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PRELIMINARY STUDY ON BOVINE TUBERCULOSIS IN NAZARETH MUNICIPALITY ABATTOIR OF CENTRAL ETHIOPIA

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ÉTUDE PRELIMINAIRE SUR LA TUBERCULOSE BOVINE A L'ABATTOIR DE LA MUNICIPALITE DE NAZARETH DANS LE CENTRE DE L'ETHIOPIE

Résumé

Une étude préliminaire a été menée d'octobre 2002 à avril 2003 à l'abattoir de la municipalité de Nazareth afin de déterminer la prévalence de la tuberculose bovine (TBB) chez 1.125 têtes de bétail sur la base de la lésion post-mortem, puis devalider la valeur diagnostique du test comparatif intradermique à la tuberculeine (TCI). La prévalence de la TBB chez les bovins abattus à l'abattoir de la municipalité de Nazareth pendant la période d'étude était de 5,15% (58/1.125) et ce, en tenant compte de l'examen post-mortem détaillé. On a constaté une bonne concordance (Kappa = 0,70) entre TCI et l'examen post-mortem détaillé. Le ratio des lésions détectées grâce à la méthode d'inspection de routine de la viande par rapport aux lésions détectées à l'aide de l'examen post-mortem détaillé était d'environ 1 : 3,2. Pour conclure, la prévalence de TBB enregistrée dans la présente étude était relativement plus forte que celle signalée dans les précédents rapports d'abattoir en Ethiopie.

Mots-clés : Tuberculose bovine, test comparatif intradermique à la tuberculeine, inspection de viande.

Summary

A preliminary study was conducted in Nazareth Municipality Abattoir from October 2002 to April 2003 to estimate the prevalence of bovine tuberculosis (BTB) in 1125 heads of cattle on the basis of post mortem lesion, and validate the diagnostic value of comparative intradermal tuberculin test (CIDT). The prevalence of BTB in cattle slaughtered in Nazareth Municipality Abattoir during the study period was 5.15% (58/1125) based on post mortem examination. A good agreement (Kappa=0.70) was observed between CIDT and detailed post mortem examination. The estimated ratio of lesions detected by the routine meat inspection method to that detected by the detailed post mortem examination was 1:3.2. In conclusion, the prevalence of BTB recorded in this study was relatively higher than the previous reports in the slaughterhouse in Ethiopia.

Key words: Bovine tuberculosis, Comparative intradermal tuberculin test, Meat inspection.

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Introduction

Tuberculosis (TB) is still the greatest single cause of human morbidity and mortality in many developing countries\(^1\). *Mycobacterium tuberculosis* is the primary cause of human TB but some cases are caused by *Mycobacterium bovis*. Bovine TB eradication campaigns in many developed countries have led to a huge reduction in the incidence of human TB caused by *M. bovis\(^2\). However, *M. bovis* is responsible for increasing animal and human health problems in several developing countries\(^3\). Most developed countries have nearly eradicated BTB or reduced to a minimum level using the test-and-slaughter policy, but in developing countries, BTB is still prevalent in animal and human populations causing great economic losses. According to Cosivi et al\(^4\), 60% of the African, 47% of the Asian and 38% of the Latin American and the Caribbean countries reported sporadic occurrence of TB. In Africa, Malawi and Mali, were described as having a high occurrence of BTB\(^4\). Of all nations in Africa, only seven apply disease control measures as part of a test-and-slaughter policy and consider BTB a notifiable disease\(^4\). But, approximately 85% of the cattle and 82% of the human population of Africa live in areas where BTB is either partly controlled or not controlled at all\(^4\). Among the East African countries, Somalia\(^5\), Djibouti\(^3\), Rwanda\(^3\), and Ethiopia\(^5-7\) have reported varying BTB occurrences. The prevalence of BTB in Ethiopia ranged from 3.4% in smallholder production systems to 50% in intensive dairy production system\(^5-9\).

Diagnosis of BTB in cattle and other susceptible species is made using history, clinical findings, tuberculin test, postmortem examinations and histopathology. *In-vitro* lymphocyte stimulation assay, interferon-gamma assay, and enzyme linked immunosorbent assay (ELISA) can also be used for the diagnosis of BTB at various levels. To confirm diagnosis, culture and biochemical properties are being used. To differentiate *M. bovis* from other members of *M. tuberculosis* complex, nucleic acid recognition methods using polymerase chain reaction (PCR) and DNA genome sequence are used\(^10-12\). Nevertheless, most of these diagnostic tools cannot be used in the field on large-scale basis except the tuberculin test, which has been used for diagnosis of BTB for more than 100 years. Limitation of this test has been recognized for many years, but the amount of published data available to validate its performance are scarce. This is primarily because the experiments to establish the sensitivity and specificity of the test are expensive and labor intensive, as they require slaughter of animals and detailed postmortem examination\(^13\). In Ethiopia, tuberculin tests have been used for the diagnosis of BTB to a limited extent. Hence, estimation of their sensitivities and specificities is of paramount importance. Similarly, post mortem examination is widely used for the detection of tuberculous lesion in slaughterhouses across the country.

Therefore, this study was formulated to estimate the prevalence of BTB in animals slaughtered in Nazareth Municipality Abattoir on postmortem examination, and validate the diagnostic value of the comparative intradermal tuberculin test.

**Materials and methods**

**Description of the study area**

Nazareth Town, located at 100km East of Addis Ababa, is one of the most populous
towns in Ethiopia, and an important multidirectional trade route. A significant number of people are involved in feedlot business, supplying cattle to surrounding butchers, abattoirs and export of live animals. As a result, cattle from different parts of the country are driven on hoof to Nazareth Town. There are about 61 legally registered butcheries in Nazareth, but more than half of the meat is supplied by back yard slaughter. Following ante-mortem examination, bleeding is carried out by cutting both jugular veins after stunning the animal by piercing with a sharp knife at the medulla oblonga. The dressing of the carcasses is carried out manually.

**Study units and sampling**

The study was conducted on small-scale feedlots of butchers, who keep animals ready for slaughter in the abattoir. Each day, on average 45 heads of cattle were slaughtered in the abattoir during the study period, which was carried out from October 2002 to April 2003. The required sample size for the validation of the test was estimated by considering the expected prevalence to be 14.8% from the previous study\(^7\). The desired precision was decided to be 5% while the confidence level was 95%. The formula described by Thrusfield\(^{14}\) was used to determine the sample size. Although the calculated sample size was 187, a few more animals were added to give a total of 205 cattle for the validation of CIDT. After obtaining the list of butcheries from Nazareth Municipality Abattoir, of the 61 legally registered butcheries, feedlots of 15 butcheries were selected randomly. Simple random sampling was used to select the study animal in each feedlot.

Additionally, a 5-year retrospective study (1999 to the first four months of 2003) report of post mortem examination records were collected and analyzed.

**Comparative intradermal tuberculin test**

Two sites on the skin of mid neck separated by 10-15 cm were shaved. The skin fold thicknesses of each injection sites were measured by caliper after which 0.1 ml (25,000IU/ml) avian PPD (D4 ER Strain, Avituber synbiotics, France) and 0.1ml (20,000 IU/ml) bovine PPD (Anstrain, Bovituber synbiotics, France) were injected into the dermis of each site. The skin fold thickness of each injection site was measured 72 hrs after injection. The result was interpreted according to OIE\(^{12}\).

**Postmortem examination**

The routine post mortem examination in Nazareth Abattoir involves the palpation and incision of the lungs and the liver, visual examination of the kidneys, lymph nodes such as tracheobronchial, mediastinal, retro-pharyngeal, pre-scapular and pre-femoral lymph nodes. For this study, in addition to the routine post mortem, mesenteric and hepatic lymph nodes and lymph nodes of the head were sliced into 2mm using surgical blade and examined for the presence of abscess, cheesy masses and tubercles\(^{15}\).

**Microscopic examination**

Suspicious tissue samples were collected for direct microscopic examination from inoculums prepared from centrifuged sediments before decontamination and stained with the Ziehl-Neelsen\(^{11,16}\) and examined under oil immersion microscope for the presence of acid fast bacilli.

**Histopathological examination**

Samples from 78 suspicious lesions were collected in 10% buffered formalin, then dehydrated in serials of alcohol and
embedded in paraffin. They were sectioned at 4 micrometers, deparaffinized in xylene, and were stained with both hematoxylin-eosin and Ziehl-Neelsen staining side by side.

Isolation of Mycobacteria

The tissue specimens for culture were collected into sterile universal bottles in saline and kept at -20°C at Nazareth Veterinary Clinic until being transported to the Institute of Pathobiology, TB laboratory for culturing. However, about 75% of the samples were not recovered because of frequent breakage of power during storage. Thirty tissue samples were recovered and processed for culturing. Each sample was homogenized using mortar and pestle. The homogenate was decontaminated with of 4% NaOH (equal volume) for 15 minutes.

<table>
<thead>
<tr>
<th>Atomic Site</th>
<th>Number of tuberculous lesions</th>
<th>Percent (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tracheobronchial lymph node</td>
<td>30</td>
<td>31.3%</td>
</tr>
<tr>
<td>Mediastinal lymph node</td>
<td>26</td>
<td>27.0%</td>
</tr>
<tr>
<td>Mesenteric lymph node</td>
<td>16</td>
<td>16.7%</td>
</tr>
<tr>
<td>Lungs</td>
<td>13</td>
<td>13.6%</td>
</tr>
<tr>
<td>Medial retropharyngeal lymph nodes</td>
<td>5</td>
<td>5.2%</td>
</tr>
<tr>
<td>Liver</td>
<td>4</td>
<td>4.2%</td>
</tr>
<tr>
<td>Hepatic lymph node</td>
<td>2</td>
<td>2.0%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>96</strong></td>
<td><strong>100%</strong></td>
</tr>
</tbody>
</table>

**Table 2:** Condemnation rate of carcasses and/or organs in Nazareth Municipality Abattoir during 1999-2003.

<table>
<thead>
<tr>
<th>Year</th>
<th>Total cattle slaughtered</th>
<th>Whole carcass</th>
<th>Lung</th>
<th>Liver</th>
<th>Heart</th>
<th>Kidney</th>
<th>Total</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1999</td>
<td>8229</td>
<td>0</td>
<td>15</td>
<td>10</td>
<td>7</td>
<td>0</td>
<td>32</td>
<td>0.38</td>
</tr>
<tr>
<td>2000</td>
<td>9101</td>
<td>0</td>
<td>17</td>
<td>23</td>
<td>2</td>
<td>0</td>
<td>42</td>
<td>0.46</td>
</tr>
<tr>
<td>2001</td>
<td>9225</td>
<td>0</td>
<td>54</td>
<td>31</td>
<td>6</td>
<td>0</td>
<td>91</td>
<td>0.99</td>
</tr>
<tr>
<td>2002</td>
<td>9518</td>
<td>4</td>
<td>104</td>
<td>76</td>
<td>10</td>
<td>38</td>
<td>232</td>
<td>2.44</td>
</tr>
<tr>
<td>2003a</td>
<td>7088</td>
<td>4</td>
<td>80</td>
<td>35</td>
<td>9</td>
<td>6</td>
<td>134</td>
<td>1.90</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>43,461</strong></td>
<td><strong>8</strong></td>
<td><strong>270</strong></td>
<td><strong>175</strong></td>
<td><strong>34</strong></td>
<td><strong>44</strong></td>
<td><strong>531</strong></td>
<td><strong>1.22</strong></td>
</tr>
</tbody>
</table>

*a*Includes the first 4 months of 2003 (January - April).
with intermittent shaking. The homogenate was then centrifuged at 3000rpm for 15 minutes. The supernatant was discarded while the sediment was neutralized with 1% HCl in phenol indicator. Neutralization was achieved when the suspension color changed from purple to yellow. After neutralization 0.1 ml of the suspension from each sample was spread on the slants of Lowenstein-Jensen (LJ). Each sample was inoculated into two LJ media, one with sodium pyruvate and the other with glycerol. The cultures were incubated aerobically at 37°C for 12 weeks, with weekly observation for growth. Whenever visible, colonies were observed, Ziehl-Neelsen staining was performed to confirm the presence of acid-fast bacilli.

Niacin test

Niacin test was conducted on seven strains of Mycobacteria that were grown on privyated LJ media. The test was conducted according to the procedure described by WHO.

Data analysis

Sensitivity and specificity were calculated and expressed in percentage. Test of agreement among different tests was calculated using Kappa statistic. Retrospective data were analyzed using regression and correlation coefficient.

Results

Prevalence of BTB and distribution of tuberculous lesion

The prevalence of BTB in cattle slaughtered in Nazareth Municipality Abattoir during the study period was 5.15% (58/1125) on the basis of detailed post mortem examination. Macroscopically, the most common changes seen in the affected organs and/or lymph nodes were the presence of circumscribed yellowish white lesions of various sizes and numbers. In two of the 58 positive animals diagnosed positive, miliary lesions were observed in the lungs. Large encapsulated nodules containing thick greenish-yellow exudates were observed in the remaining ones. The distribution of tuberculous lesion in positive animals is presented in Table 1. Larger proportion (72%) of gross lesions was observed in the thoracic cavity.

Using detailed post mortem, 5.15% (58/1125) cattle were found to contain tuberculous lesions. However, only 1.6% (18/1125) heads of cattle were detected to have TB lesions by the routine post-mortem examination. Thus, the estimated ratio of lesions detected by the routine meat inspection to the detailed necropsy procedure was 1:3.2. The estimated probability of missing an animal with lesions using the routine procedure was 69%. Moderate agreement (Kappa=0.5) was recorded between detailed necropsy procedure and routine meat inspection.

Validation of CIDT and its agreement with post mortem examination

When the doubtful animals were considered as negative, the sensitivity and specificity of the CIDT were 53.5% and 98.5%, respectively. But, when doubtful animals were considered as positive, the sensitivity and specificity of the test were found to be 70.5% and 90.9%, respectively. The doubtful animals were considered as positive reactors in calculating the maximum sensitivity (70.7%); but in calculating the maximum specificity (98.5%) doubtful animals were considered as negative. Thus, the sensitivity of CIDT was ranged from 53.5-70.7% while its specificity was ranged from 90.9-98.5%.
Test of agreement between CIDT and detailed post mortem examination indicated that of the 58 animals, 33 were tuberculin test positive and 31 were positive for both tests. There was good agreement (Kappa=0.70) between CIDT and detailed post mortem examination. Similarly, a good agreement (Kappa=0.61) was observed between post mortem and histopathological examinations.

Histopathology of the granulomatous lesions

A good agreement (Kappa=0.61) was observed between the results of post mortem and histopathological examinations. The microscopic lesions observed had a center of caseous necrosis usually with some calcification, with boundary of epithelioid cells, some of which formed multinucleated giant cells and few to numerous lymphocytes and neutrophils were observed. The lesions had a fibrous connective tissue capsule, giving the lesion a focal appearance. Parallel staining of 40 suspected samples by Ziehl-Neelsen stain demonstrated acid-fast bacilli in 28 slides, which appeared as faint pink in color inside the macrophage.

Mycobacterial culture and isolation

Growth of Mycobacteria was observed on seven pyruvate enriched LJ medium, and all of these isolates were found to be negative to niacin test. The isolates were thus classified as M. bovis strains. On the other hand, pigmented colonies were observed on four glycerol enriched LJ media and one pyruvate enriched LJ medium. These isolates were grouped as non-pathogenic Mycobacteria.

Retrospective data

The result of the retrospective data of 5 years on cattle slaughtered in Nazareth Municipality Abattoir is presented in Table 2. The condemnation rate of carcasses and/or organs due to tuberculosis infection indicated an increasing trend (0.38% to 1.9%) from 1999 to 2003 with correlation coefficient of 0.88.

Discussion

The present study reported a 5.2% of the prevalence of BTB in Nazareth Municipality Abattoir based on post-mortem examination. Similar results were reported in Southern Ethiopia" and in Addis Ababa Abattoir" through similar diagnostic methods. However, the presently recorded prevalence was low as compared to previous reports in the dairy farms in Ethiopia. This is because the prevalence of BTB has been found to be lower in beef cattle than in dairy farms. For example, a mean prevalence of 3.7 to 6.7% was reported in Argentina and a mean prevalence of 2.3% to 4.7% was recorded in Nigeria.

The results of the retrospective data showed that 1.2% of cattle slaughtered in the Nazareth Abattoir had tuberculous lesions, though these data did not include mesenteric and cranial lymph nodes. Further, it was not conducive for the meat inspector to inspect these lymph nodes due to time limitation in addition to the likelihood of missing due to poor necropsy technique during routine meat inspection.

Gross lesions were found most frequently in the lungs and associated lymph nodes. This result is similar to those reported earlier where 60% and 56%, TB lesion was reported respectively, in the lungs and associated lymph nodes. In Ethiopia, similar results were reported earlier. Corner reported that up to 95%
of cattle with visible TB lesions could be identified by examination of the lungs and associated lymph nodes. Such findings suggest that most of the TB infections are acquired by inhalation. Therefore, during slaughter, examination should focus on the lungs and associated lymph nodes.

The detailed necropsy procedure has better sensitivity in detecting lesions compared to the routine necropsy procedure. The difference in the level of sensitivity in detecting lesion between the two procedures was reported earlier\(^\text{21}\). This suggested that changes are required in the procedure of the routine meat inspection so that more lesions can be identified. Monitoring BTB prevalence by bacteriological examination may not be feasible in sub-Saharan Africa as mycobacterial culture is expensive, time consuming and often unsafe in inadequately constructed and equipped laboratories\(^\text{21}\). Therefore, in such countries, postmortem examination in the abattoirs for detection of gross lesions in combination with skin tests remains the mainstay for the diagnosis of BTB.

The sensitivity of CIDT recorded by this study was relatively lower though, it was within the range of value (32-99\%) estimated earlier\(^\text{24}\). Seventeen tuberculin test negative animals had tuberculous lesions during postmortem examination. The cause of lower sensitivity may have been due to immune suppression as result of malnutrition and transportation stress, human error\(^\text{13}\) or an inherent insensitive test. Five non-specific reactors were recorded and the causes of such non-specific reactors could be paratuberculosis, infection with or previous exposure to *Mycobacterium avium* (*M. avium*) and exposure to environmental *Mycobacterium*\(^\text{13,19}\). The specificity of CIDT was found to be high (98.5\%) as reported earlier\(^\text{13}\).

The prevalence of BTB recorded in this study was higher than previous reports from slaughterhouses in Ethiopia. The latter was attributed to the fact that routine meat inspection was found to be less sensitive than the detailed post-mortem examination. In future, it is recommended that thorough post-mortem examination be done to detect more cases of BTB to alleviate increases in human tuberculosis associated with *M. bovis*.

Acknowledgement

The financial support given by the Institute of Pathobiology for this project is highly appreciated. We would like to acknowledge Ato Yadeta Mekessa, meat inspector in the Nazareth Municipality Abattoir, for his assistance in facilitating the activities of this project. Additionally, we would like to appreciate owners of the feedlots and butchers for their willingness in accepting our project.

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ETHNOVETERINARY PRACTICES IN UGANDA: USE OF MEDICINAL PLANTS IN TREATING HELMINTHOSIS AND COCCIDIOsis IN RURAL POULTRY AND GOATS IN UGANDA

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PRATIQUES ETHNOVETERINAIRES EN OUGANDA : UTILISATION DES PLANTES MEDICINALES POUR LE TRAITEMENT DE L'HELMINTHIASE ET DE LA COCCIDIOSE CHEZ LES VOLAILLES ET LES CHEVRES VILLAGEOISES EN OUGANDA

Résumé

De nombreux éleveurs de bétail villageois confirment l'efficacité des plantes médicinales pour le contrôle des maladies chez le bétail. Une enquête transversale a été faite dans trois régions d'élevage de volailles et de chèvres villageoises dans le nord et le centre de l'Ouganda afin de recueillir des informations sur les plantes médicinales utilisées actuellement pour traiter l'helminthiase et la coccidiose. Dans la région centrale (Districts de Mpiji et de Mukono), environ 100 fermes ont fait l'objet d'études. Grâce à une combinaison de questionnaire oral, d'entretiens et d'observations, on a obtenu les informations sur les types de plantes et les parties utilisées, les méthodes d'extraction et les maladies traitées. Dix-sept plantes ont été identifiées du point de vue botanique et on a collecté les informations sur douze remèdes pour traiter l'helminthiase et la coccidiose. L'étude a montré que 42% des éleveurs utilisaient des plantes médicinales. La phytothérapie s'est avérée moins coûteuse (38%), efficace (24%), la seule alternative (23%), toujours disponible et durable (15%). Les extraits de trois des plantes les plus largement utilisées (Cassia hirsuta, Euphorbia heterophylla et Albizia coriaria) ont été évalués pour établir leur efficacité sur les nématodes caprins. Le levamisole était utilisé chez le groupe-témoin. D'après les résultats, l'extrait brut de certaines herbes avait plus de 60% d'efficacité. L'espèce de ver présente chez les chèvres était déterminée par l'identification directe des vers adultes dans le système gastro-interopéal et on a recueilli des larves des cultures fécales. Haemonchus, Trichostrongylus, Bunostomum et Oesophagostomum étaient les genres de nématode prédominants.

Summary

Many rural livestock keepers claim effective use of herbal remedies to control diseases in livestock. A cross-sectional survey was carried out in three important rural poultry and goat areas in north and central Uganda to document the current medicinal plants used to treat helminthiosis and coccidiosis. In the central region (Mpiji and Mukono Districts) about 100 farms were studied. By a combination of orally administered questionnaire, interviews and observations, information on the types of plants and the parts used, methods of extraction and the diseases treated was obtained. The plants were botanically identified and 17 plants, elaborating 12 remedies for helminthiosis and coccidiosis were documented. The studies also showed that 42% of the farmers were using medicinal plants, 38%, thought the phytotherapeutic practices were cheaper, 25%, thought they were effective, 23% thought they were readily available and 15% thought they were sustainable. Extracts of three of the most commonly used plants (Cassia hirsuta, Euphorbia heterophylla and Albizia coriaria) were evaluated for efficacy on goat nematodes. Levamisole was used in the control group. The results showed that the crude extract of some herbs had more than 60% efficacy. The worm species present in the goats were determined by direct identification of adult worms in the gastrointestinal tract and the larvae obtained from faecal cultures. Haemonchus, Trichostrongylus, Bunostomum and Oesophagostomum were the predominant nematode genera.

* Corresponding Author:
Introduction

Over 95% of the poultry stocks in Uganda are local indigenous birds, raised as scavengers in household backyard units, under free-range management systems in most rural areas. The exact number of chickens in Uganda is not known but closest estimate put the figure at 23 million, with each household keeping 2-20 birds. Goats on the other hand total about 5.5 million, kept mostly under free range and tethering systems of production in rural areas. Goats are adapted to a broad range of climatic and geophysical conditions, and are widely distributed in the country. In most rural areas of Uganda, poultry and goats are managed by women and children and do play an intermediate role in raising family capital.

Parasitic diseases, particularly helminths are a major cause of reduced livestock production in the tropics. Coccdiosis and helminthosis are among the major diseases that limit production in rural poultry. Use of modern chemotherapeutic drugs is currently seen as the most effective means of controlling livestock diseases. However, modern drugs are not available in remote rural areas, are expensive and easily lead to drug residues in food animals and their supply is not sustainable. Recently, attention has been drawn to the use of herbs in animal health care practices. Rural livestock keepers claim effective use of herbal remedies to control helminths and coccidia in livestock. The bulk of indigenous knowledge on the use of plants as medicine is disappearing fast due to socio-political changes, deforestation and urbanisation. It is, therefore, very useful to document this knowledge. The study documents some medicinal plants used in rural areas of central and northern Uganda for treating helminthosis and coccdiosis in goats and chickens. It also reports the efficacy of extracts of three of the plants against nematodes in goats.

Materials and methods

The study was carried out in Mpiigi and Mukono districts (Central Uganda) and Lira District (Northern Uganda). These are areas where the farmers keep many indigenous goats and chickens and depend on medicinal plants to control parasitic diseases.

Documentation of medicinal plants was carried out in Gomba county (Mpiigi District) and Kioga county (Lira District). A combination of orally administered questionnaire, interviews and observations on the types of plants and parts of plant used, methods of extraction and the diseases treated was carried out. Farmers were visited in their homes, market places, grazing areas and at social gatherings. A multistage cluster sampling technique was used, and data collected from 125 farmers in 12 parishes randomly selected from each study area. The formula used to derive the sample size (n) was:

\[ n = \frac{4pq}{I^2} \]

Where \( p \) is probable level of phytotherapy practice taken at 50% = 0.5
\( q = 1 - p, \quad 1 - 0.5 = 0.5 \)
\( I = \) the maximum allowable error of 8.94% = 0.0894

Therefore \( n = \frac{4 \times 0.5 \times 0.5}{(0.0894 \times 0.0894)} = 125 \)

The plants were collected and taken to the Department of Botany, Makerere Uni-
Ethnoveterinary practices in Uganda: Use of medicinal plants in treating *helminthosis* and coccidiosis in rural poultry and goats in Uganda

versity for botanical identification. Three of the commonly used plants in treatment of worms in goats (*Cassia hirsuta*, *Euphorbia heterophylla* and *Albizia coriaria*) were used in efficacy trials on goats heavily parasitised with nematodes.

An earlier study in Gomba and Kyadondo counties of Mpiigi District and Baale and Mukono counties of Mukono district involving a cross-sectional analysis of indigenous goats from 100 farms had shown a high level of nematode infection.

Table 1: Plant preparations for coccidiosis and helminthosis from Uganda.

<table>
<thead>
<tr>
<th>Plant</th>
<th>Parts (s) used</th>
<th>Method of Preparation</th>
<th>Indication</th>
<th>Animal</th>
<th>Dose per animal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. <em>Euphorbia heterophylla</em></td>
<td>Whole plant</td>
<td>Boiled in water</td>
<td>Worms</td>
<td>Goat</td>
<td>100 ml</td>
</tr>
<tr>
<td>2. <em>Aloe barbadensis</em></td>
<td>Leaves</td>
<td>Boiled in water</td>
<td>Worms</td>
<td>Chicken</td>
<td>20 ml</td>
</tr>
<tr>
<td>3. <em>Senna didyomobotrya</em></td>
<td>Leaves</td>
<td>Boiled in water</td>
<td>Worms</td>
<td>Chicken/goat</td>
<td>20 ml/100 ml</td>
</tr>
<tr>
<td>4. <em>Momordica foetida</em></td>
<td>Leaves; whole plant</td>
<td>Pounded in water; Juice given orally</td>
<td>Coccidia</td>
<td>Chicken</td>
<td>1 teaspoon thrice daily</td>
</tr>
<tr>
<td>5 <em>Cannabis sativa</em></td>
<td>Leaves</td>
<td>Squeezed in little water; Juice given orally</td>
<td>Coccidia</td>
<td>Chicken</td>
<td>As required</td>
</tr>
<tr>
<td>6. <em>Capsicum frutescens</em></td>
<td>Ripe and unripe fruits</td>
<td>Squeezed in little water; Juice given orally</td>
<td>Coccidia</td>
<td>Chicken</td>
<td>Few drops in mouth</td>
</tr>
<tr>
<td>7. <em>Cyphostema adenoclave</em></td>
<td>Whole plant</td>
<td>Squeezed in little water; Juice given orally</td>
<td>Coccidia</td>
<td>Chicken</td>
<td>1 teaspoon thrice daily</td>
</tr>
<tr>
<td>8. <em>Cassia hirsuta</em></td>
<td>Leaves; roots</td>
<td>Mixed with leaves of <em>Senna didyomobotrya</em></td>
<td>Worms</td>
<td>Goat/chicken</td>
<td>100 ml, 20 ml</td>
</tr>
<tr>
<td>9. <em>Vernonia amygdalina</em></td>
<td>Leaves; roots</td>
<td>Pounded and mixed with water; Juice given orally</td>
<td>Coccidia</td>
<td>Goat/chicken</td>
<td>As required</td>
</tr>
<tr>
<td>10. <em>Carica papaya</em></td>
<td>Roots and flowers of male plant</td>
<td>Pounded and mixed with water; Juice given orally</td>
<td>Worms</td>
<td>Goat/chicken</td>
<td>1 teaspoon thrice daily</td>
</tr>
<tr>
<td>11. <em>Sesame indicum</em></td>
<td>Whole plant</td>
<td>Crushed; sap or juice squeezed</td>
<td>Coccidia</td>
<td>Chicken</td>
<td>10 ml</td>
</tr>
<tr>
<td>12. <em>Sarcocephalus laifolias</em></td>
<td>Roots</td>
<td>Crushed; juice extracted</td>
<td>Coccidia</td>
<td>Chicken</td>
<td>Few drops orally</td>
</tr>
<tr>
<td>13. <em>Tetradenia riparia</em></td>
<td>Leaves</td>
<td>Crush add water and boil; decoction</td>
<td>Worms</td>
<td>Goat</td>
<td>150 ml</td>
</tr>
<tr>
<td>14. <em>Albizia coriaria</em></td>
<td>Bark</td>
<td>Boil in water; decoction</td>
<td>Worms</td>
<td>Goat</td>
<td>100 ml</td>
</tr>
<tr>
<td>15. <em>Carissa edulis</em></td>
<td>Roots</td>
<td>Pounded in water; Juice filtered</td>
<td>Worms</td>
<td>Goat</td>
<td>500 ml</td>
</tr>
<tr>
<td>16. <em>Lannea schweinfurthii</em></td>
<td>Leaves</td>
<td>Pounded in water; filtered juice</td>
<td>Worms</td>
<td>Goat</td>
<td>200 ml</td>
</tr>
<tr>
<td>17. <em>Azadirachta indica</em></td>
<td>Leaves</td>
<td>Crush add water and boil; decoction</td>
<td>Worms</td>
<td>Goat</td>
<td>150 ml</td>
</tr>
</tbody>
</table>
Twenty-five goats showing more than 1000 eggs per gram (epg) of faeces were used in efficacy trials. They were divided into five groups of 5 goats each. Goats were housed indoors for 14 days under conditions designed to preclude further nematode infection to allow most parasites to develop to adults. The animals were assigned treatment or control groups at random: Group 1 was the positive control. The goats were given levamisole hydrochloride (Levafas®, Norbrook laboratories Ltd, Kenya) at a dosage rate of 7.5mg/kg body weight. Groups 2,3, & 4 were given boiled herbal extracts of *Cassia hirsuta*, *Albizia coriaria* and *Euphorbia heterophylla*, respectively. The dosage varied from 200 to 300 ml of extract depending on the weight of the animal. Group 5 was the negative control and remained untreated.

The worm burden was monitored by faecal egg counts on day 1 (just before dosing) and day 7, 14 and 21 (post-dosing). The faecal samples were brought to the laboratory and either examined immediately or stored at 4°C till examined but within 48 hours of being collected. The 2nd and 3rd stage larvae were identified from cultured pooled faecal samples of individual groups. Adult worms isolated from abomasae, small intestines and large intestines of sacrificed animals were identified according to various keys and guidelines.

**Data analysis**

The data collected in the questionnaire were numerically coded and manually added into a database and analyzed using (Epi Info v.6). Only questions that had been answered by more than 80% of the farmers were analyzed. Frequency was calculated considering the total of each answer. The percentage efficacy was calculated from the worm/egg counts from the experiments on evaluation of anthelmintic activity on herbal extracts and levamisole using the following the formula:

$$\% E = \frac{c - t}{c} \times 100$$

Differences between proportions were analyzed statistically using a chi-square test, a value of $p<0.05$ being considered significant. The worm species present in the goats as determined by the direct identification of adults in gastrointestinal tract and larvae obtained from the faecal cultures were *Haemonchus*, *Trichostrongylus*, *Bu nostomum* and *Oesophagostomum*.

**Results**

The study showed that 58% of farmers were abandoning the indigenous methods of treating their animals and adopting modern veterinary practices, while those still using herbal preparations were 42%.

Treatment using herbs was considered to be cheap (38%), effective (24%), the only alternative (23%) and readily available and sustainable (15%). It was revealed that farmers who used anthelmintic in traditional grazing systems (tethering and free range) and of modern grazing systems (paddock and zero-grazing were not significantly different ($p > 0.05$). Farmers who practiced modern grazing systems (zero-grazing and paddock) were found to use anthelmintic more regularly compared to those who practiced traditional grazing systems (tethering and free range).

Seventeen (17) plants elaborating twelve remedies for helminthosis and coccidiosis were botanically identified, (Table 1). Crude extracts from three plants (*Cassia hirsuta*, *Euphorbia heterophylla* and *Albizia coriaria*) had significant anthelmintic activity on nematodes (Table 2). Percentage reductions were calculated and used to assess the efficacy of the extracts and
Ethnoveterinary practices in Uganda: Use of medicinal plants in treating helminthosis and coccidiosis in rural poultry and goats in Uganda

Table 2: Results of efficacy trials of three plants against goat nematodes.

<table>
<thead>
<tr>
<th>Name</th>
<th>Plant/antihelmintic</th>
<th>No. of goats</th>
<th>epg Xo*</th>
<th>epg Xf**</th>
<th>***Percentage reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cassia hirsuta</td>
<td>5</td>
<td>2533 ± 702</td>
<td>880 ± 302</td>
<td>68.50%</td>
<td></td>
</tr>
<tr>
<td>Euphorbia heterophylla</td>
<td>5</td>
<td>2067 ± 306</td>
<td>980 ± 584</td>
<td>64.90%</td>
<td></td>
</tr>
<tr>
<td>Albizia coriaria</td>
<td>5</td>
<td>2900 ± 964</td>
<td>1673 ± 297</td>
<td>40.10%</td>
<td></td>
</tr>
<tr>
<td>Levamisole</td>
<td>5</td>
<td>2100 ± 142</td>
<td>100 ± 200</td>
<td>96.40%</td>
<td></td>
</tr>
<tr>
<td>Untreated</td>
<td>5</td>
<td>2233 ± 550</td>
<td>2793 ± 456</td>
<td>-20%</td>
<td></td>
</tr>
</tbody>
</table>

* Arithmetic mean before treatment
** Arithmetic mean for treated goats at day 14
*** Percent reduction in epg.

the levamisole. Percent reduction of eggs per gram (epg) of faeces recorded for Cassia hirsuta (68.5%), Euphorbia heterophylla (64.9%) had highly significant percentage reductions (p<0.05). Albizia coriaria (40.1%) extract had the lowest efficacy among the herbal extracts. The worm species present in the goats as determined by the direct identification of adults in gastrointestinal tract and larvae obtained from the faecal cultures were Haemonchus, Trichostrongylus, Bunostomum and Oesophagostomum.

Discussion

Phytotherapy was still being practiced in rural areas where modern veterinary services are not well covered. In the rural areas studied, 42% of the farmers still used herbs for treating their animals because they thought they were cheap, effective and readily available. The study showed, however, that the trend was reversing as 58% of farmers no longer used herbs. This may continue for long unless governments commit themselves to encourage veterinary extension staff to use ethnoveterinary skills obtained from indigenous practices. The study revealed that many plants were in use as medicines against helminths and coccidia. Forests and grasslands of Uganda, and indeed other tropical countries are good reservoirs or gene banks of herbal medicines and have to be tapped. There is need for more research especially documentation and evaluation. Much work has been done in Uganda on herbal medicines for human beings but little in the veterinary field. The trial on the three of the plants, C. hirsuta, E. heterophylla and A. coriaria, showed considerable efficacy against the goat nematodes. Already there is growing evidence that herbal medicines are effective. The side effects or toxicity effects need study alongside therapeutic benefits. Equally required is more research in evaluating efficacy of the plants documented in this study. Subsequently, herbal remedies could be prepared and packaged for use not only by the rural farmers, but even veterinary practitioners. Planting and conservation of medicinal plants would then be encouraged both as a source of income.
and also in conservation of plant vegetation cover\textsuperscript{19}. This study is part of an on-going project but serves to show more work is needed to catalogue the potential medicinal resources still hidden in our traditional livestock keepers.

Acknowledgement

The authors wish to acknowledge the cooperation of many farmers and herbalists, the technical staff in the Departments of Botany, and Veterinary Medicine, Makerere University. The Government of Uganda, through Makerere University funded this study.

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2. Opolot J. (1998). BVM Special Project, Makerere University, Kampala

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HAEMOGLOBIN AND TRANSFERRIN (BETA-GLOBULIN) POLYMORPHISM IN INDIGENOUS KENYAN SHEEP BREEDS

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2Iowa State University, Department of Animal Science, 2255 Kildee Hall, Ames, Iowa 50011 USA.

POLYMORPHISME DE L'HEMOGLOBINE ET DE LA TRANSFERRINE (BETA-GLOBULINE) CHEZ LES RACES OVINES LOCALES DU KENYA

Résumé

Les polymorphismes biochimiques ont été décrits chez différentes races ovines, mais il y a très peu d'études réalisées sur le pelage des moutons en Afrique. Cet article décrit la variation électrophorétique de l'hémoglobine et de la transferrine chez les moutons à grosse queue et à grosse croupe au Kenya. Les fréquences de gène Hb étaient comme suit, moutons à grosse queue : HbA = 0.0115 ; HbB = 0.9885 ; moutons à grosse croupe : HbB = 1.00 ; Merino : HbA = 0.325 ; HbB = 0.675. Le locus de la transferrine a fait apparaître 15 différents phénotypes chez le Merino et leur répartition était en concordance avec l'apparition de cinq allèles (Tfa, Tfb, Tfc, Tfd, Tfe). On a trouvé 7 et 8 différents phénotypes Tf chez les moutons à grosse queue et à grosse croupe, ce qui expliquait la présence de quatre allèles (Tfa, Tfb, Tfc, Tfd). Tfd est apparu avec la plus forte fréquence. La transferrine n'était pas en conformité avec l'équilibre Hardy-Weinberg chez les moutons à grosse queue. Il faudrait entreprendre d'autres études pour évaluer les niveaux de polymorphisme dans les autres protéines sanguines et les marqueurs d'ADN et ce, afin de comprendre la diversité du pelage des moutons en Afrique.

Mots-clés : Polymorphisme du sang, hémoglobine, transferrine

Summary

Biochemical polymorphisms have been described in different breeds of sheep, but such studies on hair sheep in Africa are limited. This paper describes the electrophoretic variation of haemoglobin and transferrin in fat-tailed and fat-rumped hair sheep in Kenya. The Hb gene frequencies were: fat-tailed, HbA=0.0115, HbB=0.9885; fat-rumped, HbB=1.00; Merino, HbA=0.325, HbB=0.675. Transferrin locus showed 15 different phenotypes in Merino, their distribution being in agreement with the occurrence of five alleles (Tfa, Tfb, Tfc, Tfd, Tfe). In the fat-tailed and fat-rumped hair sheep, 7 and 8 different Tf phenotypes were found and were explained by four alleles (Tfa, Tfb, Tfc, Tfd). Tfd occurred with the highest frequency. Transferrin did not conform to the Hardy-Weinberg equilibrium in fat-tailed sheep. Further investigations are required to quantify levels of polymorphism in other blood proteins and DNA markers as an aid to understanding the diversity in hair sheep found in Africa.

Key words: Blood polymorphism, haemoglobin, Transferrin.

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Introduction

Several blood proteins have been found to exhibit heterogeneity in different farm animals and are useful in studies of evolution, parentage testing, relationship and structure of breeds. Some of the systems are particularly valuable when no family data are available because the genotypes can be determined directly. Others have been associated with reproductive and productivity traits. Haemoglobin and transferrin are some of the most thoroughly investigated blood proteins in numerous sheep breeds from various region of the world. However, such studies with regard to hair sheep found in Africa are limited. The present study describes the results of Haemoglobin (Hb) and Transferrin (TF) polymorphism in two types of hair sheep found in Kenya but widely distributed in Eastern, Central and Southern Africa.

Materials and methods

Study populations

Fat-tailed hair sheep

This type is distributed widely in Kenya and Eastern, Central and Southern Africa. In contrast to their name, these animals show a marked variation in their tail structure. In some, the tail is comparatively short, fat and carried high up in the hindquarters. In others tail is long and thick nearly touching the ground or convoluted and covered with an abundance of fat that extends almost to the tail, the remainder being comparatively thin. Majority of the fat-tailed sheep are red in colour but black and black and red animals with or without white marking on the body are common. They are kept for meat, fat and social ceremonies.

Fat-rumped hair sheep

This type is confined to the arid North and North Eastern provinces in Kenya, including Somalia. The black colour of the head and neck, and white of the body and limbs distinguish the fat-rumped sheep. They are characterised by a well-developed and folded dewlap, which may extend from the chin to the chest. During seasons of plentiful pastures, fat may be deposited on the dewlap. The tail is usually short and stumpy, taking the form of a knob between the fatty cushions on the rump, but in some animals its thinnest point may hang down on the hocks. Like the fat-tailed, it is mainly kept for meat, fat and social ceremonies.

Merino

This breed is reared under commercial ranching in Laikipia District for wool production.

Blood sampling and electrophoresis

Blood samples were obtained from 165 fat-tailed, 106 fat-rumped and 40 Merino sheep. The latter breed was used as reference population. The fat tailed sheep were sampled from Western (Kakamega District) and South Rift (Kajiado District) Kenya. The fat-rumped sheep were sampled from Northern Kenya (Isiolo District). Merino samples came from Kisima farm in Nanyuki. Fat-rumped and fat-tailed hair sheep were sampled from village flocks. Blood samples were obtained from mature animals of both sexes into vacutainer tubes having EDTA as the anti-coagulant. The plasma and Red Blood Cells were separated by centrifugation at 3000 revolutions per minute within a period of 24 hours after sampling and each pipetted into separate clearly labelled vials that were stored at 20°C until the electrophoretic studies were
carried out. Before analysis, the red cells were washed three times in 0.91% NaCl solution and then lysed with an equal volume of distilled water. Haemoglobin and Transferrin were typed using horizontal starch and discontinuous polyacrylamide gel electrophoresis, respectively. Known standards were included on each gel to ensure consistency of genotype scoring. Gene frequencies were determined from genotype frequencies by direct allele counting. Conformance to Hardy-Weinberg equilibrium was determined using \( \chi^2 \) test.

**Results**

**Haemoglobin**

Distributions of Haemoglobin phenotypes are given in Table 1. Haemoglobin phenotypes HbAA, HbAB and HbBB controlled by two codominant autosomic alleles HbA and HbB were observed. While HbB occurred in all the breeds, HbA was absent in the fat-rumped hair sheep. The frequency of HbB was higher and tending towards fixation.

**Transferrin**

Observed and expected distributions of Transferrin phenotypes in the fat-tailed, fat-rumped and Merino breeds of sheep are given in Table 2. These agree well with the expected Hardy-Weinberg equilibrium distributions for the fat-rumped and Merino sheep but not for the fat-tailed sheep. Five alleles (TfA, TfB, TfC, TfD, TfE) were observed in the Merino and only four (TfA, TfB, TfC, TfD) in the fat-tailed and fat-rumped hair sheep. TfE occurred exclusively in the Merino, while TfD occurred with the highest frequency in all the populations (Table 2). Seven Transferrin phenotypes were observed in the fat-tailed, eight in the fat-rumped and thirteen in the Merino breed of sheep. TFB was not observed in the fat-tailed sheep. In the fat-rumped sheep, all the four transferrin alleles occurred at frequencies above 0.1.

**Discussion**

The present findings with regard to the fat-tailed sheep agree with previous results on the fat-tailed Awassi and Namaqua sheep breeds\(^4,5\). The predominance of HbB over HbA has also been observed in West African and Turkish sheep breeds\(^2,8,7\). Ndamukong\(^7\), however, observed HbC in the West African dwarf sheep associated with haematological stress. The frequencies of HbA and HbB observed in the Merino

<table>
<thead>
<tr>
<th>Table 1. Observed and expected Haemoglobin phenotypes and gene frequencies in fat-tailed, fat-rumped and Merino breeds of sheep.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Haemoglobin Phenotypes</strong></td>
</tr>
<tr>
<td>Total (n)</td>
</tr>
<tr>
<td>Fat-tailed</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Fat-rumped</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Merino</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>
Table 2. Observed and expected Transferrin phenotypes and allele frequencies in fat-tailed, fat-rumpled and Merino sheep breeds.

<table>
<thead>
<tr>
<th>Transferrin Phenotypes</th>
<th>Type of sheep</th>
<th>Fat-tailed (n=165)</th>
<th>Fat-rumped (n=106)</th>
<th>Merino (n=40)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Observed</td>
<td>Expected</td>
<td>Observed</td>
</tr>
<tr>
<td>AA</td>
<td></td>
<td>0</td>
<td>1.446</td>
<td>0</td>
</tr>
<tr>
<td>AB</td>
<td></td>
<td>17</td>
<td>2.267</td>
<td>21</td>
</tr>
<tr>
<td>AC</td>
<td></td>
<td>0</td>
<td>8.344</td>
<td>0</td>
</tr>
<tr>
<td>AD</td>
<td></td>
<td>7</td>
<td>10.499</td>
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<td>-</td>
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<td>-</td>
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<tr>
<td>BB</td>
<td></td>
<td>0</td>
<td>0.842</td>
<td>6</td>
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<tr>
<td>BC</td>
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<td>4</td>
<td>8.117</td>
<td>2</td>
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<tr>
<td>BD</td>
<td></td>
<td>1</td>
<td>9.932</td>
<td>1</td>
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<tr>
<td>CC</td>
<td></td>
<td>16</td>
<td>25.898</td>
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</tr>
<tr>
<td>CD</td>
<td></td>
<td>94</td>
<td>61.743</td>
<td>43</td>
</tr>
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<td></td>
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<td>DD</td>
<td></td>
<td>26</td>
<td>35.913</td>
<td>15</td>
</tr>
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</tr>
<tr>
<td>EE</td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>$\chi^2$ Value</td>
<td></td>
<td>166.9</td>
<td>101.439</td>
<td>1.045</td>
</tr>
</tbody>
</table>

Transferrin (Beta-globulin) Allele frequencies

<table>
<thead>
<tr>
<th></th>
<th>Fat-tailed</th>
<th>Fat-rumped</th>
<th>Merino</th>
</tr>
</thead>
<tbody>
<tr>
<td>TFA</td>
<td>0.0685</td>
<td>0.132</td>
<td>0.162</td>
</tr>
<tr>
<td>TIB</td>
<td>0.0645</td>
<td>0.170</td>
<td>0.162</td>
</tr>
<tr>
<td>TIC</td>
<td>0.398</td>
<td>0.316</td>
<td>0.138</td>
</tr>
<tr>
<td>TID</td>
<td>0.469</td>
<td>0.382</td>
<td>0.475</td>
</tr>
<tr>
<td>TIE</td>
<td>-</td>
<td>-</td>
<td>0.063</td>
</tr>
</tbody>
</table>
agree with those observed by Morera et al.\(^6\) in the Spanish Merino. However, the gene frequencies observed in this study were lower. Agar et al.,\(^5\) found flocks in which HbA predominated that were generally confined to latitudes above 40°, and hypothesized that HbA may be at some disadvantage in warmer areas of the world. HbA has been observed to have a selective advantage in sheep at higher altitudes, while HbB occurs more commonly in lowland breed's\(^9\). These two observations explain the occurrence at high frequencies of HbB in indigenous sheep found in the tropics as compared to the Merino sheep, which originated from the temperate regions. Sheep having HbB have been found to show better reproductive performance, lamb production, average annual reproduction and survive drought better\(^1\).

In a limited number of studies in hair sheep in Africa, the four Tf alleles were commonly observed\(^4\).\(^6\).\(^7\). In these studies TFD also occurred at higher frequencies and could possibly be linked to a gene under selective advantage. Dellal et al.\(^10\) in a survey of indigenous sheep in Turkey found TfE to occur in Anatolian Merino and Akkaraman sheep at frequencies of 0.024 and 0.005, respectively with TfC and TfB predominating over other alleles. Electrophoretic variants occurring at low frequencies may represent, in many cases, relatively recent mutations occurring after the divergence of breeds. TfE may have originated after the divergence of the Merino and the indigenous sheep.

**Conclusion and Recommendations**

In this study, slight differences in occurrence and frequencies of the Tf and Hb genes were observed between the breeds studied. These differences if quantified on a large scale could serve to confirm the classification of the African breeds of sheep as either fat-tailed or fat-rumped. Further investigations are necessary to quantify the levels of polymorphisms inherent in other blood proteins and DNA markers that can be used as an aid to understanding the diversity and structure in hair sheep found in Africa.

**Acknowledgements**

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**References**


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PROTEIN AND LYSINE REQUIREMENTS FOR WEANER PIGS AND THEIR INFLUENCE ON SOME BLOOD PARAMETERS IN TROPICAL ENVIRONMENT

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4Faculty of Agronomy, University of Dschang.

BESOINS EN PROTEINE ET EN LYSINE DES PORCELETS SEVRES ET LEURS EFFETS SUR QUELQUES PARAMETRES SANGUINS DANS LES REGIONS TROPICALES

Résumé

Un essai a été effectué pour déterminer le taux de protéine brute et de lysine nécessaire à la croissance optimum des porcelets sevrés dans les régions tropicales et leurs effets sur quelques paramètres sanguins, selon le concept de "taux de protéine idéal", décrit par "Agricultural Research Council". Cet essai a été entrepris entre avril et juillet 2001. Quatre rations contenant 14 ; 16 ; 18 et 20% de protéine brute et respectivement 0,98 ; 1,12 ; 1,26 ; et 1,40% de lysine étaient servies aux porcelets sevrés avec un poids moyen initial de 6,22 kg pendant une période expérimentale de 56 jours. Les résultats obtenus n'ont montré aucune différence significative (P > 0,05) entre les rations contenant 18 et 20% de protéine brute et 1,26 et 1,40% de lysine lorsque la consommation alimentaire moyenne quotidienne, le gain de poids moyen quotidien, l'efficacité alimentaire, le poids moyen final et le coût des aliments/kg de gain pondéral étaient mesurés. Cependant, on a noté une nette différence (P < 0,05) lorsque l'on a comparé ces deux rations alimentaires à celles contenant 14% de protéine brute et 0,98% de lysine. En général, les paramètres sanguins n'étaient pas affectés par l'augmentation des taux de protéine et de lysine.

Summary

An experiment was conducted to determine the level of crude protein and lysine necessary for optimum growth of weaner pigs in the tropical environment and their influence on some blood parameters, using the "Ideal Protein" concept, described by the Agricultural Research Council (ARC). This was carried out between April and July 2001. Four diets containing 14, 16, 18 and 20% crude protein and corresponding to 0.98, 1.12, 1.26 and 1.40% lysine content were fed to weaner pigs with average initial body weight of 6.22 kg during 56 days experimental period. The results showed no significant difference (p>0.05) between diets containing 18 and 20% crude protein corresponding to 1.26 and 1.40% lysine, when average daily feed intake, weight gain, feed conversion ratio, final body weight and feed cost/kg weight gain were measured, but, both diets were significantly different (p<0.05) from diet containing 14% crude protein with 0.98% lysine. Blood parameters were not generally affected by increased protein and lysine levels.

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Introduction

The growth of piglets after weaning is a function of a variety of environmental factors, but primarily of dietary protein and energy\(^1\). On the other hand, lysine has also been demonstrated as essential in pig nutrition\(^2\) and appears to be the first limiting amino acid in conventional cereal-based diets\(^3,4\).

In the temperate regions, considerable research has been conducted to determine the minimum nutrient requirements of various classes of pigs\(^5,6,7\). In the humid lowlands of the tropics, the lack of information in this domain is the main constraint encountered in formulating pig diets. Despite the crude protein and lysine levels of 20% and 1.10% for 8 to 34 kg pigs and 20% and 1.02% for 8 to 23 kg pigs reported earlier\(^8,9\) as optimum protein and lysine requirement for weaner pigs, no standard recommendations for minimum nutrient requirements for different classes of pigs has yet been made in the humid lowland tropics. The formulation of pig diets is still based on recommendations made in the temperate regions where the climatic and management conditions are not the same.

The study was carried out to determine the optimum levels of crude protein and lysine in the diet of weaner pigs and their possible influence on some blood parameters, taking into consideration the concept of “ideal protein” defined by Agricultural Research Council\(^9\) as a combination of essential amino acids in which lysine is expressed as percentage of total protein. The “ideal protein” is an approximation of the ratio of amino acids in the animal body.

Materials and methods

Data presented in the present study were collected at the Institute of Agricultural Research Centre at Nkolbisson in Yaoundé. This forest region of South Cameroon is characterised by average annual temperature and precipitation of 25°C (range: 22°C to 38°C) and 1750 mm (range: 1600 to 1800 mm) respectively, with the relative humidity varying between 50 and 80% in the dry season and 70 to 90% in the rainy season.

Sixteen (16) Large white x Landrace x Duroc x Berkshire weaner pigs (8 males and 8 females), averaging 6.22 kg initial body weight (range: 4.8 to 7.5 kg) were randomly distributed into four groups of animals (2 males and 2 females / group as replicate) corresponding to four dietary crude protein and lysine concentrations. They were individually housed in a 2m x 2m concrete floor pen, equipped with feeder and drinker, and fed to appetite with water given ad libitum for 56 days experimental period in complete randomised design.

Four diets were formulated with locally available ingredients comprising of corn, cotton seed cake, fish meal, palm kernel cake, wheat middlings, etc. to provide crude protein and lysine content ranging from 14 to 20% and 0.98% to 1.40% respectively (Table 1). Dietary constituents were calculated according to INRA laboratory feed ingredients analysis\(^7\). The level of lysine in the diets was adjusted to 7% of the total crude protein as defined by the “ideal protein” concept (Table 2). Deficiencies in lysine concentration were overcome by the use of synthetic lysine (as monochlorohydride, 78.5% pure lysine). The concentration of the other essential amino
Table 1: Experimental Diets for the study on protein and lysine requirements for weaner pigs and their influence on some blood parameters in tropical environment.

<table>
<thead>
<tr>
<th>Ingredients/Protein Level (%)</th>
<th>14</th>
<th>16</th>
<th>18</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cotton Seed Cake</td>
<td>2.00</td>
<td>6.00</td>
<td>9.50</td>
<td>11.00</td>
</tr>
<tr>
<td>Fish Meal</td>
<td>4.00</td>
<td>6.00</td>
<td>8.00</td>
<td>10.00</td>
</tr>
<tr>
<td>Palm Kernel Cake</td>
<td>10.00</td>
<td>9.00</td>
<td>9.00</td>
<td>9.00</td>
</tr>
<tr>
<td>Wheat Middlings</td>
<td>27.00</td>
<td>22.00</td>
<td>18.00</td>
<td>15.50</td>
</tr>
<tr>
<td>Palm Oil</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Bone Meal</td>
<td>3.43</td>
<td>3.41</td>
<td>3.40</td>
<td>3.39</td>
</tr>
<tr>
<td>L. Lysine</td>
<td>0.47</td>
<td>0.49</td>
<td>0.5</td>
<td>0.51</td>
</tr>
<tr>
<td>Salt</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>Premix*</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Calculated nutrients composition (as fed)

<table>
<thead>
<tr>
<th></th>
<th>3028.85</th>
<th>3049.85</th>
<th>3031.60</th>
<th>3045.34</th>
</tr>
</thead>
<tbody>
<tr>
<td>Digestible energy (kcal)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude protein (%)</td>
<td>14.08</td>
<td>16.00</td>
<td>18.03</td>
<td>20.01</td>
</tr>
<tr>
<td>Crude fibre (%)</td>
<td>5.59</td>
<td>5.46</td>
<td>5.48</td>
<td>5.47</td>
</tr>
<tr>
<td>Calcium (%)</td>
<td>1.35</td>
<td>1.50</td>
<td>1.62</td>
<td>1.75</td>
</tr>
<tr>
<td>Phosphorus (%)</td>
<td>0.76</td>
<td>0.81</td>
<td>0.87</td>
<td>0.93</td>
</tr>
<tr>
<td>Calcium / phosphorus ratio</td>
<td>1.77</td>
<td>1.85</td>
<td>1.87</td>
<td>1.88</td>
</tr>
</tbody>
</table>

**Essential Amino Acids**

<table>
<thead>
<tr>
<th></th>
<th>%</th>
<th>P**</th>
<th>%</th>
<th>P**</th>
<th>%</th>
<th>P**</th>
<th>%</th>
<th>P**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysine</td>
<td>0.98</td>
<td>100</td>
<td>1.12</td>
<td>100</td>
<td>1.26</td>
<td>100</td>
<td>1.40</td>
<td>100</td>
</tr>
<tr>
<td>Methionine + Cystine</td>
<td>0.55</td>
<td>56</td>
<td>0.62</td>
<td>55</td>
<td>0.68</td>
<td>53</td>
<td>0.74</td>
<td>53</td>
</tr>
<tr>
<td>Threonine</td>
<td>0.51</td>
<td>52</td>
<td>0.58</td>
<td>52</td>
<td>0.66</td>
<td>52</td>
<td>0.74</td>
<td>52</td>
</tr>
<tr>
<td>Tryptophane</td>
<td>0.14</td>
<td>14</td>
<td>0.16</td>
<td>14</td>
<td>0.18</td>
<td>14</td>
<td>0.20</td>
<td>14</td>
</tr>
<tr>
<td>Histidine</td>
<td>0.37</td>
<td>38</td>
<td>0.42</td>
<td>38</td>
<td>0.47</td>
<td>37</td>
<td>0.52</td>
<td>37</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>0.54</td>
<td>55</td>
<td>0.62</td>
<td>55</td>
<td>0.71</td>
<td>56</td>
<td>0.79</td>
<td>56</td>
</tr>
<tr>
<td>Leucine</td>
<td>1.20</td>
<td>122</td>
<td>1.33</td>
<td>119</td>
<td>1.46</td>
<td>116</td>
<td>1.59</td>
<td>114</td>
</tr>
<tr>
<td>Phenylalamine + Tyrosine</td>
<td>1.10</td>
<td>112</td>
<td>1.27</td>
<td>113</td>
<td>1.42</td>
<td>113</td>
<td>1.58</td>
<td>113</td>
</tr>
<tr>
<td>Valine</td>
<td>0.62</td>
<td>63</td>
<td>0.82</td>
<td>73</td>
<td>0.92</td>
<td>73</td>
<td>1.02</td>
<td>73</td>
</tr>
</tbody>
</table>

**Cost/Kg (Fcfa)**

<table>
<thead>
<tr>
<th></th>
<th>161.06</th>
<th>173.64</th>
<th>184.53</th>
<th>194.86</th>
</tr>
</thead>
</table>

*Premix: composition/kg: vit a 10x 10^6 ui; vit d_3 2x 10^6 ui; e 10g; vit b_1 1g; vit b_2 0.5g; vit b_6 20mg; niacin 30g; Menadione (vit k3) 2g; acid aspartique 10g; cu 10kg; mn 80g; zn 60g; fe 50g; i 1g; co 0.15g; Sf 0.32g; antioxidant 45g; P** proportion of other essential amino acids relative to lysine; * commercial premix, vial sarl 122, rue d'aguisseau, boulogne billancourt.
acids in the experimental diets were also calculated in order to evaluate the magnitude of variation from the ideal protein.

Blood samples were obtained from the anterior vena cava of each pig at the end of the experiment and were analysed for hematocrit, white and red blood cell counts, hemoglobin, thrombocytes and blood formulae (% lymphocytes, % monocytes, % granulocytes). For simple hematocrit determination, two microhematocrit capillary tubes were immediately filled with blood collected in 5 ml medical commercial tube containing EDTA (Sodium Ethylene diamine tetra-acetate). These microhematocrit tubes were then centrifuged in Hawksley microhematocrit centrifuge at 3,000 rpm for 15 min and packed cell volume read from Hawksley microhematocrit reader (England).

For electronic apparatus determination, hematocrit, white and red blood cells, hemoglobin, thrombocytes, and blood formulae were determined by Coulter Counter apparatus (ABX micros) after 3 days storage of blood samples at 4°C in the refrigerator.

The animal performance parameters (daily feed intake, daily weight gain, and feed conversion ratio), blood parameters (hematocrit, white and red blood cell counts, hemoglobin, thrombocytes, blood formulae) and feed cost/kg weight gain were measured and recorded.

Data on animal performance and feed cost/kg weight gain, were subjected to analysis of variance for significance of difference between treatments and the Newman-Keuls test used to separate difference between means. Simple linear regression and correlation analysis were

<table>
<thead>
<tr>
<th>Essential amino acids composition</th>
<th>Percentage (%) of total protein</th>
<th>Proportion relative to lysine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysine</td>
<td>7.0</td>
<td>100</td>
</tr>
<tr>
<td>Methionine + Cystine</td>
<td>3.5</td>
<td>50</td>
</tr>
<tr>
<td>Threonine</td>
<td>4.2</td>
<td>60</td>
</tr>
<tr>
<td>Tryptophane</td>
<td>1.0</td>
<td>15</td>
</tr>
<tr>
<td>Histidine</td>
<td>2.3</td>
<td>33</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>3.8</td>
<td>55</td>
</tr>
<tr>
<td>Leucine</td>
<td>7.0</td>
<td>100</td>
</tr>
<tr>
<td>Phenylalalmine + Tyrosine</td>
<td>6.7</td>
<td>96</td>
</tr>
<tr>
<td>Valine</td>
<td>4.9</td>
<td>70</td>
</tr>
<tr>
<td>Arginine</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 3: Effects of protein and lysine levels on the performance of weaner pigs in the tropical environment.

<table>
<thead>
<tr>
<th>Treatments/Protein Levels (%)</th>
<th>14</th>
<th>16</th>
<th>18</th>
<th>20</th>
<th>±SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysine Levels (%)</td>
<td>0.98</td>
<td>1.12</td>
<td>1.26</td>
<td>1.40</td>
<td></td>
</tr>
</tbody>
</table>

Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value 1</th>
<th>Value 2</th>
<th>Value 3</th>
<th>Value 4</th>
<th>±SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of animals</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Initial body weight (kg)</td>
<td>6.27a</td>
<td>6.27a</td>
<td>6.15a</td>
<td>6.20a</td>
<td>0.05Ns</td>
</tr>
<tr>
<td>Feed intake (g/day)</td>
<td>577.44a</td>
<td>701.36a</td>
<td>894.23ab</td>
<td>1007.18b</td>
<td>192.32*</td>
</tr>
<tr>
<td>Weight gain (g/day)</td>
<td>174.12a</td>
<td>246.00a</td>
<td>354.60b</td>
<td>424.55b</td>
<td>111.43***</td>
</tr>
<tr>
<td>Feed conversion ratio</td>
<td>3.30a</td>
<td>2.81b</td>
<td>2.50bc</td>
<td>2.36c</td>
<td>0.41*</td>
</tr>
<tr>
<td>Final body weight (kg)</td>
<td>16.03a</td>
<td>19.95a</td>
<td>25.95b</td>
<td>30.00b</td>
<td>6.20**</td>
</tr>
<tr>
<td>Feed cost/kg weight gain</td>
<td>532.29a</td>
<td>489.22a</td>
<td>462.70a</td>
<td>461.81a</td>
<td>33.01 Ns</td>
</tr>
</tbody>
</table>

a,b,c: mean treatment in the same row with different superscripts differ from one another
Ns : non significant
*  : p<0.05
** : p<0.01
***: p<0.001

carried out between treatments and parameters measured\textsuperscript{10,11}. Blood parameters were compared with normal pig blood characteristics\textsuperscript{12}.

Results

Mean performance measurements are presented in Table 3. The average daily feed intake and weight gain were obtained at the highest level of crude protein (20%) and lysine (1.40%). Feed conversion ratio and feed cost/kg weight gain decreased with increased level of crude protein giving minimum feed conversion ratio and cost/kg weight gain at the highest level of crude protein and lysine. Diet containing 18 and 20% crude protein with 1.26 and 1.40% lysine concentration respectively were not significantly different (p>0.05) for feed intake, weight gain, feed conversion ratio and final bodyweight but both diets showed a significant difference (p<0.05) when compared to diet containing 14% crude protein with 0.98% lysine. There was no significant difference (p>0.05) between 16 and 18% diet for feed intake, feed conversion ratio and feed cost/kg weight gain.

Simple linear regression and correlation showed positive correlation between crude protein level (CP) and feed intake (FI) and between crude protein level and daily weight gain (DWG). Negative correlation was also
found between crude protein level and feed conversion ratio (FCR) and between crude protein and feed cost per unit weight gain (FC) according to the following computed linear equations and correlation coefficients (df = 2).

\[
FI = 74.10 \text{ CP} + 474.65 \quad r = 0.994 \quad P<0.01
\]

\[
DWG = 42.99 \text{ CP} + 431.02 \quad r = 0.996 \quad P<0.01
\]

\[
FCR = - 0.16 \text{ CP} + 5.39 \quad r = - 0.971 \quad P<0.05
\]

\[
FC = - 11.89 \text{ CP} + 888.63 \quad r = - 0.928 \quad P>0.05 \quad \text{NS}
\]

**Discussion**

The highly significant correlation coefficients suggested that a close linear relationship existed between feed intake and crude protein level. For example, the value \( r = 0.996 \) indicated that 100 \((0.966)^2\) = 99% of the variation in daily weight gain is explained by the linear function of the crude protein level, within the range of 16 to 20% crude protein.

**Table 4:** Effects of protein and lysine levels on some blood parameters of weaner pigs in the tropical environment.

<table>
<thead>
<tr>
<th>Treatments/Protein Levels (%)</th>
<th>14</th>
<th>16</th>
<th>18</th>
<th>20</th>
<th>Pig blood characteristics from Karl Otto</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysine Levels (%)</td>
<td>0.98</td>
<td>1.12</td>
<td>1.26</td>
<td>1.40</td>
<td>(12)</td>
</tr>
</tbody>
</table>

**Parameters measured**

- Number of animals: 4
- White blood cell counts X 103/mm³: 17.8±11.0, 19.6±4.8, 16.6±9.50, 20.3±2.4, 10-23
- Red blood cell counts X 106/mm³: 4.7±3.1, 6.6±6.5, 4.9±2.1, 6.7±1.1, 5-8
- Haemoglobin g/dl: 12.4±6.1, 12.5±1.1, 11.4±5.6, 13.0±2.0, 10-16
- Hematocrit %: 38.1±22.5, 43.4±4.2, 45.6±9.2, 48.5±11.8, 32-50
- Thrombocytes X103/mm³: 171.8±11.4, 192.5±114.5, 158±10.2, 225.8±92.2, 320-710

**Blood formulae**

- Lymphocytes %: 96.3±7.9, 72.4±15.4, 69.7±9.64, 67.4±2.8, 30%
- Monocytes %: 14.0±2.8, 11.9±3.7, 13.0±2.8, 12.8±3.8, 8%
- Granulocytes %: 16.7±7.5, 17.7±13.6, 17.3±17.0, 19.8±4.6, 62%

\( X±sd: \text{ mean ± standard deviation.} \)
The best performance obtained with the diet containing 20% crude protein and 1.40% lysine in this study was lower than those obtained by other workers\textsuperscript{9,13} who reported that dietary protein and lysine concentration to support maximum growth performance, for pigs weighing 8 to 20 kg live weight were 20% and 18.7% protein, respectively and 1.12% and 1.31% lysine respectively with digestible energy of 15.2 MJ/kg and 15.9 MJ/kg of diet respectively (approximately 3623 and 3800 kcal/kg).

The low performance obtained in this experiment could be explained by the low level of digestible energy used (about 3045 kcal/kg of feed) compared to the values of 3623 and 3800 kcal digestible energy reported earlier\textsuperscript{9,13} or to recommended value of 3300 kcal/kg of feed\textsuperscript{7}. The lysine energy ratios in the experimental diets, ranging from 3.23 g/Mcal to 4.59 g/Mcal were higher than the value of 3g/Mcal for 10 to 25 kg pigs recommended by INRA\textsuperscript{7} and indicating that the low performance is partially due to the low level of energy during this physiological status of the animals and suggests that the energy should not be a constraint in the diet of weaner pigs in the tropical environment.

On the other hand, the poor performance observed could also be due to the high level of crude fibre of about 5.5% in the experimental diets, compared to the value of 3 to 4.5% recommended as the maximum level in the diet for weaner pigs\textsuperscript{7}. It is well known that high concentration of crude fibre results in low utilisation of protein and low availability of amino acids in the diet\textsuperscript{14}.

This low performance could again be explained by the high concentration of some essential amino acids such as leucine and phenylalanine + tyrosine with about 22% and 16% variation respectively relative to the level of those amino acids required by the "ideal protein" and could lead to the extra heat increment originating from the catabolism of those excess amino acids as observed by other workers\textsuperscript{4,15} who reported low performance of pigs fed excess protein and excess arginine respectively.

Visual assessment of pig performance on diets with 18 and 20% protein showed well-developed ham, better conformation, sparse body hair and healthier appearance than their counterparts on diets with 14 and 16% crude protein.

Blood parameters of weaner pigs fed different levels of protein and lysine are presented in Table 4. Results showed high variation in blood parameters even in the same treatment as indicated by the standard deviation and were not affected by treatment except a slight increase of hematocrit with increased level of lysine. This observation is in agreement with a past worker\textsuperscript{16} who did not find any influence of lysine level on blood parameters but is in contrast with others workers\textsuperscript{17,18} who reported increased hematocrit and red cells counts in pigs fed supplemental lysine in corn peanut diets. It was observed that less lysine than the levels in the present experiment is required for maximum hematocrit and red cell counts\textsuperscript{17}.

The high concentration of white blood cells, high percentage of lymphocytes and monocytes found in the present study, compared to the normal pig blood parameters could suggest the high capability of the animals to withstand critical environment conditions by producing antibodies and phagocytic micro-organisms.
Conclusion

The results of this study showed that the maximum concentration of 20% crude protein and 1.40% lysine with corresponding level of 3045 kcal digestible energy/kg of feed was not high enough to support maximum growth performance (daily feed intake, daily weight gain, feed conversion ratio). This suggests further research to investigate dietary needs in order to optimise growth performance of weaner pigs in the tropical environment.

Acknowledgement

The authors gratefully acknowledge the technical assistance of Drs. F. Ekue and T.J. Shefe for collection and analysis of pig blood samples.

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PRELIMINARY LABORATORY AND FIELD TRIALS ON THE EFFECTS OF ENDOD (PHYTOLACCA DODECANDRA) ON EPIZOOTIC LYMPHANGITIS

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ESSAIS PRELIMINAIRES EN LABORATOIRE ET SUR LE TERRAIN SUR LES EFFETS DE LA PHYTOLACQUE (PHYTOLACCA DODECANDRA) SUR LA LYMPHANGITE EPIZOOTIQUE

Résumé

Une étude a été conduite pour déterminer l'effet de la phytolaque (Phytolacca dodecandra) sur la croissance de Histoplasma capsulatum var. farciminosum (HCF) dans le milieu de culture de Sabouraud et évaluer son action thérapeutique sur 52 cas de lymphangite épidémique. L'étude a été menée dans trois villes du centre de l'Ethiopie entre décembre 2001 et juin 2002. L'examen clinique, les dénombrements différentiels des globules blancs et la bactériologie étaient utilisés pour évaluer la réaction au traitement. La phytolaque stérilisée a ralenti la croissance de HCF à 1%. Une énorme différence ($X^2 = 19.05 ; P < 0.0001$) quant à la réaction au traitement a été observée entre les cas précoce et les cas avancés. Les dénombrements différentiels des globules blancs ont révélé une augmentation progressive du nombre de leucocytes neutrophiles dans les cas avancés, tandis que l'on a observé une augmentation constante des lymphocytes dans les cas précoce ayant réagi au traitement. Les résultats de la présente étude ont montré que la phytolaque ralentit la croissance de HCF dans le milieu de culture de Sabouraud.

Mots-clés : Phytolaque, lymphangite épidémique, ralentissement de la croissance, Histoplasma capsulatum var. farciminosum, action thérapeutique.

Summary

A study was conducted to determine the effect of Endod (Phytolacca dodecandra) on the growth of Histoplasma capsulatum var. farciminosum (HCF) on Sabouraud's dextrose agar and evaluate its therapeutic value on 52 cases of epizootic lymphangitis (EL). The study was carried out in three towns of central Ethiopia between December 2001 and June 2002. Clinical examination, differential white blood cell counts and bacteriology were used for the evaluation of response to treatment. Sterilized Endod inhibited the growth of HCF at 1% (W/V). A highly significant ($X^2 = 19.05 ; P<0.0001$) difference to treatment was recorded between early and advanced cases. The differential white blood cell counts showed a progressive increase in the number of neutrophils in advanced cases while lymphocytes were observed to steadily increase in the early cases that responded to treatment. The results of the present study demonstrated that Endod inhibits growth of HCF on Sabouraud's dextrose agar.

Key words: Endod, epizootic lymphangitis, growth inhibition, Histoplasma capsulatum var. farciminosum, therapeutic value

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Introduction

Ethiopia has an estimated 2.75 million horses, 5.02 million donkeys and 0.63 million mules. Because of the lack of infrastructure in the rural parts the country, most of the transportation activities are performed by the use of equines. Furthermore, as the topography of the country is not convenient for the modern transportation technology, the major means of transportation of both goods and man are equines. In certain parts of the country equines are used for ploughing. Despite all these uses, they are suffering from several diseases such as Epizootic Lymphangitis (EL), African Horse Sickness, dourine, ulcerative lymphangitis, horse mange, anthrax, rabies and babesiosis. EL is a chronic disease of equine caused by HCF and is characterized by lymphangitis and lymphadenitis. Lymphangitis as a syndrome in equine was reported in different parts of Ethiopia. The retrospective data analysis of 11 years (1984-1995) made by Bojia Endebu indicated that almost all areas visited by the final year students of the Faculty of Veterinary Medicine have problem of EL. From the questionnaire survey made among carthorse owners at Akaki and Debre Zeit towns, central Ethiopia, it was recognized that EL locally called Bitche or Nidif is the most important problem of their horses. Fungal spores are carried from infected animals by direct contact or inanimate objects such as grooming, bedding, saddlery, etc., and they gain entry through cutaneous abrasions. The organism has also been isolated from the alimentary tract of biting flies, suggesting their role in the transmission of the disease. Infection is restricted to horses, mules and rarely donkeys. The disease does not have effective treatment so far and the final fate of sick horses is to be left outdoors for scavengers.

The anti-microbial effects of Endod has been investigated by different workers, with varied results. The anti-fungal property of Endod has been studied both in vivo and in vitro. The results of this study indicated that the growth of fungi was retarded or completely inhibited by high concentration of Endod. These workers have also reported that Candida albicans, C. tropicalis and Malassezia furfur are more susceptible to Endod. Regarding HCF, the causative agent of EL in equines, no study has been done so far. Therefore, the present study was undertaken to evaluate the effect of Endod on HCF as well as to determine its therapeutic value on the cases of EL.

Materials and methods

Description of the study area

The study was conducted at three towns, Debre Zeit, Mojo and Naserath where carthorses are used for public transport and goods. These towns are located at 44 km, 70 km and 92 km southeast of Addis Ababa, with altitudes of Debre Zeit, Mojo and Naserath being 1900, 1870 and 1622 meters above sea level, respectively. The weather of Debre Zeit is hot and moist because of the presence of several lakes around it. Mojo is characterized by hot and humid weather condition while Naserath has hot and dry weather condition. The laboratory work was carried out at the Institute of Pathobiology, a biomedical research Institute of the Addis Ababa University.

Study animals and sampling

Purposive sampling was used to identify 52 (48 treatment animals and 4 control
animals) cases of EL. Of the total 52 cases, 8 were purchased from the three study towns and used. This group consisted of 4 early and 4 advanced cases, and was kept on-station under controlled management. Immediately after being recruited to the experimental site, all horses in this group were de-wormed by equine paste and vaccinated against African Horse Sickness. Land was rented at Mojo and two rooms (one for early cases and the other for the advanced cases) were built from wood and plastic sheet to protect horses from rain, sun and wind. Feeding utensils were prepared from wood and sack to avoid spill and linkage of feed materials. Crop residue locally termed as *frushka* and roughage that were bought from flour processing factories and nearby farmers constituted the feed for the experimental horses. The two feed types were fed to these horses. The other group consisted of 44 (22 early, 22 advanced) cases that were under owners' management. This group, though managed by their owners themselves had their feed types and feeding regimen similar to those under experiment.

**In vitro test**

Crude dried grounded berries of Endod (L. Herit) type 44 were used for this experiment. Different concentrations of sterilized and un-sterilized Endod were prepared in universal bottles. Sterilization of Endod was made by exposing the powder to ultraviolet (UV) light for one hour. Similar concentrations of sterilized and un-sterilized Endod were prepared and added to Sabouraud’s dextrose agar (SDA). The agar was poured into universal bottles. The concentrations of Endod used were 0.5%, 1%, 1.5%, 2%, 2.5%, 5%, 7.5% and 10% (W/V). The control constituted of media prepared without Endod. The mycelial form of HCF was inoculated into both Endod containing and control media and incubated at 26°C for two months. Any difference in growth between the treatment and control groups was observed and recorded.

**In vivo test**

**On-station experiment**

Four early and 4 advanced cases of EL were used for this experiment. Two percent concentration of Endod was topically applied to this group using sprayer for 4-8 weeks under controlled experiment.

**On-farm experiment**

Endod was applied topically to 40 cases (Debre Zeit=12, Mojo=8, Nazerath=20) owned privately by owners themselves with close supervision. Four cases, obtained from Mojo, under similar conditions served as controls. The lesions of the control group were regularly washed with water until the end of the trial.

**Application**

The preparation of crude grounded berries of Endod was made as described earlier. Briefly, one sachet (20gm) of the powder was suspended in a litre of clean tap water and was left to stay for 14 hrs. The area of the lesion was properly washed with water to remove dead tissue and cell debries from the open lesion. Then the lesion was washed/sprayed with 2% suspension of Endod. The procedure was repeated for 8 weeks.

**Methods of evaluation**

**In vitro**

Follow-up was made for the growth of HCF on SDA on weekly basis for 8 weeks. Comparison for the growth of HCF on Endod inoculated and Endod free media
were made.

In vivo

Clinical examination
As the disease causes clinically apparent lesions along the lymphatic vessels of either the forelimbs, sternum, hind limbs, neck, face, lateral sides of the body, nose, eyes or in any combination of them, clinical examination can, definitely, give satisfactory judgment as to the effect of the treatment. Hence, the effect of treatment with Endod was assessed by carefully observing and recording the disappearance of the main clinical signs especially nodules and ulcers from lymphatic system and skin of the extremities.

Differential White Blood Cell (WBC) counts
Approximately 5 ml blood sample was collected into heparinized vials from the jugular veins of the eight infected experimental horses on weekly basis. Fresh blood smears were made and stained with Giemsa stain. Differential WBC counts were made on weekly basis for 12 weeks to determine the pattern of WBC during the treatment regimen.

Microscopic examination
Samples were collected from lesions, stained with Gram’s stain and examined for microorganisms. Furthermore, wound material was streaked with loop and inoculated into nutrient broth. After 24 hrs of incubation, sub-culturing was made on nutrient agar so as to isolate any other microorganism that might have been involved in the pathology.

Statistical Analysis
Variation in the degree of response, which was measured through the observation of the healing of the lesions, to treatment between early and advanced stages of the disease was analyzed by the chi-square (χ²) test using Statistical Analysis Software (SAS)¹. Odds ratio (OR) and confidence interval (CI) were computed at 95% confidence level using the Cochran-Mantel-Haenzel (CMH) statistics in SAS¹ to estimate the effect and degree of response to treatment. A line graph was used to show the patterns of white blood cells during the treatment.

Results

Growth inhibition
UV sterilized Endod was found to completely inhibit the growth of HCF at concentrations of 1% or greater. Growth of other fungal contaminants was observed on SDA containing non-sterilized Endod.

Clinical evaluation of response to treatment
The results of the treatment trial on experimental cases showed that 4 (50%) (ID No. 002, 003, 005, 007) were completely recovered of which, 75% were at early stage of the disease when the treatment was started. Similarly, 57.5% (23/40) recovered from cases treated under owners’ management of which, 78.3% (18/23) were at early stage of the disease. On the other hand, no response was observed in 75% (15/20) of the advanced cases and in the control group. A highly significant (χ² = 19.05; P<0.0001) difference in the degree of response to treatment was recorded between early and advanced cases. The response was significantly higher [OR=21.00, CI=(4.58-96.23), P=0.0001] in early cases than in advanced ones (Table 1).

Differential WBC counts
Analysis of the results of differential white blood cell counts showed a progressive increase in the number of neutrophils (Fig.1) in advanced cases that didn’t show
any response to treatment while lymphocytes were observed to increase steadily in the early cases that were progressively recovering from the disease (Fig. 1).

**Bacterial isolation**

The bacteriological examination resulted in the isolation of *Staphylococcus, Streptococcus, Corynebacterium* and *Pseudomonas* from the lesion of the advanced cases. Table 2 shows the morphology, Gram’s stain and biochemical reactions of bacteria isolated from the lesions of cases of EL.

**Discussion**

In the present study, it was observed that Endod inhibited the growth of HCF at concentration of 1% (W/V) or above on SDA. Similarly, Endod was found to have effective therapeutic value against early cases of EL. The results of both on-station and on-farm treatment trials showed that early cases responded to Endod treatment. On the other hand, advanced cases did not respond to Endod treatment. As the case in other diseases of domestic animals, it is very difficult to cure a disease once it has attained its advanced stage. Furthermore, in this case, the advanced cases were concurrently infected with bacteria. Several bacteria such as *Staphylococcus, Streptococcus, Corynebacterium* and *Pseudomonas* were isolated from the advanced cases. Hence, failure to treatment could be due to secondary bacterial complications that may have different spectrum susceptibility to Endod. Although, these bacteria are generally considered opportunistic, their contribution to the pathology of EL needs

**Table 1:** Association between severity of the EL and the degree of response to treatment with Endod.

<table>
<thead>
<tr>
<th>Stages of disease</th>
<th>State of response</th>
<th>Total</th>
<th>X²</th>
<th>OR</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early</td>
<td>21</td>
<td>3</td>
<td>24</td>
<td>19.05</td>
<td>21.00 (4.58, 96.23)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Severe</td>
<td>6</td>
<td>18</td>
<td>24</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>27</td>
<td>21</td>
<td>48</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 2:** Genera of bacteria isolated from the lesions of severe cases of EL.

<table>
<thead>
<tr>
<th>ID. No.</th>
<th>Shape</th>
<th>Coagulase</th>
<th>Catalase</th>
<th>Gram stain</th>
<th>Oxidase</th>
<th>OF</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1</td>
<td>Cocci (Chain forming)</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>F</td>
<td><em>Streptococcus</em></td>
</tr>
<tr>
<td>4.2</td>
<td>Rod (medium)</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>F</td>
<td><em>Pseudomonas</em></td>
</tr>
<tr>
<td>8.1</td>
<td>Cocci in bunches of grapes</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>F</td>
<td>Non-pathogenic <em>Staphylococcus</em></td>
</tr>
<tr>
<td>8.2</td>
<td>Short rod</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>F</td>
<td><em>Corynebacterium</em></td>
</tr>
</tbody>
</table>

I = Inactive, F= Fermentative, OF = Oxidative / Fermentative
Fig 1: Pattern of white blood cell count of 8 experimental horses during treatment with Endod.
to be investigated. Furthermore, the identification of these microorganisms to the species/subspecies level is of paramount importance so as to determine their role in the pathology of the disease.

The results of differential white blood cell counts demonstrated progressive increase in the number of neutrophils in advanced cases while the proportion of lymphocytes was observed to increase steadily in early cases that were progressively recovered from the disease. As neutrophilia mainly indicates bacterial infection, its domination in the white blood cell count of advanced cases substantiates the fact that different bacteria were involved in advanced cases.

As far as Endod was concerned, the results of the present study have proven that it is effective against HCF. On the other hand, studies have been undertaken previously on its availability, side effect (toxicity) and cost. The results of such studies have shown that Endod can be cultivated on large-scale basis in different agroecologies of Ethiopia, which in turn signifies the existence of greater possibilities of getting Endod and using it widely for the purpose required. Consequently, reduction of cost is straightforward. Regarding its toxicity on domestic animals, previous studies have shown that domestic animals such as sheep and dog have tolerated a reasonable dose of its extract.

In conclusion, both the in vitro and in vivo results of the present study on the antifungal (HCF) effect of Endod in crude form and the results of the previous studies on its availability, toxicity and cost are promising. Therefore, further study on the extraction of active ingredient of Endod and evaluation of this extract for the treatment of EL is recommended.

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FACTORs AFFECTING PRE-WEAning MORTALITY OF RABBiTS AT DAGWOM FARMS, VOM, PLATEAU STATE

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LES FACTEURS AFFECTANT LA MORTALITÉ AVANT LE SEVRAGE DES LAPINS DANS LES FERMES DE DAGWOM A VOM DANS L'ETAT DU PLATEAU

Résumé

L'étude a été menée entre mars 1997 et décembre 1998 dans les fermes de Dagwom de l'Institut national de recherche vétérinaire à Vom dans l'Etat du Plateau. Les registres de 332 portées, qui ont produit 1.631 lapereaux, étaient examinés afin d'évaluer les effets du génotype, de l'âge de la mère, de la saison, de l'année de mise bas et de la taille de la portée à la mise bas (TPM) sur le taux de mortalité avant le sevrage de la portée (TMSP). Le TMSP moyen global était de 64,08%. Le lapin blanc de race pure de la Nouvelle-Zélande (NZ) avait le TMSP le plus élevé (74, 97%), tandis que les autres groupes avaient un taux plus faible (45,93%). La TPM était le seul paramètre qui ait beaucoup contribué (P < 0,05) aux changements de TMSP. Les taux de mortalité allant de 1 - 7 ; 8 - 21 et 22 - 42 jours étaient respectivement de 48,25 ; 9,14 et 7,05%.

Summary

The study was conducted between March 1997 and December 1998 at the Dagwom Farms of the National Veterinary Research Institute, Vom, Plateau State. Records on 332 litters which produced 1631 kids at birth, were analysed to assess the effects of genotype, parity, season, year of birth, and litter size at birth (LSB) on litter pre-weaning mortality rate (LPWMR). The overall mean LPWMR was 64.08%. Purebred Newzealand White (NZW) recorded the highest LPWMR (74.97%), while other groups had the least (45.93%). Only LSB contributed significantly (P<0.05) to the variation in LPWMR. The rates of mortality from 1-7, 8-21 and 22-42 days were 48.25, 9.14 and 7.05% respectively.

Introduction

The importance of rabbit meat and the ease of its production have been reported¹. However, a major problem facing most of the rabbit producers is the rate of kid mortality, which lowered production greatly and consequently reduced farmers' income². The causes of kid mortality include abandonment by the mother, attack of pests and predators, fright, cuts and strangulation between wire mesh as well as poor nutrition of the doe¹. The number of surviving kids is related to the number of teats per doe and that death can be caused by undernourishment due to inadequate number of teats³. It has been also reported that does sometimes eat up their kids as a result of imbalanced ration. Since kit mortality is great loss to the farmer,
considerable attention has to be paid to preventive measures in order to minimize it. The aim of this research was, therefore, to identify the major factors influencing pre-weaning mortality rate of kids of Dagwom Farms.

### Materials and methods

The experimental work was carried out at the Dagwom Farms of the National Veterinary Research Institute (NVRI), Vom, Plateau State, from March, 1997 to

| Table 1. Mean litter pre-weaning mortality rates (%) and factors affecting them in rabbits |
|---------------------------------|-----------------|
| **Factor**                      | **Mortality**   |
| Overall                         | 64.00±4.08      |
| Genotype                        | ns              |
| Local                           |                |
| ½ New Zealand White             | 67.47±3.22      |
| ¾ New Zealand White             | 68.44±3.57      |
| New Zealand White               | 63.63±3.01      |
| Others                          | 74.97±6.29      |
| Parity                          | 45.93±4.59      |
| 1                               | 62.64±5.68      |
| 2                               | 51.74±5.30      |
| 3                               | 62.38±4.72      |
| 4                               | 65.58±4.81      |
| 5                               | 68.33±5.17      |
| 6                               | 61.11±6.58      |
| 7                               | 70.64±4.93      |
| 8                               | 69.85±4.80      |
| 9                               | 59.23±7.11      |
| 10                              | 74.05±7.10      |
| 11                              | 85.38±8.48      |
| 12                              | 93.00±4.55      |
| 13                              | 42.50±17.5      |
| Season                          | ns              |
| Early wet                       | 63.48±10.7      |
| Late wet                        | 70.00±2.65      |
| Early dry                       | 63.81±3.59      |
| Late dry                        | 57.24±5.41      |
| LSB                             |                |
| 2                               | 70.0±10.7       |
| 3                               | 69.75±4.73      |
| 4                               | 68.18±4.07      |
| 5                               | 63.01±3.41      |
| 6                               | 62.03±3.86      |
| 7                               | 62.32±4.09      |
| 8                               | 49.63±9.15      |
| 9                               | 66.70±11.3      |
| 10                              | 100.00±10.00    |
| Year                            |                |
| 1997                            | 65.48±2.42      |
| 1998                            | 65.01±2.47      |

NZW = New Zealand White
LSB = Litter size at birth
* = P<0.05
ns = Not significant
December, 1998. Vom is 1280m above sea level and lies on longitude 80 45’ East and latitude 90 43’ North. Rainy season extends from April to October with peak rainfall in July / August, while the dry season starts in November and ends in March. The mean annual rainfall ranges between 1250 to 1650mm. The average air temperature ranges from 19.5 to 23.50°C. Compared to surrounding lowland areas, the climate shows characteristic coldness common at high altitudes. The climate has therefore been described as being sub-tropical.  

The breeding programme started in 1988 with the procurement of non-descript rabbits from farmers and households within and around Vom to form the foundation stock. In 1989, the Newzealand White (NZW) breed was imported and a crossbreeding programme initiated. This gave rise to the 50 and 75% NZW. Later, another group, 'Others', which included rabbits from higher order crosses and those whose original parentage could not be ascertained. Since then, these groups have undergone several generations of within-group mating.

The rabbits were housed in three enclosed rabbitries with windows providing adequate ventilation. Breeding females and males were kept separately in individual cages, while weaned bunnies were kept in cages in groups of 6 to 10 individuals. All cages were fitted with aluminium feeders and waterers. Each rabbit was identified uniquely. The rabbits were fed ad libitum, a concentrate diet containing 16% crude protein twice daily (morning and afternoon). Green forages were also given when available. Fresh clean water was available to the rabbits at all times.

Young does and bucks were considered to have attained breeding age at 210 to 240 days. Each doe was transferred to the buck's cage and restrained to ensure copulation and palpated 10 days later to determine their pregnancy status. Non-pregnant does were re-mated to the same buck, until a successful service was observed. On the 25th day of pregnancy, the nest boxes were examined. After kindling, the kids were inspected and the requisit records taken within 24 hours of kidding. The kids remained with their dams until weaning at 42 days. Young doe replacements were added to the breeding groups as needed, throughout the period of study.

The traits recorded were litter size at birth (LPWMBR) was calculated as number of kids lost before weaning, expressed as percentage of total kids born per litter. The data were transformed by the arc-sine transformation to normalize the distribution and analysed by the General Linear Model.  
The model used was as follows:  
\[ Y_{ijklm} = u + A_i + S_j + Y_{ik} + P_l + L_m + E_{ijklm} \]

Where \( Y_{ijklm} \) = Observation on single litter.  
\( u \) = Overall mean  
\( A_i \) = effect of the \( i \)th genetic group year  
(\( i = 1,5 \))  
\( S_j \) = effect of the \( j \)th season of kidding  
(\( j = 1,4 \))  
\( Y_{ik} \) = effect of the \( k \)th year of kidding  
(\( k = 1,2 \))  
\( P_l \) = effect of the \( l \)th parity (\( l = 1,13 \))  
\( L_m \) = effect of the \( m \)th litter size (\( m = 2,10 \)) and  
\( E_{ijklm} \) = random error associated with each record which is assumed to be random, independent and normally distributed with a mean of 0 and variance 2e.
Results

The genetic group least squares means for LPWMR are given in Table 1. Purebred NZW had the highest mortality rate of 74.97±6.29%, whereas others had lowest rate of 45.03±4.59%. Analysis of variance carried out showed that LSB exerted a significant (P<0.05) influence on LPWMR, while genotype, parity, season and year of birth did not. Mortality rates from birth to 7, 8 to 21 and 22 to 42 days were 48.25, 9.14 and 7.05%, respectively (Table 2).

Discussion

This genetic group means for LPWMR which ranged from 45.93±4.59 to 74±6.29% are higher than values of 24.25% for NZW rabbits1 and 35.26, 40.82 and 36% for Simonaire, NZW and Dutch breeds, respectively6.

The lack of significant variations in LPWMR between genetic groups, parity classes, seasons and years of kindling, suggest the influence of other factors on the trait. One of these factors could be the inbreeding. Past studies have shown that this type of mating reduces vigour and viability of the offspring produced6. In addition, a large proportion of early mortality had occurred as a result of starvation of kids that dropped from, cages and remained unnoticed from strangulation between wire mesh, and abandonment by mother and from predation by rodents. These causes of mortality could have masked the effects of the factors on pre-weaning death rate of kids that otherwise may have influenced it as were found by several investigations. For instance, significant variations between genotypes in pre-weaning losses of kids which was attributed to variations in milk production and mothering ability of does2-8,10. Where comparisons were made between purebred and cross bred rabbits, the latter were found to surpass the former in pre-weaning kid survival rate. The authors attributed this observation to heterotic effects brought about by non-additive gene interactions.

Assessing the effect of parity on mortality in rabbits11 found significant decline in mortality (at different ages) from second to the sixth parities and subsequent increases thereafter. They concluded that this could be due to the fact that the maternal qualities of does increased with advance in parity up to the sixth, but gradually decreased afterwards due to ageing.

Table 2. Pre-weaning mortality rates in rabbits at various stages

<table>
<thead>
<tr>
<th>Stage</th>
<th>No. dead</th>
<th>Mortality rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth 7 days</td>
<td>787</td>
<td>48.25</td>
</tr>
<tr>
<td>8 to 21 days</td>
<td>149</td>
<td>9.14</td>
</tr>
<tr>
<td>22 to 42 days</td>
<td>84</td>
<td>7.05</td>
</tr>
</tbody>
</table>
On the effect of season on pre-weaning kid mortality rate, higher kid survival during the wet season was observed which was attributed to better quality of feed resources during the season\textsuperscript{12}. On the other hand\textsuperscript{8}, the contrary was found and attributed it to chilling that causes death of young rabbits still in the nest\textsuperscript{8}. Regarding the effect of year of kidding significant variations in early loss of kids, from one year to another was attributed to changes in climatic conditions, genetic constitution of flock, management and nutrition as well as changes in disease conditions. However, such changes were not observed occur to during the period of this study, hence the lack of significant year variations.

The fact that LSB contributed significantly to the variation in death of kids agrees with past reports\textsuperscript{14,15}. However, while the authors had observed a positive relationship between LSB and LPWMR, which they attributed to the inability of small kids from large litters to compete for food and therefore susceptibility to starvation, exposure syndrome and within litter competition (for milk and feeding space), this study found the contrary in some instances. Pre-weaning losses of kids decreased significantly (P<0.05) with increase in LSB\textsuperscript{1,2,3,4,9,13,16}, but increased when the number of kids exceeded\textsuperscript{3}. This could be due to the stimulatory effect of large litter size which could elevate milk production response and increase feed intake of dam and litter which could in turn, enhance growth and survival\textsuperscript{14}.

The observed decline in mortality rate with age from birth to weaning is in conformity with past observations\textsuperscript{16}. This trend could be due to the gradual improvement of immunity, size and sight. Rabbits are usually small, hairless and sightless at birth and depend entirely on the dam for nutrition and care. Death from strangulation by wire mesh and falling from cages and predation also decreased with advance in age, since the rabbits would be too big to strangulate or escape through the wire mesh, or be attacked by rodents that are smaller.

**Conclusion**

It is clear from the result of this study that the bulk of pre-weaning death of kids occurred during the early pre-weaning. Reduced viability and vigour, starvation of kids that escaped from cages and predation by rodents, have been identified as the major causes of early kid mortality at Dagwom Farms. Therefore, in order to reduce loss of kid in the farms, the matting of closely related rabbits should be avoided by introducing new stock to increase genetic varification. In addition, cages should be repaired or new ones made to avoid the dropping of kids, while rodents should be eradicated around the rabbitries or at least measures be taken to prevent them from entering the hutches.

**References**


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SHORT COMMUNICATION

COMPARATIVE EFFECT OF LACTATION ON SOME ERYTHROCYTE BIOCHEMICAL PARAMETERS IN N’DAMA AND WHITE FULANI COWS

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The literature is replete with studies on the plasma biochemical profile of the lactating cows but few reports have been published relating erythrocyte biochemical profile in cows to their stage of lactation or pregnancy or disease\textsuperscript{1,2,3,4,5,6,7}. Little or no data are available on the influence of breed and/or physiological status on these reference values as well as the usefulness of the parameters in predicting milk yield and productivity. In the present study, the alterations that occur in the erythrocyte biochemical profile of cows, during and after lactation, were assessed in healthy N’Dama and White Fulani cows.

Fifteen adult N’Dama (eight non-pregnant non-lactating and seven lactating) and eighteen White Fulani (non-pregnant non-lactating and nine lactating) cows were used for this study. The animals were selected from a herd at Oyo, Nigeria during the late dry season (i.e. January to March, 1997). The age of the cows ranged from 3-7 years while their calves ranged from 2-4 months. The cows were raised on pastures with brewer’s dried grain supplementation. Water was supplied \textit{ad litem}. They were dewormed with Mebendazole (15mg/kg), prophylactically treated against trypanosomosis with diminazene diaceturate (5mg/kg) and vaccinated against rinderpest and haemorrhagic septicaemia, according to the farmers.

Blood samples were collected by jugular venipuncture into EDTA-containing evacuated tubes. Erythrocytes were separated from the plasma and buffy coat by centrifugation at 3000x g for 10 minutes. The plasma and buffy coat were removed by aspiration using long-tipped Pasteur pipettes\textsuperscript{8}. This procedure was reported twice by alternate suspension and centrifugation in 140mm lithium chloride to obtain plasma and leukocytes free erythrocyte. The haematocrit of the washed erythrocyte was determined, after thorough mixing, and 10% erythrocyte suspension, in de-ionised water, prepared. The haemolysate was then vortex-mixed, and a 2ml aliquot of the lysate stored at 20°C until required for analysis.

Enzymes, lipids, total proteins, cation and metabolites analyses were performed using an automated analyser (Boehringer Mannheim Hitachi 704E Auto-analyser).

Statistical significance of differences between breeds were established by the analysis of variance (ANOVA) procedure while within breed as well as within physiological status levels of significance were determined by the students t-test procedure\textsuperscript{9}.

The results of the erythrocyte biochemical analyses in the White Fulani and N’Dama cows are as presented in Table 1.

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The eNa and eK exhibited non-significant variations (P>0.05) between the breeds, although their values were generally higher in the White Fulani than in the N'Dama.

The eAP and eAST were significantly increased (P<0.05) in the lactating N'Dama relative to the dry N'Dama and White Fulani. The eALT value was also higher in the lactating N'Dama relative to the other groups, albeit non-significantly (P>0.05).

The ANOVA showed no significant alteration in the values of eBUN and erythrocyte creatinine across breeds. However, N'Dama breed had higher eBUN value while White Fulani breed had higher creatinine concentration.

The cholesterol concentrations were fairly stable across the breeds, although the White Fulani cows had higher values than the N'Dama. The triglycerides levels varied non-significantly between breeds (P>0.05) with the White Fulani having higher value.

Total protein concentration was stable across the breeds and generally above 64 G/L except in the lactating White Fulani with

### Table 1: Mean ±s.em erythrocyte concentrations of biochemical parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>N'Dama Cows</th>
<th>White Fulani Cows</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dry</td>
<td>Lactating</td>
<td>Dry</td>
</tr>
<tr>
<td>eNa (mmol)</td>
<td>14.57±0.571</td>
<td>13.29±0.68</td>
<td>15.43±0.571</td>
</tr>
<tr>
<td>eK (mmol)</td>
<td>2.614±0.214</td>
<td>2.443±0.163</td>
<td>2.943±0.24</td>
</tr>
<tr>
<td>eBUN (mmol)</td>
<td>10.43±0.721</td>
<td>9.535±0.742</td>
<td>5.762±0.989</td>
</tr>
<tr>
<td>eCreatinine (µmol)</td>
<td>30.94±4.42</td>
<td>28.75±2.21</td>
<td>30.056±3.586</td>
</tr>
<tr>
<td>eAP (U/L) ***</td>
<td>131.86±11.99</td>
<td>242±23.15</td>
<td>69.14±5.51</td>
</tr>
<tr>
<td>eAST (U/L) ***</td>
<td>39±2.69</td>
<td>88.71±7.53</td>
<td>31.7±2.1</td>
</tr>
<tr>
<td>eALT (U/L)</td>
<td>42.14±1.64</td>
<td>58.86±3.56</td>
<td>28.57±2.1</td>
</tr>
<tr>
<td>eTotal protein (G/L)</td>
<td>64.7±1.9</td>
<td>65.14±0.91</td>
<td>65±4.6</td>
</tr>
<tr>
<td>eCholesterol</td>
<td>2.557±0.296</td>
<td>2.468±0.116</td>
<td>3.015±0.239</td>
</tr>
<tr>
<td>eTriglycerides</td>
<td>0.676±0.023</td>
<td>0.77±0.058</td>
<td>1.044±0.057</td>
</tr>
<tr>
<td>eNa (mmol)</td>
<td>5.721±0.355</td>
<td>5.571±0.424</td>
<td>5.476±0.557</td>
</tr>
</tbody>
</table>

+++ P<0.01 (within N'Dama breed)

+ P<0.05 (within White Fulani breed)

☆ P<0.05 (breed lactating groups), P<0.001 ☆ ☆ ☆

XP<0.05 (between dry groups); XXP<0.01; XXX P<0.001

*** P<0.05 (between breed) Values with different superscripts in the same horizontal row are significantly different.
a value below 60 G/L.

Lactation non-significantly decreased eNa concentrations in both the N'Dama (-8.0%) and White Fulani (-5.2%), relative to their respective dry group. It also non-significantly reduced eK in the N'Dama (-6.54%) but increased eK in the White Fulani (5.7%). The lactating White Fulani experienced more reduction in the eNa/eK ratio (-7.8%) than the lactating N'Dama (-2.62%) when compared to their respective non-lactating group.

The erythrocyte levels of BUN and creatinine were non-significantly affected by lactation (P>0.05). Although creatinine and BUN were reduced (-7.14% and -8.3%, respectively) during lactation in the N'Dama, they were increased in the White Fulani (5.9% and 19.3%, respectively) relative to their respective dry group.

The concentrations of eAP, eAST and eALT were affected significantly by lactation in both breeds (P<0.05). However, the within N'Dama effect was more than the within White Fulani effect.

Cholesterol concentration was non-significantly lowered (P>0.05) by lactation in both breeds. However, triglycerides increased in the N'Dama (14.7%) but decreased in the White Fulani (-14%) relative to their respective dry group.

The erythrocyte total protein concentration was not affected by lactation in the N'Dama cow. However, the lactation tended to induce a non-significant reduction (P>0.05) in the value of erythrocyte total protein in the White Fulani (-7.85%) relative to the non-lactating White Fulani group.

The erythrocyte levels of BUN, AP, AST and ALT were significantly higher in the lactating N'Dama relative to the lactating White Fulani cows (P<0.05). The eNa, eK, erythrocyte creatinine and lipids were higher in the lactating White Fulani than the lactating N'Dama, albeit, non-significantly (P>0.05). Erythrocyte total protein and eNa/eK ratio were higher in the lactating N'Dama but not significantly different from the lactating White Fulani group (P>0.05).

Erythrocyte concentrations of BUN, triglycerides, AP, AST and ALT varied significantly between the non-lactating N'Dama and non-lactating White Fulani cows (P<0.05). The values of eNa to eK ratio and erythrocyte creatinine tended to be higher in the dry N'Dama than in the non-lactating White Fulani. However, the concentrations of erythrocyte Na, K, total protein and cholesterol were non-significantly lower (P>0.05) in the dry N'Dama relative to the dry White Fulani cows.

The observed tendency towards a slight decrease in the mean eNa level and a sharp increase in the eK level in the better milk producing White Fulani cows relative to the poorer milk producing N'Dama are supported by the findings of earlier workers. The increased eK indicates a healthy erythropoietic response to the decreased packed cell volume associated with lactation. The greater rate of increment in the value of eNa to eK ratio in the lactating White Fulani than in the lactating N'Dama confirms the trend in the eK and eNa and also is supported by the observation by an earlier worker that aging red blood cells have higher Na to K ratio. The decreases in both eK and eNa in the lactating N'Dama relative to its dry control, unlike the White Fulani, indicated that lactation is more stressful to the poor milk producing N'Dama cows than the better milk producing White Fulani cows.

The concentrations of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase enzymes have been reported to be stable
in the high producing dairy cows contrary to the observed alterations in the erythrocyte concentrations of AST, ALT and AP in the present study. However, the values of all the enzymes under investigation were higher in the poor milk producing N'Dama than the better producing White Fulani. Thus, better milk producers are apparently poor enzyme producers, since the plasma concentrations of these enzymes in the White Fulani cows were reported to be equally lower than those of the N'Dama cows.

Although the erythrocyte total protein levels were stable in both breeds of cows, the erythrocyte of lactating White Fulani experienced more alteration than the erythrocyte of N'Dama cows. This alteration may be due to the reported lactation-induced plasma hypoproteinaemia. The proteins would have been diverted into the processes of milk production.

The plasma cholesterol level has been reported to be higher in the lactating White Fulani than dry White Fulani while the plasma triglycerides concentration was reportedly higher in the lactating N'Dama than the dry N'Dama. The observed reversal in the erythrocyte concentrations of these parameters might be associated with their being differentially mobilized from the erythrocyte into plasma and subsequently into the milk product.

The results show that differences in breed and physiological status could alter the profiles of some biochemical parameters in the indigenous cows. These alterations could be further investigated as a tool in the dairy industry and breeding programmes.

References


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SHORT COMMUNICATION

A CASE OF CLINICAL SWINE OEDEMA DISEASE ASSOCIATED WITH FOOD-BORNE TOXIGENIC ESCHERICHIA COLI IN FINISHER PIGS

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Pig industry is acquiring increasing importance in western Kenya as an alternative source of income. However, the level of knowledge on their management is still in its infancy within the area. Occasionally disease outbreaks occur in pigs that are attributed mainly to trypanosomes and Escherichia Coli. Haemolytic E. coli has been reported to cause diarrhoea in neonatal pigs and oedema disease in weaned pigs. Oedema disease usually occurs three days to two weeks after weaning, but it is seen occasionally in piglets. It is considered the primary cause of mortality in pigs between weaning and the market. The disease occurs as sporadic outbreaks affecting up to 50% of pigs within a group with a change of diet being a predisposing factor. The affected pigs develop nervous symptoms, exhibit sudden death and at necropsy subcutaneous oedemas of the gastro-intestinal tract is observed\(^1\). Culture of intestinal tract contents is the most common method of diagnosing the causative agent of oedema disease in swine. Confirmation, however, requires more information, such as history, clinical signs, histopathology, and bacterial characterisation. Recent advances in molecular biology have now made it possible for diagnostic laboratories to differentiate between pathogenic and non-pathogenic E. coli\(^4\). The occurrence of oedema disease was reported by other workers in the central parts of Kenya, with no other studies reported elsewhere\(^2,3\).

The current study reports on an outbreak of the disease in a pig farm in western Kenya. The pigs described in this study originated from an intensive commercial farm in Malaba town adjacent to river Malaba on the Kenya / Uganda border in March to April 2001. The farm had on average a population of 65 pigs at any one time. The pigs were fed on commercial finisher mash supplemented by the use of market-waste. Prior to the outbreak, the pigs had been fed on contaminated and discarded rice declared unfit for human consumption. Following reports of sudden deaths in pigs, an exploratory survey was carried out to determine the cause of the disease. Clinical observations were made and some of the animals in extremis sacrificed and post-mortem done. Bacteriological culture and isolation was done from the intestinal contents of freshly dead pigs\(^1\).

The clinical signs were initially observed eight days after feeding on the discarded rice. The affected pigs were aged between 6-9 months and were first suspected as having trypanosomosis, which is endemic in the area. They were, however, not
responsive to trypanocidal drug administration. The observations were made at different times on the pigs either dead or in extremis where they were sacrificed.

The affected pigs were the thrifty and fast-growing individuals of a group under conditions of excellent management and nutrition. Initially, nine were found dead suddenly. Simultaneously, seven others exhibited nervous symptoms, including staggering, head tilting, stumbling and falling, assumption of a "dog sitting" position, lying on the sternum, or on the side while making continuous kicking movements. Some showed swollen eyelids noted as hyperaemia around the eye. The morbidity rate among the pigs was 65% and mortality around 15% (16/59). Those pigs that did not succumb to disease recovered after a few days. The course of the disease lasted between two and five days, but recurred among some of the weaned pigs two weeks later. In this second outbreak, the pigs walked slowly, wandered aimlessly around the edge of the pen, with the head slightly elevated and/or tilted to one side. Clinical examination of the sick pigs revealed marked dyspnoea, bluish-red discolouration of the skin, incoordination and difficulty in walking. Bacteriological examination of the gut contents of two freshly dead pigs yielded pure cultures of haemolytic *E. coli*.

A total of five pigs were examined at post mortem. Externally, these pigs were in good body condition and finish. The gross changes consisted of subcutaneous oedema of the head and ventral abdomen. Other findings included marked oedema of the mesentery of the spiral colon, oedema of the brain, enlargement and dark red coloration of the liver, gastro-enteritis, hyperaemia and haemorrhages in the kidneys and heart, oedema of the lungs and the wall of the gall bladder. On the basis of the history, clinical signs, isolation of haemolytic *E. coli* and the post-mortem findings, a diagnosis of oedema disease was made.

In this study, the disease was managed through the immediate withdrawal of the incriminated rice feed, whilst simultaneously increasing the use of wheat bran that was readily available. This was supported by the administration of sulphonamides (Quinabic®, Abic, Jerusalem, Israel) for five days. The clinical signs and pathology were similar to those already reported. The many body parts including the kidneys presented hyperaemia and haemorrhages, due to the effect of toxins produced by the concomitant microflora. The morbidity and mortality was consistent with the results of others. The *E. coli* toxin results to fluid accumulation especially in the brain, where swelling can result in destruction of some brain tissue and, in many cases, death of the animal. Various symptoms, such as staggering gait, head tilting, odd lying and sitting positions are indicative of the disease's presence. In this study, diarrhoea was not a major finding as in other studies involving weaners or younger piglets. It would appear that in older pigs the toxaemia is a more important consequence of the disease than the enteric effect. This is in agreement with past finding but not with those of others. However, an age-related immunity is suggested as the possible explanation of this difference in presentation.

In this study, the rice was the culprit for introducing infection and may have been contaminated by free ranging pigs or in the manner of storage or handling. This is what
resulted in the initial outbreak whereas the secondary one may have resulted from contamination of the commercial feed or water by those pigs that were initially infected in the herd. Practitioners have long known that decreasing the amount of feed given after weaning can control oedema disease. Changing the diet will alter the growth conditions for bacteria in the intestine, and it may allow other types of bacteria to proliferate and replace the strains of *E. coli* that cause Oedema disease. Treatment with antibacterial drugs also may help prevent additional cases. However, treatment of pigs that have clinical symptoms, however, is often ineffective among piglets. Further studies need to be done on the disease to establish the full extents of its epidemiology and the diversity among the infective isolates of *E. coli* in the country.

References


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SHORT COMMUNICATION

ISOLATION OF CORYNEBACTERIUM PEDICUM IN POULTRY IN GHANA


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The poultry industry in Ghana is besieged with a host of constraints, among them being poor husbandry practices and feed ingredients contaminated with microorganisms1,2 resulting in infections and toxicity. Despite widespread use of expensive anti-microbial agents by farmers to combat the microorganisms and associated diseases, respiratory and intestinal infections persist. This causes high mortality3 and other economic losses.

In Ghana, isolation and identification of aetiologic agents have been conducted and reported at the Veterinary Services Department and Animal Research Institute (A.R.I.) after post-mortem examinations as confirmatory procedure. Organisms frequently isolated for causing respiratory diseases include Haemophilus spp., Staphylococcus spp and Mycoplasma spp. Similarly, among those responsible for enteric infections are Salmonella spp, Enterobacter spp, Escherichia coli as well as protozoa such as Eimeria spp4. Record on isolation of Corynebacterium spp in poultry is unavailable. However, the organism has been isolated from raw milk produced on the Accra Plains of Ghana5 and also from the liver of a sheep with multiple abscesses6. Necrotic/ulcerative, mycotic or non-specific enteritis have been described to be among the most common diseases which affect the intestinal tract of poultry in Ghana and elsewhere7,8. Although ulcerative enteritis in poultry caused by Corynebacterium spp. have been rare and therefore been described as "uncommon pathological" conditions, outbreaks caused by Corynebacterium pedicum have been reported in several birds including geese, turkey, chicken and pigeons. The fastidious nature of the organism might explain its rare isolation8.

A poultry farmer purchased six hundred pullets in April, 1999 at point of lay (20 weeks old). The birds were kept on raised coops with wire net floor. Five days after receiving the birds he started recording mortality without observing any clinical signs. There were eight deaths recorded within two weeks. The dead birds were sent to a veterinarian and enteritis was diagnosed. Chlortetracycline was recommended for treatment but mortality persisted.

Later the farmer reported observing "outstretched" legs among the birds. He also reported that when he opened up some of the dead birds, there were unbroken eggs both in and outside the reproductive track. The condition persisted for about two months resulting in total mortality of 42 carcasses were then taken to the A.R.I.
Veterinary section for further investigation and identification of the causative agent.

On gross examination, the carcasses appeared to be in good condition with few of them showing slight emaciation. The livers showed yellow and gray spotty to large non-discreet shaped areas and the edges were thickened. Among other findings were haemorrhagic enteritis and ulcers in many portions of the intestines especially the duodenum. The ulceration appeared hollow-like and in various shapes and sizes.

The ovaries were found to be haemorrhagic. About 50% of dead birds had hardened whole egg retained in the reproductive track. Most of the retained eggs were found in the cloaca while the lungs were also congested.

Lungs, liver and ovaries were aseptically collected at post-mortem into sterile petri dishes for microbiological examination. Retained whole eggs were also taken for culture.

Surfaces of the organs were seared with hot spatula and incisions made with sterile scalpel blade. Direct smears were made and stained by Gram stain. The eggs were swabbed with tincture methiolate 1:10,000 and punctured with sterile scalpel blade.

Cultures were made unto blood agar and MacConkey agar and incubated at 37°C for 18-24 hours aerobically. The organism was sensitive to Streptopen and Chloramphenicol, but it was resistant to Tetracycline, Erythromycin, Neomycin and Colistin sulphate. Colonial, cell morphology and Gram stain were used as basis to identify isolates. Wet preparations were made from portions of intestines and examined microscopically. Portions of the intestines with severe ulceration were also sent for coccidial examination.

The intestinal smears did not show *Eimeria spp.* while the smears from the visceral samples showed Gram positive, straight or slightly curved rods. There was no growth on MacConkey agar. Pure culture was obtained on blood agar, which showed beta-haemolytic, double layer clearance, grayish-brown, flat with spreading mucoid colonies. Stained preparation showed Gram positive straight or slightly curved rods with rounded ends occurring in singles and arranged in Chinese-like letters, characteristic of *C. pedicium* as described by Peckham (1960). The organism being host specific was thus identified and confirmed as *C. pedicium*. Penicillin and Streptomycin combination was used in treatment. The disease was controlled after four weeks. Mortality was 54 birds (9%) during a period of 12 weeks.

In this report, the disease occurred among birds between 20-24 weeks of age with 9% mortality. Earlier workers have reported it to occur in birds between the ages of 4-12 weeks and often associated with coccidiosis. This outbreak, however, was not found to be associated with coccidiosis as mentioned previously. The isolation of *C. pedicium* from ovaries and whole-retained eggs in this study confirms the presence of *Corynebacterium* spp among other bacteria from diseased internal organs and joints of poultry. Some of these disease conditions include respiratory tract disorders and arthritis. Since *C. pedicium* has been isolated from joints of birds with arthritis, it could be deduced that the observed paralysis, described by the farmer as “outstretched legs”, was caused by *C. pedicium*. It is suggested that the organism could be incriminated in various poultry infections in
Ghana. Care should, therefore, be taken for its isolation, diagnosis and treatment in many infectious diseases in poultry. It appears that this is the first time \textit{C. pediculum} is being confirmed in poultry in Ghana.

\textbf{References}


6. Opoku-Pare personal communication - Veterinary Services Laboratory Accra.


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EFFECT OF OSMOTIC STRESS ON THIRD STAGE LARVAE OF *Dictyocaulus filaria*, *Haemonchus contortus* and *Trichostrongylus colubriformis*.

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Nematode larvae generally succumb to osmotic stress. Depending upon the concentration of the solution, responses vary as concerns the larval stage or the species involved. For instance, prompt inactivation of *Nippostrongylus muris* and *Ancylostoma caninum* occurred to a low hypertonic concentration\(^1\). Also Croll et al.\(^2\) found a very prompt inactivation of *A. tubaeformae* under a slight osmotic stress. Wharton et al.\(^3\) has also reported a progressive inactivation of *Trichostrongylus colubriformis* under osmotic stress. Both first and second stage larvae of *Dictyocaulus filaria* and the first stage larvae of *Haemonchus contortus* and *T. colubriformis* succumbed to osmotic stress in short durations (Melaku, Unpublished observations). Inactive larvae under osmotic stress show varying degrees of body collapse\(^2,3,4\). It is more serious in the first stage larvae than in the second stage larvae\(^4\). Infective larvae coil in response to osmotic stress; and the type of coiling is helical where both longitudinal and lateral twistings are involved\(^5\). The most important energy consuming process of free-living larvae nematodes is their activity\(^5\). This is especially very important in free-living larvae of *D. filaria* as they do not feed in their stay out of the host. Sparing their limited energy is therefore very vital, especially to the infective stage or its vaccine derivative.

First stage larvae of *D. filaria* were collected by a modified Baermann technique from faeces of naturally infected lambs. The larvae were suspended in boiled and cooled tap water for 5 days to harvest the infective (third stage) larvae. Third stage larvae of both *H. contortus* and *T. colubriformis* were collected from faeces of lambs with mixed infections after 7 days of storage in plastic bags under room temperature to allow larval development (free-living larvae of both parasites are coprophagus).

Fresh infective larvae of the three parasitic nematodes were immersed in 0.2, 0.5M Sodium chloride (NaCl) solutions and in water as control in duplicates. Larvae were observed promptly after immersion, after 24 hours of immersion (all 3 species) and at day 10 and day 15 for *D. filaria* larvae in 0.5 and 0.2M NaCl solutions, respectively. Follow-up of the other two parasites was abandoned after the 24 hours. Percentage of larval coiling was made for larvae in 0.5 M NaCl at the 24 hour (for *H. contortus* and *T. colubriformis*) and at day 10 for *D. filaria*, because at those moments inactivity was irreversible in both cases. The percentage was calculated from double counts of each tube of the duplicates.

Examination of infective larvae of the three parasites upon immersion in both
hypertonic solution concentrations showed a general disturbed and increased, but transient activity. This may be a flight response to altered environment. Larvae of all the 3 parasites were observed at the 24 hrs of suspension. Inactivation of both *H. contortus* and *T. colubriformis* was observed and it was complete after 24 hrs of immersion under the two concentrations. No effect had been observed on the third stage larvae of *D. filaria*. They were not completely inactivated until after day 5 or day 15 of immersion in 0.5M and 0.2M NaCl solutions, respectively. Proportions of larval coiling were made at 24 hrs of immersion for both *H. contortus* and *T. colubriformis* infective larvae and at day 10 for *D. filaria* larvae, both groups in 0.5M NaCl concentrations. As responses of both *H. contortus* and *T. colubriformis* larvae were similar their reactions are treated as one. The proportion of coiling on an average were 77% for *D. filaria* but lower, 43% for *H. contortus* and *T. colubriformis* infective larvae. The rest of the larvae were stretched or crescent shaped with their bodies collapsed. The findings of this study are in conformity with several other reports. For example, free-living larvae of *Ancylostoma tubaeforme*², *A. caninum* and *Nippostrongylus muris*³ were promptly inactivated with accompanied body collapse in hypertonic solutions of even lower concentrations than used in this study. Also Wharton *et al.*³ showed that *T. colubriformis* infective larvae became progressively coiled and inactive. Melaku⁴ has demonstrated prompt inactivation of both first and second stage larvae of *D. filaria* under 0.2 and 0.25M NaCl solutions, but that there was extraordinary osmotic resistance by the third stage larvae. The type of coiling of all the three nematode infective larvae was helical. This was formed by simultaneous coiling of the longitudinal and lateral axes of the larval body. The helical coil was rather perfect being mainly cylindrical for *D. filaria* larvae, but it was imperfect, being predominantly conical (with a wide base at one end and pointed at the other) in both *H. contortus* and *T. colubriformis* larvae. Again it was observed that the whole length of the larval body of these two latter nematodes may not at times be involved in coiling. The extraordinary osmotic resistance of the infective larval stage of *D. filaria* is worth noting.

The helical coiling posture of nematode larvae may help reduce outward pressure on the larval hydrokeleton thus reducing the amount of energy needed to maintain the coiling. The permeability of the body cuticle of the infective larvae may as well be reduced sparing the larvae further damage due to hypertonicity. Helical coiling under osmotic stress is not, however, a feature shared by both first and second stage larvae of *D. filaria*⁴. Even flat coiling to other stimuli is not a feature of these larval stages and the first larval stages of both *H. contortus* and *T. colubriformis* (Melaku, Unpublished observation).

The success of cryopreservation of certain nematode larvae⁶⁷ might have been due to their ready susceptibility to even lower hypertonic solution concentrations. Prior dehydration of infective larvae (a necessary process for successful cryopreservation) with hypertonic solutions or cryopreservatives before plunging into the deep freezer is easier to attain in other nematode species. But if cryopreservation of infective larvae of *D. filaria* or its vaccine derivative is required, a very strong hypertonic solution or cryopreservative need to be used and for a far longer pe-
period. This may, however, compromise with larval energy, because larvae may remain active until the desirable level of dehydration is attained. The failure of successful cryopreservation of *D. viviparus* third stage larvae may possibly have been due to a failure of successful prior dehydration. Infective larvae or the vaccine of *D. filaria* after dehydration may be kept under conditions where larval activity remain minimal, viz low, above zero temperatures, under darkness and where mechanical stimulus such as shaking is avoided.

An undue energy wastage by infective third stage larvae of *D. filaria* or its vaccine derivative upon prior dehydration for successful cryopreservation can be bypassed by using third stage larvae developed from second stage larvae in hypertonic solution of about 0.2M NaCl concentration. This is because fresh infective larvae cultured in osmotic solution are already lethargic and tend to coil. Such infective larvae therefore can be irradiated and promptly suspended in a cryopreservative (of choice and concentration) and can be directly plunged into a deep freezer. This regime is thought to be better and it is suggested it be tested and followed in the future, should use of a vaccine be desirable in Ethiopia or elsewhere.

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**References**


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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.

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Deux exemplaires des articles doivent être adressés à Monsieur le Rédacteur en Chef, Bulletin de la Santé et de la Production Animales en Afrique, Union Africaine/Bureau interafrique 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.

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L'introduction expose le but de la recherche.
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