. On day 49, all the animals were treated and released since both species had eliminated parasites from the peripheral blood circulation.

Anaemia
The mean PCV values for buffaloes and N'Damas are presented in figure 4B. For the first two weeks there were no major differences between the two species. After this period, the buffaloes maintained their PCV level above 30% throughout the experimental period whereas N'Damas PCV levels declined as the disease progressed. The N'Damas had their lowest mean PCV of 20% at four weeks post-infection but gradually recovered to above 25% at the end of experimental period.
Figure 4: Mean parasitaemia (A) and PCV (B) in four buffaloes (triangles) and four N’Dama cattle (closed circles) following infection with *T. vivax* transmitted by *G. m. centralis*.
Leukocyte Subpopulations

As in the *T. congoense* infection, an unidentified cell population appeared in the four buffaloes, but not in the N'Dama cattle. On one occasion, this population was purified by sorting in flow cytometry and large granular leukocytes were found to be present. However, the nature of the cells was difficult to establish without further characterization.

Discussion

This study compared buffaloes and N'Dama cattle in their ability to respond to *T. congoense* and *T. vivax* infections. The results show that although both groups effectively dealt with the *T. congoense* infection, buffalo controlled the disease better than N'Dama cattle. In the *T. vivax* experiment, buffalo controlled the infection fairly well whereas the N'Dama did not. The superiority of the buffalo was expressed at different levels. Firstly, the prepatent period in the buffalo was almost twice as long as in N'Dama cattle. This difference may be due to the presence of non-immune factors which may inhibit proliferation of metacyclic parasite forms in the skin of the buffalo. Secondly, the buffalo showed a superior ability to control parasitaemia and thirdly, it suffered less from anaemia.

In the *T. congoense* infection, parasitaemia levels in buffaloes were a hundred-fold lower than in N'Dama cattle between 20 to 80 post-infection. Two buffaloes (7240 and 7448) were significantly superior than N'Dama in their ability to control parasitaemia. However, one buffalo (7181) which developed diarrhoea from other causes than the trypanosome infection in the third week of infection, had the same parasitaemia as N'Dama cattle. This observation suggests that when buffaloes are stressed by disease other than physiological or environmental factors (starvation, drought) they may show similar clinical signs of trypansomiasis, such as a drop in PCV, like N'Dama cattle. The parasitaemia levels recorded in buffalo during this study were higher than those reported in buffalo in our previous studies\(^{(6,20)}\).

Differences in parasitaemia between N'Dama and buffalo were also observed in the *T. vivax* infection, but were not as large as in the *T. congoense* infection. Parasitaemia in this experiment were higher than those reported earlier\(^{(5)}\), and may be attributed to the different strains of *T. vivax* as in both studies.

The PCV during the *T. congoense* infection decreased in both species up to day 20 post-infection. After that period, buffalo recovered while the N'Dama kept deteriorating. Neither buffalo nor N'Dama suffered from severe anaemia (PCV below 20%). This difference was even more pronounced in the *T. vivax* infection. The N'Damas suffered from severe anaemia 4 weeks post-infection, while buffalo had normal PCV levels. Furthermore, one of the N'Damas had a very low PCV of 17% and had to be treated to save its life. This observation suggests that N'Dama cattle may be less resistant to East African strains of *T. vivax* than to West African strains\(^{(1,2,25)}\). Additional field studies in N'Dama in East Africa require to elucidate on this observation.

Since neutralizing metacyclic antibodies appeared much later in Boran cattle than in buffaloes, it was speculated that they might contribute to the higher resistance of the buffalo to the disease\(^{(6,20)}\). However, in our experiments we could not correlate the kinetics of neutralizing antibodies with the greater resistance of the buffalo, since they appeared even earlier in the N'Dama than in the buffalo group. Their contribution to resistance cannot be ruled out completely, as their levels and affinities were not followed during the infections.

Absolute neutrophil counts were higher in buffalo than N'Dama and during infection, their numbers increased in buffalo, but decreased in the N'Dama. Also monocytes were slightly increased in buffaloes. Neutrophils and monocytes have an important role in clearing parasites from the system by phagocytosis\(^{(12)}\), and could be one of the reasons for the lower parasitaemia in buffalo.

When monitoring lymphocyte sub-populations in buffalo and N'Dama, changes were noted as observed before in cattle\(^{(5,26)}\): levels of CD2+T-cells drop, while the levels of B and γδ
T-cells increase. Such changes do not seem to be correlated with resistance to trypanosomiasis in N'Dama or in buffalo.

In buffaloes, a population of cells which co-purified with the lymphoid cells but of unknown origin appeared in the course of these experimental infections. These cells were recognized by their weak staining with some lineage-restricted antibodies, but the staining patterns were different compared to those of known lymphoid and myeloid lineage cells. On one occasion, this population was sorted by flow cytometry and analysed after Giemsma staining. They contained large granular lymphocytes, but most of the cells were not identified on the basis or morphology. This population of cells did not appear in infected N'Dama. Their numbers increased soon after the drop in PCV levels in each individual buffalo. It is possible that they are immature erythroid cells that appear in the blood before they complete their maturation, in response to the developing anaemia. Characterization of this cell population may give us an indication on the differences between the responses of buffaloes and cattle.

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References


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PASSIVE SENSITIZATION OF RAT MAST CELLS WITH SERUM FROM CATTLE SENSITISED TO BITES OF RHIPICEPHALUS APPENDICULATUS

M.G. BINTA*, E.A. MUSHI AND F. RURANGIRWA
International Centre for Insect Physiology and Ecology,
P.O. Box 30772, Nairobi, Kenya.

SENSIBILISATION PASSIVE DES MASTOCYTES DE RAT AVEC DU SERUM DE BOVINS RENDUS SENSIBLES AUX PIQURES DE RHIPICEPHALUS APPENDICULATUS

Résumé
Les mastocytes pétonéaux du rat ont été passivement sensibilisés avec du sérum de bovins exposés aux piqûres de Rhipicephalus appendiculatus et avec du sérum de bovins indemnes de tiques. On a noté l'ampleur de la dégranulation suite à l'application d'un antigène spécifique préparé avec des larves de tique.

La dégranulation des mastocytes était plus accentuée avec les sérum des bouvillons rendus sensibles aux tiques qu'avec les sérum de bouvillons exempts de tiques. Le potentiel relatif de dégranulation du sérum d'un bouvillon reconnu auparavant comme étant très résistant aux tiques était de 100 % à une dilution du sérum 0 jusqu'à 10^5. Le potentiel de dégranulation des mastocytes de rat était attribué aux anticorps hétérophiles, très probablement IgG1. Les changements morphologiques dus à l'antigène extrait des tiques étaient plus accentués chez les mastocytes de rats rendus passivement sensibles par le sérum des bouvillons vulnérables auparavant aux piqûres des tiques.

Summary
Rat peritoneal mast cells were passively sensitized with serum from cattle exposed to the bites of Rhipicephalus appendiculatus and from tick-naive cattle. The extent of degranulation following application of a specific antigen prepared from tick larvae was recorded.

Mast cell degranulation was higher with the serum from steers sensitised to ticks than serum from tick-naive steers. The relative degranulating potential of the serum from a steer previously shown to be highly resistant to ticks was 100% at 10^-1 through to 10^-5 serum dilution. The degranulating potential of the rat mast cells was attributed to heterophile antibodies, most likely IgG1. The morphological changes induced by the tick extract antigen were more marked in the rat mast cells passively sensitised by serum from steers previously sensitised to tick bites.

Introduction
Immediate hypersensitivity reaction type 16 has been observed by the authors when cattle previously exposed to ticks were introduced in paddocks infested with R. appendiculatus. Involvement of reaginic antibodies in this immunological phenomenon in cattle 17 and rabbits 14 has been alluded to previously.

Although IgG1 in rabbits has been implicated, the nature of this antibody in cattle is unknown. Both bovine IgG1, and IgG2, mediate passive cutaneous anaphylaxis in cattle 11. The existence of a bovine reaginic antibody has been documented 5, 7, 8, 12, 16. Furthermore, cross reactivity between bovine IgE and human IgE has been demonstrated 17, 12.

There is need to develop a test to screen for acquired resistance to tick bites. The rat mast cell degranulation technique initially described by Nielsen et al 13 which relies on the passive sensitisation of the rat mast cells with bovine serum was adopted. In the same study, an attempt was made to demonstrate the possible involvement of cytotoxic antibodies in this reaction which stimulates the immediate hypersensitivity reaction associated with tick bite.
Materials and Methods

Animals
A total of 9 pure-bred *Bos taurus* steers were used in the study. These were divided into 3 arbitrary groups, namely, A, B and C.

Three steers assigned to group A were naturally sensitised by multiple exposure to the larvae of *R. appendiculatus* for a period of two weeks following a described procedure. Assessment of their tick resistant status was done using an intradermal test and feeding 100 nymphae on the ears of the steers as described before. One of these three steers was an Ayrshire (steer 49). The criteria for tick resistant steers was the positive skin test and recovery of a low percentage of poorly fed replete nymphae. These three steers, especially steer 49, characteristically manifested cutaneous hypersensitivity reactions to larvae when released into tick infested paddocks. Group B steers previously infested with 1,000 larvae of *R. appendiculatus* on their ears as described before for rabbits, were used. The ear bag was stuck on the ear with a non-irritant and non-toxic adhesive glue.

Cattle in Group B were allowed a relatively higher percentage of ticks to feed on them than steers in group A by feeding 100 nymphae on the ears as described for the rabbit hosts. In addition, they elicited only a slight cutaneous hypersensitivity reaction to the bites of larval ticks.

Group C steers were used as controls. These had been reared on a tick-free farm and were therefore considered to be tick-naive. They were negative on the skin test and allowed at least 95% of the nymphal ticks to feed to repletion.

Collection of serum
All of the 9 steers were bled for serum. The 6 test steers were bled 1 week after the larval tick infestation and 24 hours after the 100 nymphal test. The serum of individual animals thus collected was stored separately at -20°C in aliquots of 1 ml, until ready for use. There was no pre-infestation serum from Group A steers.

Serum treatments
Aliquots of undiluted serum from cattle groups A, B and C, whole serum from steer 49 diluted in Hank’s Balanced Salt Solution (HBSS) at a dilution of 10⁻⁶ were used.

One microliter of serum from the steers in groups A, B and C were each treated with an equal volume of various rabbit anti-bovine immunoglobulin fractions. These were as follows: rabbit anti-bovine – whole globulin, rabbit anti IgG₁, rabbit anti IgG₂, rabbit anti IgM (Miles Laboratories, New York, USA).

Treatment of serum with mercaptoethanol
Two millilitres of serum from steer 49 (Group A) were mixed with an equal volume of 0.1% 2-mercaptoethanol.

Heat treatment
Serum from steer 49 (Group A) was heated at 56°C for 2 hours.

Treatment of serum with ammonium Sulphate
Ten millilitres of serum from steer 49 and a steer from Group C were each precipitated with a 33% saturated Ammonium sulphate at 4°C. The precipitate from each serum sample was reconstituted with 2ml Hank’s Balanced Salt solution.

Rat mast cell degranulation test
Rings of 1 cm in diameter were marked on a microscope slide for use in the modified version of the test previously described. The technique for preparing and enriching the rat mast cell fraction has been cited in literature. Washed rat mast cells were counted in a haemocytometer and the number adjusted to 10⁶ cells.

Suspensions of the mast cells were put on to the microscope slides in large enough quantities to cover 1 cm diameter ring drawn on the slide. The mast cells were thus made to adhere to the slides by incubating the slides at 37°C for 15 minutes instead of 1 hour at 37°C as done by Nielsen et al. in a humid chamber. This was considered optimal for the reaction. The slides
were then washed with HBSS. The slides bearing the mast cells each had one of the serum treatments applied to them.

These preparations were applied in aliquots of 50 µl which was enough to cover the cells. The slides were then incubated in humid chambers at 37°C for 5 minutes. The slides were gently washed with HBSS and then stained either with 1% toluidine blue or 10% (w/v) Giemsa4 and then examined microscopically for degranulation.

The larval tick allergen was prepared as previously described by Blinta et al and the protein concentration determined after the method of Lowry et al.10 A checker board titration of the larval tick allergen serially diluted in HBSS, 10⁰ through 10⁻⁶ dilution was carried out against serum (10⁰–10⁻⁸) from steer 49 only. Equal quantities of the allergen and the serially diluted serum were all used in the degranulation test. Polymyxin B sulphate (6.51 U/ml) in 1 ml quantities was applied to control slides of fixed mast cells at 37°C for 5 minutes.

This was to provide the 100% degranulation control. The assessment of the percentage degranulation of individual mast cells in any slide preparation was achieved by examining the slides under the microscope using the (x 40) magnification (Leitz, Germany).

Three representative fields were examined and the average degranulation determined.

**Result**

When polymyxin B sulphate was incubated with rat mast cells fixed on slides and then examined microscopically, there was 100% degranulation of all mast cells. This type of degranulation was complete and was characterized by total extrusion of the intracellular granules into the surrounding medium.

When the larval tick extract was applied to the mast cells together with bovine serum, two types of morphological changes occurred in the mast cells. Firstly, the use of undiluted serum from Group A steers resulted in complete disruption of the cell membrane and extrusion of the granules into the medium. The intensity of this reaction was comparable to the degranulation that occurred with polymyxin B sulphate.

Secondly, when serum from group B steers was used, there was disorganization of the granules within the cells. The granules moved to the periphery of the cell without being extruded.

Serum from steer 49 of group A gave 100% degranulation from 10⁰ through to 10⁻⁵ dilution of serum using undiluted antigen. At a serum dilution of 10⁻⁸, degranulation was reduced by 1%. Between 10⁻⁷ and 10⁻⁹ serum dilutions, the degranulation was reduced by 10%. In contrast, serum from Group C steers induced only 1% degranulation of the mast cells.

Heat treatment of undiluted serum for 2

<table>
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**Table 1**: The degranulating potential of tick-resistant bovine serum, Hanks balanced salt solution, and the tick extract on the mast cells.
hours or its treatment with 2-mercaptoethanol induced only a 1% degranulation of mast cells in the presence of the tick tissue antigen. The mast cells which were treated with serum from tick-naive animals did not induce any degranulation.

Treatment of serum from a highly resistant steer with either anti-bovine IgG, or rabbit anti-whole bovine gamma globulin reduced the degranulating potential of the test serum by 99%. However, when whole bovine serum from steer 49 was treated with rabbit anti-bovine IgG₂, rabbit anti-bovine IgM or rabbit anti-bovine IgA separately, this had negligible visible effects on the integrity of the mast cells. Bovine IgE was not available for use in this experiment.

**Discussion**

Serum from the three groups of cattle of varying degrees of exposure to the tick *R. appendiculatus* were used. Using the previously established criteria, the three groups of cattle were categorized as: highly tick resistant (group A), moderately tick-resistant (B), and tick-naive (group C). The serum samples from these three groups of cattle were used in the mast cell degranulation technique. This technique relies on the passive sensitization of rat mast cells with bovine serum in the presence of a specific antigen. In this study, the serum from group A steers (highly tick-resistant) induced maximum (100%) degranulation of mast cells in the presence of the larval tick allergen. In contrast, no mast cell degranulation was induced by serum from tick-naive steers.

Failure of the serum from steers with minimal tick exposure (group B) to induce significant degranulation could probably be attributed to the absence of certain humoral factors in their serum. These factors could well have been immunoglobulins. The possible involvement of the latter in this phenomenon was confirmed by the abrogation of the mast cell degranulation response using anti-serum prepared against specific bovine immunoglobulins.

Heat treatment of the serum and its chemical reduction with mercaptoethanol which normally eliminates activity due to IgM and IgE but not the IgG class antibodies did not interfere with the degranulating potential of the serum. These two treatments are known to disrupt the disulphide bonds in the IgM molecule thus inactivating it.

The degranulating activity of this inactivated serum could have been attributed to an IgG class of antibody, most likely IgG₁. The involvement of the latter was confirmed by the observation that treatment with anti-IgG₁ and IgG₂ separately reduced the degranulating potential of the serum.

The exclusion of IgM from this reaction was confirmed by the failure to induce degranulation using immunoglobulin M and also serum treated with anti-IgM.

There may have been a possibility of IgE playing a role in this reaction. Unfortunately, bovine IgE and anti-IgE were not used in this study. Since the mast cells degranulation reaction in this study simulates this *in vivo* allergic reaction, the possibility of IgE involvement was not very remote. The involvement of IgE in the cutaneous allergic reaction in tick resistant cattle has previously been described. However, in this study, the heterophil antibodies of IgG subclass as opposed to reagin were implicated in the rat mast cell degranulation reaction.

There is a strong possibility that the rat mast cell degranulation technique used in this study could be used as one of the tests for screening tick-sensitized cattle in tick control programmes and experiments.

**Acknowledgement**

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**Reference**

Passive sensitization of Rat Mast Cells with Serum from Cattle Sensitized to bites of Rhipicephalus appendiculatus 37


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PLANT POISONING CASES AMONG LIVESTOCK IN BOTSWANA, 1982–1991

BINTA M.G.; ADOM E.K.; DITEKO, T.* AND MUSHI E.Z.**
National Veterinary Laboratory, Private Bag 0035, Gaborone, Botswana

CAS D’INTOXICATION VEGETALE CHEZ LE BETAIL AU BOTSWANA: 1982–1991

Résumé
Au total, on a enregistré 182 cas d’intoxication végétale chez le bétail domestique et la faune au Laboratoire national vétérinaire pendant la période 1982–1991. Le plus grand nombre de cas d’intoxication végétale (68%) s’est produit entre 1984–1986. L’incidence moyenne annuelle était de 18,2 ± 2,9 avec la plupart des cas survenus durant les années de sécheresse 1982–1987. L’incidence saisonnière a montré que la majorité des cas se sont produits entre mai et septembre. Le taux de répartition des trois principales espèces de plantes concernées était comme suit: Pavetta harboui (49,7%), Urigina sanguinea (15,3%) et Dichapetulum cymosum (10,4%). Les autres espèces de plante représentaient chacune moins de 2%. Des espèces de plantes non-identifiées constituaient pour 20,2% de l’ensemble des cas enregistrés.
Il faudrait, par conséquent, entreprendre d’autres études pour déterminer les principes actifs des espèces de plantes non-identifiées. Par ailleurs, il est impératif d’élaborer un programme rigoureux d’éradication des plantes toxiques dans tout le pays pour réduire les pertes de bétail.

Summary
A total of 182 outbreaks of plant poisoning involving livestock and wildlife were recorded at the National Veterinary Laboratory from 1982–1991. The highest number of plant poisoning cases (68%) occurred between 1984–1986. The mean annual incidence was 18.2 ± 2.9 with most of the cases occurring during the drought years 1982–1987. The seasonal incidence showed that the majority of the cases occurred between May and September. The percentage distribution of the three main implicated plant species was as follows: Pavetta harboui (49.7%), Urigina sanguinea (15.3%) and Dichapetulum cymosum (10.4%). The other plant species contributed less than 2% each. Unidentified plant species accounted for as much as 20.2% of the total outbreaks.
Consequently, further studies are warranted to determine the active principles in the unidentified plant species. Furthermore, it is imperative to establish a stringent poisonous plant eradication programme countrywide to curb stock losses.

Introduction
Although most of Botswana is semi-arid and desert especially in the south west of the Country, the terrain still sustains several potentially toxic plants.

These toxic plants are responsible for high mortalities in domestic and wild ungulates. Pavetta harboui has been proved to be toxic to cattle. Its toxicity was similar to the better known plant Pachystigma pygmæum widely occurring in Southern Africa. Both plants are implicated in the “sudden death” syndrome known as “gousiekte” (Afrikaans). The syndrome is ingestion of sufficient quantities of the plant.

The pathognomonic histological lesion of progressive myocarditis with subsequent fibrosis has also been demonstrated. Rutin, a flavon cardiac glycoside and other principles such as ursolic acid are also suspected.

Dichapetulum cymosum is widely distributed in the sandveld in Botswana. The young leaves have been shown to be more toxic than the mature ones. The active principle has been identified as monofluoroacetate. Other fluorine compounds with questionable toxicity have been identified in the leaves as w-fluorooleic and w-fluoropalmitic acid. The
monofluoroacetate interferes with Kreb's cycle, mainly with the enzyme aconitase hydratase culminating in cardio-pulmonary embarrassment\textsuperscript{10,18}. The latter is exacerbated by the animal drinking water. \textit{Urginea sanguinea} like other tulips such as \textit{Homeria glauca} are associated with a syndrome referred to as "slangkop" (Afrikaans) characterized by severe diarrhoea, dehydration and occasional bloat\textsuperscript{17,22,24}. A cardiac glycoside has been extracted from the flowering heads which are often implicated in intoxications\textsuperscript{22}. \textit{Crotalaria burkeana} belonging to the family leguminosae has also been implicated in this study. Crotalariosis has been described before in Southern Africa\textsuperscript{6}. Alkaloids\textsuperscript{1} and ursolic acid\textsuperscript{4} were among the possible toxic agents isolated from \textit{crotalaria} species.

The present study also mentions \textit{Sorghum vulgare} as one of the causes of plant toxicity. \textit{Sorghum} species have been known to contain a cyanogenetic glycoside, dhurrin\textsuperscript{2,9,11}. Upon ruminal hydrolysis, hydrocyanic acid a potent cellular asphyxiant is released\textsuperscript{9}. \textit{Sorghum} species are also known to contain toxic quantities of nitrates and nitrites\textsuperscript{3,5,12}.

\textit{Amaranthus retroflexus}, abundant in the rainy season in Botswana, has been associated with nitrate and nitrite poisoning\textsuperscript{9}. This paper discusses the epidemiology of plant poisoning in the ten-years period 1982–1991. The inciting factors and current control strategies are discussed.

### Materials and Methods

Whole plant specimens, leaves from suspected plants and ruminal contents from poisoned animals were submitted to the National Veterinary Laboratory for identification.

Also, pieces of tissues, in particular the heart, collected from poisoned animals were submitted to the laboratory fixed in formal saline for histopathological studies.

### Results

The results are summarized in Table 1 and Figure 1. Most of the cases of poisoning occurred in 1984 (25.7\%). The annual mean incidence was 18.2±12.9. No plant poisoning outbreaks were reported in 1989. \textit{Pavetta harbori} was implicated in 49.7\% of the outbreaks for the ten-year period. This species of toxic plants constituted 53.2\% of the total number of outbreaks for the year 1984. On the other hand, \textit{Urginea sanguinea}, \textit{Dichapetulum cymosum} and \textit{Sorghum vulgare} were responsible for 15.3\%, 10.4\% and 11.6\% of the total number for the ten years respectively. The other species of plants namely \textit{Amaranthus retroflexus}, \textit{cestrum species}, \textit{Homeria glauca}, \textit{Crotalaria burkeana} and \textit{Datura stramonium} each accounted for 0.6\% of the outbreaks for the ten-year period. Outbreaks due to \textit{Pavetta harbor} and \textit{Dichapetulum cymosum} were

<table>
<thead>
<tr>
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<th></th>
<th></th>
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<tbody>
<tr>
<td>Paveta harbori</td>
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<td>12</td>
<td>25</td>
<td>16</td>
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<td>4</td>
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<td>0</td>
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<td>0</td>
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<tr>
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<td>1</td>
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<td>0</td>
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<td>0</td>
<td>3</td>
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<td>0</td>
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<td>0</td>
<td>0</td>
<td>1</td>
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<tr>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
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</tr>
<tr>
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<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
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<td></td>
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<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.6</td>
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<tr>
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<td>0</td>
<td>0</td>
<td>8</td>
<td>8</td>
<td>7</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>12</td>
<td>37</td>
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</tr>
<tr>
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<td>12</td>
<td>18</td>
<td>47</td>
<td>19</td>
<td>31</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>13</td>
<td>23</td>
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<td></td>
</tr>
<tr>
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<td>6.6</td>
<td>9.8</td>
<td>25.7</td>
<td>10.4</td>
<td>16.9</td>
<td>5.5</td>
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<td>7.1</td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>
confined to the sandveld. *Urginea* species and other bulbs being rampant in the southern part of the country especially, Kanye District, were implicated in the plant poisoning outbreaks there. A moderately high percentage (20.2%) of the outbreaks were attributed to unidentified toxic plants. Most of the poisoning cases occurred between May and September. Histopathological examination of the heart muscle consistently showed a myocarditis with fibrosis in cases due to *Pavetta harborii*.

**Figure 1:** Plant poisoning cases among livestock
Discussion
During the ten-year period, 1982–1991, 182 outbreaks of plant poisoning were reported in Botswana. The highest number of confirmed outbreaks was in 1984, half of which were caused by Pavetta harborii. The other outstanding plants implicated in these outbreaks were Urginea sanguinea and Dichapetalum cymosum. The most probable explanation for this could be severe drought experienced in the country between 1983 and 1987. The scanty rainfall resulted in poor pastures low in essential nutrients. The situation aggravated by lack of amply supplementary feeding forced the animals to eat poisonous plants such as Pavetta harborii and Dichapetalum cymosum which have a well developed rooting system while Urginea sanguinea and the other tulips are bulbous thus storing food for long periods.

These adaptations ensure their survival and perpetuation even under severe drought which periodically threatens the country. Moreover, Pavetta harborii and Dichapetalum cymosum are perennials which thrive well in the sandveld, an area which extends into the Kalahari desert where normal vegetation is substituted by sand. The other reason which could account for these plant poisoning outbreaks is the malpractice of deliberate setting of veld fires. This is often done during drought or in early spring (July–November) to stimulate the growth of palatable plants. Unfortunately, it is often the toxic plants such as the Urginea species with stored food reserves or those with a well established rooting system such as the Pavetta and Dichapetalum species which sprout first. This makes the animals more vulnerable to poisoning. A common observation in Botswana and Southern Africa is that whenever land is laid bare either by veld fires of drought, it is poisonous plants which flourish soon after the first rains. Another contributing factor is the unrestricted movement of livestock and wildlife migration in search of better grazing and water. Cattle are also often trekked to slaughter houses. High cattle mortalities have occurred as the animals cannot instinctively avoid eating unfamiliar poisonous plants flourishing in another terrain.

Under certain circumstances, poisonous plants such as Dichapetalum cymosum and Datura stramonium blend so well with innocuous grass species that animals inadvertently eat them indiscriminately. Normally the repulsive smell of Datura stramonium would scare animals away.

The ease with which Dichapetalum cymosum lends itself to eradication techniques\(^{15}\) has resulted in fewer outbreaks than either Pavetta harborii or Urginea sanguinea. The complex rooting system of Pavetta harborii makes herbicide application ineffective. The tulips on the other hand are bulbous and uprooting off the heads is a labour-intensive exercise.

The high ambient temperatures coupled with the cold frosty nights often damage the young sorghum crop and some cyanogen species of grass known to contain cyanogenic glycosides. The outbreaks associated with hydrocyanic acid were caused by young wilted, stock trampled or frost damage Sorghum vulgare. The leaves were more toxic than the stalk.

In order to curb stock losses caused by poisonous plants, it is suggested that proper identification of the toxic plant species and their active principles must be done. Secondly, fencing off tracts of land in which these plants are growing could be helpful. Of Paramount importance is the public awareness on these dangers of veld fires.

Supplementation of stock during drought and winter as well as the use of herbicides to rout out the noxious plants should be done. Awareness on palliative treatment to be instituted in clinical cases could save lives.

Acknowledgement

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References

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THE POSSIBILITY OF MAKING SILAGE FROM "WILD SORGHUM" (SORGHUM ARUNDINACEUM)

J.E. FLEISCHER AND A.M. TACKIE
Department of Animal Science, University of Ghana, P.O. Box 226, Legon, Accra, Ghana

POSSIBILITE DE FAIRE DE L'ENSIAGE AVEC DU "SORGHO A CROISSANCE SPONTANEE" (SORGHUM ARUNDINACEUM)

Résumé

Une expérience a été conduite pour étudier la composition chimique et la valeur nutritive de l'ensilage préparé avec le sorgo à croissance spontanée traité ou non-traité, et en combinaison avec Leucaena leucocephala. "Le sorgo à croissance spontanée" (Sorghum arundinaceum) récolté dix semaines après que les pousses nouvelles sont apparues, a été utilisé pour l'expérience. Après la récolte, la masse de sorgo a été coupée en morceaux de 2 - 3 cm environ, puis on a mis un kilogramme de ces morceaux dans des sacs en polyéthylène que l'on a fermés par la suite. Il y avait quatre traitements présentés comme suit: (i) le sorgo à croissance spontanée (Sorghum arundinaceum: SA) non traité (T1); (ii) "SA" séché au soleil pendant six heures (T2); (iii) "SA" traité pour contenir 0.5% d'acide formique (T3) et (iv) "SA" en combinaison avec Leucaena leucocephala dans la proportion de 7 contre 3 (T4). La masse de sorgo a été stockée pendant 28, 56, 84 et 112 jours puis soumise à une analyse chimique et à une analyse de digestibilité de la matière sèche in vitro

La teneur en matière sèche a baissé dans tous les traitements avec une période d'ensilage avancée. Le mélange avec l'acide formique a considérablement réduit (P < 0,05) le pH et l'azote ammoniacal, mais il a augmenté (P < 0,05) les acides organiques totaux par rapport aux autres traitements. L'hydrogène cyanuré (HCN) a beaucoup diminué (P < 0,01) avec la période d'ensilage avancée. Le séchage au soleil (T2) ainsi que la combinaison avec Leucaena leucocephala (T4) ont réduit la teneur en HCN.

Il a été conclu que "le sorgo à croissance spontanée" peut être ensillé et que les caractéristiques d'ensilage sont améliorées soit par le séchage, soit par l'addition d'acide formique. La valeur nutritive du "SA" est améliorée par l'addition de Leucaena leucocephala.

Abstract

An experiment was conducted to study the chemical composition and nutritive value of silage made from "wild sorghum" untreated or treated as well as combined with leucaena. "Wild Sorghum" (Sorghum arundinaceum) harvested after ten weeks regrowth was used. The plant material after harvest was chopped to about 2 – 3cm long and one kilogramme of that was stuffed into double-lined polythene sacks. There were four treatments as follows: (i) untreated "wild sorghum" (T1), (ii) "Wild sorghum" wilted in the sun for six hours (T2), (iii) "Wild sorghum" treated to contain 0.5% formic acid (T3), (iv) "Wild Sorghum" combined with leucaena in the ratio of 7:3 (T4). The material was stored for 28, 56, 84 and 112 days after which it was analysed chemically and for digestibility of dry matter in vitro (IVDMD).

Dry matter content decreased in all treatment with advancing period. Mixing with formic acid significantly lowered (P < 0.05) pH and ammonia nitrogen but increased (P < 0.05) total organic acids content compared to the other treatments. HCN significantly decreased (P < 0.01) with advancing ensiling period. Sun-drying (T2) and also combination with leucaena (T4) both decreased the HCN content.

It was concluded that the "wild sorghum" can be ensiled and that the ensiling characteristics are improved by either wilting or addition of formic acid. The nutritive value of the "wild sorghum" is improved by the addition of leucaena.
Introduction

The productivity of ruminant livestock in Ghana largely depends on the natural grassland for their complete nourishment. The productivity and quality of the natural grasslands are described elsewhere. During the dry season cattle are said to lose as much as 11% of their body weight. Thus, attempts are being made to overcome the dry season feeding problem by the use of crop residues and agro-industrial by-products or by the use of silage.

The “Wild Sorghum” (Sorghum arundinaceum) which belongs to the sorghum family has the potential of being used as silage material. Initial work carried out with the “wild sorghum” has shown that the dry matter (DM) yield is about 10.0 t DM ha⁻¹ over a growth period of 10–12 weeks. This is enough to satisfy the DM requirement of nine livestock units (300 kg live weight) over a period of five months. It has also been found that its low crude protein (6% DM) can be overcome by combining it with leucaena (Leucaena leucocephala de Witt).

The objective of this study was therefore to evaluate the chemical composition and nutritive value of silage made from “wild sorghum” untreated and also when combined with leucaena.

Materials and Methods

The experiment was carried out between January and December, 1990. “Wild Sorghum” (Sorghum arundinaceum) cutting were used to establish the field, and leucaena (Leucaena Leucocephala de Witt) was collected from a naturalized plot that has existed for over twenty (20) years and is slashed about thrice in a year. The land preparation and management has been described elsewhere.

The herbage material was harvested by hand with a cutlass at about 5 cm above ground level. Leucaena was also harvested with a cutlass and where necessary combined with the “wild sorghum”. Both grass and legume were chopped to 2–3 cm length using cutlass.

Silage Preparation

One polythene sack of thickness 0.15 mm was placed into another and was used as a silo. Each of these silos contained one kilogramme of the silage material. There were four treatments of the “wild sorghum” before ensiling. These include the following:

i) Untreated “Wild sorghum” (T1)
ii) “Wild sorghum” wilted in the sun for six hours (T2)
iii) “Wild sorghum” treated to contain 0.5% formic acid by weight (T3)
iv) “Wild sorghum” combined with leucaena in the ratio of 7:3 (T4).

These silos were tightly sealed and placed in a drum for storage. The silos were opened after 28, 56, 84 and 112 days of storage. Thus the design was a 4 x 4 factorial design in two replicates.

On opening a silo, samples were divided into two portions. One portion was stored in a freezer. The other portion was then dried in the conventional oven at 70°C for 48 hours for dry matter determination. It was then ground through a 1 mm sieve with a Wiley mill and stored until analyses.

Analyses

The ground samples were used for neutral detergent fibre (ADF), cellulose and acid detergent lignin (ADL) determination according to the method of Goering and Van Soest and in vitro dry matter digestibility according to the method of Minson and McLeod.

Some of the fresh samples were used for analyses of crude protein (CP) and hydrogen cyanide (HCN) according to the methods outlined by A.O.A.C., for pH and also total organic acids (TOA) and volatile basic ammonia (VBN) according to Makham.

The results were analysed by a two-way analysis of variance and difference were separated by the Duncan’s multiple range test.
Results

The chemical composition of the forage materials before ensiling are shown in Table 1. "Wild sorghum" had significantly (P < 0.05) lower crude protein content and IVDMD, but higher cell wall constituents compared to those of leucaena. Consequent upon these differences the combined product of "wild sorghum" also had a relatively high value of HCN which was reduced to almost 70% by combining with leucaena.

The characteristics of silage as influenced by ensiling period and treatment is shown in Table 2. Even though dry matter content decreased with advancing ensiling period in all silages except the formic acid treated silage i.e., T2, these decreases were not significant (P > 0.05). Wilted significantly (P < 0.05) increased the dry matter content, but no significant differences (P > 0.05) were observed among other treatments.

**Table 1:** Chemical composition of "wild sorghum", leucaena and their combination prior to ensiling (DMB)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dry Matter</th>
<th>Crude Protein</th>
<th>NDF (%)</th>
<th>ADF</th>
<th>Cellulose ADL</th>
<th>IVDMD</th>
<th>HCN mg kg⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;Wild Sorghum&quot;</td>
<td>24.41a 2.61a</td>
<td>77.76a</td>
<td>47.41a</td>
<td>36.27a</td>
<td>6.97a</td>
<td>47.17a</td>
<td>354.8</td>
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<tr>
<td>Leucaena Leucocephala</td>
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<td>37.67b</td>
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<td>14.46b</td>
<td>5.67b</td>
<td>66.24b</td>
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<tr>
<td>&quot;Wild Sorghum&quot; Leucaena Combination</td>
<td>26.03ab 24.01ab 66.89ab</td>
<td>33.47ab 25.81ab 6.21ab</td>
<td>59.55ab 252.00</td>
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<td></td>
</tr>
</tbody>
</table>

1. NDF = Neutral detergent fibre; ADF = Acid detergent fibre; ADL = Acid detergent lignin; IVDMD = In vitro dry matter digestibility; HCN = Hydrogen cyanide
2. Figures in the same column with different letters are significantly different (P < 0.05)

**Table 2:** "Wild Sorghum" Silage Characteristics as Influenced by Ensiling Period and Treatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Days of Ensiling</th>
<th>Dry Matter (%)</th>
<th>pH</th>
<th>Total organic Acid (mg)</th>
<th>NH₄ N (mg)</th>
<th>Hydrogen Cyanide (mg)</th>
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</thead>
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<tr>
<td>Untreated</td>
<td>282</td>
<td>24.01a</td>
<td>4.96a</td>
<td>5.21</td>
<td>106.06ab¹</td>
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<tr>
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<td>56</td>
<td>23.09a</td>
<td>4.85a</td>
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<tr>
<td>84</td>
<td>23.12a</td>
<td>5.08a</td>
<td>13.1a²</td>
<td>129.87ab¹</td>
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<tr>
<td>112</td>
<td>20.96a</td>
<td>5.18a</td>
<td>17.8a³</td>
<td>133.00ab²</td>
<td>250.2a²</td>
<td></td>
</tr>
<tr>
<td>Sorghum with fomric acid</td>
<td>56</td>
<td>23.96a</td>
<td>4.24b</td>
<td>11.2b¹</td>
<td>84.10a¹</td>
<td>297.4a¹</td>
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<tr>
<td>forming 0.5%</td>
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<td>23.45a</td>
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<td>19.1b³</td>
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<td>Sorghum wilted in the sun for 6 hours</td>
<td>28</td>
<td>38.82b</td>
<td>5.00a</td>
<td>22.4b³</td>
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<td>14.4a²</td>
<td>152.47b³</td>
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</tr>
<tr>
<td>Sorghum combined with leucaena in a ratio of 7:3</td>
<td>28</td>
<td>24.24a</td>
<td>5.08a</td>
<td>18.0a³</td>
<td>199.35c¹</td>
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<tr>
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<td>5.23a</td>
<td>14.0a³</td>
<td>282.24c³</td>
<td>98.8c⁴</td>
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</tr>
</tbody>
</table>

LSD 4.98 0.57 2.53 24.77 17.58

1. Figures in the same column under similar periods but with different superscript show significant treatment differences (P < 0.05).
2. Figures in the same column in the same treatment block but with different superscript figures show significant periodic differences (P < 0.05)
Even though pH of silage rose slightly with increasing ensiling period, no significant difference (P>0.05) was observed. Addition of formic acid significantly (P<0.05) decreased the pH compared to the others. There was however, no significant difference between the other treatments which were generally high, being almost 5.0 or above.

Total organic acids significantly increased (P<0.01) with advancing ensiling period. Formic acid treated “wild sorghum” (T2) had the highest content of total organic acid and this was significantly different (P<0.05) from the other treatments. There was however, no significant difference (P>0.05) in the content of total organic acids among T1, T3 and T4.

Ammonia nitrogen content significantly increased (P<0.01) as the storing period increased. The significant increase was evident up to eighty-four (84) days after which it either remained the same or increased slightly depending on the treatment. T4 gave the highest NH2-N level and this was significantly different (P<0.01) from that of T2 but not T1.

The HCN content significantly decreased (P<0.01) with increased ensiling period. Compared to the unensiled, the HCN content of the silage reduced by between 50% and 70% by the 12th day. T4 gave the lowest HCN content which was significantly lower (P<0.01) than those of the other treatments. Even though T2 had HCN level lower than those of T1 and T3 at all times, no significant difference (P<0.05) was found among them.

The chemical composition and in vitro dry matter digestibility of silage as influenced by ensiling period and treatment is shown in Table 3. For crude protein, no significant differences (P>0.05) were observed among the ensiling period. However, significant differences (P<0.01) were established among treatments. T4 had the highest crude protein contents which was significantly different (P<0.01) from all others. On the contrary, even though T2 had the lowest crude protein content, it was not significantly different (P>0.05) from either of those of T1 or T3.

Neutral detergent fibre (NDF) decreased with increasing ensiling time. The decrease

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Days of Ensiling</th>
<th>Crude Protein %</th>
<th>Neutral Detergent Fibre %</th>
<th>Acid Detergent Fibre %</th>
<th>Cellulose %</th>
<th>Acid Detergent lignin %</th>
<th>IVDMD %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>28</td>
<td>6.21a</td>
<td>76.9a</td>
<td>49.64a</td>
<td>37.65ab</td>
<td>7.07a</td>
<td>45.0ac</td>
</tr>
<tr>
<td>Sorghum</td>
<td>56</td>
<td>6.10a</td>
<td>74.13acd</td>
<td>48.98a</td>
<td>37.03ab</td>
<td>6.97a</td>
<td>41.0ab</td>
</tr>
<tr>
<td></td>
<td>84</td>
<td>6.18a</td>
<td>73.70bcd</td>
<td>47.37a</td>
<td>36.78ab</td>
<td>7.05a</td>
<td>39.4b</td>
</tr>
<tr>
<td></td>
<td>112</td>
<td>6.20a</td>
<td>73.46bcd</td>
<td>48.07a</td>
<td>35.94ab</td>
<td>6.83a</td>
<td>38.9b</td>
</tr>
<tr>
<td>Sorghum with formic acid</td>
<td>28</td>
<td>6.10a</td>
<td>76.80a</td>
<td>47.85a</td>
<td>36.58ab</td>
<td>6.93a</td>
<td>46.0ac</td>
</tr>
<tr>
<td>Sorghum with formic acid</td>
<td>56</td>
<td>6.10a</td>
<td>73.80bc</td>
<td>46.28a</td>
<td>35.68ab</td>
<td>7.34a</td>
<td>41.8b</td>
</tr>
<tr>
<td>Sorghum wilted for 6 hours</td>
<td>84</td>
<td>5.95a</td>
<td>73.76bc</td>
<td>46.61a</td>
<td>36.17ab</td>
<td>7.05a</td>
<td>41.4b</td>
</tr>
<tr>
<td>Sorghum wilted for 6 hours</td>
<td>112</td>
<td>5.78a</td>
<td>72.85bc</td>
<td>45.41a</td>
<td>36.09ab</td>
<td>6.91a</td>
<td>40.9b</td>
</tr>
<tr>
<td>Sorghum wilted in the sum for 6 hours</td>
<td>56</td>
<td>5.65a</td>
<td>76.9a</td>
<td>48.38a</td>
<td>37.93a</td>
<td>6.73a</td>
<td>42.9bc</td>
</tr>
<tr>
<td>Sorghum combined with leucaena in the ratio 7:3</td>
<td>84</td>
<td>5.57a</td>
<td>71.36bc</td>
<td>46.04a</td>
<td>35.65ab</td>
<td>6.84a</td>
<td>41.8bc</td>
</tr>
<tr>
<td>Sorghum combined with leucaena in the ratio 7:3</td>
<td>112</td>
<td>5.52a</td>
<td>71.95bcd</td>
<td>46.75a</td>
<td>36.00ab</td>
<td>6.77a</td>
<td>42.2bc</td>
</tr>
<tr>
<td>LSD</td>
<td>28</td>
<td>23.90b</td>
<td>61.07e</td>
<td>35.24b</td>
<td>27.79c</td>
<td>6.27b</td>
<td>48.3a</td>
</tr>
<tr>
<td>LSD</td>
<td>56</td>
<td>23.32b</td>
<td>62.50e</td>
<td>34.66b</td>
<td>26.78c</td>
<td>6.24b</td>
<td>45.5c</td>
</tr>
<tr>
<td>LSD</td>
<td>84</td>
<td>22.99b</td>
<td>60.82e</td>
<td>35.83b</td>
<td>24.91c</td>
<td>6.19b</td>
<td>42.7bc</td>
</tr>
<tr>
<td>LSD</td>
<td>112</td>
<td>22.15b</td>
<td>60.88e</td>
<td>34.25b</td>
<td>24.97c</td>
<td>6.05b</td>
<td>42.4bc</td>
</tr>
</tbody>
</table>

1. Figures in the same column with different letters are statistically significant (P<0.05)
was statistically significant (P<0.05). Inclusion of leucaena significantly reduced (P<0.01) the NDF content. Though T2 had slightly lower NDF content compared to those of either T1 or T3 the difference were not significant (P>0.05).

Acid detergent fibre (ADF), cellulose and acid detergent lignin (ADL) were not significantly (P>0.05) influenced by increasing ensiling period. No significant differences (P>0.05) were found among T1, T2 and T3 in any of the parameters. Except for cellulose which was significantly lower (P<0.05), these three silages were significantly higher (P<0.01) than T4 in ADF and ADL.

In vitro dry matter digestibility significantly decreased (P<0.05) with advancing storing period. T4 silage had the highest IVMDM values compared to the others. However, except for the initial ensiling period when T4 was significantly (P<0.05) higher than that of T3, none of the differences was significant (P>0.05).

Discussion

The relatively lower crude protein content but higher cell wall constituents of “wild sorghum” compared with leucaena is consistent with published results\(^2\). Consequently, combining the grass and legume improves the overall quality of the grass which forms the bulk of the ruminant feed. This is reflected in the improvement in IVMDM of the combined product (60% of 47%) which could serve as a production feed. There was the added advantage of reducing the HCN content of the grass since leucaena is not known to contain cyanogenic glucoside\(^1\).

The pH of the silage was relatively high in this study probably because of the low water soluble carbohydrate content\(^1\) which resulted in low total organic acid content. Furthermore, the effect of wilting\(^1\) and the addition of leucaena which has a high buffering capacity\(^1\) may also have contributed to it. On the contrary, the addition of formic acid reduced the pH considerably\(^1\). In many trials, wilting has resulted in increased pH, and butyric acid was grossly reduced indicating that secondary fermentation is either limited or absent. Even though, in the present experiment, the various acids were not examined, the general trend of high pH and the increase in total organic acids compared to the control or untreated would suggest that secondary fermentation was restricted.

The \(NH_3-N\) content increased with increasing ensiling period as a result of proteolysis\(^2\). The low \(NH_3-N\) content of formic acid treated silage was due to the improved acidification which prevented proteolysis. On the contrary, the relatively higher \(NH_3-N\) values obtained with the wilted silage compared to the untreated is at variance with the result of others\(^3\). It appears that in as much as the type of protein to start with may be involved\(^3\), some amount of secondary fermentation might have occurred in this experiment.

The finding that ensiling reduced the level of HCN content in all treatment with advancing ensiling period agrees with published results\(^4\). Furthermore, the HCN content as reduced by sun-drying has also been observed with forage sorghum\(^4\) and with cassava\(^5\).

The greatest potential barrier to use of sorghum is the HCN content. Moran\(^6\) indicated that the actual 24 hr tolerance of a cow to HCN probably varied between 15 and 50 mg HCN per kilogramme body weight. However, Hatch\(^7\) pointed out that rapid intake of plant material to the equivalent of 4.0 mg HCN per kg body weight is toxic to cattle or sheep. It would therefore depend on the palatability of the feed and also the satiety state of the animal. At 3% body weight consumption the HCN derived from these silages would range between 0.89g and 2.74g. Since the animal does not consume all its feed at once, it may not prove toxic\(^8\). Nevertheless, more work needs to be done to reduce the HCN to acceptable levels.

The IVMDM of “wild sorghum” combined with leucaena was slightly better than the others because of the leucaena which had higher digestibility value. That wilted silage had a slightly lower value than the others is consistent with published results\(^12\).\(^2\)

It is concluded that “wild sorghum” can be ensiled. The ensiling characteristics are enhanced by sundrying or adding formic acid.
Sun-drying also greatly reduces the potential toxicity and the nutritive value of the material is improved by combining with leucaena.

References

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THE PRODUCTIVITY OF MUTURU CATTLE (BOS BRACHYCEROS) UNDER
THE TRADITIONAL VILLAGE MANAGEMENT SYSTEM: HERD HEALTH

D.V. UZA, N.N. UMUNNA1 AND E.O. OYEDIPE2

Department of Animal Production, University of Agriculture, PMB 2373 Makundi, Nigeria

PRODUCTIVITE DES BOVINS MUTURU (BOS BRACHYCEROS) DANS UN SYSTEME
TRADITIONNEL D’EXPLOITATION VILLAGEOISE: SANTE DU TROUPEAU

Résumé
Une étude sur la santé du troupeau a été menée chez les bovins Muturu élevés dans un système
traditionnel d’exploitation villageoise au sud de la savane guinéenne dans l’Etat de Benue au
Nigeria. Au total, l’enquête portait sur 362 troupeaux composés de 2490 têtes de bovins Muturu
trypanotolérants répartis comme suit: 49,2% de vaches; 21,2% de taureaux; 26% de veaux et 3,6%
de castrats.
Très peu de signes de maladie ont été observés, seule la diarrhée (27%) sévissait pendant
la saison des pluies. Très peu de fermiers, 14,6% et 10,8% respectivement, ont rencontré des cas
d’avortement et de mortalité de veau dans leurs troupeaux. Des cas de mises bas difficiles
(dystocie) ont été signalés chez quelques troupeaux (13,3%). La plupart des éleveurs (76,5%) ne
vaccinaient pas leur bétail. Il y avait le même pourcentage d’éleveurs qui traitaient leur bétail
(44,2%) et qui ne le faisaient pas (45%). Ceux qui traitaient leurs animaux avaient recours aux
métodes traditionnelles, à savoir les herbes (81,8%).
Il a été conclu que les bovins Muturu sont des animaux robustes et il semblerait que dans
le système traditionnel d’exploitation villageoise, la maladie ne constitue pas une entrave à la
production animale. Il a été proposé de tirer profit des ces caractéristiques exceptionnelles et ce
faisant, d’accroître la productivité grâce à l’amélioration du système d’exploitation.

Abstract
A herd health study was conducted in Muturu cattle reared under the traditional village manage-
ment system in the Southern Guinea Savanna of Benue State, Nigeria. A total of 362 herds
involving 2490 head of trypanotolerant Muturu cattle made up of 49.2% cows, 21.2% bulls, 26%
calves and 3.6% castrates were investigated.
Signs of disease were very few and only diarrhoea (27%) occurred during the rainy season.
Only few farmers, 14.6% and 10.8% respectively experienced abortions and calf mortality in their
herds. Cases of difficult birth (dystocia) were reported only in a few herds (13.3%). Most farmers
(76.5%) did not vaccinate their animals. Those who treated their animals (44.2%) and those who
did not treat (45.%) were about the same. Those who treated, used traditional methods, i.e. herbs
(81.8%).
It was concluded that Muturu cattle were hardy animals and that under the traditional village
management system, it appeared disease was not a constraint militating against production. It was
suggested that advantage be taken of these unique characteristics to effect increased productivity
through improved management.

Introduction
The major cause of animal protein shortage in
several areas of Africa appears to be the low off-
take rates for the continent. In cattle, for ex-
ample, off-take rate for Africa was 12.5% com-
pared to the world average of 18.5%1. In addi-
tion, total volume of meat output is low due to
low animal weights. Several factors such as
disease, environmental hazards, genetics and
nutrition may limit Africa’s animal production2,3.
Of these, disease is probably the most impor-
tant factor limiting productivity. A host of dis-

1 Address: International Livestock Research Institute; Addis Ababa, Ethiopia.
2 Address: National Animal Production Research, Institute, Ahmadu Bello, University, Zaria, Nigeria.
eases and parasites plague these animals. In Nigeria for example, the tsetse fly which is the vector of the dreaded trypanosomiasis has infested those areas which coincide with good forage growth and availability of water. Since most animal species succumb to the disease, these areas cannot be used extensively for livestock farming. However, breeds that are trypanotolerant can be successfully reared in these areas. Efforts must be made in exploiting the Muturu which is the only "pure-bred" trypanotolerant cattle indigenous to Nigeria. Since this breed is little studied\cite{5}, its health attributes if any, are also not understood. This study is therefore aimed at exploiting the health status of Muturu herds in Benue State with a view to assessing the contribution of the breed to Nigeria's beef industry.

**Materials and Methods**

Data were obtained through the administration of questionnaires to farmers of 362 herds made up of 2490 head of Muturu Cattle during the rainy season months of May–July 1990 and the dry season months of November–December 1991 as previously described\cite{6}.

The data obtained were subjected to simple frequency contingency table analysis and descriptive statistics computing mean percentages.

**Results**

362 herds involving 2490 head of Muturu cattle made up of 49.2% cows, 21.2% bulls, 26% calves and 3.6% castrates were studied.

Signs of disease were very few with diarrhea (27.1%) occurring in the rainy season (Table 1). Only few farmers experienced abortions (Table 2), calf mortality (Table 3) and difficult birth (dystocia) (Table 4) in their herds. Most farmers did not vaccinate their animals (Table 5). Those farmers who treated and those who did not treat their animals were about the same (Table 6). Those who treated, used traditional methods i.e. herbs (81.8%).

**Table 1:** Signs of disease in Muturu cattle

<table>
<thead>
<tr>
<th>Disease sign</th>
<th>Yes</th>
<th>No</th>
<th>Total response</th>
<th>Rainy</th>
<th>Dry</th>
<th>None</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhoea</td>
<td>98(27.1)</td>
<td>264(72.9)</td>
<td>362(100.0)</td>
<td>34(9.4)</td>
<td>18</td>
<td>5(5.0)</td>
</tr>
<tr>
<td>Not eating and drinking</td>
<td>44(12.2)</td>
<td>317(87.8)</td>
<td>361(100.0)</td>
<td>12(3.3)</td>
<td>10</td>
<td>2(2.8)</td>
</tr>
<tr>
<td>Skin problem</td>
<td>51(14.1)</td>
<td>310(85.9)</td>
<td>361(100.0)</td>
<td>21(3.3)</td>
<td>17</td>
<td>4(4.7)</td>
</tr>
<tr>
<td>Foot problem</td>
<td>15(4.2)</td>
<td>346(95.8)</td>
<td>361(100.0)</td>
<td>5(1.4)</td>
<td>5</td>
<td>1(1.4)</td>
</tr>
<tr>
<td>(foot-rot)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abortion</td>
<td>15(4.2)</td>
<td>346(95.8)</td>
<td>361(100.0)</td>
<td>7(2.0)</td>
<td>4</td>
<td>1(1.1)</td>
</tr>
<tr>
<td>Persistent coughing</td>
<td>35(9.7)</td>
<td>326(90.3)</td>
<td>361(100.0)</td>
<td>6(1.7)</td>
<td>25</td>
<td>6(6.9)</td>
</tr>
<tr>
<td>Circling and twisting of neck</td>
<td>9(2.5)</td>
<td>352(97.5)</td>
<td>361(100.0)</td>
<td>2(0.6)</td>
<td>3</td>
<td>0(0.8)</td>
</tr>
<tr>
<td>Blood in urine</td>
<td>21(5.8)</td>
<td>340(94.2)</td>
<td>361(100.0)</td>
<td>19(5.2)</td>
<td>0</td>
<td>0(0.0)</td>
</tr>
<tr>
<td>Worm in faeces</td>
<td>32(8.9)</td>
<td>329(91.1)</td>
<td>361(100.0)</td>
<td>4(1.1)</td>
<td>17</td>
<td>4(4.7)</td>
</tr>
<tr>
<td>Swollen joints</td>
<td>11(3.1)</td>
<td>350(97.0)</td>
<td>361(100.0)</td>
<td>2(0.6)</td>
<td>3</td>
<td>0(0.9)</td>
</tr>
<tr>
<td>Sudden death</td>
<td>10(2.8)</td>
<td>351(97.2)</td>
<td>361(100.0)</td>
<td>5(1.4)</td>
<td>4</td>
<td>1(1.1)</td>
</tr>
</tbody>
</table>

*Figures in parenthesis are percentages*
### Table 2: Occurrence of abortion in Muturu cattle

<table>
<thead>
<tr>
<th>Response</th>
<th>Number of respondents</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>52</td>
<td>14.6</td>
</tr>
<tr>
<td>No</td>
<td>225</td>
<td>62.2</td>
</tr>
<tr>
<td>No response</td>
<td>84</td>
<td>23.2</td>
</tr>
<tr>
<td>Total</td>
<td>362</td>
<td>100.0</td>
</tr>
</tbody>
</table>

### Table 3: Calf mortality in Muturu cattle

<table>
<thead>
<tr>
<th>Response</th>
<th>Gboko</th>
<th>Guma</th>
<th>Gwer</th>
<th>Katsina</th>
<th>Kon</th>
<th>Kwande</th>
<th>Makurdi</th>
<th>Ushongo</th>
<th>Vandeikya</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>20</td>
<td>4</td>
<td>7</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>39</td>
</tr>
<tr>
<td>(1.67)</td>
<td>(0.00)</td>
<td>(0.00)</td>
<td>(5.56)</td>
<td>(1.11)</td>
<td>(1.94)</td>
<td>(0.00)</td>
<td>(0.28)</td>
<td>(0.28)</td>
<td>(10.83)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>74</td>
<td>17</td>
<td>42</td>
<td>45</td>
<td>18</td>
<td>39</td>
<td>2</td>
<td>12</td>
<td>3</td>
<td>252</td>
</tr>
<tr>
<td>(20.56)</td>
<td>(4.72)</td>
<td>(11.67)</td>
<td>(12.50)</td>
<td>(5.00)</td>
<td>(10.83)</td>
<td>(0.56)</td>
<td>(3.33)</td>
<td>(0.83)</td>
<td>(70.00)</td>
<td></td>
</tr>
<tr>
<td>No response</td>
<td>16</td>
<td>7</td>
<td>6</td>
<td>22</td>
<td>0</td>
<td>10</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>69</td>
</tr>
<tr>
<td>(4.44)</td>
<td>(1.94)</td>
<td>(1.67)</td>
<td>(6.11)</td>
<td>(0.00)</td>
<td>(2.78)</td>
<td>(0.83)</td>
<td>(0.83)</td>
<td>(0.56)</td>
<td>(19.17)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>96</td>
<td>24</td>
<td>48</td>
<td>87</td>
<td>22</td>
<td>56</td>
<td>5</td>
<td>16</td>
<td>6</td>
<td>360</td>
</tr>
<tr>
<td>(26.67)</td>
<td>(6.67)</td>
<td>(13.33)</td>
<td>(24.17)</td>
<td>(6.11)</td>
<td>(15.56)</td>
<td>(1.39)</td>
<td>(4.44)</td>
<td>(1.67)</td>
<td>(100.0)</td>
<td></td>
</tr>
</tbody>
</table>

Figures in parenthesis are percentages

### Table 4: Incidence of difficult birth (dystocia) in Muturu cattle

<table>
<thead>
<tr>
<th>Response</th>
<th>Number of respondents</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>48</td>
<td>13.3</td>
</tr>
<tr>
<td>No</td>
<td>269</td>
<td>74.3</td>
</tr>
<tr>
<td>No response</td>
<td>45</td>
<td>12.4</td>
</tr>
<tr>
<td>Total</td>
<td>362</td>
<td>100.0</td>
</tr>
</tbody>
</table>

### Table 5: Vaccination status of Muturu cattle in Benue State

<table>
<thead>
<tr>
<th>Response</th>
<th>Gboko</th>
<th>Guma</th>
<th>Gwer</th>
<th>Katsina</th>
<th>Kon</th>
<th>Kwannde</th>
<th>Makurdi</th>
<th>Ushongo</th>
<th>Vandeikya</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>27</td>
<td>0</td>
<td>4</td>
<td>20</td>
<td>12</td>
<td>1</td>
<td>0</td>
<td>5</td>
<td>3</td>
<td>72</td>
</tr>
<tr>
<td>(7.50)</td>
<td>(0.00)</td>
<td>(1.11)</td>
<td>(5.56)</td>
<td>(3.33)</td>
<td>(0.28)</td>
<td>(0.00)</td>
<td>(1.39)</td>
<td>(0.83)</td>
<td>(20.00)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>69</td>
<td>22</td>
<td>39</td>
<td>62</td>
<td>10</td>
<td>54</td>
<td>5</td>
<td>11</td>
<td>3</td>
<td>275</td>
</tr>
<tr>
<td>(19.17)</td>
<td>(6.11)</td>
<td>(10.83)</td>
<td>(17.22)</td>
<td>(2.78)</td>
<td>(15.00)</td>
<td>(1.39)</td>
<td>(3.83)</td>
<td>(0.83)</td>
<td>(76.39)</td>
<td></td>
</tr>
<tr>
<td>No response</td>
<td>0</td>
<td>2</td>
<td>5</td>
<td>5</td>
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<td>1</td>
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<td>13</td>
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<tr>
<td>(0.00)</td>
<td>(0.56)</td>
<td>(1.39)</td>
<td>(1.39)</td>
<td>(0.00)</td>
<td>(0.28)</td>
<td>(0.00)</td>
<td>(0.00)</td>
<td>(0.00)</td>
<td>(3.61)</td>
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<td>(6.67)</td>
<td>(13.33)</td>
<td>(24.17)</td>
<td>(6.11)</td>
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<td>(1.39)</td>
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Figures in parenthesis are percentages
Table 6: Treatment of Muturu cattle in Benue State

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<th>Gboko</th>
<th>Guma</th>
<th>Gwer</th>
<th>Katsina</th>
<th>Kon</th>
<th>Kwande</th>
<th>Makuri</th>
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<td>(13.33)</td>
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<td>(15.56)</td>
<td>(1.39)</td>
<td>(4.44)</td>
<td>(1.67)</td>
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</table>

* Figure in parenthesis are percentages

Discussion

Signs of disease

The very few signs of disease in Muturu cattle as reported in this study as well as the animal’s tolerance to trypanosomiasis and other diseases such as Streptothricosis and tickborne diseases such as heartwater (Cowdria ruminantium), anaplasmosis, endemic piroplasmosis and babesiosis indicated the hardy nature of this cattle breed. Diarrhoea (27.1%) was the highest disease sign reported mainly during the rainy season and may be as a result of consumption of lush pasture during this season and therefore not of much consequence. Muturu cattle are however reported to be much more susceptible to rinderpest than Zebu, while it was noted that the animals were also highly susceptible to foot-rot. In this study, 95.8% of the farmers did not observe foot-rot in their Muturu herds.

Abortions and calf mortality

The few abortions and low calf mortality reported in this study is in agreement with ILCA/FAO who reported that diseases and premature mortality were uncommon in the Muturu. According to Ferguson however, the most important cause of deaths among young stock was helminthiasis with a few sporadic cases of anthrax. In this study however, only very few farmers reported worms in faeces (8.9%), an indication of helminthiasis while sudden death (2.8%) which may point to anthrax was also low.

Abortions and calf mortality are of great economic significance as they are associated with reproductive wastage, infertility, lowered milk yield, prolonged breeding intervals in Zebu breeds in Nigeria. In traditionally managed Zebu herds, for example, Okoh reported a very high abortion rate of 15–49% in the neighbouring States of Bauchi, Gongola and Plateau. The few abortions and low calf mortality in the Muturu is further evidence of the breed’s outstanding asset that should be fully exploited.

Vaccination and treatment

Vaccination

Contact between Muturu cattle farmers and veterinary extension staff is poor in Benue State. In the present study most farmers did not report to veterinary extension workers when their animals fell sick. This poor contact may probably be responsible for farmers not vaccinating their animals. The non-vaccination may also be attributed to the fact that since Muturu cattle rarely fell sick, the farmers had no pressing need to contact veterinary extension staff. In Nigeria, annual vaccination of livestock is usually carried out routinely. During this time, cattle are vaccinated against rinderpest, contagious bovine pleuropneumonia (CBPP), anthrax, haemorrhagic septicaemia and blackquarter.
Rinderpest and CBPP vaccination is compulsory. These vaccinations are usually carried out in Zebu cattle especially the Bunajii (White Fulani) which are predominantly raised under transhumance conditions. There is no organized vaccination for Bos taurus cattle including the Muturu which are raised under sedentary conditions.

In traditionally managed nomadic herds of White Fulani cattle, Okoh et al. reported low vaccination coverage for anthrax, haemorrhagic septicaemia and blackquarter and attributed this to poor extension services. The authors suggested that a considerable number of the herds were not protected and may be fully susceptible in the wake of a disease outbreak. Poor rinderpest vaccination coverage in the nomadic herds was responsible for the emergence of a virulent strain of rinderpest virus which decimated cattle stock and wildlife in Nigeria during the last decade. Since Muturu cattle are known to be susceptible to rinderpest, the findings in this study that most farmers did not vaccinate their animals posed a great risk of rinderpest outbreak since the animals were not protected. There is urgent need for veterinary extension services to address this important issue of vaccination in Muturu cattle particularly against rinderpest and CBPP which are compulsory in Nigeria.

Treatment

In Benue State, Muturu cattle farmers who are of the Tiv tribe, treated their animals themselves using traditional methods, i.e. herbs. The use of modern western methods by veterinary staff in treating the animals were extremely low. Since diarrhoea was the most common problem of Muturu, one may suppose that these traditional treatments were probably mostly directed against this ailment. It was not possible to get the farmers to disclose the names of the herbs they used and against which diseases because of the cultural practices of secrecy surrounding the use of traditional medicine.

Traditional methods of treatment of diseases among nomadic Fulani animals is very popular. Okoh et al. reported that the nomadic Fulani used local herbs called "bagaruwa" (Acacia arabica) to treat foot and mouth disease, "bafedoge" and "madaci" (Khaya senegalensis) for treatment of rinderpest and common salt for the treatment of diarrhoea in cattle, sheep and goats. The authors further reported that among the Fulani, some herdsmen were not willing to disclose the details of the methods of the local curative and preventive treatment carried out on their animals and said this was a secret handed to them by their grandfathers.

There is need to carry out thorough investigations into the traditional methods that the Tiv people of Benue State used in treating their Muturu cattle as these may have contributed to the reasonable health status of the traditionally managed stock. Perhaps, some of these remedies may have some scientific basis and could supplement or in fact be the sole cure for certain tropical livestock diseases.

References

References


Received for publication on 11th January, 1996
SERO-PREVALENCE OF GUMBORO AND NEWCASTLE DISEASES IN LOCAL CHICKENS (GALLUS GALLUS DOMESTICUS) IN IBADAN, NIGERIA

'OEYEWO,M, K.A. 'OOGUNDIPE, G.A.T. AND 2DURAIJYE, O.A.
Departments of 'Veterinary Public Health and Preventive Medicine, and 2Veterinary Microbiology and Parasitology, University of Ibadan, Ibadan, Nigeria.

SERO-PREVALENCE DE LA MALADIE DE GUMBORO ET DE LA MALADIE DE NEWCASTLE CHEZ LES POULETS LOCAUX (GALLUS GALLUS DOMESTICUS) A IBADAN AU NIGERIA

Résumé
Une étude sérologique a été menée en vue de déterminer la prévalence de la maladie de Newcastle (MNC) avec le test d'inhibition de l'hémagglutination et la prévalence de la maladie infectieuse de la bourse (MIB) avec le test de précipitation du gel d'agar, chez les poulets locaux à Ibadan. Parmi les 221 sérum examinés, 38,01% et 44,34% étaient positifs pour MNC et MIB, les anticorps étant très probablement acquis à la suite d'infection naturelle. Ce qui a confirmé, par ailleurs, l'état enzootique de MNC et MIB chez les poulets locaux, porteurs de germes de ces maladies. Il est recommandé d'élaborer un programme national de lutte contre MNC et MIB.

Summary
A serological survey was conducted to determine the prevalence of Newcastle disease (NCD) using Haemagglutination Inhibition Test (HIT) and Infectious bursal disease (IBD) using the qualitative Agar gel precipitation Test (AGPT) in local chickens in Ibadan. Of the 221 sera screened, 38,01% and 44,34% had positive NCD and IBD, antibodies which were most probably acquired from natural infection. This further confirmed the enzootic and carrier status of local chickens for these diseases. A national NCD and IBD control programme is recommended.

Introduction
Newcastle disease and infectious bursal disease (or Gumboro) are the two most economically important, highly contagious viral disease of poultry in Nigeria. The losses from either of the diseases are usually in terms of high morbidity and mortality rates which could reach 30% for IBD and 100% for NCD1. In addition, IBD is known to cause retarded growth rate and poor immunological response to vaccines in survivors due to the destruction of the bursa of Fabricius.

Newcastle disease was first reported in Nigeria in 19522 while the first report of IBD was in 19693. Further incidences of the two disease were described by other authors4,5,6,7,8. The two diseases have since become enzootic throughout Nigeria.

The extensively managed free-roaming indigenous local chickens (Gallus gallus domesticus) with an estimated population of 124 million, 92% of the total chickens in Nigeria, is believed to act as reservoirs and carriers of NCD, IBD and other infections to themselves and to the more susceptible exotic poultry breeds in commercial enterprises9.

The local chickens in Nigeria do not receive any immunoprophylactic treatment, yet 60.3% (44/73) of those screened9 were positive for IBD antibodies. Similarly, 41.04% (307/748) of local chickens screened in 1986 were positive for NCD while virulent NCD viruses were isolated from the cloacal swabs of apparently normal local chickens9. Many confirmed outbreaks of NCD and IBD have been reported in local chickens in Zaria11,12.

This paper reports the current prevalence of NCD and IBD antibodies in local chickens in Ibadan in south-western Nigeria.

Materials and Methods
Blood samples were collected from a total of 221 apparently healthy local chickens in five local government areas of Ibadan. The approximate ages of the chickens ranged from

Corresponding author
Table 1: Parameters of the goats before and after the outbreak

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Time* (BF/AF)</th>
<th>Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BF</td>
<td>AF</td>
</tr>
<tr>
<td>Weight (Kg)</td>
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<td>II 6.1, 5.8, 7.6</td>
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<tr>
<td>PCV (%)</td>
<td>I 26.3, 26.0, 29.5</td>
<td>II 26.0, 29.5</td>
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<tr>
<td>Oocysts/gram</td>
<td>I 633,325, 75</td>
<td>II 66,650, 75</td>
</tr>
<tr>
<td>Strongyle/gram</td>
<td>I 125, 100, 125</td>
<td>II 112.5, 125, 125</td>
</tr>
<tr>
<td>Strongyloides/gram</td>
<td>I 125, 125</td>
<td>II 125, 125</td>
</tr>
</tbody>
</table>

*BF = Before outbreak (day 0)
AF = After outbreak (day 32)

Among the four species of Eimeria encountered, E. aroingi and E. ninakohyakimova appear to be the commonest and most important contributors to the outbreak. These species are very prevalent and have been previously associated with acute coccidiosis in young goats in Nigeria and elsewhere. E. intricata and E. parva are not very pathogenic but could complicate diagnosis in mixed infections.

The results of this investigation emphasize the need to prophylactically dose randomly acquired range-reared animals against coccidian parasites before they are brought together under intensive conditions of management. More importantly, this outbreak further illustrates the dangers of using such randomly acquired animals of unknown history for scientific research.

References


Received for publication on 12th July, 1994
SHORT COMMUNICATION

EFFECT OF SEVIN (1 - NAPHTHL METHYL CARBAMATE) ON BLOOD CHOLINESTERASE OF CATTLE

A.K. BASU

Institute of Animal Health and Veterinary Biologicals, Calcutta, India

In earlier communication, it was reported that three applications with 0.1 and 0.2 per cent of the acaricide Sevin (1 - naphtyl methyl carbamate) as a spray was effective on ticks and lice of cattle and buffaloes, while 0.3, 0.4 and 0.5 per cent produced similar effects with only two applications\(^1\). In the present communication, an attempt was made to determine the toxicity of the drug in cattle at different concentrations using the activity of blood cholinesterase as an index.

Six groups of animals, comprising five cattle in each group, were selected at random. Group I to group V were sprayed once with Sevin (carbamate compound) at 0.1, 0.2, 0.3, 0.4 and 0.5 per cent respectively. Group VI received Malathion (organophosphorous compound) once at 0.5 per cent. Only one concentration of effective dose of Malathion was used because that concentration of the compound is widely used as acaricide. The cholinesterase activity of blood of each group of cattle was determined in sample taken at pre- and post-treatment (after an hour of spray) of acaricide. Blood sample (10ml) was collected in heparinised vigour bottle under aseptic conditions from the jugular vein and transported to the laboratory on ice. In laboratory plasma samples were then separated by centrifugation with relative centrifugal force (RCF) of 1258 g for 20 minutes and stored thereafter at 4°C for 24 hours. The cholinesterase activity was determined following the technique of Michal\(^2\) given here under.

The enzyme in an aliquot of diluted plasma is allowed to act on acetylcholine in a standard buffer solution for a measured time (usually 1 - 2 hours). The pH of the mixture is measured at the beginning and at the end of this time. The action of the enzyme on the substrate produces acid which lowers the pH of the mixture. The rate of change in pH is therefore a measure of enzymatic activity.

The cholinesterase activity of the sample in units of pH per hour is calculated as follows:

\[ \Delta \text{pH/hr} = \frac{(\text{pH}_1 - \text{pH}_2 - b)}{t} \]

where \(\text{pH}_1\) and \(\text{pH}_2\) are the initial and final pH readings respectively, \(t\) is the time in hours between the mixing with acetylcholine and the time of reading \(\text{pH}_2\) and \(b\) and \(f\) are correction factors (Table 1), \(b\) being a correction for non enzymatic hydrolysis of substrate, and \(f\) correcting for the effect of pH change on enzyme activity relative to buffer capacity.

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<th>pH2</th>
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Present Address: Dr. A. K. Basu, B-5/52, Kalyani – 741235, West Bengal, India
Table 2: Effect of Sevin and Malathion on cholinesterase activity of blood of cattle

<table>
<thead>
<tr>
<th>Name of acaricides</th>
<th>Group</th>
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<th>ChE activity before treatment</th>
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<tr>
<td>Sevin</td>
<td>I</td>
<td>0.1</td>
<td>0.73 ± 0.02</td>
<td>0.665 ± 0.05</td>
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<tr>
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<td>II</td>
<td>0.2</td>
<td>0.75 ± 0.007</td>
<td>0.655 ± 0.005</td>
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<td>III</td>
<td>0.3</td>
<td>0.77 ± 0.02</td>
<td>0.715 ± 0.025</td>
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<td></td>
<td>IV</td>
<td>0.4</td>
<td>0.925 ± 0.015</td>
<td>0.83 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>V</td>
<td>0.5</td>
<td>0.84 ± 0.15</td>
<td>0.74 ± 0.08</td>
</tr>
<tr>
<td>Malathion</td>
<td>VI</td>
<td>0.5</td>
<td>0.99 ± 0.0102</td>
<td>0.76 ± 0.07</td>
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</table>

The results of plasma cholinesterase estimates before and after treatment with the acaricides are presented in Table 2. The decreases in the cholinesterase activity in the groups treated with Sevin were consistent but the differences between samples collected before and after treatment were not statistically significant. O'Kelly observed that subcutaneous administration of the drug Neguvon and Sevin to cattle resulted in a significant drop of blood cholinesterase activity. But in the present study, there was no significant difference in cholinesterase activity probably because of the fact that the drug Sevin was used as a spray which was absorbed through the skin.

It appears from the results of this study that Sevin can be used safely as a spray to control ticks and lice as it has no adverse effect on cholinesterase.

**References**


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SHORT COMMUNICATION

PREVALENCE AND CAUSES OF MORTALITY IN NEWLY BORN OUDA AND BALAMI LAMBS IN NORTHERN NIGERIA

E. B. OTESILE AND O.O. ODUYE
Department of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria

Lamb mortality is one of the most important causes of reduced productivity of sheep establishments all over the world. A study by the International Livestock Centre for Africa (ILCA) showed that the chances of survival of lambs up to one year of age was only 55% with most deaths occurring during the neonatal period, that is, up to one month of age. Most neo-natal deaths take place during the first week of life. Thus in Australia, Dennis found that 86.6% of all neonatal deaths occurred during the first 72 h of life.

This communication is on the prevalence and causes of mortality during the first week of life among two breeds of Fulani sheep, the Ouda and the Balami. Both breeds of sheep are native to the northern Guinea savannah and the Sahel zone of tropical Africa. They have been selected for multiplication by the Federal Government of Nigeria to boost the national meat supply. The mature Ouda female weighs about 45 kg while the Balami is slightly heavier.

The investigation took place at the Tuma and Ladanawa farms of the Sheep Meat Production Project of the Federal Department of livestock and Pest Control Services, Katsina, Nigeria. Batches of about 300 sheep were bred monthly by natural mating between the months of April and July 1982 and lambings took place during the months of September to December 1982. The sheep were maintained on gamba grass (Andropogon gayanus) supplemented with groundnut cake or cotton seed cake at the rate of 400 g per pregnant or lactating ewe per day. Water was provided ad. libitum.

Newborn lambs were ear-tagged and weighed to the nearest 0.05 kg within 24 h of parturition with a baby scale (Waymaster, England). A total of 408 live-born lambs, comprising 225 Ouda and 183 Balami, were monitored for survival during the first seven days postpartum. Carcasses of all dead lambs were subjected to post mortem examination. Samples were collected from infected tissues for the isolation and identification of bacteria. Each sample was seeded into five percent sheep blood and McConkey agar plates as well as Robertson's cooked meat medium. A set of blood agar plates and Robertson's cooked meat medium was incubated anaerobically in a GasPak system (BBL Cockeysville, US) for 24 – 48 h after which subcultures were made from the Robertson's cooked meat medium onto fresh blood agar plates. The blood agar plates were then incubated anaerobically for 24 – 48 h. Another set of blood and McConkey agar plates were incubated in air at 37°C for 24 h. Bacterial isolates were identified after the methods described by Cowan. The differences between mortality rates were evaluated by Chi square analysis.

Of the 225 live-born Ouda lambs studied, 21.3% died during the first seven days of life while 14.2% of the 183 Balami lambs studied died during the same period. There was no significant difference (P>0.05) between the mortality rates of the two seeds.

Mortality among Ouda male and female lambs was 25.9% and 17.1% respectively during the period of study; the corresponding figures for Balami lambs were 17.6% and 11.2% respectively. In neither of the two breeds was the mortality rate among the male significantly (P>0.05) higher than that in the females. The difference between the mortality rates for Ouda single (19.1%) and twin-lambs (31.0%) was not significant (P>0.05). Among Balami lambs, the mortality rate for twins (42.3%) was significantly (P<0.05) higher than that for singles (9.6%).
The most common cause of mortality was starvation, accounting for 29.7% of all deaths (Table 1). Desertion of the newborn lamb by the dam on pasture, was the chief cause of starvation. Septicaemia (20.3%) was the second most important cause of death. The bacteria associated with cases of septicaemia were Escherichia coli (7 isolates), Pasteurella haemolytica (3 isolates), Staphylococcus aureus (2 isolates), Salmonella dublin (2 isolates) and Clostridium perfringens (1 isolate). Inability to stand up and suckle due to low birth weight was responsible for 17.6% of all deaths. All lambs with birth weights of less than 2.0 kg died within 48 h of birth. Acute broncho-pneumonia was diagnosed in 12.2% of all cases. Multiple bacterial isolates were often recovered from pneumatic lungs. The significant species were: Pasteurella haemolytica (6 isolates), P. multocida (5 isolates), Corynebacterium pyogenes (3 isolates), Streptococcus zooepidemicus (2 isolates) and Pseudomonas aeruginosa (1 isolate).

The mortality rates obtained in the Ouda (21.3%) and Balami lambs (14.2%) in the present study were higher than that in West African Dwarf lambs during the first week of life\(^6\). These relatively high rates suggest the need for measures that can reduce mortality among newly born Ouda and Balami lambs. In this study, sex did not significantly (P>0.05) influence mortality rate, although males of both breeds had higher mortality rates. It is possible that the relatively small sample sizes were responsible for a lack of statistical significance. Prolificacy, the average number of lambs born live per birth, were low for both the Ouda (1.1) and the Balami (1.08). Mortality rates among twin lambs were higher than those among single lambs for both breeds. This indicates that, in the area of study, selection for twinning is not desirable in both breeds\(^8\).

The control of the four conditions that were responsible for 79.8% of all deaths (starvation, septicaemia, low birth weight and pneumonia), will result in a significant reduction in lamb mortality and hence improve flock productivity. The withdrawal of ewes from pasture at parturition and their confinement indoors up to about one week post-partum, can result in a significant decline in cases of desertion of lambs\(^8\). The same measure will also decrease the incidence of infections including septicaemia since the newborn lamb will have ready access to antibodies-rich colostrum. Low birth weights were usually encountered among twin lambs in this study. Artificial nursing of such lambs can increase their chances of survival\(^10\).

| Table 1: Causes of mortality in Ouda and Balami lambs during the first seven days of life |
|-----------------------------------------------|----------------|---------|
| Ante-mortem cause                              | No. of cases |         |
| 1. Starvation due to:                          |               |         |
| a) Desertion by dam                            | 11            | 14.9    |
| b) Lack of or inadequate milk secretion        | 6             | 8.1     |
| c) Sickness and/or death of dam                | 5             | 6.8     |
| Sub-total                                      | 22            | 29.7    |
| 2. Septicaemia                                 | 15            | 20.3    |
| 3. Low birth weight                            | 13            | 17.6    |
| 4. Bronchopneumonia                            | 9             | 12.2    |
| 5. Dystocia                                    | 5             | 6.8     |
| 6. Diarrhoea                                   | 5             | 6.8     |
| 7. Omphalohlebitis                             | 2             | 2.7     |
| 8. Strangulation                               | 1             | 1.4     |
| 9. No diagnosis                               | 2             | 2.7     |
| Total                                         | 74            | 100     |
References


Received for publication on 19th July 1994
THE EFFECT OF SUBSTITUTION OF FISH MEAL BY BREWERS' YEAST IN BROILER STARTER RATIONS IN THE TROPICS.

N. P. OKPOKWASILI*

Department of Animal Science, University of Nigeria, Nsukka,
Enugu State, Nigeria.

Fish meal is a vital ingredient in poultry rations. Local fish meal is poorer in quality compared to that imported\(^1\). The high cost of fish meal importation increases the cost of poultry production since poultry feed accounts for about 50 – 70% of the total cost\(^2\). The high cost of importation of fish meal would justify the use of by-products of plant origin to replace fish meal partly or wholly in poultry rations. Because brewers' yeast contains 35–42% of highly digestible protein\(^1\), it could thus be an excellent substitute for fish meal. The objectives of this study were, therefore, to determine if fish meal can be replaced by brewers' yeast in order to reduce the cost of poultry feed and to assess the optimum replacement level of fish meal by brewers' yeast with no detrimental effects on the performance of broiler chicks.

Four basic rations were formulated by varying the proportions of yellow maize, fish meal, brewers' yeast, palm kernel cake, spent grain soybean meal, bone meal, vitamin/mineral premix, palm oil and salt; all calculated to contain the same levels of crude protein (24%) and metabolisable energy (3.03k cal/g) (4,5). The four rations (A–D) contained varying levels of fish meal and brewers' yeast as follows:

A: 100% fish meal + 0% brewers' yeast
B: 66.67% fish meal + 33.33% brewers' yeast
C: 33.33% fish meal + 66.67% brewers' yeast
D: 0% fish meal + 100% brewers' yeast

The study was undertaken in the Metabolism Laboratory of the University of Nigeria farm, Nsukka. The temperature of the room was 32°C before the arrival of the birds\(^6\). A total of sixty, 8 – day old Hypeco Goldline Broiler birds from the University hatchery were used for the trial. Water and the experimental rations were supplied to the birds ad libitum for four weeks\(^7\). The parameters monitored were average daily weight gain, average daily feed intake, feed conversion efficiency and total estimated feed cost. The rations were subjected to proximate analysis using methods outlined by the Association of Official Analytical Chemists\(^8\). All data were analysed statistically using Analysis of Variance, ANOVAR\(^9\) and means separated by standard error. Differences between means were determined using Duncan's Multiple Range Test\(^10\).

The study showed that complete replacement of fish meal in starter ration D resulted in depression of live weight gain in the birds (Table 1). This finding is in close agreement with the report of Edgar and Hons\(^11\) where micro-organisms (conventional sources of protein) were replaced with high levels of yeast in broiler rations and reduced growth by 39%. With regards to feed intake, birds on the ration with 100% fish meal consumed 27% more food than those on 100% brewers' yeast. The greater consumption of the former could be attributed to the attractive fish meal flavour. The feed consumption of birds on 100% fish meal ration was significantly (P<0.05) higher than those on rations with 33.33, 66.67 and 100% fish meal substitution. The feed conversion efficiency (Table 1) of birds on fish meal-based ration A was significantly (P<0.05) higher than those on yeast-based ration D. Birds on rations A, B and C containing different levels of fish meal 100, 66.67 and 33.33% respectively had significantly the same feed conversion efficiency. This result is in agreement with the findings of Avila and Ballou\(^12\) that the feed efficiency of birds receiving different levels of fish meal in their rations were similar. The total estimated cost of feed

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Table 1: Effect of replacement of fish meal by brewers' yeast on broiler performance

<table>
<thead>
<tr>
<th>Rations</th>
<th>Mean Values</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Average</td>
<td>Average</td>
<td>Average</td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td></td>
<td>daily live</td>
<td>daily feed intake</td>
<td>daily feed conversion</td>
<td>Cost</td>
<td>Total</td>
</tr>
<tr>
<td></td>
<td>weight</td>
<td>per g/bird</td>
<td>per g/bird</td>
<td>efficiency*</td>
<td>N**</td>
</tr>
<tr>
<td>A: 100% fish meal+</td>
<td>12.76a</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0% brewers' yeast</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>B: 66.67% fish meal +</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>33.33% brewers' yeast</td>
<td>9.19bc</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>C: 33.33% fish meal +</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>66.67% brewers' yeast</td>
<td>8.94b</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D: 0% fish meal+</td>
<td>7.73*</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>100% brewers' yeast</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>SE ±</td>
<td>0.95</td>
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</tr>
</tbody>
</table>

Means bearing different superscript in the vertical columns are significantly different (P<0.05).

* Calculated as:
Average daily live weight gain (g)

** N = Naira

$1 = N85

(Table 1) consumed by birds on fish meal-based ration A was significantly (P<0.05) higher than those on rations with 33.33, 66.67 and 100% fish meal substitution. Rations B, C, and D were lower in price than ration A by 3.41, 6.82 and 10.27% respectively. It had already been noted above that Ration D with 100% brewers' yeast (no fish meal) was associated with decline in live weight gain, feed intake and feed conversion efficiency of the birds.

The results confirm that fish meal protein is superior to brewers' yeast protein. It is therefore recommended that brewers' yeast should not replace more than 66.67% of dietary fish meal protein in broiler starter rations.

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Objet
Le Bulletin de la Santé et de la Production animales en Afrique contient des articles de recherches originales traitant d'activités en matière de santé et de production animales visant à assurer le développement de l'industrie animale et une meilleure utilisation des ressources du bétail en Afrique. Le Bulletin est un périodique trimestriel.

Présentation des articles
Deux exemplaires des articles doivent être adressés à Monsieur le Rédacteur en Chef, Bulletin de la Santé et de la Production Animales en Afrique, Organisation de l'Unité Africaine/Bureau interafricain des Ressources animales, P.O. Box 30786, Nairobi, Kenya.
Un article ne peut être soumis pour publication que s'il n'a pas encore été proposé ailleurs; il fera l'objet de quelques modifications par le Comité de Rédaction.

Genres d'articles publiés dans le Bulletin
- des communications originales
- des brèves communications
- analyse des articles proposée par le Rédacteur
- des éditoriaux
- le courrier des lecteurs
- analyse d'ouvrages
- informations et annonces

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Les manuscrits doivent respecter les conditions suivantes:
Le titre doit être concis et ne pas dépasser plus de 15 mots, il est suivi du (des) nom(s) de l'auteur (ou des auteurs) et des établissements ou le travail a été effectué, ainsi que de l'adresse pour les correspondances si elle n'est pas la même.
Le résumé ne doit pas excéder 200 mots. Son texte bref et concis comprendra les principaux résultats et la (les) conclusion(s) de l'étude.
L'introduction expose le but de la recherche.
Le matériel et les méthodes utilisés.
Les résultats présentés brièvement.
Un débat sur l'importance de l'article.
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