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BACTERIAL FLORA ASSOCIATED WITH MASTITIS IN SHEEP AND GOATS IN ZARIA (NIGERIA) AREA

A. A. ANYAM AND J.O. ADEKEYE*

Artificial Insemination Unit, National Animal Production Research Institute
Ahmadu Bello University, P.M.B. 1096, Shika-Zaria, Nigeria
*Department of Pathology & Microbiology, Faculty of Veterinary Medicine
Ahmadu Bello University, Zaria, Nigeria

LA FLORE BACTERIENNE ASSOCIEE A LA MASTITE CHEZ LES OVINS ET LES CAPRINS DANS LA REGION DE ZARIA AU NIGERIA

Résumé
On a examiné la flore bactérienne des échantillons de lait d’animaux souffrant de mastite et de ceux en bonne santé chez les races caprines rousses de Sokoto et de Kano, et chez la race ovine Yankasa aux marchés de Shika, Samaru et Zango. Les bactéries suivantes ont été isolées de 56 échantillons de lait de chèvres atteintes de mastite: Staphylococcus aureus 13 (31,70%); Micrococcus sp. Bacillus sp. (114.7%); E. Coli et Klebsiella sp. 7,3% chacun, tandis que Staphylococcus aureus, Micrococcus sp. et Moraxella sp. 11,1% chacun et Bacillus sp. (22,2%) ont été isolés de 20 échantillons de lait de chèvres en bonne santé.

Notre étude n’a signalé aucun cas de mastite chez les ovins, alors que 56% des caprins examinés souffraient de cette maladie. Seuls 2 (4.4%) des 45 échantillons de lait ovin ont laissé apparaître une croissance bactérienne (Micrococcus sp.), le reste 43 (95.6%) était stérile. La mastite clinique chez chèvres était souvent associée à la présence de Staphylococcus aureus.

Mots-clés: mastite, bacéterien, glandes mammaires, ovins et caprins.

Abstract
The bacterial flora of mastitic and normal milk samples of Sokoto red and Kano brown breeds of goats and Yankasa breed of sheep in Shika, Samaru and Zango market were examined. The following bacteria were isolated from 56 mastitic goat milk samples: Staphylococcus aureus 13 (31.70%); Micrococcus sp 6 (14.6%); Bacillus sp (14.7%); E. coli and Klebsiella sp (7.3%) each whereas Staphylococcus aureus, Micrococcus sp and Moraxella sp (11.1%) each and Bacillus sp (22.2%) were isolated from the 20 normal goat milk samples.

Our study revealed no cases of mastitis in sheep whereas 56% of the goats examined had mastitis. Only 2 (4.4%) out of the 45 sheep milk samples yielded bacterial growth (Micrococcus sp) while the remaining 43 (95.6%) were sterile. Clinical mastitis in goats was found to be largely associated with the presence of Staphylococcus aureus.

Keywords: mastitis, bacterial, mammary glands, sheep and goats.

Introduction
Mastitis has been defined as inflammation of the mammary gland caused by microbial infection or undue stress on the mammary tissue or both(1). It is characterized by physical, chemical and bacteriological changes in the glandular tissue(2). Clinically, the disease is identified by swelling, induration of mammary glands and noticeable change in milk colour and presence of clots. The disease affects all species of animals. It however, assumes major economic importance only in dairy cattle. Testing for and treatment of mastitis in Nigeria is not yet routine in many herds as in developed countries.

Many infectious agents are incriminated as causative agents of mastitis in farm animals. The most common ones reported include: Staphylococcus aureus, Streptococcus sp, Corynebacterium pyogenes, pasteurella multocids, Escherichia coli, Corynebacteria pseudotuberculosis (3,4,5,6,2).
Although the population of sheep and goats in Nigeria is increasing, their importance lies more in meat than milk production. In spite of the nutritive value of the milk, very little quantity is consumed by man and this is why problems associated with goat milk production have not been investigated to any great extent (4,6). Thus, the objective of this work was to study the bacterial flora of sheep and goat mastitis in Zaria and its environs.

**Materials and Methods**

**Areas of survey**
The study was carried out in Shika, Samaru, Zango and Tudun Wada 'sheep - and - goat' market in Zaria, Kaduna State, Nigeria. The study area lies in the northern part of the Guinea Savanna between latitude 10° and 12° North of the equator and between longitude 7° and 8°.

**Animals**
The animals used for this study were divided into four groups: the first group were flocks herded by small boys in the morning and returned to their sheds in the evening. The second group were those kept around houses roaming the streets while the third group were those found in government and institutional farms. The last group comprised goats/sheep brought to the market for sale at the 'sheep-and-goat' market along Kano road in Zaria. As a result of the variation in management of these animals, the survey was done on individual animals.

**Collection of milk for bacteriological examination**
The mammary glands were examined for evidence of enlargement, hyperthermia, pain, consistency and patency of lactiferous duct. Milk was then obtained from the mammary gland whether infected or not infected (control). The mammary glands were washed with soap and water, then dried with a towel and milked into sterile bottles after stripping. Milk samples were placed in an ice pack flask and sent immediately to the laboratory where they were stored in the refrigerator at 4°C until cultured. In cases where teats were blocked, milk samples were aspirated with a sterile hypodermic needle and 10 ml syringe.

**Bacteriological examination**
Milk samples were centrifuged at 1000 x g for 10 minutes and the sediment inoculated into 5% defibrinated blood agar, MacConkey agar and Eosin methylene blue (EMB).

Plates were incubated at 37°C aerobically for 24 - 72 hours and then examined for bacterial growth. Isolates were subcultured and biochemically tested according to standard bacteriological procedure (7,8).

**Results**

**Sheep**
Forty-five sheep were examined and no mastitic mammary gland were observed. Of the 45 milk samples from apparently normal sheep mammary glands, only 2 (4.4%) had bacterial growth while the remaining 43 (95.6) were sterile. These two isolates were *Micrococcus sp.*

**Goats**
A hundred adult female goats were examined and fifty-six mammary glands were found to be mastitic. Fifteen goats of the mastitic group had bilateral enlargement of the mammary gland while the rest had either left or right halves enlarged. Sometimes both halves were unequally enlarged. There was no significant difference between the infection rate of either the right or left half.

Visual inspection and palpation of the mastitic mammary gland showed the following conditions at varying degrees: teat laceration, sloughing of the skin of the mammary glands, induration, blockage of the lactiferous duct and teat cisterns. Milk obtained from the affected mammary glands had varying appearances such as opaque, white watery, light to dirty brown and watery while others were thick with fibrin clots. An abscess on the mammary gland of one goat ruptured during sampling. Milk samples from non-mastitic (control) goats were of normal appearance.

Table 1 shows the bacterial organisms and
Table 1: Relative frequency and types of bacteria in normal and mastitic goat udders

<table>
<thead>
<tr>
<th>Type of Udder Specimens examined</th>
<th>Number of</th>
<th>S. aureus</th>
<th>Micrococcus sp</th>
<th>E. coli</th>
<th>Aeromonas sp</th>
<th>Moraxella sp</th>
<th>Shigella sp</th>
<th>Bacillus cereus</th>
<th>Streptococcus sp</th>
<th>Klebsiella sp</th>
<th>Citrobacter sp</th>
<th>Corynebacterium sp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>20</td>
<td>3(33.3)</td>
<td>3(33.3)</td>
<td>-</td>
<td>-</td>
<td>1(11.1)</td>
<td>-</td>
<td>2(22.2)</td>
<td></td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Mastitic</td>
<td>56</td>
<td>13(31.7)</td>
<td>6(14.6)</td>
<td>3(7.3)</td>
<td>2(4.0)</td>
<td>4(9.7)</td>
<td>6(14.6)</td>
<td>1(2.4)</td>
<td>3(7.3)</td>
<td>1(2.4)</td>
<td>2(4.0)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>76</td>
<td>16(32.0)</td>
<td>9(18.0)</td>
<td>3(6.0)</td>
<td>2(4.0)</td>
<td>1(2.0)</td>
<td>8(16.0)</td>
<td>1(2.0)</td>
<td>3(6.0)</td>
<td>1(2.0)</td>
<td>2(4.0)</td>
<td></td>
</tr>
</tbody>
</table>

Figures out of brackets indicate number of isolate
Figures in brackets indicate percentage

The frequency of isolation from the mastitic and normal milk samples. Eight milk samples of the mastitic mammary glands had multiple isolates. It can be seen that the most frequently isolated bacterium was *Staphylococcus aureus* (13.7%). Others included *Micrococcus sp* and *Bacillus sp* each 14.6%, *Shigella sp* 9.7%, *E. coli* 7.3%, *Klebsiella sp* 7%, *Corynebacterium sp* 4.9%, *Aeromonas sp* 4%, *Streptococcus sp* 2.4%, and *Citrobacter sp* 2% from the mastitic milk samples while *Micrococcus sp* and *Staphylococcus aureus* had the same frequency of occurrence in the non-mastitic milk samples. The only other organism found in normal milk was *Moraxella sp*. All bacteria isolated from mastitic milk samples except *S. aureus*, *Bacillus sp* and *Micrococcus sp* were absent from normal milk samples. There was no significant difference in the rate of occurrence of mastitis between the goat breeds.

Discussion

The result of this work shows that sheep mastitis is not common in Zaria area. The fact that only 2 out of 45 milk samples yielded bacteria growth indicated that there were no subclinical infections. The *Micrococcus sp* isolated from the two samples may be considered normal flora. Similar observations have been reported before.

Goats on the other hand had a big problem of mastitis. Of the goats examined, 56% had mastitis. The study indicated that *Staphylococcus aureus* was the most common isolate or agent causing mastitis in goats. This agrees with the findings of other workers. Isolation of *S. aureus* from normal milk probably indicates latent infection. Other workers observed that *C. pyogenes* was the common isolate in the United Arab Republic followed by *Staphylococcus spp* while *Staphylococcus sp* was reported as the commonest isolate in India. Contrary to other findings that *Streptococcus sp* was the next important pathogen causing mastitis in goats, only one case of mastitis in our present study was due to *Streptococcus sp*. The difference could be due to different methods used in the isolation or they might have been the actual pathogens at the time of sampling, although the presence of *Streptococcus sp* in cases of mastitis in Zaria has been reported.

*Corynebacterium sp* was isolated in only two cases in this study. Others reported that *Corynebacterium sp* was the most common pathogen causing goat mastitis in the United Arab Republic. Similar findings have been reported. Results from this study did not indicate *Corynebacterium sp* as a common pathogen of goat mastitis in Zaria area. *E. coli* was recorded in 7.3% of the mastitic goats. Similar findings have been reported. Since *E. coli* is a normal flora in the intestinal tract of both animals and humans, we regarded it as an opportunistic invader.

*Bacillus sp* (14.6%), *Aeromonas sp* (4.8%), *Klebsiella sp* (7%) and *Citrobacter sp* (2.4%) were encountered in this study; their importance as the primary causes of
mastitis in goats in this environment needs further investigation. It is likely that they were contaminants.

References

CURRENT STATUS OF BOVINE TUBERCULOSIS IN TWO STATES OF NORTHERN NIGERIA

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

SITUATION ACTUELLE DE LA TUBERCULOSE BOVINE DANS DEUX ETATS AU NORD DU NIGERIA

Résumé

La prévalence totale de la tuberculose bovine dans les deux États était de 0,58% (1643/282,975). L’État du Plateau a connu une prévalence plus forte de 0,68% (949/139,422), tandis que l’on a enregistré un taux de 0,48% (694/143,553) dans l’État de Kaduna. Les lésions étaient plus répandues dans les poumons des bovins affectés, mais moins visibles dans la plèvre et le péritoine. Les informations recueillies des interviews ont montré que la maladie est endémique dans la zone d’étude et a des conséquences considérables sur l’économie et la santé publique.

Le test et la méthode d’abattage que l’on envisage d’appliquer au Nigeria, pourrait être le moyen le plus sûr de ramener à un niveau plus faible la prévalence de la maladie.

Abstract
A prospective survey was carried out in two Northern states of Nigeria to determine the current status of bovine tuberculosis in slaughtered cattle between 1989 and 1993 inclusive. Tuberculous-like lesions obtained at meat inspection were collected and sent to the laboratory for preliminary identification using the acid-fast technique. In addition, personal interviews were conducted with the abattoir managers and butchers in the study area to determine factors that may be contributory to the current status of the disease.

The overall prevalence of bovine tuberculosis in the two states was found to be 0.58% (1643/282,975). Plateau state recorded a higher prevalence of 0.68% (949/139,422) while Kaduna state recorded 0.48% (694/143,553). The lesions were most commonly located in the lungs of the affected cattle and least in the pleura and peritoneum. Information obtained from the interviews indicated that the disease is endemic in the study area with considerable economic and public health significance.

The test and slaughter policy, yet to be embarked upon in Nigeria, may be the surest option for reducing the prevalence of the disease to a tolerable level.

Introduction
Tuberculosis is, by far, the most important zoonotic disease of all warm-blooded mammals that presents a particularly serious problem in cattle in developing countries where it is said to have been eliminated through the test and slaughter policy. In cattle, the most important causative agents are Mycobacterium bovis and M. tuberculosis which are difficult to distinguish clinically (2a).

The disease is characterized by progressive development of tubercles in any of the organs in most species, abscess formation with resulting caseation and calcification, cachexia and high fatality (3). It is a density dependent disease most commonly transmitt-
ted through inhalation and ingestion of contaminated food and water. Thus infected humans and animals constitute the principal reservoirs for human infections.

Reports in Nigeria (2a,2b,4,4.6.7) have confirmed the existence and importance of the disease in the indigenous cattle population. The present study specifically investigates the current status of bovine tuberculosis based on the acid-fast staining technique, and the organ distribution in slaughtered cattle in Kaduna and Plateau states of Northern Nigeria. It also seeks to determine other factors that are likely to be contributory to the current status of the disease in the affected states through personal interviews with abattoir managers and butchers.

Materials and Methods

Between 1989 and 1993, a survey was conducted at fourteen randomly selected abattoirs in Kaduna and Plateau states of Northern Nigeria to determine the current status of bovine tuberculosis in slaughtered cattle. Characteristic nodular lesions that had a gritty sensation on sectioning during routine meat inspection were collected, packed in ice and sent to the laboratory in Zaria within 48 hours for preliminary identification using the acid-fast technique.

The organ location of the lesion encountered was identified accordingly and recorded.

In the laboratory, the lesion was aseptically cut open and the caseous exudate smashed and smeared onto a clean glass slide, fixed in alcohol and stained with 5% carbonyl fuschin(2a). The slide was decolorized with acid alcohol and counter-stained with methylene blue dye. Excess stains were washed off with water after which it was air-dried and examined under the microscope for slim, slender pinkish rods (acid-fast) in a bluish background.

In addition, personal interviews were conducted with the abattoir managers and butchers within the study area to obtain necessary information as regards on-the-job experience, frequency of meat inspection, who is involved in meat inspection, whether tuberculosis is a common finding, method of disposal of condemned parts or carcasses and the sources of cattle offered for slaughter. The butchers were also asked whether they could identify suspect tuberculosis lesions and if any of them ever had chronic cough. All responses to the interviews were tabulated and subjected to statistical analysis.

Results

The prevalence of bovine tuberculosis in the two states surveyed as revealed by the acid-fast technique was 164/282,975 (0.58%). Plateau state recorded a higher prevalence, 949/139,422 (0.68%), than Kaduna state, 694,143,553 (0.48%) within the study period (Table 1). The year 1990 recorded the highest annual prevalence in each state understudy: 193/28,591 (0.68%) and 118/15,336 (0.77%) for Kaduna and Plateau States respectively. In both cases, there was a steady increase in the prevalence rates throughout the study period. The quarterly prevalence on the other hand, indicated the highest number of cases in the second quarter (April–June) of each year.

The distribution of the lesions was site-dependent (P<0.05, X²) as over 67% (1104/1643) of the lesions that gave positive results were located in the lungs while about 27.6% (453/1643) of such lesions were encountered in the lymph node or liver. Less than 6% of the positive lesions were located in the Pleura or peritoneum (Table 2).

The responses of the abattoir managers to the questionnaire indicated that over 80% of those interviewed were experienced veterinarians/meat inspectors (with a minimum of 5 years experience) and were familiar with the practice of meat inspection. It also indicated that routine meat inspection was being conducted on a daily basis, and that tuberculosis-like lesions were a common finding. All animals offered for slaughter were either from within the states or from neighbouring states. Interviews with the butchers on the other hand indicated that over 50% of them had a minimum of 10 years experience on the job and were capable of identifying suspect tuberculosis lesions during carcass processing. None of them accepted having chronic cough.
Table 1: Prevalence of bovine tuberculosis in Kaduna and Plateau States of Northern Nigeria (1989-93)

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Kaduna</td>
<td>No. slaughtered</td>
<td>27,430</td>
<td>28,591</td>
<td>28,283</td>
<td>29,943</td>
<td>29,306</td>
</tr>
<tr>
<td></td>
<td>No. positive (% positive)</td>
<td>97(0.35)</td>
<td>193(0.68)</td>
<td>138(0.49)</td>
<td>140(0.47)</td>
<td>126(0.43)</td>
</tr>
<tr>
<td>Plateau State</td>
<td>No. slaughtered</td>
<td>29,8282</td>
<td>15,336</td>
<td>34,672</td>
<td>39,677</td>
<td>29,919</td>
</tr>
<tr>
<td></td>
<td>No. positive (% positive)</td>
<td>169(0.57)</td>
<td>118(0.77)</td>
<td>236(0.68)</td>
<td>270(0.68)</td>
<td>156(0.52)</td>
</tr>
<tr>
<td>Total</td>
<td>Positive (%)</td>
<td>266(0.46)</td>
<td>311(0.71)</td>
<td>374(0.59)</td>
<td>410(0.59)</td>
<td>282(0.48)</td>
</tr>
</tbody>
</table>

Table 2: Organ location of tuberculous lesions encountered at slaughter in Kaduna and Plateau States (1989-93)

<table>
<thead>
<tr>
<th>Organ location</th>
<th>No. positive</th>
<th>% positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lungs</td>
<td>1104</td>
<td>67.2%</td>
</tr>
<tr>
<td>Pleura/peritoneum</td>
<td>86</td>
<td>5.2%</td>
</tr>
<tr>
<td>Liver/lymph node</td>
<td>453</td>
<td>27.6%</td>
</tr>
<tr>
<td>Total</td>
<td>1643</td>
<td>100%</td>
</tr>
</tbody>
</table>

Discussion

The overall prevalence rate of 0.58% for bovine tuberculosis as revealed in this study is on the increase as compared to previous studies in the Northern region of Nigeria. The steady increase in prevalence may probably be due to the fact that tuberculin testing and subsequent elimination of infected animals is not a routine practice in Nigeria as of now, as one would have desired, probably because of the fear of its financial implication, especially in the areas of paying compensation to the owners. In most parts of the world where this test and slaughter policy is practiced, it is uncommon to detect such a relatively high prevalence at meat inspection.

The quarterly prevalence was highest for the second quarter (April–June) of each year and this corresponds to the peak of acute water shortage just before the rains, as experienced in the study area. During this period, there is a large concentration of animals at stagnant water points and hence the ease of aerosol transmission. The predominant mode of transmission of the disease is by inhalation and this is evident in the present study as over 67% of the positive lesions were found located in the lungs of the affected cattle. Findings in this study also suggest that the oral route of transmission may be of significance in the indigenous Nigerian cattle as over 27% of the positive lesions were encountered in the lymph node (especially the mesenteric lymph node) and/or the liver. This finding agrees with previous reports.

The responses of the abattoir managers and the butchers to the questionnaires indicates that over 80% of those interviewed had adequate experience in their respective jobs, and were capable of recognising tuberculous lesions, and the fact that such lesions were a common finding goes to suggest that tuberculosis is endemic in the study area. This is supported by the fact that less than 25% of total suspected lesions submitted to the laboratory for analysis tested negative for Mycobacterium species. Apart from other diseases such as nocardiosis and coccidioidomycosis which produce similar granulomas, it is also possible that the circumscribed tubercles became sterile with age.

Furthermore, the time lag between collection and analysis of the sample, especially given the hot climatic conditions in the study area may account for the negative results.
Tuberculous parts or carcasses were partially or totally condemned depending on the magnitude of the spread and this amounts to substantial economic loss and a reduction in the amount of animal protein available for human consumption.

Acknowledgment

The authors are grateful to the staff of Veterinary Division, especially the abattoir managers, in the study areas for their co-operation throughout the study period. The Financial support provided by the Ahmadu Bello University, Zaria, is hereby well appreciated.

References


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MICROBIAL DEVELOPMENT AND SOME CHEMICAL CHANGES DURING THE MAKING OF ERGO, A TRADITIONAL ETHIOPIAN FERMENTED MILK

MOGESSIE ASHENAFI
Department of Basic Sciences, Awassa College of Agriculture
P.O. Box 5, Awassa, Ethiopia

DEVELOPPEMENT DES MICROBES ET QUELQUES CHANGEMENTS CLINIQUES OBSERVES PENDANT LA PREPARATION DE L’ERGO, UN LAIT TRADITIONNEL ETHIOPIEN FERMENTE

Résumé
Le développement des microorganismes pendant la fermentation de l’Ergo a été examiné en utilisant du lait cru collectée de huit fermes laitières à Awassa. Le dénombrement initial d’organismes mésoophiliques variait entre 10^4 cfu/ml et 10^6 cfu/ml. Pour la plupart des échantillons, l’Ergo était prêt après 24 h et le dénombrement était en moyenne > 10^6 cfu/ml. Les coliformes, les bactéries de l’acide lactique et les ferment avaient respectivement en moyenne des dénombrements initiaux de < 10 - 10^4 cfu/ml, 10 - 10^4 cfu/ml et < 10 cfu/ml. À la fin de la fermentation, ces chiffres s’élevaient respectivement à 10^5 cfu/ml, 10^5 cfu/ml et 10^5 cfu/ml. La teneur moyenne en pH et en acides lactique de l’Ergo était respectivement de 4,3 et 0,88%. Une forte variabilité a été observée entre les échantillons pour ce qui est du dénombrement et des valeurs. La microflore pendant la fermentation a été dominée dans la plupart des cas par les lactobacilles ayant la forme de cocciobacilles.

Abstract
The development of microorganisms during Ergo fermentation was studied using raw milk collected from eight dairy farms in Awassa. Initial aerobic mesophilic count varied between 10^4 cfu/ml and 10^6 cfu/ml. In most samples, Ergo was formed at 24 h and the average count was > 10^6 cfu/ml. Coliforms, lactic acid and bacteria and yeasts had average initial counts of <10-10^4 cfu/ml, 10^4-10^5 cfu/ml, and < 10 cfu/ml, respectively and their count at completion of fermentation rose to 10^5 cfu/ml, 10^5 cfu/ml and >10^6 cfu/ml, respectively. Average pH and lactic acid content of Ergo was 4.3 and 0.88%, respectively. High variability was seen in count and values between samples. The microflora during fermentation was dominated in most cases by cocciobacillus-shaped lactobacilli.

Introduction
For centuries a significant proportion of milk has been consumed in the form of freshly prepared fermented products. Basically, this is milk that is soured by a lactic acid fermentation. In some fermented milk, a citric acid fermentation produces neutral flavour compounds, primarily diacetyl, and certain volatile flavourful organic acids. In some instances, a small amount of ethyl alcohol is present.

Several different species of microorganisms are used in the making of fermented milks. The consistency of fermented milk varies, ranging from the easily pourable to that having a thick, jelly-like structure. Its composition also varies. Some may have essentially similar composition to that of whole milk or skim milk, while in some cases it may be fortified with additional milk solids. Various flavors or fruits are often added. In many countries, the composition of fermented milk is specified by regulation.

There are a variety of fermented milk products in the world. Among the well-known fermented milk types are yoghurt, acidophilus milk, cultured butter milk and other region-specific types such as kefir and kumiss. Oberman lists about 25 different types of fermented milk products. In most cases the starter microflora is defined. There are, however, fermented milk products such as Prokis (Asia, Africa, Europe), Zhentitsa (East Carpathian mountains), Taet-Mjoelk and Kjadder milk (Scandinavia), and Sos-tej (Hungary) where fermentation is effected by unknown
or undefined mixed microflora. The Ethiopian Ergo may thus be classified as one of those products, whose fermenting microflora is undefined or unknown. The purpose of this study was to examine the development of microorganisms during the natural fermentation of Ergo and to see changes in some of its chemical parameters.

Materials and Methods

Collection of samples
One litre of milk was collected from milking utensils in a sterile bottle from each of eight dairy farms in Awassa town. Samples were immediately brought to the laboratory and allowed to ferment naturally at 25°C.

Microbiological analyses
Fermenting milk was sampled at 0, 6, 12, 24, and 36h and the following microbiological and chemical parameters were determined.

Aerobic mesophilic count: Appropriate dilutions were surface-plated on pre-dried surfaces of China Blue Lactose Agar (Oxoid) for total count and count of lactose-fermenters.

Coliform count: Appropriate dilutions were surface-plated on pre-dried surfaces of Violet Red Bile Agar (VRB) (Oxoid) and plates were incubated at 30 to 32°C for 24 hours. Pink colonies with bile precipitation around them were counted as coliforms.

Yeasts: Appropriate dilutions were surface plated on pre-dried surfaces of chloramphenicol-Bromphenol Blue Agar: 0.5% yeast extract; 2% glucose; 0.01% chloramphenicol; 0.001% bromophenol blue; 1.5% agar. pH was adjusted to 6.0–6.4. Plates were incubated at 25–27°C for 3 to 5 days.

Lactic acid bacteria (LAB): Appropriate dilutions were plated in duplicates on LSD (Oxoid) agar and aerobically incubated at 30°C for 5 days.

pH and titratable acidity: The pH of fermenting milk was measured using a digital pH meter. A volume of 0.9 ml of fermenting milk was titrated against 0.1N NaOH to determine % titratable acidity as lactic acid. Organoleptic tastes were carried out by laboratory staff to evaluate the taste and flavor of the final product.

Differentiation of microflora
Microbial differentiation was done for only 5 of the samples. Ten to twenty colonies were picked at random from countable plates of China Blue Lactose Agar and LSD agar. The isolates were examined microscopically, purified by repeated plating and differentiated using the following tests: Gram reaction was tested by the KOH method of Gregersen. Shape, motility and presence of spores were determined using phase contrast microscopy. The method of Kovacs was applied to test for cytochrome oxidase, and catalase formation was detected using 3% H₂O₂. The oxidative and fermentative properties of the isolates were investigated by the O/F test of Hugh and Leifson. Based on these tests, the isolates were tentatively identified to the genus level.

Isolates from LSD were also examined microscopically and tested for their catalase activity. Catalase negative and non-sporing rods and cocci were considered as lactic acid bacteria.

Results
The initial aerobic mesophilic count of milk sampled from milking utensils of the eight dairy farms ranged from 10⁴ cfu/ml to 10⁶ cfu/ml (Fig. 1). There was a constant increase in aerobic mesophilic counts and at 24h, most samples had a thick jelly-like structure (Ergo) and the average count was > 10⁵ cfu/ml. Coefficient of variation (V) was 15.6% for counts at 0h, but this was lower for the remaining sampling times (V < 7%). The initial count of coliforms was highly variable (V > 30%) in the samples ranging between <10 and 10⁴ cfu/ml (Fig. 2). Coliform count increased in the first 12h, and the average count reached 10⁶ cfu/ml, although this was variable within samples (V > 10%). At 24h and thereafter, the coliform count decreased gradually following a fall in pH below 4.3.

The initial count of lactic acid bacteria in the various samples varied markedly (V = 15%), ranging between 10⁴ cfu/ml and 10⁶ cfu/ml (Fig. 3). A count of 10⁶ cfu/ml was reached at 12h with slight variation between samples.
Fig 1. Variations in counts of aerobic mesophilic bacteria during Ergo fermentation from various milk samples (line represents mean values).

Fig 2. Variations in counts of coliforms during Ergo fermentation from various milk samples (line represents mean values).

Table 1: Changes in dominant bacteria in four fermenting Ergo samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Time (hr)</th>
<th>LAB LAB*</th>
<th>Gram* cocci</th>
<th>Gram* rods</th>
<th>Gram* rods</th>
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<td>30</td>
<td>30</td>
<td>10</td>
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*LAB = Lactic acid bacteria
Fig. 3. Variations in counts of lactic acid bacteria during Ergo fermentation from various milk samples (line represents mean values)

Fig. 4. Changes in pH and lactic acid content in fermenting Ergo from various milk samples (line represents mean values)

After 24h, a slight decrease in LAB count was detected in 50% of the samples. LAB count reached a level of $10^9$ cfu/ml or higher at different times in the different samples.

Initial yeast count in most samples was <10 cfu/ml. Final counts of $>10^5$ cfu/ml were detected in many samples; whereas in some samples the count remained <10^4 cfu/ml (data not shown).

Average initial pH of the raw milk samples was 6.7 and showed little variation between samples. The drop in pH was gradual (an average of 0.5 units) until 12h, and a sharp fall was observed thereafter. Most samples reached pH values of 4.3 or lower at and after 24h (Fig. 4).

Average initial titratable acidity for the samples was 0.16%, the highest value being 1.8% (Fig. 4). Marked increase in titratable acidity was seen at 12h and thereafter.

Titratable acidity values of more than 1% were recorded for some samples at different
times. The highest average value was, however, 0.88%

Most samples had favourable Ergo taste at 24h. There were, however, instances where different samples with pH4.0 were evaluated as good, too sour or “flat”. There were also cases where samples with 0.69% lactic acid were found to taste flat, samples with 0.61% or 0.82% to taste good and samples with 0.70% to taste sour.

A total of 286 isolates from China Blue Lactose Agar were differentiated to the genus level. In four of the five samples, which were analyzed for their microbial flora, lactic acid bacteria and gram positive cocci (mainly micrococci) constituted more than 60% of the initial microflora (Table 1). During souring, the lactic acid bacteria dominated the microflora followed by gram negative rods, consisting of mainly enterobacteria.

Among the 150 strains isolated from LSD medium, coccobiculli in pairs dominated the initial lactic acid flora and remained dominant up to 24h. In one sample, however, streptococcidi dominated the initial lactic acid flora until 12h and then a shift towards coccobiculli was observed (data not shown).

**Discussion**

Variations in the initial counts and types of the microflora in raw milk resulted in dissimilarity in the microbial development during the fermentation of Ergo from raw milk in the various samples. Whereas some samples showed their highest count at 12h, others showed this at 36h. Thus the reason why Ergo has varying consistency and taste from place to place and from time to time, could be due to the large variation in the initial count and type of microflora and the rate of development of this microflora during souring.

Although the pH and lactic acid content of Ergo varied from sample to sample in this study, the values were within the limits for fermented milk types (pH3.8-4.6; L.A. 0.6-1.3) as given by FIL-IDF(2)

This study has shown that the rate of coliform growth was rather low and the highest count reached was only about 10^6 cfu/ml. In addition there was also a constant decline in coliform count after 24h. This may be due to the low pH, the level of lactic acid and possible presence of small quantities of other organic acids and other inhibitory components which provide unfavorable environment. The rapid disappearance of coliforms by the pH is also noted in yoghurt fermentation and storage(2).

Titrable acidity is one of the parameters used to control the end point of fermentation as in the case of cultured buttermilk and acidophilus milk(1). This is practical when the type of the starter cultures and their behavior during fermentation is well-understood. In a product like Ergo, however, where fermentation occurs naturally and the role of the fermenting flora is not controlled, it is difficult to use parameters like titratable acidity to control the fermentation.

The development of lactic acid bacteria is desirable during Ergo fermentation. These are actually the starter cultures which produce lactic acid and the other flavor components. A product containing insufficient acid may taste “flat” because the highly flavored diacetyl and volatile acids are not formed in appreciable amounts until late in the fermentation(1). In a mixed culture fermentation like Ergo, careful control of the balance between the acid producers and the flavour types must be maintained. For cultured buttermilk, an incubation temperature of 21 – 22°C is essential, yoghurt is fermented at 42 – 45°C and acidophilus milk is produced at 38 – 40°C(1). Thus, in order to produce a well-accepted and uniform Ergo, the starter microorganisms and the appropriate incubation temperature which produce an acceptable flavour and acidity must be determined.

It is too early to define the lactic acid flora of Ergo at this level, although coccobiculli-shaped lactobacillus dominated in most of the samples. The dominance of streptococcidi in one sample indicated lack of uniformity in the nature of the lactic acid flora. The type and dominance of the lactic acid flora may vary in the various ecological zones of the country depending in the nature of the initial flora, degree of contamination, temperature of incubation and various other factors.

The presence of micrococci as part of the
dominant flora may not have significance in Ergo because they are usually important as psychrotrophic spoilage microorganisms and Ergo is not traditionally stored at low temperatures for longer periods; it is rather consumed fresh. *Micrococcus* spp. have a high intracellular protease and peptidase activity and are among the psychrotrophic bacteria which exhibit proteolytic activity in dairy products. Presence of coliforms in Ergo is indicative of fecal contamination. Obviously, the low pH of Ergo may rapidly inactivate coliforms. However, some pathogens, like *Salmonella, Listeria monocytogenes* and *Staphylococcus aureus* may withstand the adverse effects of low pH and multiply. Despite general belief that the low pH in Ergo could control the proliferation of undesirable microorganisms, previous studies have indicated that the dangers of infection with *Salmonella, S. aureus*, and *L. monocytogenes* from fresh Ergo must not be undermined. The higher count of yeasts in Ergo may be undesirable. They may be troublesome, for many are unaffected by the lactic acid. In fact, they utilize it and grow well in association with the normal culture organisms, thereby raising the pH and producing yeasty and fruity off-flavours, often with gassiness from CO₂.

There is perhaps not much to do about the microbiological quality of Ergo, if Ergo remains to be produced on a household level. Use of an old Ergo as a starter culture for a boiled and cooled milk may help to produce a more or less uniform and safe product. But if Ergo is to be produced on a factory level, some tasks should be undertaken beforehand. First, as many lactic acid bacteria as possible have to be isolated from Ergo produced in the various ecological zones of the country. These cultures have to be identified and various combinations of them may then be used for a controlled fermentation of Ergo. The organoleptic quality of the product in relation to the various combinations may be determined. Those combinations having favourable organoleptic quality may then be tested for their sensitivity to phases before they are used for large-scale production. It may then be possible to define Ergo in terms of its microbiology and biochemistry.

Acknowledgment

The technical assistance of Haile Alemayehu and Tsigereda Bekele is acknowledged.

References

EVIDENCE OF EQUINE INFLUENZA VIRUS OCCURRENCE IN NIGERIA: NATION-WIDE SERO-EPIDEMIOLOGICAL SURVEY

C.A.O. ADEYEFA1,2,4, C. HAMBLIN1, A.A. CULLINANE3 AND J.W. McCULEY1
1. Institute for Animal Health, Pirbright Laboratory, Ash Road, Pirbright GU24 0NF, Surrey, U.K.
2. Department of Veterinary Medicine, University of Ibadan, Nigeria.
4. Author to whom correspondence should be addressed at the Department of Veterinary Medicine, University of Ibadan, Nigeria.

PREUVE DE LA PRESENCE DU VIRUS DE LA GRIPPE EQUINE AU NIGERIA: ENQUETE SERO-EPIDEMIOLOGIQUE DANS TOUT LE PAYS

Résumé
Il existe très peu d’informations sur l’épidémiologie de la grippe équine au Nigeria et en Afrique tropicale en dépit des mouvements réguliers/occasionnels des chevaux dans cette région pour les manifestations équestres. A la suite de l’isolement des virus de la grippe équine du Nigeria, une enquête sérologique des anticorps de la grippe équine a été menée dans tout le pays pour déterminer l’épidémiologie de la grippe équine au Nigeria. Selon les résultats de cette enquête, la grippe équine sévit dans la majeure partie du Nigeria avec un taux d’incidence de 18% en moyenne et les virus de la grippe équine se sont répandus depuis longtemps dans la population équine de ce pays.

La technique ELISA décrite dans la présente étude a permis de reconnaître, chez les chevaux, la différence entre l’infection aigue et les réactions à l’anticorps après la vaccination.

Abstract
There is paucity of information on the epidemiology of equine influenza in Nigeria and tropical Africa despite regular/occasional movement of horses into this region for equestrian events. Following isolation of equine influenza viruses from Nigeria, a nation-wide serological survey of equine influenza antibodies was carried out to determine the epidemiology of equine influenza in Nigeria. The results of this study indicate that equine influenza occurs in most parts of Nigeria with an average of about 18% incidence rate and that equine influenza viruses have been circulating among equine populations in Nigeria for a long time.

The competition ELISA described in this study appears to be useful in discriminating between acute infection and post-vaccination antibody response in horses.

Introduction
Influenza A viruses are widespread in nature and cause disease in man, animals and birds(4). These viruses are classified according to the antigenic differences between their haemagglutinin (HA) and neuraminidase (NA) glycoproteins. So far, 14HA and 9NA subtypes of influenza A virus have been identified(2) none of which are serologically cross-reactive. Two subtypes cause clinical disease in horses, the H7N7 and H3N8 subtypes referred to as equine-1 and equine-2 respectively. Both have been reported to circulate individually in horse populations in the world with the exception of Australia(3) while both had previously co-circulated in some horses(4). Although the last recorded isolation of H7N7 virus from horses in the field was in 1978(5), there is serological evidence to suggest continued circulation of the virus, (Webster, R.G. unpublished data). However, the mechanism of virus maintenance in horses is not clear but it seems more likely that the viruses are passed continuously to susceptible horses(5).

There is paucity of information on the epidemiology of equine influenza in tropical Africa. Recently, we reported the first known outbreak of equine influenza in Nigeria among polo horses.
from which 3 influenza viruses of H3N8 subtype was independently isolated\(^7\). Studies on molecular characterisation of these viruses indicated that their genes are all equine in origin and that the HA is most closely related to recent U.S.A and European isolates\(^6\).

However, the exact origin/source of these viruses is unknown. It is possible that they were already circulating among the equine populations in Nigeria prior to this outbreak. Durojaiye and Denya in 1982 (personal communication) observed antibodies to both equine-1 and equine-2 viruses in some Nigerian horses. Alternatively, it is probable that the viruses were brought into the country with horses recently imported from Argentina or United Kingdom where there have been recent outbreaks of equine influenza\(^6\). Also, preliminary investigation in 1991 revealed antibodies to equine-2 influenza virus in horses not involved in the outbreak that we recently reported. The purpose of this study was, therefore, to further elucidate the epidemiology of equine influenza through a nation-wide serological survey. Comparative studies were also carried out with post-natural infection, pre- and post-vaccination equine sera from Ireland. We report here the results of our seroepidemiological survey and the competitive enzyme immunoassorbent assay (ELISA) test developed for this study.

**Materials and Methods**

**Virus antigen**

Equine-2 prototype A/Eq/Miami/63 (H3N8) virus was grown in 11-day-old embryonated hen eggs and purified by differential sedimentation through 15, (60% sucrose gradients in Beckman swing-out) 28 and 40.1 rotors. Virus concentration which was 20 mg/ml was determined at 260 nm wavelength in a UV spectrophotometer. The purified virus was used as an ELISA antigen at a concentration of 1 ng/ml.

**Equine sera**

A total of 375 equine sera were tested for antibodies to equine 2 influenza virus of H3N8 subtype. These comprised 181 horse and 6 donkey sera collected between Jan. 1990 and Oct. 1991 from various parts of Nigeria; 4 post-natural infection, 45 pre-vaccination and 45 post-vaccination sera collected between January and June 1992 at the Irish equine Center, all tested in Pirbright, as well as additional 42 horse and 52 donkey sera collected between July and December 1993 in Nigeria and tested in Ibadan. The sources, number and dates of collection of the Nigeria equine sera are shown in table 2.

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**Table 1: Correlation between HI and ELISA results of 94 Irish and 94 Nigerian equine sera.**

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## Evidence of Equine Influenza virus occurrence in Nigeria

### Table 2: Dates of collection, sources, number and percentage positive in Nigerian equine sera tested by competion ELISA

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### Fig 1. Map of Nigeria showing areas of sampling and the geographical spread of equine influenza virus activity
Competition ELISA
Monoclonal antibody (Mab) against the HA of A/Eq/Miami/63 (H3N8) was used in competition ELISA developed for this test. H1 clone of the Mab against the coding region of A/Eq/Miami/63 HA was used at a dilution of 1:2500.00. The ELISA was performed in Dynatech flexible ELISA plates. The optimal dilutions of test antigen and Mab were determined by checkerboard titrations in 0.05M carbonate bicarbonate buffer pH9.6. Test Sera were used at dilutions of 1.5.

Briefly, 50 µl per well of antigen diluted in carbonate bicarbonate buffer was passively absorbed onto the solid phase of ELISA plates which were then incubated at 37°C for 2 hours. Plates were washed 5 times with PBS pH 7.6 by flooding and emptying the wells which were blotted dry to remove residual washing buffer. 50 µl of 1:5 dilution of each test serum prepared in PBS containing 0.05% Tween 20 (PBST) blocking buffer was added to duplicate wells of polystyrene microwell transfer plates. Duplicate wells of column 11 of the transfer plates also received 50 µl per well of a known strongly positive convalescent antiserum, a weekly positive control antiserum and a negative control horse serum diluted in PBST while column 12 received 100 µl of PBS only. 50 µl of Mab diluted 1:250,000 in PBST was simultaneously added to each well in columns 1–11 of the transfer plates and mixed on an orbital shaker for 5 minutes. The contents of the transfer plates were then transferred into correspondingly marked ELISA antigen plates and incubated for 1 hour at 37°C on an orbital shaker after which they were washed and blotted dry. 2gm ovalbumin was dissolved in 40ml PBST which was used to dilute anti-mouse IgG horse radish peroxidase conjugate at 1:1000. 50 µl of conjugate/ovalbumin in PBST was added to each well and incubated for 1 hr at 37°C on a shaker. After washing, 50 µl/well of orthophenylene diamine/H₂O₂ was added. Colour development was rapid and was stopped after 10 minutes by addition of 50 µl/well of 1.25M H₂SO₄. Plates were read spectrophotometrically at 492nm with Titertek(R) Multiscan ELISA plate reader. The value for no competition (maximum colour) was obtained from the mean absorbance values at 492nm in column 12 and measures the interaction between mouse antiserum and influenza virus. Positive reactions were recorded when test sera in duplicate wells showed less than 50% inhibition of the mean absorbance value recorded in column 12 (the 8 virus control wells).

Haemagglutination inhibition test (HI)
HI was performed in microtiter plates with 1% chicken red blood cells by standard method. The 94 Irish equine sera and the additional 94 Nigerian equine sera were tested for HI antibodies. All sera used in HI test were pre-treated with potassium metaperiodate to preclude any non-specific inhibition. HI titres are expressed as reciprocals of the highest serum dilutions inhibiting 4HA units of virus.

Results
Only 29 of 181 horse sera collected between January 1990 and September 1991 in Nigeria were positive for ELISA antibodies while none of the 6 donkey sera was positive. However, 6 horse and 5 donkey sera respectively out of the 42 horse and 52 donkey sera collected between July and December 1993 in Nigeria were positive for ELISA antibodies. Of the 94 Irish horse sera, 1 of 4 natural infection sera, 2 of 45 pre-vaccination and 39 of 45 post-vaccination sera were positive by ELISA.

The percentages of positive sera by competition ELISA varied considerably among Nigerian equine sera and ranged between 9.1 and 50%. Although sera from some sources were negative, there was no part of the country where there were no positive serum samples. One of the 2 donkey sera from Ilorin was positive, indicating 50% positive, followed by horse sera from University of Ibadan Vet. Teaching Hospital (40%), Ibadan Polo Club (29.6%), Jos Polo Club (28.5%), Lagos Polo Club (25%) and Sokoto horse market (19.04%). Other positive samples ranged from 5.5 to 12.5% including those from Bia, Kano, Kaduna, Ilorin and Nsukka from where local information indicates that a few of horses sampled there were brought from Yola on the mid-northeastern border.
In the HI tests, 6 Nigerian horse and 6 donkey sera were positive for HI antibodies out of the 6 horse and 5 donkey sera positive by ELISA with HI titers ranging from 1 – 640. With the Irish horse sera, 33 out of 39 positive by ELISA were also positive by HI among the 45 post-vaccination sera while none of the 4 post-infection sera and only 2 of the 45 pre-vaccination sera were positive by HI as they were by ELISA with HI titers ranging from 16 – 512. Table 1 shows the correlation between HI and ELISA results of the Irish and Nigerian equine sera while Table 2 shows the ELISA results of Nigerian equine sera. The geographical spread and percentage positives of equine influenza 2 virus antibodies in Nigeria are shown in Fig. 1.

Discussion

The results of our nation-wide serological survey indicate that equine influenza occurs in most parts of Nigeria as there was no region of the country where there were no positive serum samples. Although the study of the circulation of equine influenza viruses within horse populations in any geographical area relies mostly on the detection of serum antibodies to these viruses, precise knowledge of the antigenic identity of the viruses prevalent in the areas under study is of inestimable value. We have previously isolated 3 equine-2 (H3N8) influenza viruses from sick horses involved in a recent outbreak of disease in Ibadan, Nigeria during a polo tournament in January 1991 (7) and studies are in progress to characterise the viruses at the molecular level to determine the origin of the virus genes. Some of the animals involved in this outbreak comprised indigenous and imported horses. Some of the sera collected from these horses and some collected from different parts of the country long before and long after the outbreak of disease showed antibodies to equine-2 virus. This suggests previous exposure of horses with positive sera to equine-2 (H3N8) influenza virus which implies that the virus(es) might have previously circulated and are still circulating in Nigerian equine populations.

Furthermore, a sustained presence of antibodies in peripheral circulation is characteristic of the immunological response to equine-2 influenza virus. Our detection of antibodies in sera collected long before the clinical outbreak of disease further supports the possibility of previous exposure of some of the horses to the virus. Alternatively the viruses we isolated were probably introduced to susceptible animals in Ibadan by the recently imported horses from endemic regions with the stress of transportation and the unfavourable dusty weather conditions in Ibadan at the time of outbreak acting as predisposing factors.

The Irish equine sera presented another picture entirely. Only 1 (25%) of the 4 post-clinical natural infection sera was positive by ELISA while only 2 (4.4%) of 45 pre-vaccination and 39 (66.6%) of 45 post-vaccination sera were positive by ELISA. These results correlated well with HI results although the ELISA test was more sensitive in detecting antibodies in 6 more sera than the HI test. The competition ELISA described in this study appears to be very useful in discriminating between acute infection and post-vaccination antibody responses in horses as indicated by the results of the Irish equine sera. However, the low percentages of positive sera detected by the test in the Nigerian sera could be due to the Mab used which was directed against a region of the HA and thus probably recognised a single or fewer epitopes on the antigen. A/Eq/Newmarket/76 polyclonal antiserum gave a higher percentage positive of positive sera when used in the ELISA test and on a limited number of randomly selected sera (data not shown).

Therefore, where influenza glycoprotein specific Mabs are not available for competition ELISA for extensive epidemiological investigations of influenza, the use of polyclonal antiserum may still yield useful results.

Acknowledgement

We are grateful to Dr. A. I. Daneji of Dept. of Vet. Medicine, Usman Dan Fodio University, Sokoto and Dr. V. O. Shoyinka, Dept. of Vet. Pathology, University of Nigeria, Nsukka for equine sera collected in July – December, 1993. The handling assistance of Mr. Tunde Mabawonku,
Dept. of Vet. Medicine, University of Ibadan during 1990 – 1991 sample collection is also acknowledged with thanks. Dr. Adeyefa was supported by the Association of Commonwealth Universities Academic Staff Fellowship tenable at the Pitbrigt Laboratory of the Institute for Animal Health, U.K. from January to November 1992.

References


PRELIMINARY EVIDENCE OF THE OCCURRENCE OF BLUE-TONGUE (BT) VIRUS INFECTION IN TANZANIAN SHEEP AND GOATS

J.M.K. HYERA AND V.H. LYARUU
Department of Virology, Animal Diseases Research Institute (ADRI)
P.O. Box 9254, Dar es Salaam, Tanzania.

PREUVÉ PRELIMINAIRE DE L’INCIDENCE DE L’INFECTION PAR LE VIRUS DE LA FIEVRE CATARRHALE CHEZ LES OVINS ET LES CAPRINS EN TANZANIE

Résumé
Au total, 292 sérum ovins et 1,173 sérum caprins recueillis de différentes localités géographiques de la Tanzanie ont été examinés pour établir la présence d’anticorps au virus de la fièvre catarrhale a l’aide de la technique ELISA (titrage avec immunosorbant lié à une enzyme). Des prévalences d’anticorps d’environ 74% (216/292) et 77% (908/1,173) ont respectivement été enregistrées chez les ovins et les caprins. Ces résultats sont discutés par rapport aux conclusions des autres enquêtes sérologiques conduites ailleurs.

Summary
A total of 292 sheep and 1,173 goat sera collected from different geographical locations of Tanzania were investigated for antibodies to Blue-tongue (BT) virus by use of the indirect Enzyme Linked Immunosorbent Assay (ELISA) technique. Antibody prevalence of approximately 74% (216/292) and 77% (908/1,173) were established respectively in sheep and goats. These results are discussed in relation to other serological findings reported elsewhere.

Introduction
Blue-tongue (BT) is an infectious non-contagious arthropod-borne viral disease of domestic and wild ruminants to which sheep are particularly most susceptible. The principal vectors of BT virus are biting midge or gnats of the genus Culicoides\(^{(1,2)}\).

BT was for a long time thought to be restricted to Africa particularly south of the Sahara\(^{(3,4,5,6,7)}\) but the disease has now been recognized in many countries outside Africa\(^{(2,8,9,10,11,12,13)}\). In East Africa (Kenya, Uganda, Tanzania, Rwanda and Burundi) BT virus and the respective vectors have received much attention in Kenya\(^{(11,14,15)}\). Little or nothing at all appears to have been done in relation to BT virus and/or its vectors in the other East African countries. This communication presents serological evidence on the occurrences of BT virus infection in Tanzania sheep and goats.

Materials and Methods
Sera
Blood samples from individual sheep and goats were collected by puncture of Vena jugularis using vacationer tubes. Samples were held at room temperature overnight to coagulate and thereafter the sera harvested individually; distributed in sterile bijou bottles which were properly labelled and stored at \(-20^\circ\text{C}\) until use.

Antibody detection
Group-specific antibodies to BT virus in the various test sera were investigated by the indirect Enzyme Linked Immunosorbent Assay (ELISA) following the procedure described by Anderson\(^{(16)}\). All test sera were investigated at a single dilution of 1 in 5.

The BT ELISA kit inclusive BT antigen, positive control serum, and rabbit anti-mouse IgG labelled with horse radish peroxidase were obtained from the Animal Health Institute (AHI)
Pirbright, through the courtesy of Dr. John Anderson. The substrate used was 0-
phenylenediamine (OPD) supplied to ADRI along with the rinderpest ELISA kit by the
International Atomic Energy Agency (IAEA), Vienna, Austria. The optical densities were
measured at 492nm using a standard ELISA reader.

**Statistical Analysis**

Point prevalence was used in this investigation. Prevalence was defined as the percentage of
sera which exhibited group-specific antibodies to BT virus. The standard (SE) of the respective
percentages were calculated by statistical methods\(^{17}\). To validate the significance of the
difference between percentages the number of standard deviations (SD) were worked out by
dividing the percentage difference by the average SE of the percentages being compared.
The probability table of P. Armitage\(^{17}\) was used to ascertain the level of significance.

**Results and Discussion**

Tables 1 and 2 present the prevalence of BT virus infections respectively in sheep and goat
populations studied in different geographical locations of Tanzania. Overall, group-specific antibodies to BT virus were detected in 216 of 296 (73.9 ± 5.0%) sheep and 908 of 1,173
(77.4 ± 2.4%) goat sera tested indicating that the infections are common in the areas studied.
The prevalence appeared to differ between geographical locations (Tables 1 and 2) and
age groups; in the latter the percentage of sero-positive animals tending to increase with

### Table 1: Prevalence of antibodies to Blue-tongue (BT) virus in Tanzania sheep sera sampled between 1991–1992

<table>
<thead>
<tr>
<th>Source of sera</th>
<th>Number of sera tested (n)</th>
<th>Number of sera positive</th>
<th>Prevalence (%) ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arusha</td>
<td>135</td>
<td>109</td>
<td>80.7 ± 6.7</td>
</tr>
<tr>
<td>Same</td>
<td>119</td>
<td>79</td>
<td>66.4 ± 8.5</td>
</tr>
<tr>
<td>Tanga</td>
<td>38</td>
<td>28</td>
<td>73.7 ± 14.0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>292</strong></td>
<td><strong>216</strong></td>
<td></td>
</tr>
</tbody>
</table>

SE = Standard error within 95% confidence limits

### Table 2: Prevalence of antibodies to Blue-tongue (BT) virus in Tanzanian goat sera sampled between 1991–1992

<table>
<thead>
<tr>
<th>Source of sera</th>
<th>Number of sera tested (n)</th>
<th>Number of sera positive</th>
<th>Prevalence (%) ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arusha</td>
<td>119</td>
<td>75</td>
<td>63.0 ± 8.7</td>
</tr>
<tr>
<td>Same</td>
<td>66</td>
<td>59</td>
<td>88.4 ± 2.4</td>
</tr>
<tr>
<td>Tanga</td>
<td>181</td>
<td>123</td>
<td>67.9 ± 6.8</td>
</tr>
<tr>
<td>Mtwara</td>
<td>207</td>
<td>121</td>
<td>58.5 ± 6.7</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>1,173</strong></td>
<td><strong>908</strong></td>
<td></td>
</tr>
</tbody>
</table>

SE = Standard error within 95% confidence limits

### Table 3: Prevalence of antibodies to Blue-tongue (BT) virus in sera of various age groups of Tanzanian sheep sampled between 1991–1992

<table>
<thead>
<tr>
<th>Age in years</th>
<th>Number of sera tested (n) (a)</th>
<th>Number of sera positive</th>
<th>Prevalence (%) ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 1(^{10})</td>
<td>22</td>
<td>12</td>
<td>(54.5)(^{b})</td>
</tr>
<tr>
<td>1 - 2</td>
<td>95</td>
<td>66</td>
<td>69.5 ± 9.5</td>
</tr>
<tr>
<td>2 - 3</td>
<td>15</td>
<td>14</td>
<td>(93.3)(^{b})</td>
</tr>
<tr>
<td>&gt; 3</td>
<td>13</td>
<td>11</td>
<td>(84.6)(^{b})</td>
</tr>
</tbody>
</table>

a) Sample pool from Arusha, Same and Tanga; b) Approximately 6 - 11 months old; c) Not significant (n < 30);
SE = Standard error within 95% confidence limits
advancing age (Table 3 and 4). The overall prevalence in sheep was however insignificantly lower than that observed in goats (SD = 0.946; P > 0.05); a finding that seems to suggest that the two species of ruminants possibly have similar susceptibility to the virus. This is perhaps true especially if one takes into consideration the fact that all the breeds studied are native with no exotic European blood.

It is estimated that there are 3,080,147 sheep and 6,443,666 goats in Tanzania\(^{18}\). No vaccination programme against BT virus either in sheep or in goats is in practice in Tanzania. Hence, the occurrence of group-specific antibodies in the sera of the local sheep and goats has arisen as a consequence of either natural infection with the virus or of passive immunization with maternal antibodies.

Clinical BT is generally seen amongst wool sheep and their crosses with native hair sheep; the disease occurring particularly after weaning to the age of 2 years\(^{16}\). Hair sheep populations do show evidence of challenge by many strains of BT virus but clinical manifestation of the disease has not been encountered\(^{15}\). Almost 100% of the Tanzanian sheep population is native hair type. Consequently, clinical BT does not seem to occur in Tanzania. Neither before nor during and/or after the present study, has clinical BT disease been encountered in Tanzania flocks of sheep.

Geographically, Kenya is amongst the closest neighbours of Tanzania. In Kenya, BT is enzootic, the infection with BT virus having been confirmed in domestic ruminants (sheep, goats and cattle) and in various species of wild ruminants, the wildebeests\(^{16}\) — migrating extensively between the two countries inclusive\(^{15}\). Therefore, the occurrence of BT virus infection in Tanzanian sheep, goats and possibly in other species of ruminants (domestic or wild) is not surprising. However, studies are needed to increase our knowledge of the epizootiology and pathogenesis of BT virus among different animal populations, in particular the domestic and wild ruminants in Tanzania.

### Acknowledgments

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### References


### Table 4: Prevalence of antibodies to Bluetongue (BT) virus in sera of various age groups of Tanzania goats sampled between 1991-1992

<table>
<thead>
<tr>
<th>Age in years</th>
<th>Number of sera tested (n)</th>
<th>Number of sera positive</th>
<th>Prevalence (%) ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 1(^{18})</td>
<td>124</td>
<td>82</td>
<td>66.1 ± 8.3</td>
</tr>
<tr>
<td>1 - 2</td>
<td>419</td>
<td>359</td>
<td>85.7 ± 3.3</td>
</tr>
<tr>
<td>2 - 3</td>
<td>123</td>
<td>102</td>
<td>82.9 ± 6.6</td>
</tr>
<tr>
<td>&gt; 3</td>
<td>203</td>
<td>187</td>
<td>92.1 ± 3.7</td>
</tr>
</tbody>
</table>

a) Sample pool from Arusha, Same and Tanga; b) Approximately 6 - 11 months old; SE = Standard error within 95% confidence limits.

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THE ROLE OF AGAR GEL DIFFUSION TEST (AGDT) IN THE DIAGNOSIS AND ERADICATION OF CONTAGIOUS BOVINE PLEUROPNEUMONIA (CBPP)

*AMEH, J.A., NAWATHE, D.R. AND EMMANS, I.E.
Faculty of Veterinary Medicine, University of Maiduguri, Maiduguri–Nigeria

ROLE DU TEST DE DIFFUSION EN GELOSE DANS LE DIAGNOSTIC ET L’ERADICATION DE LA PERIPNEUMONIE CONTAGIEUSE BOVINE (PPCB)

Résumé

Le test de diffusion en gélose a été utilisé pour détecter l’antigène de la périnéumonie contagieuse bovine (PPCB) dans les prélèvements de poumon des bovins abattus à l’abattoir de Maiduguri entre novembre 1990 et avril 1991.

Selon les examens post-mortem effectués entre novembre 1990 et avril 1991 à l’abattoir de Maiduguri, sur 20.165 bovins abattus :964 (4,8%) montraient des lésions pulmonaires et 51 (0,25%) avaient des lésions macroscopiques caractéristiques de PPCB. Au total, 104 prélèvements de poumon avec des lésions pneumoniques ont été recueillis. Sur 30 prélèvements avec des lésions caractéristiques de PPCB, 23 (76%) avaient une réaction positive, tandis que sur 74 prélèvements où l’on dépistait une pneumonie autre que PPCB, 2 (2,7%) étaient positifs; soit sur 104 prélèvements 25 (24%) étaient positifs pour PPCB.

On a déduit de la présente étude que la péripneumonie contagieuse bovine demeure un problème grave au Nigeria en dépit de l’existence d’un pogramme d’eradication.

Abstract

Agar gel diffusion test was used to detect Contagious Bovine Pleuropneumonia (CBPP) antigen in the lung samples from cattle slaughtered at Maiduguri abattoir between November 1990 and April 1991.

Post-mortem examinations conducted between November 1990 and April 1991 at the abattoir in Maiduguri showed that of 20165 cattle slaughtered, 964 (4.8%) had lung lesions, and 51 (0.25%) had gross lesions suggestive of CBPP. A total of 104 lung samples with pneumonic lesions were collected. Out of 30 lung samples with lesions typical of CBPP, 23 (76.7%) gave positive reaction while out of 74 lung samples with pneumonia other than CBPP, 2 (2.7%) were positive. Thus, out of 104 samples 25(24%) were positive for CBPP. It is concluded from this study that CBPP is still a serious problem in Nigeria in spite of the eradication programme.

Introduction

Contagious bovine pleuropneumonia is still serious in Tropical Africa, Spain, Portugal, France, Italy, Middle East and India(1-3). Tropical Africa being a large area however, it remains the great focus of CBPP(3-4). Previous communication by Nawathe(2) reported on the resurgence of CBPP in Nigeria. CBPP is caused by the small colony form of Mycoplasma mycoides var. mycoides(MmSC)(4). Griffin(5) and Karst(6) recommended the use of Agar Gel Diffusion test for the diagnosis of CBPP. It was observed that the test could be used for examination of antigens in the lungs from cattle at abattoirs and tracing the source of any lesions(5).

Karst(6) reported that the method is used in slaughter surveys in order to detect foci of infection for the final proof of eradication. The complement fixation test cannot be regarded as ideal for field application when facilities are limited and is more suitably applied in a laboratory(5). Also, heavily contaminated tissues unsuitable for cultural examination, could be examined by this test(7). It was reported(3) that no single test can detect CBPP at all stages of the disease.

This paper reports on the use of AGDT in the diagnosis of CBPP in Maiduguri.

*Corresponding author
Materials and Methods

Collection of Samples
Bi-weekly visits were made to Maiduguri abattoir between November 1990 and April 1991. A total of 104 pneumonic lung samples were collected.

One portion was kept fresh on ice and another was preserved in 10% formalin. Triangular filter paper strips were soaked in lung exudates, labelled and air-dried.

The agar gel diffusion test (AGDT) for detecting CBPP antigens in the lung samples was performed as described by Griffin[9]. The immune serum was obtained from sheep instead of rabbits. The serum was supplied by Mr. G. Pam of NVRI, Vom, together with negative serum and control antigens.

Results
A total of 104 pneumonic lung samples from cattle were collected and examined by AGDT. Between November 1990 and April 1991, 20165 cattle were slaughtered; 964 (4.8%) had pneumonia, and 51 had gross lesions suggestive of CBPP.

Of the 104 pneumonic lungs examined, 30 had lesions of CBPP and 23(76.7%) of these gave positive reaction to AGDT, while 2(2.7%)

Table 1: Incidence of contagious bovine pleuropneumonia (CBPP) and other pneumonias at Maiduguri Abattoir for the period November 1990 to April 1991. ***

<table>
<thead>
<tr>
<th>Month</th>
<th>Year</th>
<th>No. of Cattle Slaughtered</th>
<th>Total No. and % of cattle showing Pneumonic lesions</th>
<th>No. and % of cattle showing CBPP lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>November</td>
<td>1990</td>
<td>3,480</td>
<td>174(5.0)</td>
<td>17(9.9)</td>
</tr>
<tr>
<td>December</td>
<td>1990</td>
<td>3,399</td>
<td>170(5.0)</td>
<td>16(9.4)</td>
</tr>
<tr>
<td>January</td>
<td>1991</td>
<td>3,453</td>
<td>170(4.9)</td>
<td>4(2.4)</td>
</tr>
<tr>
<td>February</td>
<td>1991</td>
<td>3,169</td>
<td>148(4.7)</td>
<td>8(5.4)</td>
</tr>
<tr>
<td>March</td>
<td>1991</td>
<td>3,381</td>
<td>135(4.0)</td>
<td>3(2.2)</td>
</tr>
<tr>
<td>April</td>
<td>1991</td>
<td>3,233</td>
<td>167(6.1)</td>
<td>3(1.5)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>20,165</td>
<td>964(4.8)</td>
<td>51(0.25)</td>
</tr>
</tbody>
</table>

Figures in parentheses denote percentages.
* Data from the slaughter house record in Maiduguri.
*** These figures do not reflect the actual number of outbreaks as some may not be detected or recognised by the slaughter house inspectors, or are not reported.

Table 2. Results of AGDT for CBPP antigens in Pneumonic Lung Samples from Maiduguri Abattoir

<table>
<thead>
<tr>
<th>Months/Year collected samples</th>
<th>No. Samples with CBPP lesions</th>
<th>No. Positive</th>
<th>No. Sample other than CBPP</th>
<th>No.(%) Positive</th>
<th>Total samples tested</th>
<th>No. (%) positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>November 1990</td>
<td>9</td>
<td>9 (100)</td>
<td>29</td>
<td>0 (0)</td>
<td>38</td>
<td>9 (23.7)</td>
</tr>
<tr>
<td>December 1990</td>
<td>5</td>
<td>5 (100)</td>
<td>16</td>
<td>0 (0)</td>
<td>21</td>
<td>5 (23.8)</td>
</tr>
<tr>
<td>January 1991</td>
<td>6</td>
<td>2 (33.3)</td>
<td>8</td>
<td>2(25.0)</td>
<td>14</td>
<td>2 (14.3)</td>
</tr>
<tr>
<td>February 1991</td>
<td>2</td>
<td>2 (100)</td>
<td>6</td>
<td>0 (0)</td>
<td>8</td>
<td>2 (25.0)</td>
</tr>
<tr>
<td>March 1991</td>
<td>5</td>
<td>2 (40.0)</td>
<td>9</td>
<td>0 (0)</td>
<td>14</td>
<td>2 (14.3)</td>
</tr>
<tr>
<td>April 1991</td>
<td>3</td>
<td>3 (100)</td>
<td>6</td>
<td>0 (0)</td>
<td>9</td>
<td>3 (33.3)</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>23 (76.6)</td>
<td>74</td>
<td>2 (2.7)</td>
<td>104</td>
<td>25 (24.0)</td>
</tr>
</tbody>
</table>

Figures in parentheses denote percentages.
from the rest of the 74 lung samples with pneumonia were positive to AGDT.

Both formalin-treated and soaked filter paper strips also gave lines of precipitation. The lines of precipitation of filter paper strips soaked in lung exudates took more time to develop as well as, but not as sharp as the fresh samples. They required a longer incubation time in the humid chamber.

Discussion

Karst\(^6\) reported that of the many serological tests for diagnosis of CBPP, the only two tests reliable enough and capable of dealing with big numbers of samples are the complement fixation test and AGDT. The AGDT for detecting CBPP antigens is specific and easy to perform in the field and also appears to be adequate for initial survey work\(^2\). It has been used in slaughter surveys in order to detect foci of infection or for proof of final eradication\(^6\). The results of this study showed that CBPP is still present – 25(24\%) positive cases. Epidemiological data available\(^2\) also support this observation.

Other serological tests such as CFT, counterimmunoelectrophoresis, histochemistry and isolation of the causal agent, to mention just a few are cumbersome, time-consuming and expensive for routine diagnosis\(^2\). AGDT is a simple test that can be applied on the field. The two positive samples from penumonic lungs other than those of CBPP observed in this study may have resulted from early cases of CBPP, and not recognised by macroscopic examination\(^5\).

A campaign against CBPP however, will not become a success unless the diagnostic side is working well\(^6\). The AGDT is simple to perform, requiring a minimum of equipment, is suitable for use by field staff, and is considered a useful diagnostic aid in the control and eradication of CBPP.

The control of CBPP is not given the same attention as rinderpest probably because of the insidious nature of CBPP. Nawathe\(^2\) reported that the disease has a high debilitating effect on the animals, immunosuppression, poor growth, reproductive failures and is prone to intercurrent diseases.

The control and eradication of CBPP is presently hampered by the dwindling national revenue as a result of the structural adjustment programme (SAP) introduced in Nigeria in 1986. The position of Borno State, sharing boundaries with three African countries (Niger, Chad and Cameroon) puts it at a disadvantage. The state has the highest concentration of livestock, especially cattle, in Nigeria. Griffin\(^7\) observed that if a reduction in incidence could be achieved in Borno, then an improved situation elsewhere might follow. This is true since CBPP is a disease of moving cattle. Provost\(^6\) reported that the logical consequence is that efforts to eradicate CBPP must concentrate, on a priority basis, on those regions where extensive animal husbandry is practised.

During the period of study, only 3 outbreaks were reported in the area, but the number of positive cases observed in this study shows that most of the outbreaks were not reported. It is concluded from this study that CBPP is still a serious problem in Nigeria and coordinated regional efforts in eradicating the disease should be intensified to rid the region of the disease.

References


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EFFECTS OF CYCLOPHOSPHAMIDE IN RABBITS

M.G. BINTA*, E.Z. MUSHI AND F.R. RURANGIRWA

International Centre for Insect Physiology and Ecology, P.O. Box 30722, Nairobi, Kenya
National Veterinary Research Institute
Kenya Agricultural Research, P.O. Box 32, Kikuyu, Kenya

EFFETS DU CYCLOPHOSPHAMIDE CHEZ LES LAPINS

Résumé

L’administration par voie intraveineuse du cyclophosphamide (100 mg/kg de poids vif) a eu une action immunodépressive chez les lapins. Ceci a été caractérisé par une leucopénie lymphopénique due à la déplétion des lymphocytes des ganglions lymphatiques, de la rate et du thymus. Le niveau d’immunosuppression dépendait de la dose. Toutefois, les doses supérieures à 200 mg/kg de poids vif étaient mortelles pour ces animaux.

Abstract

Intravenous administration of cyclophosphamide (100 mg/kg body weight), induced immunosuppression in rabbits. This was characterised by a lymphopenic leucopenia resulting from depletion of lymphocytes from lymph nodes, spleen and thymus. The degree of immunosuppression was dose dependent. However, doses of over 200 mg/kg body weight were fatal in this species.

Introduction

Cyclophosphamide (Cy) is a cytostatic and mitostatic drug with potent cytotoxicity for rapidly dividing cells\(^6\). The mitostatic effects of Cy on lymphoid cells is more marked against short-lived B-lymphocytes than against the long life T-lymphocytes\(^7\). The depletion of B-lymphocytes has been shown to lead to diminished humoral antibody response in mice, rats, guinea pigs, chickens, dogs, pigs, cattle and man\(^1\). Cyclophosphamide has been widely used in studies on the pathogenesis of disease processes in various animal species\(^6\).

The rabbit as an animal model in disease pathogenesis has been used in many instances. However, the use of Cy in the rabbit is not well documented. Previous authors have used a single dose of 100 mg/kg body weight to induce immunosuppression\(^8\). Attempts to use this single dose to induce immunosuppression were unsuccessful as rabbit mortality was high.

The purpose of the present study was to investigate the effects of Cy on both the humoral and the cellular immune responses of rabbits, a common experimental model.

* Present address: National Veterinary Laboratory, P/Bag 0035, Gaborone, Botswana

Materials and Methods

Experimental animals

Outbred New Zealand white rabbits weighing about 2.0 kg body weight were used.

Drug

Cyclophosphamide powder (Endoxan-Asta Werke Ag Fabrik, D-480 Biolefeld, 14, Germany) was dissolved in sterile de-ionised water containing Penicillin and Streptomycin. These were calculated to give each rabbit 200 I.U. penicillin and 200 ug streptomycin.

Cy administration

Three outbred New Zealand White rabbits were used. Cyclophosphamide powder (Endoxan-Asta) (Asta-Werke Ag. Chemische Fabrik, D-480, Biolefeld, 14 Germany) was used. The Cy powder was dissolved in sterile de-ionised water containing penicillin and streptomycin calculated to give each rabbit 200 iu penicilllin and 200 ug streptomycin.

Each rabbit was injected intravenously with 20mg/kg body weight cyclophosphamide. Each rabbit was injected daily for the first 7 days and thereafter on alternate days.
Collection of blood and serum
Bleeding of the rabbits was done by venipuncture of the marginal ear vein. Blood was collected in bijou bottles containing a drop of 20% EDTA solution. Clotted blood was used for serum. The total and differential leucocyte counts were done on the whole blood samples.

Collection of lymphoid tissue
Lymphoid tissues were collected from CT treated rabbits. These included the thymus, spleen and lymph nodes. All the tissues were fixed in formal saline, sectioned at 5 μm and stained with haematoxylin and Eosin (H&E) using conventional procedures.

Immunization of rabbits with bovine serum albumin (BSA)
Cy-treated and normal rabbits were immunized with bovine serum albumin (BSA, BDH Chemicals, Poole, England) to determine the effect of Cy on the primary antibody response. Each rabbit was inoculated intravenously with 8 mg BSA 7 days post-initiation of Cy administration.

Passive haemagglutination assay to determine antibody response to BSA
The passive haemagglutination test was used to assay the antibody response. BSA was coupled to three times washed red blood cells (SRBC) collected as described before in 0.85% sterile physiological saline using chromic chloride as described by Gold(4). One percent of the sensitized cell suspension was used in normal saline.

The rabbit serum was heat inactivated (56°C, 30 min) and then diluted in doubling dilutions in microtitre plates. An equal volume (50/μl) of the 1.0% sensitized SRBC was added to the serum dilutions and the plates were shaken and incubated at 37°C for 1 hour. The plates were removed and put at 4°C for another hour after which the agglutination was read.

Jerne Plaque Assay
A slide technique of the Jerne Plaque assay was used after the modification of Cunningham and Stennberg (1968). Spleen cells were collected from rabbits four days after immunization with 2 ml of 20% SRBC. The spleen cells were prepared by teasing the organ through a stainless gauze. Aseptic precautions were taken. The lymphocytes were added to molten 4% agarose and spread on a slide. The slides were incubated at 37°C for 45 minutes. The haemolytic plaques were counted using a light microscope at x1 magnification.

Results
Tolerance of rabbits to Cy
Preliminary trials of the drug in rabbits using a single large dose of more than 100 mg/kg body weight resulted in the death of all the rabbits. Furthermore, when rabbits were injected with a dosage regimen less than 100 mg/kg the rabbits were inadequately suppressed. The use of small repeated doses (10 mg/kg) given intravenously daily for a week was sufficient to induce and maintain a leucopenia culminating in the death of the rabbits.

Effect of Cy on leucocytes
In the first experiment where Cy was administered daily for 9 days, its short-term effects on leucocytes are depicted in Figure 1. A change in total leucocyte count was observed 24 hours after the first dose of Cy. The total count was generally less than 50% (4000 leucocytes/mm³ of the initial count from 6 days post-inoculation (~p.i.)

The leucopenia was due to total lymphocytopenia. In an attempt to study the long-term effects of Cy on rabbit leucocytes, Cy was administered daily for the first seven days. Subsequently, injections of the drug were given every alternate day for 40 days.

Once again the initial fall in leucocyte count was evident by day 7 p.i (Figure 1). The leucocyte numbers remained less than 50% of the initial count. When Cy was administered on alternate days, the leucocyte counts tended to rise slightly but to not more than 505 of the initial count.

Histological changes of lymphoid tissues
The most significant histological change was the marked depletion of cells from all the
lymphoid organs examined. In the thymus, the cortex it markedly depleted and the cortical medullary junction was not clear. The lymph nodes displayed a marked depletion of lymphocytes from follicles, germinal centres and the region of the cortico-medullary junction.

These changes were evident in a rabbit killed 2 days p.i. but were most severe in a rabbit killed on day 9 p.i. The spleen showed slightly less depletion of lymphocytes but the lymphoid sheath was devoid of cells, particularly at the periphery.
Primary antibody response
Cy-treated rabbits immunized 4 days earlier with SRBC had a few antibody forming cells (Table 1) as determined by the Jerne Plaque assay. Similarly the mean antibody titres against BSA were diminished in the Cy-treated rabbits (Figure 2).

<table>
<thead>
<tr>
<th>Rabbit no</th>
<th>Treatment</th>
<th>Mean PFC/10^6 to cells/chamber</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cy*</td>
<td>4.73 ± 3.8</td>
</tr>
<tr>
<td>2</td>
<td>Cy*</td>
<td>128.67 ± 22</td>
</tr>
<tr>
<td>3</td>
<td>Control</td>
<td>200.8 ± 40</td>
</tr>
</tbody>
</table>

* Mean ± s.d.; PFC = Plaque forming cells

Figure 2. Antibody titre to BSA
Table 2. Haemagglutination antibody titres to sheep red blood cells of Cyclophosphamide-treated (Cy+) and control rabbits

<table>
<thead>
<tr>
<th>Rabbit No.</th>
<th>Treatment</th>
<th>Days post-immunization with SRBC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>M1</td>
<td>Cy+</td>
<td>&lt;2*</td>
</tr>
<tr>
<td>M2</td>
<td>Cy+</td>
<td>&lt;2</td>
</tr>
<tr>
<td>M3</td>
<td>Cy+</td>
<td>&lt;2</td>
</tr>
<tr>
<td>M4</td>
<td>Cy+</td>
<td>&lt;2</td>
</tr>
<tr>
<td>M5</td>
<td>Control</td>
<td>&lt;2</td>
</tr>
<tr>
<td>M6</td>
<td>Control</td>
<td>&lt;2</td>
</tr>
</tbody>
</table>

*Reciprocals of end points

Discussion

Small repeated doses of Cy resulted in adequate immunosuppression of the treated rabbits using this regimen; the mortality rate of the rabbits was nil.

In contrast, the large dose was toxic. The depression of total leucocytes and lymphocytes in Cy-treated rabbits was similar to that in other species (1). The depression was attributed to the mitostatic and direct toxic effects of Cy on rapidly multiplying cells such as lymphocytes. It was shown that a single injection of 80 mg/kg Cy led to the depletion of B-lymphocytes in the blood and lymphoid organs of rabbits (8).

In this study, the effect of Cy on T and B cells was not investigated. Most likely, the long-term administration of Cy involved both T and B areas. Antibody formation to BSA and SRBC was suppressed in Cy-treated rabbits. Humoral antibody formation to those antigens has been shown to be depressed by Cy administration in rats, mice, chickens, dogs, pigs and cattle (5).

It was suggested that suppression of the humoral response could have been due to a lack of cells able to differentiate into antibody-secreting cells.

Another plausible explanation was that since SRBC is a T-cell dependent antigen, suppression of antigen formation could have been due in part to the T-cell depletion.

Thus, the Cy-induced destruction of immunocompetent cells and the subsequent suppression of antibody formation can be used to study the humoral effector mechanism in the rabbit model.

Acknowledgements

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References


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AN ABBATTOIR SURVEY OF THE PATHOLOGICAL CONDITIONS OF THE BOVINE SCROTUM AND ITS CONTENTS IN OYO STATE, NIGERIA

M. O. AKUSU
Department of Veterinary Surgery and Reproduction, University of Ibadan, Ibadan, Nigeria

ENQUETE MENEE DANS UN ABATTOIR SUR LES CONDITIONS PATHOLOGIQUES DU SCROTUM BOVIN ET SES CONTENUS DANS L'ETAT DE OYO AU NIGERIA

Résumé
Une étude a été a l'abattoir de Bodija à Ibadan, en vue de déterminer les conditions pathologiques du scrotum bovin et ses contenus. Au total, 103 taureaux ont été choisis au hasard pour entreprendre l'enquête.

La dégénérescence testiculaire et calcaire (51,5%) était la condition pathologique la plus fréquente affectant les testicules, avec une incidence plus élevée chez les taureaux plus âgés. De même, les cas d'adhérence (40,8%) entre les testicules et les tuniques dépendaient de l'âge. L'incidence d'hypoplasie testiculaire était de 10,7%, le testicule gauche étant le plus souvent affecté.

Les conditions pathologiques de l'épididyme ont été observées dans 23,3% des prélèvements examinés, parmi lesquels il y avait 13,7% de cas de spermiostase. Les infections parasitaires du scrotum et/ou ses contenus étaient dues à l'infestation par les tiques (39,8%), la besnoitiose (15,5%), la demodicose (1,9%) et l'onchocercose (1%).

Abstract
An abattoir survey to determine the pathological conditions affecting the bovine scrotum and its contents was carried out at the Bodija abattoir in Ibadan. A total of one hundred and three bulls were randomly selected for the study.

Testicular degeneration and calcification (51.5%) represented the most common pathological condition affecting the testes and incidence was higher in older bulls. Similarly, the incidence of adhesion (40.8%) between the testes and the tunics was age-related. The incidence of testicular hypoplasia was 10.7%, the left testis being more frequently affected.

Pathological conditions of the epididymis were observed in 23.3% of the materials out of which spermiostasis accounted for 13.7%. Parasitic conditions of the scrotum and/or its contents were tick infestation (39.8%) besnoitiosis (15.5%), demodicosis (1.95) and onchocerciasis (1%).

Introduction
The productive performance of the Nigeria zebu breeds of cattle has long been recognised as being poor. The factors responsible for this are genetical, nutritional, environmental and infectious. Earlier reports have largely centered on the female. However, observations in some West European countries, American and Australia showed that pathological conditions of the testes and epididymides are major causes of bull wastage.

This study is aimed at determining some of the pathological conditions that may contribute to infertility in the Nigerian zebu bulls.

Materials and Methods
The materials used for this study were derived from the trade cattle which arrived at the abattoir from the Northern parts of the country. The animals were either trekked, railed or transported in lorries to the abattoir where antemortem examination by veterinary officials was performed. The animals could therefore be regarded as being in relatively good physical condition prior to slaughter.

One hundred and three pairs of intrascrotal testes were randomly collected. As a rule, between three and five pairs were collected on defined days. Each of the animals was aged by
dentition, and animals were grouped as follows: young bulls (1–3 years); mature bulls (3–7 years); old bulls (7 years).

Immediately after slaughter, the scrotum was severed at its neck and placed in a well-insulated box containing ice, maintained at 4–10°C and transported to the laboratory within 5 minutes after slaughter.

The scrotum was thoroughly examined for the presence and distribution of ectoparasites, nodular lesions, scarring, excoriations or fresh wounds. Scrotal samples measuring about 2 x 1 cm were taken and fixed in 10% buffered formal saline or freshly prepared aqueous Bouin’s fluid.

Tunical Vaginalis: The degree and distribution of adhesions between the tunics and the testis and/or the epididymis was scored (Table 1).

<table>
<thead>
<tr>
<th>Grade</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 or 1 (None or fine)</td>
<td>No adhesion on both testes or fine adhesions which are easily detached when testes are raised by holding onto the tunica vaginalis.</td>
</tr>
<tr>
<td>2 (Moderate)</td>
<td>Testes can be raised from a level surface with the aid of the tunica vaginalis. Fibrous strands can only be detached with the finger and adhesion marks remain on testes.</td>
</tr>
<tr>
<td>3 (Severe)</td>
<td>Fibrous strands or bands can only be detached from testes with the aid of a scissors; if forcefully pulled, will result in tear into testes parenchyma.</td>
</tr>
</tbody>
</table>

Testis and Epididymis: These structures were removed through an extended incision on the scrotum. After gross examination, the epididymis was carefully dissected from the testis while the spermatic cord was dissected off at the attached border of the testis.

Each testis was then bisected along its longitudinal plane by a midsaggital incision. The cut-surfaces were examined for bulging, colour and moisture. Several intratesticular incisions, about 1 cm apart were made radially on the bisected testis. Tissue samples 2 x 1 cm were taken routinely from the dorsal, equatorial and ventral aspects of each testis, while epididymal samples were taken from the caput, corpus and cauda epididymis. Tissue samples of about 1 cm in length were also taken from the spermatic cord. All tissues were fixed in freshly prepared aqueous Bouin’s fluid and processed routinely for paraffin-wax embedding. Tissues were sectioned at 5–7 microns and stained with haematoxylin and eosin (H & E) and in some cases with Periodic Acid Schiff (PAS), Von Kossa Stain and Brown and Brenn (B & B) techniques for Gram stain.

**Results**

1. **Scrotum**: Nodular lesions measuring 1–2.5 cm were frequently observed on the scrotum and were often associated with tick bites. Ticks were observed on the scrotum of 39.8% of the bulls and bulls of all age groups were affected. The tick species were mostly *Boophilus decoloratus* and *Ambyloma variegatum*. Other tick species observed infrequently were *Rhipecephalus appendiculatus* and *Hyaloma spp*. In light and moderate tick infections (less than 10 and 11–40 ticks respectively) of the scrotum, the posterior aspect of the scrotal neck was a preferential site for tick attachment. However, in heavy tick infestation (more than 40 ticks) the middle and ventral parts of the scrotum, particularly the posterior aspect were affected.

2. **Tunica Vaginalis**: Adhesion between the visceral tunic and the testes and/or the epididymis was observed in 40.8% of the bulls and the incidence increased with age (Table II). Adhesions were either unilateral or bilateral, focal or extensive, strandlike or bandlike. Adhesions mostly involved the tunic and the dorsal part of the testis on one hand and the lateral or posterior aspects of the caput epididymis on the other. Several and extensive adhesions were associated with degeneration and/or calcification of the testis. Such testicular changes were not usually observed in fine and moderate adhesions.
Table 2. Effect of Age on Incidence of Testicular Adhesion in 103 Bulls

<table>
<thead>
<tr>
<th>Age group</th>
<th>Number of Bulls affected</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Moderate</td>
<td>Severe</td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td>Young (1-3 yrs)</td>
<td>1(1.0%)</td>
<td>1(1.0%)</td>
<td>2(1.9%)</td>
<td></td>
</tr>
<tr>
<td>Mature (3-7 yrs)</td>
<td>7(6.8%)</td>
<td>5(4.9%)</td>
<td>12(1.7%)</td>
<td></td>
</tr>
<tr>
<td>Old (7 yrs)</td>
<td>16(15.5%)</td>
<td>12(11.7%)</td>
<td>28(27.2%)</td>
<td></td>
</tr>
<tr>
<td>Total %</td>
<td>24(23.3%)</td>
<td>18(17.5%)</td>
<td>42(40.8%)</td>
<td></td>
</tr>
</tbody>
</table>

3. Testis: (a) Degenerative changes in the testicular parenchyma were observed in 34.9% and the incidence was higher in old bulls (Table 2). Grossly, degenerated testes were characterised by flabbiness and a dry cut surface which did not bulge in severe cases. Histologically, the changes in the seminiferous tubules and the interstitium were of two forms. In mild and moderately affected testes (10-20% or 21-50% of seminiferous tubules affected) there was pyknosis of nuclei and vacuolation of cytoplasm of spermatocyte layer and formation of spermatidic giant cells. The basement membrane was apparently normal, and there was no quantitative increase in interstitial connective tissue. The severe form (>50% of seminiferous tubules affected) resulted in variable tubular diameters, collapse of some tubules and increase of interstitial connective tissue. Within the tubules spermatogonia and sertoli cells were the only surviving cells. Some cases showed sloughing and desquamation of the epithelium, wavy thickening and fragmentation of basement membranes and formation of interstitial sperm granuloma. Lymphocytes and plasma cells were sometimes present in the interstitium.

(b) Calcification: Testicular calcification was observed in 16.5% of the bulls examined; 4.9% in mature bulls and 11.6% in old bulls. It was usually bilateral, focal or diffused. A gritty sound was noticed when a midsagittal incision was made. The cut surface might not bulge but appeared wet. Calcified areas appeared as pinpoint white or grey hard areas and when several of these coalesced they formed a larger mass. Histologically, masses of apparently dead spermatozoa were found buried in an irregular purple-staining calcified mass. Adjacent seminiferous tubules were either normal or severely degenerated.

c) Testicular Hypoplasia: Unilateral testicular hypoplasia was observed in 10.7% of the bulls; 8.7% left-sided while 2% were right-sided. Histologically, the germinal epithelium of hypoplastic testes ranged from normal to cases where there were only spermatogonia and sertoli

Table 4. Distribution of 14 Cases of Spermiositosis according to age and site of Epididymis affected

<table>
<thead>
<tr>
<th>Age</th>
<th>Site</th>
<th>Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young</td>
<td>CCP 4</td>
<td>3 SPC, 1 PDA</td>
</tr>
<tr>
<td></td>
<td>RCP 2</td>
<td>2 PDA</td>
</tr>
<tr>
<td></td>
<td>RCD 1</td>
<td>1 SPC</td>
</tr>
<tr>
<td>Mature</td>
<td>RCD 1</td>
<td>1 SPC</td>
</tr>
<tr>
<td>Old</td>
<td>LCP 2</td>
<td>1 SPC, 1 PDA</td>
</tr>
<tr>
<td></td>
<td>RCP 3</td>
<td>2 SPC, 1 PDA</td>
</tr>
<tr>
<td></td>
<td>BIL 1</td>
<td>1 SPC</td>
</tr>
</tbody>
</table>

SPC: Spermiositosis  
PDA: Poorly Defined Areas
LCP = Left caput  
RCP = Right caput  
RCD = Right caput  
BIL = Bilateral

Table 3. Effect of Age on Incidence of Testicular Degeneration

<table>
<thead>
<tr>
<th>Age Groups of bulls</th>
<th>No of bulls</th>
<th>Degree of Degeneration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mild 10-20%</td>
<td>Moderate 21-50%</td>
</tr>
<tr>
<td>3 years</td>
<td>19</td>
<td>2(1.9%)</td>
</tr>
<tr>
<td>3-7 years</td>
<td>38</td>
<td>6(5.8%)</td>
</tr>
<tr>
<td>7 years</td>
<td>46</td>
<td>6(5.8%)</td>
</tr>
</tbody>
</table>

Note: Degeneration observed in at least one of a pair of testis
cells. Formation of spermatidic giant cells was also observed. The basement membrane was generally normal.

d) Orchitis and Periorchitis: These conditions were observed histologically. Chronic interstitial orchitis was observed in old bulls. There was severe degeneration of the testicular parenchyma. The interstitium was heavily infiltrated by lymphocytes, plasma cells and giant cells.

Chronic periorchitis was observed in one mature bull. There was mild testicular degeneration. Neither bacteria nor parasitic organisms were identified in these chronic inflammatory conditions.

4. Epididymis

a) Sperm-stasis: Two forms of sperm retention were observed in the caput epididymis. Spermatocele (which had an incidence of 8.7% and situated either on the free surface or attached surface of the caput epididymis) consisted of well defined whitish or creamish cystic cavities measuring about 2mm - 3cm in diameter. The fluid content of these cystic structures was sperm cells with detached heads and coiled tails. The Gram stained smear did not reveal any bacteria.

b) Poorly Defined Areas (PDA): This form of sperm retention occurred in the caput epididymis in 4.9% of the bulls and were recognised only after histological sections were studied. Table IV shows that these cystic conditions were observed in bulls of all ages and were mostly unilateral in the caput. The lumen of these ducts were not dilated and the epithelium was often normal. The content of the lumen was a few sperm cells arranged randomly or in whorls.

In the severe category, there was heavy intraluminal stasis of sperm causing apparent pressure atrophy of the epithelium of the dilated efferent ductule. Adjacent ducts were atrophied or collapsed with resultant interstitial fibrosis, mononuclear cell infiltration or formation of sperm granuloma.

c) Epididymitis: Gross enlargement of the epididymis was not observed. However, mononuclear infiltration in the cauda epididymis was observed in 9.7% of the bulls and was usually unilateral. The epithelium of the epididymis was normal in most cases.

d) Dermatoses: The disease causing dermatoses of the scrotum were besnoitiosis (15.5%), demodicosis 2% and onchocerciasis (1%). The gross lesions of besnoitiosis consisted of pin-point nodules. The presence of the cysts of the organism did not appear to affect the fertility of the bulls. Adult females and microfilariae of Onchocerca sp. and Demodex folliculorum were observed in histological section of the scrotum. The contents of the scrotum were not affected by these parasites.

Discussion

The tick species found on the scrotum and the predominance of Boophilus sp in trade cattle in Nigeria were also reported by earlier works.

The most frequent determinatosis of the bovine scrotum observed in this survey was besnoitiosis in contrast to streptothricosis reported to be the most important clinical dermatoses of cattle. The seasonal incidence of streptothricosis might be a factor, apart from the fact that we were only concerned with the scrotum. Furthermore, while spontaneous cure is possible in streptothricosis, cyst forms of Besnoitia besnoiti persist for life in the host. The fact that out of the 16 bulls observed with cysts of Besnoitia besnoiti it was in three animals that the gross lesions were found, seemed to confirm that the cases encountered were inapparent infections.

It was not evident from this study if the dermatoses observed had any effect on the fertility of the bulls. Genital involvement of besnoitiosis is reported to cause sterility. However, normal fertility has been reported in heifers bred to chronically infected bulls. The incidence of adhesion observed in this investigation was higher than the reports of Australian workers. While mild adhesions were not associated with pathological conditions of the testes, severe and extensive adhesions were usually found in testes showing degenerative changes. The age incidence and the distribution of adhesions on the lateral aspect of the testes and epididymides would suggest trauma.
as an important causal factor. The incidence of testicular degeneration observed was higher than was reported for the zebu bulls in Northern Nigeria\(^6\), probably due to the relatively younger bulls studied by these workers. The changes in the seminiferous epithelium were similar to earlier reports \(^8\,12\).

The incidence of testicular hypoplasia (10.7%) might not represent the actual picture and it is considered to be in the lower limit. This notwithstanding, it was higher than reports in Australian and Indian bulls\(^6,8,20\). The observation that left testes suffered more from hypoplasia was in agreement with earlier findings\(^8\). The incidence (23.3%) of the pathological conditions of the epididymis in this study was higher than that reported elsewhere\(^11,20\). The gross and histological appearance of spermiostasis was similar to the report of other workers\(^2,6,13\). The observation that most of the cystic conditions in the bulls were unilateral\(^18\) would suggest that affected bulls can be fertile unlike in goats where it is usually bilateral. However, the congenital nature of spermiostasis makes it an important congenital abnormality.

The available literature is rather scarce on the causes of epididymitis but these include extravasation of spermatozoa\(^14\), Brucella organisms\(^18\) and viruses such as EPIVAG\(^15\) and \textit{T. Brucei} \(^7\). The cases observed in this investigation were probably due to non-specific secondary infections, trauma and tick-bites.

\begin{center}
\textbf{References}
\end{center}

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EFFET DE L'ACIDE ASCORBIQUE SUPPLEMENTAIRE SUR LA PERFORMANCE DES POULES EN MILIEU TROPICAL

B.M. ORUWARI, O.O. MBERE AND B.T. SESE

Animal Science Department, Rivers State University of Science and Technology, P.M.B. 5080, Port Harcourt, Rivers State, Nigeria

EFFETS DES ALIMENTS CONTENANT UN COMPLEMENT D'ACIDE ASCORBIQUE SUR LA PERFORMANCE DES POULES PONDEUSES EN LILIEU TROPICAL

Résumé

Une expérience a été conduite en vue de déterminer les effets de l'inclusion d'acide ascorbique (AA) dans les aliments sur la performance des pondeuses en milieu tropical. AA du sang, la consommation alimentaire, le gain pondéral et la mortalité ont également été suivis de près pour connaître les effets de compléter les aliments des poules avec 0, 1, 1.5 ou 2 g AA/kg d'aliments.

Les résultats ont montré que les pondeuses ont besoin d'un supplément AA en milieu tropical. Dans les conditions climatiques qui prévalent, l'addition de 1 g AA/kg d'aliments était très favorable à la production d'œufs. Le supplément AA a beaucoup amélioré (P < 0.05) le poids de l'œuf, son contenu et les qualités de la coquille (Unités Haugh et épaisseur de la coquille, respectivement), AA du sang, la consommation alimentaire, le gain pondéral et le taux de mortalité par rapport aux sujets-témoins. S'agissant de l'entretien et de la performance normale des poules, le supplément AA avait tendance à réduire le stress dû à la non-adaptation au climat, ce qui a permis à l'état physiologique des poules vivant en milieu tropical de devenir normal.

Summary

An experiment was conducted to investigate the effects of dietary ascorbic acid (AA) on the performance of laying hens in a tropical environment. Blood AA, feed intake, body weight gain and mortality were also monitored in determining the effects of supplementing the diets for the hens with 0, 1, 1.5 or 2 g AA/kg diet.

The results showed that laying hens have a requirement for supplemental AA in a tropical environment. Under the prevailing climatic condition, addition of 1.0 g AA/kg diet was most beneficial to egg production. Supplemental AA significantly (P < 0.5) improved egg weight, interior egg and shell qualities (Haugh Units and Shell thickness, respectively), blood AA, feed intake, body weight gain and mortality compared to the control. In regard to the maintenance and normal performance of the hens, supplemental AA tended to ameliorate the non-adaptive climatic stress conditions, thereby normalising the physiological status of the hens under the tropical environment.

Introduction

The ameliorative effects of ascorbic acid (AA) on physiological stress which is specific to the hypothalamic-pituitary-adrenal axis occur because AA appears to reduce the synthesis of glucocorticoids, and that it may shift adrenal steroidogenesis in favour of sex steroid synthesis(1). This supported the findings that adrenal corticosterone reduced hepatic and renal AA levels, and enhanced AA utilization(2). High environmental temperature is a stressor which has been reported to impair the synthesis of AA in heat-stressed laying hens whose plasma AA was consequently decreased(3,4). Furthermore, Perek and Kendler(3) and Thorton(5) found that the requirement for AA would increase whenever a stressor such as high climatic condition is imposed, and that supplementation might be necessary. Also, its involvement in integrity maintenance and normal functioning of the adrenal has been reported(6).

Supplementation of layer diets with AA increased plasma, AA(7), periodically improved egg production and egg weight(8,9), egg shell thickness and/or interior egg quality(8,10). Supplementation of AA was found to be effective when hens were fed limited calcium(11) and it has been
found to increase plasma calcium\textsuperscript{12}. Also, the effects of AA supplementation of chicken diets on mortality\textsuperscript{10,13} and on feed consumption, and body weight\textsuperscript{14,15} have been reported. Ascorbic acid supplementation appears to be of great benefit when hens are exposed to either an environmental or any other overt stressor in all the studies\textsuperscript{16}. Since those experiments were conducted in artificially heated environments, or only in the summer, there is a real need to study the effects of AA on laying hens in a tropical environment. It was the objective of this study to examine the effects of dietary AA supplementation on the performance of laying chickens in a tropical environment.

**Materials and Methods**

The research was conducted at the University of Science and Technology, Nkpolu, Port Harcourt, animal research farm in the months of December to March. This is the South-Coast of Nigeria where the Atlantic sea breeze ameliorates the climatic conditions. Temperature and humidity peak during these months.

Two hundred hens (Babcock strain) of 18 weeks in lay with about the same weight were used. They were randomly assigned to individual cages, measuring 40 x 40 x 46 cm, in a two tier cage system in an open-sided house. The cages were equipped with horizontal drinkers and feeders. Clean fluorescent bulbs were placed over the cages to give equal light distribution on all the tiers. The caged hens were randomly assigned to 20 partitions of ten hens each. Five of these partitions formed a replicate and were assigned to one of the four dietary treatments in a completely randomized design. The laying hens were given 16 hours of light per day.

Three of the four dietary treatments were made from the basal diet which is the control, treatment A. The composition of the basal diet is given in Table 1. Graded levels of dietary AA, 1, 1.5 or 2 g/kg in the basal diet formed the test treatments B, C, and D respectively. The various levels of L-AA (Hopkin and Williams, Chadwell Heath, Essex, England) were mixed thoroughly in the control diet with a small electric mixer. Micro-mixing of the L-AA supplemented diets was done twice weekly, the diets being stored in sealed black polyethylene bags at room temperature to avoid degradation of the vitamin. The diets contained (17.5\%), 175g protein and (280.15) kcal/kg, 11.72 Mj/kg, and the various levels of AA were added to make the test diets at the expense of no ingredient.

Experimental diets and water were provided ad libitum. The birds were fed manually with care to ensure no feed wastage. Feed consumption in each replicate was monitored weekly by differences between the served and the remaining quantities. All eggs produced by each bird were collected and labelled daily, and weighed weekly to determine the average egg production and egg weight of treatment groups. Body-weight gain was determined by weighing birds at the start and end of the experiment.

**Analysis**

Blood samples were taken on the last day of the four 28-day periods of the experiment for AA determinations by the method of Roe\textsuperscript{17}. Blood samples were taken from 5 birds in each replicate. All eggs collected during the fourth period were stored for 7 days and evaluated for albumen quality in Haugh Units. The egg shells of these eggs were oven-dried for determination of egg shell quality by measuring the thickness at different points with an "Avil micrometer". Percent mortality was calculated for each treatment group at the end of the experiment.

Relative humidity and ambient temperature of the open-sided experimental house were recorded daily at about 07.00, 13.00 and 18.00 hrs. All data were subjected to analysis of variance, and orthogonal polynomial contrasts \textsuperscript{18} were used to determine the most beneficial level.

The following model was used:

\[ Y_{ij} = u + T_i + E(ij) \]

where\( Y_{ij} \) representing each observable measurement

\( u \) represents treatment effect

\( i = 1, 2, 3, 4 \) levels of AA

\( E(ij) \) represents residual random term.
Results

The mean temperatures at 07.00, 13.00 and 18.00 hr were 27.9°C, 42°C and 40°C, respectively, and the mean relative humidity (RH) at these recording times were 53, 77, 70, respectively (Table 2). Wide diurnal temperature in weather parameters were recorded in this study and all recorded temperatures during the experimental periods were above 22.2°C. However, the study was conducted in the South-Coast of Nigeria where the sea breeze usually ameliorates the weather.

Average egg production, egg weight, Haugh units, shell thickness are shown in Table 3. Treatment B (1gAA/Kg) yielded the highest egg production among the AA supplemented diets, although each of these diets yielded significantly (P<.05) higher than the control. Haugh units and shell thickness showed linear (P<.05) response to the levels of AA supplementation, indicating that as AA levels increased, these parameters increased. Average blood AA, feed intake, body weight, and mortality are shown in Table 4. Treatment differences (P<.05) were obtained in all of the treatments with a linear (P<.05) response, except that, that of mortality was inverse, decreasing as AA supplementation increased. As feed intake increased, egg production, egg weight, body weight gain and livability increased in accordance with plasma AA and supplemental AA (Tables 2 and 8).

Table 1: Composition of basal diet

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount (g/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>500.0</td>
</tr>
<tr>
<td>Palm kernel meal</td>
<td>45.0</td>
</tr>
<tr>
<td>Soya bean meal (450g protein/kg)</td>
<td>40.0</td>
</tr>
<tr>
<td>Groundnut cake (450g protein/kg)</td>
<td>90.0</td>
</tr>
<tr>
<td>Fish meal (880g protein/kg)</td>
<td>30.0</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>40.0</td>
</tr>
<tr>
<td>Brewers dried grain</td>
<td>124.0</td>
</tr>
<tr>
<td>Brewers dried yeast</td>
<td>40.0</td>
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<tr>
<td>Calcium carbonate</td>
<td>50.0</td>
</tr>
<tr>
<td>Bone meal</td>
<td>33.5</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>1.0</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>4.0</td>
</tr>
<tr>
<td>Vitamin/trace mineral premix²</td>
<td>2.5</td>
</tr>
</tbody>
</table>

Composition (per kg)

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein (analyzed, g)</td>
<td>175</td>
</tr>
<tr>
<td>ME (calculated, Mj/kg)</td>
<td>11.72</td>
</tr>
<tr>
<td>Ca (calculated, g)</td>
<td>35.6</td>
</tr>
<tr>
<td>P (calculated, g)</td>
<td>7.0</td>
</tr>
<tr>
<td>Lysine (calculated, g)</td>
<td>82.7</td>
</tr>
<tr>
<td>Methionine + Cystine (calculated, g)</td>
<td>65.5</td>
</tr>
</tbody>
</table>

¹1, 1.5 or 2g AA/kg were added to make the test diet at the expense of no ingredient.
²Containing per kg vitamin and mineral mixtures:
0.12g retinol, 15mg cholecalciferol, 1.36g DL-α-tocopherol, 1g manadione bisulphite, 1.5g riboflavin, 5g nicotinic acid, 1.5g pantothenic acid, 4mg cobalamin, 75g chlorine chloride, 62.5g antioxidant; 12.5g Fe, 40g Mn, 25 Zn, 1g Cu, 0.6g I, 0.1g Co, 0.05g Se

Table 2: Average temperature (°C) and Relative Humidity

<table>
<thead>
<tr>
<th></th>
<th>December</th>
<th>January</th>
<th>February</th>
<th>March</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recording Hr.</td>
<td>07.00 13.00 18.00</td>
<td>07.00 13.00 18.00</td>
<td>07.00 13.00 18.00</td>
<td>07.00 13.00 18.00</td>
<td>07.00 13.00 18.00</td>
</tr>
<tr>
<td>Temperature</td>
<td>27.11 40.50 38.90</td>
<td>27.90 40.10 39.10</td>
<td>28.10 42.90 40.00</td>
<td>28.49 44.50 42.00</td>
<td>27.9 42.00 40.00</td>
</tr>
<tr>
<td>Humidity</td>
<td>51.00 74.00 69.00</td>
<td>52.00 76.00 69.00</td>
<td>53.50 78.00 71.00</td>
<td>55.50 80.00 73.00</td>
<td>53.00 77.00 70.00</td>
</tr>
</tbody>
</table>
Table 3. Effect of Dietary Ascorbic Acid on Egg Production and Quality

<table>
<thead>
<tr>
<th>Dietary Treatment</th>
<th>Egg Production (Hen-day%)</th>
<th>Egg Weight (g)</th>
<th>Haugh Units</th>
<th>Shell Thickness (microns)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (A)</td>
<td>57.22± 4</td>
<td>55.71± 1.2</td>
<td>68.54± 1.7</td>
<td>255±14.3</td>
</tr>
<tr>
<td>A+0.1 AA/kg(B)</td>
<td>79.86± 2.5</td>
<td>62.79± 1.3</td>
<td>83.81± 1.9</td>
<td>325±18.8</td>
</tr>
<tr>
<td>A + 1.5 AA/Kg(C)</td>
<td>74.57± 2.3</td>
<td>63.56±1.4</td>
<td>87.14±2.3</td>
<td>335±22.2</td>
</tr>
<tr>
<td>A + 2g AA/kg(D)</td>
<td>71.27±2.1</td>
<td>64.21±1.6</td>
<td>90.76±2.5</td>
<td>355±22.2</td>
</tr>
</tbody>
</table>

1 Data are means ± SEM of 5 replicated of 10 hens each.

*a,b,c,d* Means within same column with different superscript are significant (P<0.05)

Table 4. Effect of Supplementing Layer Diet with Ascorbic Acid on Blood, Feed Intake, Body Weight and Mortality

<table>
<thead>
<tr>
<th>Dietary Treatment</th>
<th>Blood AA (mg%)</th>
<th>Feed Intake (g/d)</th>
<th>Body Weight gain (g)</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (A)</td>
<td>0.96±0.33</td>
<td>96.45±3.3</td>
<td>15.75±1.1</td>
<td>36.0±0.8</td>
</tr>
<tr>
<td>A+1g AA/kg(B)</td>
<td>1.55±0.41</td>
<td>110.51±4.1</td>
<td>45.83±2.1</td>
<td>6.0±0.2</td>
</tr>
<tr>
<td>A + 1.5 AA/Kg(C)</td>
<td>2.36±0.45</td>
<td>114.61±4.5</td>
<td>54.56±2.3</td>
<td>2.0±0.1</td>
</tr>
<tr>
<td>A + 2g AA/kg(D)</td>
<td>2.75±0.51</td>
<td>119.93±4.8</td>
<td>66.71±2.5</td>
<td>2.0±0.1</td>
</tr>
</tbody>
</table>

1 Data are means ± SEM of 5 replicated of 10 hens each.
a,b,c,d Means within same column with different superscripts significant (P<0.05)

Discussion

The observed daily average temperature was generally above the normal thermoneutral range for egg production. Also the relative humidity (RH) observed in this study was relatively high enough to be concurrent with the temperature to cause the expected environmental stress. Environmental conditions constituting thermal stress in poultry become significant when diurnal mean temperature departs in either direction from 22.2°C (approximately) and when there is a sudden change in temperature in either direction from that to which the animals have become conditioned. Nonetheless, significant mortality rates in control hens than in AA-treated hens maintained in a summer environmental temperature of 27 to 31°C with an average RH of 75% have been reported. In accordance with the finding that changes in plasma AA in chickens are transient because AA is not actually stored by tissues of the body, supplemental AA has been reported to modify the incidence of heat-associated mortality. It was found that AA supplementation (500 and 1000 ppm) reduced heat-associated mortality and that this effect of supplementation was most evident in females. The significant liner (P<0.05) response of AA supplementation to mortality in this study demonstrated that supplemental AA reduced mortality in the laying hens, and therefore, supported the finding that AA should be considered as a potential pharmacological agent to reduce stress-related economic losses in the poultry industry. The significantly high egg production in response to AA supplementation in this study demonstrated that laying chickens have a requirement for ascorbic acid. The result also indicated that AA supplementation in diets of lays may have a definite metabolic effect in ameliorating non-adaptive climatic stress conditions.

The observed significant egg weight in this study agreed with previous results. A decrease in egg weight due to high environmental temperature has been reported. The progressive linearity in interior egg quality as measured by Haugh units and that of shell thickness in this study agreed with the results of and. Later studies have also reported that supplemental AA improved albumen quality in Haugh units and shell thickness as
found in this study. The improvement in Haugh units in this study could be explained by the interaction of dietary AA with dietary protein, whereas for that of egg shell thickness, increase in intestinal absorption of calcium and egg shell maintenance by supplemental AA could be used as an explanation.

Supplemental AA linearly increased plasma AA as supplementation increased in this study as it was previously reported. Whether the administration was via water or feed, it has been reported that plasma levels of AA are transitory and that levels of supplementation of 100 ppm or less did not give significant change in plasma AA.

The obtained linear response of feed intake and body weight gain to AA supplementation tended to indicate that dietary AA normalised the physiological status of the hens under the tropical condition, probably through amelioration of the observed climatic stress. In this study, the hens must have been in normal physiological status to be able to have an increased feed intake, weight-gain, egg production, egg weight, and liveability as evidence by the reduced mortality in the AA supplemented treatments. Although, Kechik and Sykes reported that feed AA supplemented diets did not affect feed intake of heat-stressed birds, significantly high feed intake and body weight gain in AA treated broilers have been reported as found in this study. Specifically, the body weight gain results of this study agreed with previous findings but disagreed with the reports. The differences may be caused by the strains used, AA levels tested and the environmental stress experienced by chickens.

References
PERFORMANCE OF VARIOUS CROSSES OF MPWAPWA CATTLE

M.G. UDO(1), M. MGHENI AND OLA SYRSTAD(2)
Department of Animal Science and Production, Sokoine University of Agriculture
P.O. Box 3004, Morogoro, Tanzania

PERFORMANCE DES DIVERS CROISEMENTS AVEC DES BOVINS MPWAPWA

Résumé

On a examiné les dossiers relatifs au croisement transversal: CT (Bos taurus x Mpwapwa), au croisement de retour; CR (Mpwapwa x CT) et au croisement inter se (CR X CR) des animaux d'un troupeau élevé à l'Institut de recherche sur la production animale à Mpwapwa en Tanzanie. Les paramètres examinés étaient les poids à la naissance, au sevrage (75 jours), à 252 jours et à 504 jours; l'âge au premier vêlage, la production laitière, la durée de la lactation et l'intervalle des vêlages.

Les animaux issus de croisement transversal avaient une performance meilleure que ceux des autres groupes génétiques pour tous les paramètres étudiés, à l'exception du poids à la naissance et de l'intervalle des vêlages. S'agissant de la production laitière, la différence était de 500 à 700 kg. Les veaux issus de taureaux Frisons et Ayrshire pesaient plus lourd que ceux des taureaux Jersey. Les vaches accouplées avec des taureaux Frisons produisaient plus de lait/lactation (environ 200 kg) que celles accouplées avec des taureaux des autres races, mais elles avaient aussi des intervalles des vêlages légèrement plus longs. Il n'y avait pas de différence significative entre les groupes "croisement de retour" et "croisement inter se" pour tous les paramètres étudiés.

Les résultats de la présente étude confirment les rapports antérieurs selon lesquels la forte production laitière des croisements Bos taurus x Bos indicus ne peut s'expliquer uniquement par les effets des gènes additifs et de la prépondérance de gènes dominants.

Abstract

Records of crossline (Bos taurus x Mpwapwa), backcross (Mpwapwa x crossline), and inter se (backcross x backcross) animals in the herd in the Livestock Production Research Institute at Mpwapwa, Tanzania, were analysed. Traits considered were weights at birth, weaning (75 days), 252 days and 504 days of age; age at first calving; lactation yield; lactation length and length of calving interval.

The crossline outperformed the other genetic groups in all traits except birth weight and calving interval. The difference in lactation yield was 500 to 700 kg. Calves sired by Frisian and Ayrshire bulls had higher weights than calves with Jersey sires. Cows by Frisian bulls produced more milk per lactation (about 200 kg) than cows sired by bulls of the other breeds, but also had slightly longer calving intervals. There was no important difference between the backcross and inter se groups in any of the traits studied.

The results support previous reports in suggesting that lactation yield of Bos taurus x Bos indicus crosses cannot be explained by additive gene effects and dominance alone.

Introduction

Mpwapwa cattle are a synthetic dual-purpose breed developed in Livestock Production Research Institute (LPRI) at Mpwapwa in central Tanzania. The breed has received about 60% of its inheritance from improved dairy breeds originating in the Indian subcontinent (Sahiwal and Red Sindhi), about 30% from African zebu breeds (Boran and Tanzanian shorthorn zebu), and the remaining 10% from European dairy breeds (mainly Ayrshire). The Mpwapwa breed comprises only about 1,000 head in two major herds.

Since 1968 a part of the Mpwapwa herd has been used for an experiment in which a random sample of females was mated to

Present address
(1) Department of Animal Science, University of Juba, P.O. Box 321/1, Khartoum, Sudan;
(2) Norwegian Centre of International Agricultural Development (NORAGRIC), The Agricultural University of Norway, P.O. Box 5002, 1432 As, Norway, (to whom all correspondence should be sent)
Friesian, Ayrshire or Jersey bulls. The offspring from these matings were termed the crossline. Female offspring were backcrossed to Mwapwa bulls. Subsequently, male and female backcrosses were mated together to produce the inter se group. A historical account of the breeding programme was given by Getz et al.\(^1\)

Dairy performance of Mwapwa, crossline and backcrosses was examined by Mkonyi\(^2\). The crossline calved at a younger age than Mwapwa, produced about 30% more milk per lactation, and had shorter calving intervals.

Backcrosses were intermediate between the parental groups in all traits, but closer to Mwapwa in milk yield. Msanga\(^3\) compared weights for age of the same genetic groups\(^4\). The crossline was heavier than the other groups at all ages, but the differences were rather small. In neither of these studies were the three crosslines separated by breed of sire. No comprehensive study involving the performance of inter se has been carried out. This, together with the accumulation of more data and better computational facilities, justifies a new comparison of the various crosses to Mwapwa cattle.

**Material and Methods**

**Site of experiment**

The data used for the study were copied from the files at LPRI, Mwapwa. The institute is located at 6°20' S, 36°30' E, about 1,100 m above sea level. The climate is semi-arid, with average annual rainfall of about 660 mm. The distribution is unimodal, most rain falling from December to May. However, the amount and distribution of rain varies widely from year to year. Average maximum and minimum temperatures are 27.5° and 11.5°C respectively\(^5\).

**Herd management**

Seasonal breeding is practiced at the institute, resulting in two major seasons of calving: from December to February and June to August. Calves are separated from their dams shortly after birth, and fed milk (about 4 litres per day) from buckets up to 75 days of age. They also have access to hay and concentrates. After weaning, the calves graze natural and improved pasture with little or no supplementation. Heifers are usually exposed to bulls during the first breeding season after 25 months of age. Cows are milked twice a day. They graze improved pasture on a rotational grazing system. Hay is fed during periods of pasture shortage. Cows in milk are given small amounts of concentrates at milking time.

**Data collection**

The records used in this study were those collected routinely in the institute. They included date of birth, weaning, 252 days (36 weeks) and 504 days (72 weeks) of age. For females entering the milking herd, all calving dates, dry dates and lactation yields were collected. From these data age at first calving, calving intervals and lactation lengths were computed. No attempt was made to estimate missing records. The study covers the period 1975 to 1990.

**Statistical analysis**

Records on body weights were analysed according to a statistical model including the fixed effects of genetic group (three crosslines, backcross, inter se), period of birth (195-79, 1980-84, 1985-90), season of birth (dry or wet) and age of dam <3, 4-5, 6-7, 8-9, and > 10 years):

\[
Y_{ijklm} = u + A_i + B_j + C_k + D_l + e_{ijklm}
\]

where

- \(Y_{ijklm}\) = the body weight of an individual calf at birth, weaning, 252 or 504 days of age
- \(u\) = overall mean
- \(A_i\) = effect of the \(i\)-th genetic group, \(i = 1.5\)
- \(B_j\) = effect of the \(j\)-th period of birth, \(j = 1, 2, 3\)
- \(C_k\) = effect of the \(k\)-th season of birth, \(k = 1, 2\)
- \(D_l\) = effect of \(l\)-th sex, \(l = 1, 2\)
- \(e_{ijklm}\) = a random element associated with the weight of the \(ijklm\)-th calf.

A similar model was used for analysis of
age at first calving, lactation length, lactation yield and calving interval, except that period and season of birth were replaced by period and season of calving, and sex was replaced by parity (the latter was not relevant in the case of age at first calving). Interactions among main effects were not considered. Coefficients of correlation between various traits were estimated from the residual (error) sums of squares and crossproducts.

Results

The analyses of variance of weights indicated that season of birth and sex had significant effects on weight at all ages. Males were consistently about 5% heavier than females, while the ranking of the two seasons was reversed from 252 to 504 days. Period of birth and age of dam had significant effects on all weights except weight at weaning. Calves born 1985–90 were lighter than calves born in earlier periods, and weights increased by increasing age of dam.

Weights were significantly influenced by genetic group at all ages. Least square means are reported in Table 1. Friesian and Ayrshire crosses were heavier than Jersey crosses. Backcrosses and inter se did not differ significantly at any age, both were inferior to all crosslines at 252 and 504 days.

Coefficients of correlation between weights of the same calf at different ages were low to moderately high. Birth weight was significantly correlated with weaning weight (r = 0.17), but not with post-weaning weights. Correlations among weaning and post-weaning weights were all highly significant, ranging from 0.31 to 0.45.

All traits related to lactation (age at first calving, lactation yield, lactation length and length of calving interval) were significantly influenced by period of calving. Age at first calving and calving interval increased from period 1 and 2 to period 3, while lactation yield and lactation length declined. None of these traits was affected by season of calving. Parity had a significant effect on lactation yield and calving interval, but not on lactation length. Lactation yield increased from first to third parity and then levelled out, while calving interval showed a declining trend by increasing parity number.

Genetic group had a highly significant effect on all the four traits. Least square means are presented in Table 2. The main difference was between the crossline on one side and the backcrosses and inter se on the other, the crossline were younger at first calving (2 to 4 months), had higher lactation yields (500 to 700 kg), longer lactations (20 to 30 days) and longer calving intervals (about 20 days).

Among the crosslines, Friesian crosses had the highest yields, but also the longest calving intervals. The three lines were similar in age at first calving and lactation length. Backcrosses and inter se differed significantly only in lactation length (P = 0.03). Milk yield per day of calving interval ranged from 4.85 kg in the Friesian crossline to 3.41 kg in the inter se.

Age at first calving was not significantly related to first lactation yield or length. A high correlation (r=0.61) was found between lactation yield and lactation length, while the corre-

<table>
<thead>
<tr>
<th>Genetic group</th>
<th>Birth wt. kg</th>
<th>Weaning wt kg</th>
<th>252 d. wt kg</th>
<th>504 d. wt kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crosslines</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Friesian</td>
<td>29.7±0.4</td>
<td>65.1±1.6</td>
<td>108.1±2.5</td>
<td>161±3.8</td>
</tr>
<tr>
<td>Ayrshire</td>
<td>29.02±0.5</td>
<td>61.8±1.6</td>
<td>109.2±2.7</td>
<td>163.6±4.0</td>
</tr>
<tr>
<td>Jersey</td>
<td>28.1±0.4</td>
<td>58.3±1.3</td>
<td>100.9±2.2</td>
<td>150.1±3.3</td>
</tr>
<tr>
<td>Backcross</td>
<td>29.5±0.2</td>
<td>60.3±1.1</td>
<td>97.5±1.2</td>
<td>146.4±1.9</td>
</tr>
<tr>
<td>Inter se</td>
<td>28.9±0.2</td>
<td>58.2±0.9</td>
<td>96.0±1.1</td>
<td>148.3±1.6</td>
</tr>
<tr>
<td>Overall mean</td>
<td>28.9</td>
<td>59.1</td>
<td>97.9</td>
<td>147.7</td>
</tr>
<tr>
<td>Standard dev.</td>
<td>4.3</td>
<td>9.9</td>
<td>18.9</td>
<td>27.4</td>
</tr>
<tr>
<td>No. of records</td>
<td>1416</td>
<td>782</td>
<td>928</td>
<td>790</td>
</tr>
</tbody>
</table>
Table 2: Least square means for age at first calving, lactation yield, lactation length and length of calving interval

<table>
<thead>
<tr>
<th>Genetic group</th>
<th>Age at 1st calving, mths</th>
<th>Lactation yield, kg</th>
<th>Lactation length, days</th>
<th>Calving interval, days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crosslines</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Friesian</td>
<td>39.1±0.9</td>
<td>2132±38</td>
<td>292±4</td>
<td>439±9</td>
</tr>
<tr>
<td>Ayrshire</td>
<td>38.6±1.1</td>
<td>1927±43</td>
<td>291±5</td>
<td>425±7</td>
</tr>
<tr>
<td>Jersey</td>
<td>39.3±1.0</td>
<td>1909±38</td>
<td>289±4</td>
<td>412±7</td>
</tr>
<tr>
<td>Backcross</td>
<td>42.7±0.5</td>
<td>1422±26</td>
<td>258±3</td>
<td>402±6</td>
</tr>
<tr>
<td>Inter se</td>
<td>41.7±0.5</td>
<td>1404±34</td>
<td>268±4</td>
<td>412±7</td>
</tr>
<tr>
<td>Overall mean</td>
<td>41.1</td>
<td>1572</td>
<td>273</td>
<td>418</td>
</tr>
<tr>
<td>Standard dev.</td>
<td>5.2</td>
<td>511</td>
<td>59</td>
<td>83</td>
</tr>
<tr>
<td>No. of records</td>
<td>330</td>
<td>1276</td>
<td>126</td>
<td>947</td>
</tr>
</tbody>
</table>

Table 3. Proportion Bos taurus inheritance and proportion of maximum Bos taurus vs. Bos indicus heterozygosity in various genetic groups of the Mwapawwa herd

<table>
<thead>
<tr>
<th>Genetic group</th>
<th>Expected proportion (in %) of Bos taurus</th>
<th>Heterozygosity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mwapawwa</td>
<td>10</td>
<td>18</td>
</tr>
<tr>
<td>Crossline</td>
<td>55</td>
<td>90</td>
</tr>
<tr>
<td>Backcross</td>
<td>32.5</td>
<td>54</td>
</tr>
<tr>
<td>Inter se</td>
<td>32.5</td>
<td>44</td>
</tr>
</tbody>
</table>

The correlation between lactation yield and calving interval was low, but nevertheless highly significant (r = 0.14).

Discussion

The superior performance of the crosslines as compared with backcrosses and inter se might be ascribed to their larger proportion of Bos taurus inheritance and/or to more heterosis. Expected proportions of taurus genes and proportion of maximum taurus vs. indicus heterozygosity in various genetic groups are set out in Table 3. The two proportions are almost completely confounded, and their effects can therefore not be separated in the present data. For traits related to dairy performance previous studies indicated that both are important. Syrstad(7), reviewing a large number of crossbreeding experiments in the tropics, estimated that an increase of one percentage unit in proportion of Bos taurus in the crosses on average was accompanied by an increase of 10.75 kg in lactation yield. This corresponds to a difference of 242 kg in lactation yield between the crosslines and the backcrosses (55 and 32.5% Bos taurus respectively). In the same study heterosis in F1 was estimated at 432 kg. Of this 389 kg (90%) would be expected to be retained in the crosslines and 233 kg (54%) in the backcrosses, a difference of 156 kg. Thus the two genetic effects combined account for about 400 kg of the difference between the crosslines and the backcrosses. By the same reasoning, the expected difference between crosslines and inter se would be about 440 kg.

It has been suggested that lactation performance of various Bos taurus x Bos indicus crosses cannot be explained by additive gene effects and dominance (heterozygosity) alone, but that also epistasis (recombination loss) might contribute. The present study supports this hypothesis, as the backcrosses were poorer than expected from the additive + dominance model. Syrstad(7) suggested that the simplest genetic model which explains the results is one which assumes that Bos taurus animals are homozygous for one or more sets of dominant genes which are complementary to one another. Of the dominance x dominance interactions in Bos taurus (two independent loci) all will be intact in the crossline (and in all other crosses having one Bos taurus parent), while only 31% will be retained in the backcrosses (i.e. only 31% of the backcrosses will receive an intact pair of interacting taurus genes in one of the gametes from which they originate, see 8). Unfortunately records of pure Mwapawwa animals were not included in the present study. Mkonyi(2)
found that the crossline exceeded Mwapwa by 500 kg of milk in first lactation and by 773 kg in second lactation. In our study the difference between the crossline (the three sire lines combined) and the backcrosses was about 570 kg. Taken together this means that the backcrosses are only slightly better than Mwapwa in lactation yield.

The lack of significant differences between backcrosses and their *inter se* is not surprising, as the groups are identical in additive genetic merits, and only slightly different in heterozygosity and epistatic combination retained (54 vs 44% and 31 vs 205, respectively). These results suggest that if the targeted lactation yield of 2270 kg (10) shall be achieved then the proportion of *Bos taurus* inheritance has to be increased above the 32.5% level in the backcrosses and *inter se*.

Inspite of the ability of the camel to lactate, helminthiasis has been found to be the second major cause of economic loss in camel production (14). It has been established that all of the internal parasites of camel, gastrointestinal nematodes are the most serious economic consequence (15). Nematodes in camels cause the clinical signs of anorexia, general debility, reduced growth rates and milk yields, increased calving intervals, unthriftiness, assassination, emaciation, and consumption of large amounts of sand (pea). *Haemonchus contortus* is the commonest and most pathogenic internal parasite of the camel (16). However, in Iraq and Kuwait (17), Trichobilharzia spiralis was the most prevalent helminth parasite in all the camels examined. The parasites was found to be more common in calves. Limited studies on the prevalence of gastrointestinal helminth of camels in Kenya have been published (18).

This report studies the prevalence of gastrointestinal helminth in different age groups and sexes of camels raised in a mixed communal grazing environment as assessed by worm egg counts, per gram of faeces (EPG) and coprodiagnosis and identification of recovered larvae.

The total of 255 faecal samples from camels belonging to 27 farmers in Lariol Division.

References

Camels continue to be an integral component of the ecosystem in which the vegetation of the marginal lands can be converted to human food. This is because, all over the world, camels have been found to be superbly adapted to the harsh arid environment. In spite of this, camels are susceptible to a number of viral, bacterial, mycotic, protozoal and parasitic diseases\(^1\).

In spite of the aridity of the camel habitat, helminthiasis has been found to be the second major cause of economic loss in camel production\(^2,3\). It has been established that of all the internal parasites of camels, gastrointestinal nematodes are of the most serious economic consequence\(^4,5\). Nematodiasis in camels causes the clinical signs of diarrhoea, general debility, reduced growth rates and milk yields, increased calving intervals, inappetance, anaemia and consumption of large amounts of sand (pica)\(^6\). *Haemonchus longistipes* is the most common and most pathogenic internal parasite of the camel\(^7-10\). However, in Iraq and Kuwait\(^11,12\) *Trichostrongylus probolurus* was the most prevalent helminth parasite in all the camels examined. The parasite was found to be more common in calves. Limited studies on the prevalence of gastrointestinal helminths of camels in Kenya have been published\(^13,14\).

This study reports the prevalence of gastrointestinal helminths in different age groups and sexes of camels raised in a mixed communal grazing environment as assessed by worm egg counts per gram of faeces (EPG) and coproculture and identification of recovered larvae.

The total of 255 faecal samples from camels belonging to 27 farmers in Loroki Division,
tapeworm were visible in the faecal droppings especially those of female adults and calves. Eggs of *Fasciola spp* were identified in a few of the camels. This is the first time that fascioliasis is being reported in camels in Kenya.

The rise in worm egg-counts during the wet season in camels has been reported previously in Kenya\(^{7,8,13,14}\). High amounts of rainfall are known to favour the survival and transmission of infective larvae of the pasture. Throughout our study period there was a high prevalence rate of nematodiasis. This agrees with the findings of earlier workers\(^{2,3,6,16}\) that nematodes are the commonest and the most pathogenic parasites of camels. The study showed that *Haemonchus spp* was the most prevalent and this finding has been reported in camels in this country.

The fairly high level of cestodes recorded has been reported in earlier studies\(^ {3,11,17}\). This study reports for the first time the occurrence of fascioliasis in camels in Kenya. However, this condition has been reported in camels in other countries\(^ {5,10}\).

This study confirms the importance and prevalence of helminths in camels. The authors recommend a communal worm control
programme that will involve administration of anthelminitics to 70% of all classes of livestock just before the onset of the long rains that occur usually from March to June.

References

SHORT COMMUNICATION

COMPARISON OF PHOSPHORUS CONTENT IN BOVINE JUGULAR AND COCCYGEAL BLOOD

BEIGHLE, D.E.
Department of Animal Health, School of Agriculture
University of Bophuthatswana, Mmabatho 8681
South Africa

Despite the limitations of using serum phosphorus (P) as an indicator of the P status in the bovine\(^1\), it is still used in the screening of animals thought to be P deficient because blood is easily and quickly collected from animals in the field and analyzed in the laboratory.

Previous research\(^2\) found that blood from the jugular vein contained less P than blood from the coccygeal vessels in cattle and Teleni, Dean and Murray\(^3\) confirmed that the mean tail blood P was 12% higher than the mean jugular blood P. The purpose of this research was to confirm previous reports related to these differences in light of ever improving laboratory techniques and methods, and to alert current research workers to differences which can exist in blood P values from the same animal.

Ten Friesian oxen were used in the experiment, and samples were taken weekly for 6 sampling periods and then monthly for 6 sampling periods.

Blood was taken from the jugular immediately after collection from the coccygeal vessels using an 18 gauge bleeding needle, collected in a vacuum blood tube and allowed to clot. Blood was refrigerated within an hour of collection, and serum was removed within 24 hours. Serum was stored at \(-10^\circ\)C until analysis and 10% Trichloracetic acid was used to precipitate the protein. Serum was analyzed using the method of Fiske and Subbarow\(^4\). Data was analyzed with the Statistical Analysis System\(^5\) using T test of comparisons within animals and not between animals.

Throughout the experiment the mean concentration of P in the jugular vein was less than that taken from the coccygeal vessels. The largest difference was seen among animals bled once a month where jugular blood 7.466 mg/dl P compared to 7.95 mg/dl P in the blood taken from the tail (p = 0.0001) (Table 1). This difference was less than 7%, compared to those bled once a week where the difference was less than 2%. During weekly sampling, jugular blood had 7.052 mg/dl P compared to 7.17 mg/dl P in blood from the tail (p = 0.137) (Table 1). When all samples were compared the difference was less than 4% with the jugular blood having 7.218 mg/dl P compared to 7.482 mg/dl P in tail blood or a difference of 0.264 mg/dl (p = 0.0001) (Table 1).

Reports of normal content of P in bovine serum and plasma have ranged from 4 to 9 mg%\(^6\) and 5.5 to 8.9 mg%\(^7\). Engles\(^8\) reported plasma P up to 7.91 mg/dl and Read et al\(^9\) reported serum inorganic P between 4 and 8 mg/dl. Results presented here are in agree-

| Table 1: Mean Jugular and Tail Blood Phosphorus Mg/dl |
|-----------------|-----|-----|
| Weekly sampling | Jugular | Tail | Difference |
| 7.052           | 7.170 | 0.118 |
| SE=0.16         | SE=0.16 | P=0.137 |
| Monthly sampling | 7.466 | 7.950 | 0.484 |
| SE=0.23         | SE=0.21 | P=0.0001 |
| All samples     | 7.218 | 7.482 | 0.264 |
| SE=0.14         | SE=0.13 | P=0.0001 |

...ment and demonstrate less of a discrepancy between jugular and coccygeal blood P than has previously been reported\(^8\). This could have been due to the order in which the samples were collected. It has been shown that excitement can cause an increase in blood P\(^9\). The
collection of the tail samples could have caused an increase in P in the blood which was later collected from the jugular vein and resulted in less difference in the two values. This confirms the dangers in the use of blood to establish the P status of the bovine(1). In addition, improved laboratory techniques and instrumentation could also have been responsible for the decrease in the discrepancy in the 2 values. Researchers should therefore still take care not to compare jugular and coccygeal blood results and should take cognizance of factors which can cause a variation in Blood P concentrations.

Acknowledgements

Sincere appreciation is expressed to Mr. E.S. Medupe and Mr. M. Raito for assistance with laboratory analysis and collection of samples, Mr. B. Moncho for feeding of animals and cleaning of holding pens, Ms. L. Kgobe for data capture and Mr. S. Ntabi for the statistical analysis.

References

CORRELATION BETWEEN ERYTHROCYTIC VALUES AND EGG PRODUCTION IN OLD LAYERS

E.K. AWOTWI AND G.S. ABOAGYE
Department of Animal Science, University of Ghana, Legon, Ghana

It has been observed that there are differences in erythrocytic values between laying and non-laying birds[1,2,3,4,5]. These differences are presumably due to differences in hormonal status. Oestrogen administration, for instance, has been found to result in a decline in the haematocrit of many species including chicken[2,6]. The difference in erythrocytic values between laying and non-laying birds has been utilized to identify unproductive turkey breeder-hens[4]. The most popular system for rearing poultry in Ghana and other West African countries is the deep litter system, where all the birds are kept in one room. With this system, unproductive hens cannot be identified and this becomes a problem especially in flocks over one year old where egg production is low. The aim of this study therefore, was to assess the use of haematocrit, haemoglobin concentration and total red blood cell count in identifying unproductive birds in a flock of old layer hens.

Blood samples for analysis were taken from forty-five HYSEX BROWN layers kept at the University of Ghana’s Agricultural Research Station (A.R.S. Legon). Blood samples were taken from each bird at 40, 50, 60 and 70 weeks of age. All the birds used for the study were kept in individual cages. The egg production records of individual hens were kept from 40 to 70 weeks. Standard haematological assay procedures were utilized in determining packed cell volume (PCV), haemoglobin concentration (Hb) and total red blood cell count (RBC) for each blood sample. Simple linear correlations between egg production over the experimental period (30 weeks) and mean values for the three haematological parameters were determined.

The total number of eggs produced by the birds at ten-weekly intervals are shown in Table 1. Egg production declined as the birds grew older. Table 2 shows the mean erythrocytic values for the old layers at different ages. The mean PCV value for the birds at 40 weeks was significantly lower (P<0.05) than at 50, 60 and 70 weeks. There was a trend of increasing PCV

<table>
<thead>
<tr>
<th>Age of birds (wks)</th>
<th>No of Birds</th>
<th>Total number of eggs produced</th>
<th>Average No of eggs/bird/wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>41 - 50</td>
<td>45</td>
<td>2,327</td>
<td>5.17</td>
</tr>
<tr>
<td>51 - 60</td>
<td>45</td>
<td>1,781</td>
<td>3.96</td>
</tr>
<tr>
<td>61 - 70</td>
<td>45</td>
<td>1,006</td>
<td>2.24</td>
</tr>
</tbody>
</table>

Table 2. Erythrocytic Values (Mean ± S.D.) in Hysex Brown Layer Chicken at Different Ages

<table>
<thead>
<tr>
<th>Parameter</th>
<th>No of Birds</th>
<th>40</th>
<th>50</th>
<th>60</th>
<th>70</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCV (%)</td>
<td>45</td>
<td>27.66 ±4.29a</td>
<td>33.29 ±3.42</td>
<td>35.44 ±2.90</td>
<td>36.54 ±3.12b</td>
</tr>
<tr>
<td>Hb (gm%)</td>
<td>45</td>
<td>11.28 ±0.93</td>
<td>11.21 ±1.23</td>
<td>10.02 ±1.44</td>
<td>9.95 ±1.42</td>
</tr>
<tr>
<td>RBC (x106/mm3)</td>
<td>45</td>
<td>2.23 ±0.30</td>
<td>2.43 ±0.26</td>
<td>2.53 ±0.32</td>
<td>2.58 ±0.37</td>
</tr>
</tbody>
</table>

Means with different superscripts are significantly different (P<0.05)
Table 3. Correlation Between Ethrocytic Values and Egg Production in Old Hysex Brown Layer Chickens

<table>
<thead>
<tr>
<th>Parameter</th>
<th>No of Birds</th>
<th>Range</th>
<th>Mean ± S.D.</th>
<th>Correlation with Egg Production</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCV (%)</td>
<td>45</td>
<td>26.50 - 36.88</td>
<td>33.49 ± 2.30</td>
<td>0.0964 (NS)</td>
</tr>
<tr>
<td>Hb (gm %)</td>
<td>45</td>
<td>8.33 - 12.38</td>
<td>10.63 ± 0.83</td>
<td>0.0558 (NS)</td>
</tr>
<tr>
<td>RNC (x106/mm3)</td>
<td>45</td>
<td>1.74 - 2.93</td>
<td>2.45 ± 0.23</td>
<td>0.2577 (NS)</td>
</tr>
</tbody>
</table>

NS: Not significant (P > 0.05)

and RBC and decreasing Hb as the birds grew older. The correlations between the three erythrocytic values measured and egg production are shown in Table 3. All three correlation values were low and statistically not significant (P > 0.05).

The PCV values obtained for the birds at 50, 60 and 70 weeks were similar to the 34.03—35.22% reported for younger birds of the same strain [7]. It is interesting to note that the lowest mean PCV value of 27.66% was recorded during the period of high egg production. Other workers have reported of a significantly lower PCV in laying than non-laying birds [49]. Even though PCV tended to increase as egg production declined, the correlation between PCV and egg production was very low and not significant (P > 0.05). This indicates that PCV values cannot be used to identify unproductive old layers. This finding is in contrast to that of Proudman and Wentworth [4], who found that PCV was significantly correlated with the level of egg production in turkey breeder-hens.

Even though there was a trend of declining Hb values as the birds grew older and egg production declined, the differences in Hb values at 40, 50, 60 and 70 were not significantly different (P > 0.05). Oyewale and Fajimi [5] did not also find any significant differences between Hb values for laying and non-laying guinea hens. In contrast, Winter [1] reported of a lower level of Hb in laying hens while Paulsen et al. [8] found laying turkey hens to have higher Hb values than non-laying birds.

Even though the differences between RBC values of the birds at the different ages were not statistically significant (P > 0.05), the values tended to increase as the birds grew older and egg production declined. Results of studies on the relationship between RBC values and egg production have not been consistent. While some authors [5,9] have found no significant difference in RBC values between laying and non-laying birds, others [2,3] have found non-laying birds to have higher RBC counts than laying birds.

The low and statistically non-significant correlations between PCV, Hb, RBC and egg production observed in the present study indicate that in old layer birds, erythrocytic values cannot be used as an indicator of egg production.

References

SHORT COMMUNICATION:

NUTRITIVE VALUE OF "WILD SORGHUM" FORTIFIED WITH LEUCAENA (LEUCAENA LEUCOCEPHALA DE WIT. LAM.)

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

In an earlier work to study the "wild sorghum" (Sorghum arundinaceum) as a potential forage crop, it was observed that the dry matter yield, crude protein content (CP) and in vitro dry matter digestibility (IVDMD) at six weeks of primary growth were 0.25 t ha⁻¹, 19% and 67% respectively. However, while the dry matter yield increased to 10.2 t ha⁻¹, the CP and IVDMD values decreased to 3% and 39% respectively at the fourteenth week of growth. One way to overcoming the low quality and improving intake of such forages is to use leguminous browse and shrubs as supplements since these are relatively high in both crude protein content and digestibility. One such shrub that can be used is leucaena (Leucaena leucocephala de Wit LAM). Leucaena produces between 2 and 9 t Dm ha⁻¹ of forage with a crude protein content of between 17% and 30%. Its IVDMD values range between 60% and 70%. The objective of this work was to study the nutritive value of the "wild sorghum" fortified with leucaena.

Leucaena (Leucaena leucocephala) was collected from a nearby naturalised plot where effluent flows. This plot is slashed about thrice in a year and has been there for the past twenty years. Secondary and tertiary growths of the "child sorghum" were harvested by hand with a cutlass at 5 cm above ground level. In both instances, regrowth period was ten (10) weeks while leucaena was cut after ten to twelve weeks of regrowth. After harvesting the "wild sorghum" and leucaena, they were manually chopped into lengths of 2–3cm using a cutlass. Samples were dried while sub-samples were mixed in the ratio of 7:3 of grass, legume and dried. Drying was done in the oven at 70°C for 48 hours. After drying, the samples were ground through 1.00 mm sieve with a Wiley mill and stored until analyses.

The ground samples were analysed for the following: crude protein (CP) and hydrogen cyanide (HCN) according to the methods of A.O.A.C⁴ neutral detergent fibre (NDF), acid detergent fibre (ADF), cellulose and acid detergent lignin (ADL) according to the methods of Goering and Van Soest⁵. In vitro dry matter digestibility (IVDMD) was done according to the method of Minson and McLeod⁶.

Dry matter yield of the secondary growth was 9.71 t Dm ha⁻¹ and though this was lower than the 10.07 t DM ha⁻¹ for the tertiary growth the difference was not significant (P>0.05).

Chemical composition of the "wild sorghum", leucaena and their combination are shown in Table 1. Dry matter content of the secondary growth harvest was significantly lower (P>0.01) than those of the tertiary growth harvest for both "wild sorghum" and leucaena. This was reflected in the combined product.

"Wild sorghum" had an average crude protein value of 6% which was significantly (P<0.01) lower than that of the sole leucaena (35%) or that of leucaena combined with "wild sorghum" (24%).

The values for NDF, ADF and cellulose of the "wild sorghum" were significantly higher (P<0.01) than those of either sole leucaena or leucaena combined with "wild sorghum." Similarly, the difference between the last two in these cell wall constituents was significant (P<0.01). Though "wild sorghum" again had the highest ADL content, the value was comparable to that of leucaena combined with "wild sorghum" and these were significantly higher (P<0.05) than that of sole leucaena.
Table 1: Chemical composition of "wild sorghum", leucaena and their combination

<table>
<thead>
<tr>
<th>Analytical Component</th>
<th>Treatment</th>
<th>Crude Protein</th>
<th>NDF</th>
<th>ADF</th>
<th>ADL</th>
<th>Cellulose</th>
<th>HCN</th>
<th>IVDMD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>[Wild Sorghum]</td>
<td>5.85</td>
<td>76.60</td>
<td>45.60</td>
<td>23.71</td>
<td>6.90</td>
<td>392.00</td>
<td>354.80</td>
</tr>
<tr>
<td></td>
<td>[Leucaena]</td>
<td>6.12</td>
<td>78.92</td>
<td>49.21</td>
<td>31.01</td>
<td>7.04</td>
<td>347.50</td>
<td>354.80</td>
</tr>
<tr>
<td></td>
<td>[Combined]</td>
<td>6.12</td>
<td>78.92</td>
<td>49.21</td>
<td>31.01</td>
<td>7.04</td>
<td>347.50</td>
<td>354.80</td>
</tr>
</tbody>
</table>

Sole leucaena had the highest IVDMD values (66%). This value was significantly higher (P<0.01) than those of leucaena combined with "wild sorghum" (60%) which was in turn significantly higher than that of sole "wild sorghum" (47%).

The dry matter yield of the “wild sorghum” is comparatively higher than the average yield from the natural grasslands and compares favourably with a lot of the sown pastures. For the two regrowths, together with the primary growth yield of 9.5 t DM ha\(^{-1}\) at twelve weeks growth period\(^1\) it appears that over the growing season of thirty-two weeks a total harvestable dry matter yield of some 30 t can be obtained. This is about twice to ten times the natural grassland\(^7\). The average daily intake for a 300 kg live weight grazing animal is about 2.5 kg dry matter per 100 kg live weight per day. This will amount to about 1125 kg of dry matter over a five-month dry season, which is the average length of the dry season period in this part of the country. It would therefore appear that one hectare of the “wild sorghum” grown over a period of thirty-two weeks would be able to sustain about twenty-seven (27) animals as indicated above over the five-month period.

The crude protein content of 6.0% of the “wild sorghum” is below the minimum level of 7% below which intake declines in beef animals\(^5\). Consequently intake would be expected to be below normal. Nevertheless the value is still greater than the values obtained with the natural grasslands in the dry season\(^9\). The grazing animal requires 156g digestible protein for every kilogramme dry matter intake for optimum performance. Thus for the livestock described above the required DCP would be about 1170g. Dermarquilly and Weiss\(^10\) estimated that the crude protein level needed to provide that amount of DCP is about 16.25%. When however, “wild sorghum” was fortified with the leucaena the protein level was raised to about 24%. Thus, it appears that inclusion level of the leucaena would have to be less than 30%.

The IVDMD value of the “wild sorghum” obtained in this experiment compares favourably with the results obtained with primary growth\(^1\). Similarly leucaena also had an IVDMD value
comparable to that in the literature. The minimum IVDMD level below which production is grossly affected is about 50% for tropical forages\textsuperscript{11}. Thus the IVDMD value of 47% of “wild sorghum” is just below this level. However, when combined with leucaena this value is very much enhanced to 60%, a value which makes it a potential production feed.

The HCN content of the “wild sorghum” is higher than 200 ppm considered as the minimum lethal dose for livestock\textsuperscript{12}. Thus, this potential toxicity threatens the potential usefulness of the plant as animal feed. However, combining the “wild sorghum” with leucaena reduces the level considerably, though efforts must still be made to bring it below the 200 ppm level.

References

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STAFF LIST

Director
Dr. W.N. Masiga, B.V.Sc., Dip.Bact., Ph.D., D.Sc. (Honoris Causa)

Chief Animal Health Officer
Dr. A.G. Tall, Docteur Veterinaire

Chief Livestock Projects Officer
K.M. Katondo, B.Sc., M.Sc.

Chief Animal Production Officer
Dr. J.T. Musiime, B.V.M., Dip. P.V.M., M.Sc., Ph.D.

Documents Officer
M.A.S. Machani, B.A. (Hons)

Translators
M. Ranaivoson
P.E. Dadzie
RECOMMANDATIONS AUX AUTEURS

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 interafrican 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

Format des articles
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 où 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.

Remerciements éventuels.

Bibliographie : les références bibliographiques doivent être numérotées dans l'ordre, telles qu'elles apparaissent dans le texte. L'identification des références dans le texte se fera à l'aide de numéros (entre parenthèses) et non pas par les noms des auteurs.

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Le nom du pays, l'année faisant l'objet du rapport, puis le nom du service ou de l'organisation, le numéro de la première page.

Si le même auteur est cité plus d'une fois, ses publications seront indiquées dans l'ordre chronologique dans la liste bibliographique et s'il y a plus d'une publication, les lettres "a, b, c, ..." seront ajoutées aussi bien dans la liste bibliographique que dans le texte.

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