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

Introduction stating the purpose of the work.

Materials and Methods used.

Results presented concisely.

Discussion of significance.

Acknowledgements.

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Tables should be limited and number of headings restricted. A massive table is difficult to read even if it can be reproduced. Tables and figures should be numbered consecutively. Table 1 etc., or Fig. 1 etc., respectively, and attached at the end of the text. References to tables and figures in the text should be by number and not to "table below" or "figure below". Coloured illustrations are reproduced only at the author(s) expense.

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STUDY ON PARASITES OF FISH AT LAKE AWASSA, ETHIOPIA

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ENQUETE SUR LES PARASITES DE POISSON AU LAC AWASSA EN ETHIOPIE

Résumé

Une enquête a été menée sur les parasites de poisson au lac Awassa entre janvier et mars 2003. Au cours de l'étude, au total 600 poissons, qui représentent trois espèces: Oreochromis niloticus (n = 300), Clarias gariepinus (n = 200) et Barbus (n = 100) ont été choisis au hasard et ont fait l'objet d'un examen externe et interne approfondi pour détecter la présence des vers parasitaires. Sur l'ensemble des poissons examinés, 474 (79%) étaient affectés par un parasite ou plus. Les métabasiques encapsulées (plus petites) Clinostomum étaient présentes dans la cavité branchiale de 227 (75,7%) O. niloticus, tandis que l'on a aperçu Euclinostomum dans la cavité branchiale, le rein, le foie et la vésicule biliaire de 90 (30%) O. niloticus. Les métacercaires non-encystées issues du cerveau et les Eustrongylides larvaires encapsulés issus de la musculature étaient recueillis de 45 (22,5%) et 33 (16,5%) C. gariepinus respectivement. Des cestodes adultes Bothriocephalus et Proteocephalus étaient présents chez l'espèce Barbus avec des taux de prévalence de 35 (35%) et 18 (18%) respectivement. Les nématodes larvaires Amblycerae étaient recueillis de 82 (41%) C. gariepinus et 33 (33%) Barbus. Des parasites Contracaecum étaient recueillis de 119 (39,67%) O. niloticus, 161 (80,5%) C. gariepinus et 36 (36%) Barbus. Il s’est avéré que les parasites Argulus africanus et Dolops ranarum affectent toutes les trois espèces de poisson échantillonnées. Au cours de la présente étude, il était possible d’identifier, de décrire et d’évaluer la pathogénicité des helminthes les plus courants et des parasites de poisson dans le lac.

Mots-clés: Lac Awassa, poisson, parasites, prévalence.

Summary

A survey was carried out on parasites of fish at Lake Awassa in the period between January to March 2003. During the study, a total of 600 fish representing three species Oreochromis niloticus (n=300), Clarias gariepinus (n=200) and Barbus species (n=100) were randomly sampled and examined thoroughly both externally and internally for the presence of parasitic worms. Of the total fish examined, 474 (79.0%) were affected by one or more parasites. Encapsulated Clinostomum species (smaller) metacercaire were found in the branchial cavity of 227 (75.7%) O. niloticus and Euclinostomum species in the branchial cavity, kidney, liver and gallbladder of 90 (30.0%) O. niloticus. Uncysted digenean metacercaiae from brain & encapsulated larval Eustrongylides from musculature were recovered from 45 (22.5%) and 33 (16.5%) C. gariepinus respectively. Adult cestodes Bothriocephalus and Proteocephalus species were found in Barbus species with prevalence rate of 35 (35%) and 18 (18%) respectively. The larval nematode parasites Amblycerae species were recovered from 82 (41%) C. gariepinus and 33 (33%) of Barbus species. Contracaecum species were recovered 119 (39.67%) O. niloticus 161 (80.5%) C. gariepinus and 36 (36%) of Barbus species. The crustacean parasites Argulus africanus and Dolops ranarum were found to affect all the three different fish hosts sampled. During this study it was possible to identify, describe and assess the pathogenic significance of the most common helminth and crustacean parasites of fish in the lake.

Key words: Lake Awassa, Fish, Parasites, Prevalence.

*Corresponding author E-mail: esyima_n@yahoo.com
Introduction

Ethiopia, a land locked country, depends on its inland water bodies for fish supply to its population. The country's water bodies (major lakes and reservoirs) cover an estimated total surface area of 7,334 km² and small water bodies cover 275 km². The main rivers elongate to some 7,185 km inside the country. Despite the huge volume of water bodies in Ethiopia, their contribution to the national economy is marginal, mainly due to man made and environmental constraints.

During the 1980's researches on the etiology, therapy & control of diseases in cold-water fish cultured in Europe & North America gained momentum & developed fast toward a level of sophistication equaling that of veterinary sciences of farm animals. All of the major groups of animal parasites are found in fish, and apparently healthy wild fish often carry heavy parasite burdens. Knowledge of specific fish hosts greatly facilitates identification of parasites with marked host and tissue specificity, while others are recognized because of their common occurrence and lack of host specificity.

So far there is no comprehensive study conducted on parasites of fish in Ethiopia. Along with the growing interest in the development of fish farming in the different parts of the country, where water supply is reliable, there is an increasing awareness of the importance of fish disease as one of the major detrimental factors in culturing fish. The presence of parasites in fish is very common, although the majority of them do not have direct public health significance they represent unaesthetic appearance thus cause rejection & complaint from the buyer &/or customer.

Therefore, this study was conducted with the objectives of identifying the most common parasites of fish in the lake and to obtain baseline data on parasites of fish, which may aid in the formulation of future policies and strategies concerning fish diseases prevention and control.

Materials and Methods

Description of study area

Lake Awassa the smallest rift valley lake is situated 275km south of Addis Ababa at 6°33' - 7°33'N and 38°22' - 38°29'E and at an altitude of 1680 meters above sea level. The lake has surface area of 88.2 km², maximum and mean depth of 22m and 11.75m respectively. It has a shore line length of 54.9 km and maximum length of 15.85 km with maximum and mean width of 7.90 km and 5.56 km respectively. It has a volume of 1.036x10⁸ m³ and water level fluctuation of 2.162cm and water pH ranges from 8.7-9.05. Water temperature of the lake ranges between 18°C - 26°C and its Secchi disc depth varies between 70 - 80cm. Its main inlet water is from the swampy source area called Shallo through the Tikur Wuha River, but it has no obvious surface out let. There are three economically important fish species in the lake that include Nile tilapia (Oreochromis niloticus), Catfish (Clarias gariepinus) and Barbus species.

Fish samples collection

A total of 600 different fish samples that included O. niloticus 300, C. gariepinus 200 and Barbus species 100 were examined from January to March 2003 for the presence of helminth & crustacean parasites of fish in the lake. Fish samples were collected from the lake every other day and all the fish specimens were thoroughly examined
both externally and internally while fresh within six hours of capturing. During examination, parasites recovered were washed and cleaned in normal saline and later transferred to appropriate fixatives. Cestode and trematode parasites were fixed in AFA (Alcohol-Formalin-Acetic Acid). Crustacean copepods were fixed in 70% ethyl alcohol or 4% formalin and parasites collected were put in labeled flat bottom

<table>
<thead>
<tr>
<th>Fish host</th>
<th>Parasite species recovered</th>
<th>Site of localization</th>
<th>No. of fish infected</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>O. niloticus</em></td>
<td><em>Clinostomum</em> species</td>
<td>(Smaller) Branchial cavity</td>
<td>227</td>
<td>75.67</td>
</tr>
<tr>
<td>(n=300)</td>
<td>(Digenean)</td>
<td>(Larger) Between cranium &amp; pharyngeal teeth</td>
<td>34</td>
<td>11.33</td>
</tr>
<tr>
<td>“</td>
<td><em>Euclinostomum</em> species</td>
<td>Branchial cavity, liver, kidney, gallbladder</td>
<td>90</td>
<td>30.0</td>
</tr>
<tr>
<td>(Digenean)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>“</td>
<td><em>Contracaecum</em> species</td>
<td>Pericardial cavity</td>
<td>119</td>
<td>39.67</td>
</tr>
<tr>
<td>(Nematode)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>C. gariepinus</em></td>
<td>Digenean metacercariae</td>
<td>Cranial cavity (Brain tissue)</td>
<td>45</td>
<td>22.5</td>
</tr>
<tr>
<td>(n=200)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>C. gariepinus</em></td>
<td><em>Contracaecum</em> species</td>
<td>Mesentery</td>
<td>161</td>
<td>80.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>“</td>
<td><em>Amplicaecum</em> species</td>
<td>Mesentery</td>
<td>82</td>
<td>41.0</td>
</tr>
<tr>
<td>(Nematode)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>“</td>
<td><em>Eusstrongylides</em></td>
<td>Musculature</td>
<td>33</td>
<td>16.5</td>
</tr>
<tr>
<td>(Nematode)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>O. niloticus</em></td>
<td><em>Argulus africanus</em></td>
<td>Gill, fins, oral cavity</td>
<td>16</td>
<td>5.33</td>
</tr>
<tr>
<td>(crustacean)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>“</td>
<td><em>Dolops ranarum</em></td>
<td>Gill, fins, oral cavity</td>
<td>25</td>
<td>8.33</td>
</tr>
<tr>
<td>(crustacean)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>C. gariepinus</em></td>
<td><em>Argulus africanus</em></td>
<td>Gill, fins, oral cavity, skin</td>
<td>89</td>
<td>44.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>“</td>
<td><em>Dolops ranarum</em></td>
<td>Gill, fins, oral cavity, skin</td>
<td>127</td>
<td>63.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Skin</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 2  Prevalence of helminth and crustacean parasites from Barbus species at Lake Awassa, Ethiopia.

<table>
<thead>
<tr>
<th>Fish host</th>
<th>Parasite species recovered</th>
<th>Site of localization</th>
<th>No. of fish infected</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barbus species</td>
<td>Bothriocephalus species</td>
<td>Intestinal lumen</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>(n=100)</td>
<td>(Cestode)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proteocephalus</td>
<td>Intestinal lumen</td>
<td>18</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>(Cestode)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amplicaeicum</td>
<td>Mesentery</td>
<td>33</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>(Nematode)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Contracaecum</td>
<td>Mesentery</td>
<td>36</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>(Nematode)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Argulus africanus</td>
<td>Gill, fins, oral cavity</td>
<td>6</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>(Crustacean)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dolops ranarum</td>
<td>Gill, fins, oral cavity</td>
<td>17</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>(Crustacean)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

tubes containing the specific fixative agent.

During the process of trematode parasite collections from the fish, encapsulated larval trematode metacercariae were released manually from their cysts, fixed in appropriate fixatives and after staining with hematoxylin & cleared in xylene, specimens were permanently mounted.

Encysted larval nematode parasites were carefully released from their cysts and then fixed in hot formalin to ensure their relaxation and preserved in 4% formalin mixed with 5% glycerin to avoid accidental drying. Preserved parasites were cleared in lactophenol and then observed under low power magnification. Live specimens of branchiuran copepods and those fixed in 4% formalin were examined under stereomicroscope after staining with cotton blue and clearing in lactic acid.

Nematodes were cleared in lactophenol for 24 hours and examined while in the case of trematodes and cestodes diagnosis was made from permanently mounted specimens. The identification of each parasite to its generic level was made using the techniques of Byklovskaya et al., Paperna and Schmidt as guidelines.

Results

Digenean parasites such as Clinostomum species larger, and smaller were recovered from the branchial cavity of 34 (11.5%) and 227 (75.7%) of O. niloticus respectively. In addition to their localization in the branchial cavity (Table 1),
Euclinostomum species were recovered from the kidney, liver and gallbladder of 90 (30%) O. niloticus. The other digenean metacercariae (recovered from the cranial cavity) and Eustrongylides species were found only in C. gariepinus and cestodes such as Bothriocephalus and Proteocephalus species were exclusively recovered from Barbus species (Tables 2). Amplicaeicum species were found in 82 (41%) of C. gariepinus and 33 (33%) of Barbus species while Contracaecum species were recovered from all fish hosts. Euclinostomum species, the other digenean metacercariae (recovered from the cranial cavity), Eustrongylides species and Proteocephalus species were new geographic records for the lake. The external crustacean fish parasites commonly called fish lice, Argulus africanus and Dolops ranarum were recovered from all fish hosts with different intensities of infestation.

Discussion

Larval helminth parasites belonging to the genera Clinostomum, Euclinostomum, Amplicaeicum, Contracaecum, Eustrongylides and other digenean species are known to occur in most African fresh water fishes8. Adult Cestodes such as Bothriocephalus and Proteocephalus species accumulate in the anterior intestine that may cause obstruction or perforation to the intestine seriously affecting the health of the fish hosts and this may also induce high mortalities11. The definitive hosts of Clinostomum and Euclinostomum species are known to be herons, darters, cormorants and pelicans9. It is very likely that the large population of piscivorous birds, which gather and feed around the lake, harbor the adult stages of the above mentioned parasites.

Simultaneously, these birds could also play a significant role in maintaining the life cycle of these parasites. Clinostomum and Euclinostomum species were recovered from the different organs of the fish in an encysted form without any apparent clinical effects. Although the number of parasitic burden was low up to a maximum of 5, Euclinostomum species when found in large numbers in vital organs like the kidney can directly interfere with its normal function.

The digenean metacercariae recovered from the cranial cavity of the catfish was actively motile which can easily spread to the other parts of the fish during evisceration. Therefore the identity and nature of this parasite needs to be further studied. The awareness for zoonotic diseases originating from raw fish eating among fishermen and customers is very little although raw fish eating is very common in the area. Prevalence of nematodes in Clarias gariepinus and Barbus species is higher because they are too large to be prey for birds and serves as parasite tank. Helminth parasites can cause damages such as compression and disruption of vital organs including the gonads leading to sterility, eyes leading to blindness, poor growth rate and unthriftiness especially in young fish when they are found in large numbers in their body cavities and some times they can even cause human diseases12,13.

The digenean Euclinostomum species and the nematodes Contracaecum & Eustrongylides species were newly recovered additions to the parasitic fauna of fish compared to previous studies conducted on the lake2,14. This shows the dynamic nature of parasitism and the need to conduct an exhaustive and extensive study, taking into consideration all the seasons and
representative areas of the lake. Almost all fish caught were eviscerated and processed along the shore and all washed material is again released into the lake causing recontamination that in turn increases parasite burden per fish and facilitates further transfer between fish.

The maximum number of *Contra caecum* species found in the pericardial cavity of the heart was 2 and since the size of the larval worm was big it can affect the normal pumping activity of the organ. Moreover, extensive areas of hemorrhages and congestion were observed in the mesentery of fish infected by both Contra caecum and *Amplicae cum* species. On the other hand *Eustrongylides* species are long reddish worms, therefore the presence of large number of the parasite in the musculature can result in consumer rejection although, fewer number of the parasite were recovered from the musculature of *Clarias garipienus*.

Coproparasitologic study among 150 children under the age of 15 engaged in fishing and fish processing in Awassa revealed a heterophyid infection rate of 2.1%, a parasite known to be transmitted by eating raw fish\(^\text{15}\). In Egypt in a village near Lake Manzala up to 90% of the school children and 22% of adults were infected by *Heterophyes heterophyes*\(^\text{9}\). However, in this study we did not encounter any parasit of that kind in the fish sampled. In Lake Awassa where raw fish eating habit is extremely popularized, the prevalence rate of infection by zoonotic parasites of fish is expected to be very high. This suggests the need for a more concerted effort to be undertaken to know the actual magnitude of the problem both in humans and fish so that effective prevention and control methods could be instituted.

Paperna\(^\text{9}\) reported few cases of laryngopharyngitis infection due to *Clinostomum complanatum* infection of the larynx in human in the near east. Hence, periodic assessments to determine the prevalence of zoonotic diseases related with the consumption of fish are therefore very important. Fish parasites do have not only economic impact but also public health importance. Creating public awareness among society about consumption of raw fish and its possible consequences should be encouraged. Favorable situation should be arranged and sufficiently studied publications should be widely distributed.

Localized hemorrhages and inflamed areas were seen in the oral cavity, gills and fins at the site of attachment of *Argulus africanus* of all fish examined which is caused by the piercing proboscis stylet and the action of the lytic enzyme that it secretes. However, due to the low number of parasites present and the continuous attaching and detaching activity of the parasites no extensive epithelial damage was seen.

The majority of the fish that carried the *Bothricephalus* species had empty intestine packed with the parasite which could interfere with the normal digestion and absorption of food that in turn affect the food efficiency and growth rate of the fish.

The study conducted at Lake Awassa may contribute to enrich the existing scanty body of knowledge on parasites and diseases of fish in Ethiopian fresh water. It may be wise to extend the study further rather than giving intuitive conclusion based on preliminary surveys to point out clearly the impact of parasites on fish host, production and fisheries activities. With the ever-increasing demand for animal protein, fish could play a significant role in fulfilling some of these needs in particular the demand for high quality proteins. Full
exploitation of lakes like Awassa and others is based on conservation of natural resource and utilization for artificial fish rearing systems. In this regard the importance of diseases would be very high and this preliminary study may contribute its share in creating awareness about the importance of fish parasites during culturing. Proper waste disposal method should be implemented to avoid the existing contamination of the lake and break recycling of parasites of fish. The potential pathogenic effect of the parasites to the fish population in the lake, piscivorous animals and public health is considered to be significant as the result of habit of raw fish consumption, poor sanitary measures and lack of awareness about fish parasites. Associated with the improper human waste disposal, study should be conducted on zoonotic bacterial pathogens and parasites of fish from the lake.

References


Received for publication on 16th March, 2007
The study conducted at Lake Andres should continue to monitor the existing community fishing practices for their impact on fish stock and the environment. It may be wise to extend the study further in order to gather more information based on preliminary surveys. It is crucial to fully understand the impact of fishing on fish stock, population, and ecosystem. With the ever-increasing demand for aquaculture products, it could play a significant role in filling some of these needs, in particular the demand for high-quality proteins. Full
Efficacy of aqueous extract of Carica papaya leaf in the control of coccidiosis in chicken

S.M. Odeyinka, P.O. Babalola, E.O. Akinfala and B.O. Oyebanji.

Department of Animal Science, Obafemi Awolowo University, Ile-Ife, Nigeria

Efficacité de l’extraït aqueux de la feuille de Carica papaya pour le contrôle de la coccidiose chez les poulets

Résumé

L’objet de l’étude était d’examiner l’efficacité de l’extraït aqueux de la feuille de papaye (Carica papaya) pour le traitement de l’infection coccidiennne chez les poulets. Trois cents jeunes coqs âgés de 4 semaines étaient utilisés pour la présente étude. Les poulets expérimentaux étaient répartis en cinq groupes de vingt plus infectés trois fois chacun. Chaque groupe était infecté par un ovocyste sporulé de la culture de coccidie que l’on a mise dans de l’eau potable et servie aux poulets. L’extrait aqueux brut de feuilles de papaye a été administré par voie intramusculaire à raison de 2ml, 3ml et 4ml/kg aux trois groupes respectivement. Le sulfamide était injecté à la dose de 0,5ml/kg de poids vif au 4ème groupe et le 5ème groupe non traité servait de groupe-témoin.

Les résultats de l’étude ont montré qu’il n’y avait pas de différence significative quant à la consommation alimentaire et au gain pondéral des poulets soumis aux différents traitements (P<0,05). Les poulets du groupe 5 avaient une mortalité beaucoup plus forte que ceux des autres groupes. L’extrait aqueux brut de feuille de papaye est efficace contre la coccidie en fonction de la dose administrée bien que le sulfamide ait donné de meilleurs résultats avec les paramètres utilisés. L’extrait aqueux à la dose de 4ml/kg peut être recommandé pour le traitement de la coccidiose dans les zones reculées où les médicaments ordinaires ne sont pas disponibles.

Summary

The study was designed to investigate the potency of Pawpaw (Carica papaya) leaf extract (aqueous) in the treatment of coccidial infection in chicken. Three hundred cockerel chicks (aged four weeks) were used for this study. The experimental chicks were divided into five groups (treatments) of twenty each and replicated thrice. Each group was infected with sporulated oocyst of the cultured coccidian through drinking water. The crude Pawpaw leaves extract was administered intramuscularly at the rate of 2ml, 3ml and 4ml/kg to three groups respectively. Sulpha drug was injected at 0.5ml per kg body weight to the fourth group and the fifth group was not treated, to serve as control.

The results of the study showed that there were significant differences in the feed intake and weight gain of chicks on different treatments overtime (P<0.05). The chicks in treatment five had significantly higher mortality than all the chicks in other treatments. Crude Pawpaw leaf extract has activity against coccidion organism in a dose dependent manner though the sulpha drug gave a better result using the parameters considered. The extract at 4ml/kg can be recommended for treatment of coccidiosis in remote areas with no access to standard drugs.

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Introduction

Coccidiosis is a disease caused by coccidia, parasitic protozoans, commonly observed in digestive tract of domestic animals. It is a major disease of the poultry industry. Coccidia infection increases mortality, reduces growth and feed efficiency in chicken\(^1\). Coccidia of the genus *Eimeria* causes coccidiosis in chickens and Turkeys. These *Eimeria* parasites are host and organ specific\(^2\). They multiply in the intestinal tract and cause tissue damage with resultant interruption of feeding and leading to dehydration, blood loss and increased susceptibility to other disease agents\(^3\).

The number of infective coccidia consumed by the host is a primary factor as to the severity of the resulting infection. An infection may be mild enough to go unnoticed while a large infective dose of coccidia may produce severe lesions that can cause death. They are easily transmitted from one house to another on contaminated boots, clothing, free flying birds, equipment, feed sacks, insects and rodents\(^4\). Coccidiosis usually occurs in growing birds and young adults. It is seldom seen in the birds under three weeks or in mature birds. Signs of an outbreak include birds that are pale, droopy, tend to hurdle, consume less feed and water, have diarrhoea and may become emaciated and dehydrated. Laying hens will experience a reduction in rate of egg production\(^5\).

According to Shane\(^6\), it is estimated that the cost of preventing Coccidiosis and the losses which occur as a result of infection amount to $ 300 million annually in both industrialized and tropical countries. Despite the availability of many anti-coccidial drugs, infection caused by species of *Eimeria* continues to be a source of significant economic loss to the poultry industry. After two decades in which the use of ionophorous antibiotics gave unparalleled control of coccidiosis, drug resistance is once again tipping the balance in favour of the parasites\(^7\). The conventional drugs in use include Sulphadimidine, Sulphamethazine, sulphadoxine, nitrofurazone, amprolium and a host of others. There is an increased acceptance by the poultry industry worldwide of live vaccines to control coccidiosis.

The drugs though proved to be effective, have been found to be expensive and scarce especially in the local areas where the greater percentage of the poultry farmers inhabit. This necessitates the quest for a locally available and cheaper medication of relatively equal potency as the conventional drugs.

Many successes have been recorded with various parts of *Carica papaya* in the treatment of livestock diseases. *Papaya* latex had been tested against *Ascaris suum* and found to be efficacious, it was also reported that papaya leaf extract is used as prophylaxis against malaria\(^8\). This study is designed to test the efficacy of pawpaw (*Carica papaya*) leaf extract in controlling coccidiosis.

Material and Methods

a. Experimental Chickens

A total of three hundred and nine cockerel (309) chicks aged four weeks were acquired from a commercial poultry farm in Ikirun, Osun State of Nigeria. The housing system used was deep litter using wood shavings as the liter material; the pens were fumigated using formalin/Potassium permanganate vapour two days before the chicks were housed. There was record of vaccination against Gumboro disease at first
and third weeks, Newcastle disease (NCD I/O, NDV lasota booster) at day old and three weeks respectively. Anti – stress (Biovit) was administered in drinking water for two days. Nine chicks picked at random were sacrificed for pre – treatment sample collection and were ascertained to be free of coccidia in the digestive system or any lesion characteristic of coccidiosis. The remaining chicks (300) were randomly distributed into five treatments of sixty chicks each.

b. Infective Materials

Eighteen coccidal infected dead birds from a small poultry farm in Iree Nigeria were used. The intestinal and caeca contents were collected in two different containers. The debris was removed as much as possible to give near homogenous material. 2.5% Potassium dichromate solution was used for sporulation of the oocysts according to the method described\(^\text{9}\). This sporulated oocyst is the stage of infectivity of the coccidia organism. The prepared intestinal contents were mixed with 2ml potassium dichromate solution after the debris was removed A filter paper was soaked in the Potassium dichromate solution and placed at the bottom of the petri dish. A thin layer of the intestinal content was smeared on the laid filter paper after which a little of the potassium dichromate solution was sprinkled on top. This same process was carried out on the caecal contents. The Petri plates were all incubated between 29\(^\circ\)c and 30\(^\circ\)c in a humidified incubator for two days. The contents of the Petri dishes for both intestinal and caecal scraping were standardized using the Mc Master slide chambers. The concentration of oocysts in the culture was counted using a modification of the method described by Long and Rowell\(^\text{10}\).

c. Preparing the Pawpaw leaf extract

The freshly plucked pawpaw leaves were washed clean with water, chopped into small sizes. The leaves were then boiled at the temperature of 100\(^\circ\)c in water at a ratio of 50g per litre of water. The boiling was done for fifteen minutes after which the supernatant was decanted. The liquid was allowed to cool at room temperature for six hours and then stored in the refrigerator till administered on the experimental chicks.

d. Infecting the Chicks with Oocysts

The infection was carried out when the chicks were 28 days old while the pre – treatment diagnosis was done at 27 days old. To get the intestinal and caecal oocysts into the experimental chicks, each bird was made to drink 2ml of intestinal oocysts suspension and 1ml of caecal oocysts suspension to ascertain infection with the intestinal and Caecal strains of coccidia. This was done by using syringe to drop the liquid in the mouth of each bird to ascertain uniform level of exposure. The stage was replicated thrice to increase the level of precision of the experiment.

e. Treatments Administration

The prepared extract was injected intramuscularly into groups 1, 2 and 3 chicks at the rate of 2mls, 3mls and 4mls/kg respectively while sulphamethazine sodium (injectable solution), a widely used and effective anticoccidial drug, was administered on the fourth group of chicks intramuscularly at the rate of 0.5ml /kg for four consecutive days starting from six hours after exposure and subsequent ones at twenty – four hours interval. Group 5 chicks served as the control experiment therefore no drug or extract was
administered on them. Feed intake, consistency and presence of coccidial oocysts in faecal droppings, physical appearance of the chicks and weight gain were the parameters investigated.

**Results and Discussion**

**Feed intake**

Table 1 shows the average feed intake in grams. There was reduction in the feed intake of Groups 1, 2, 3 and 5 chicks till day 10 post infection and Group 4 chicks till day 4 post infection instead of progressive increment in feed intake expected as the bird ages, this is in agreement with the findings of Long and Jeffers who observed that chickens stop feeding and huddle together for warmth after the 3rd day of Coccidial infection. The treatment 4 birds, though experienced ephemeral reduced average feed intake, had increased feed intake from the 6th day. Treatment 5 chicks had the greatest reduction in feed intake. The stoppage of feed by some chickens in treatments 1 and 5 was in agreement with findings of Mc Dougald and Reid that following the multiplication of the genus *Eimeria* of the protozoan parasites in the intestinal tract, there is tissue damage with resultant interruption of feeding and digestive processes or nutrient absorption among other things. The high potency of the sulpha drugs against Coccidia might have caused the improved feed intake observed in Group 4 compared to the other treatments. This could have stopped the development of the oocysts into sporozoites before a lot of

<table>
<thead>
<tr>
<th>Days Post infection</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
<th>T5</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>2&lt;sup&gt;nd&lt;/sup&gt; day</td>
<td>83.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>82.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>83.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>84.7&lt;sup&gt;h&lt;/sup&gt;</td>
<td>83.3&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.38</td>
</tr>
<tr>
<td>4&lt;sup&gt;th&lt;/sup&gt; day</td>
<td>81.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>81.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>82.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>82.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>83.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.40</td>
</tr>
<tr>
<td>6&lt;sup&gt;th&lt;/sup&gt; day</td>
<td>80.9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>81.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>80.6&lt;sup&gt;d&lt;/sup&gt;</td>
<td>86.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>82.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.10</td>
</tr>
<tr>
<td>8&lt;sup&gt;th&lt;/sup&gt; day</td>
<td>82.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>81.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>80.9&lt;sup&gt;d&lt;/sup&gt;</td>
<td>86.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>82.1&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1.04</td>
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<tr>
<td>10&lt;sup&gt;th&lt;/sup&gt; day</td>
<td>71.4&lt;sup&gt;d&lt;/sup&gt;</td>
<td>82.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>81.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>90.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>80.1&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3.05</td>
</tr>
<tr>
<td>12&lt;sup&gt;th&lt;/sup&gt; day</td>
<td>82.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>82.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>82.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>91.8&lt;sup&gt;d&lt;/sup&gt;</td>
<td>76.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.48</td>
</tr>
<tr>
<td>14&lt;sup&gt;th&lt;/sup&gt; day</td>
<td>82.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>82.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>83.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>92.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>78.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.22</td>
</tr>
<tr>
<td>Overall mean</td>
<td>80.57&lt;sup&gt;b&lt;/sup&gt;</td>
<td>82.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>82.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>87.93&lt;sup&gt;a&lt;/sup&gt;</td>
<td>80.97&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.33</td>
</tr>
</tbody>
</table>

SEM: Standard error of the mean.
a b c: means within each row with different superscripts are significantly different (P<0.05)

**Table 2: Clinical observations on the experimental chicks**

<table>
<thead>
<tr>
<th>Days post Treatment</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
<th>T5</th>
</tr>
</thead>
<tbody>
<tr>
<td>2&lt;sup&gt;nd&lt;/sup&gt; day</td>
<td>A</td>
<td>a</td>
<td>A</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>4&lt;sup&gt;th&lt;/sup&gt; day</td>
<td>b, c, d</td>
<td>b, c, d</td>
<td>b, c, e</td>
<td>b, c, d</td>
<td></td>
</tr>
<tr>
<td>6&lt;sup&gt;th&lt;/sup&gt; day</td>
<td>c, d, e, f</td>
<td>b, c, d, e, f</td>
<td>b, c, d, e</td>
<td>b, c, d, e, f, g</td>
<td></td>
</tr>
<tr>
<td>8&lt;sup&gt;th&lt;/sup&gt; day</td>
<td>d, e, f, g, h</td>
<td>d, e, f</td>
<td>b, c, d, e</td>
<td>b, c, d, e, f, g, h</td>
<td></td>
</tr>
<tr>
<td>10&lt;sup&gt;th&lt;/sup&gt; day</td>
<td>G</td>
<td>g</td>
<td>g</td>
<td>a</td>
<td>g, h</td>
</tr>
<tr>
<td>12&lt;sup&gt;th&lt;/sup&gt; day</td>
<td>g, i</td>
<td>g, i</td>
<td>g, i</td>
<td>a</td>
<td>g, i</td>
</tr>
<tr>
<td>14&lt;sup&gt;th&lt;/sup&gt; day</td>
<td>i</td>
<td>g, i</td>
<td>g, i</td>
<td>a</td>
<td>i</td>
</tr>
</tbody>
</table>

a = Normal  b = depression  c = huddling
d = low feed intake  e = diarrhoeic feaces
f = haemorrhagic feaces  g = mortality recorded
h = off feeding observed in some chicks.
i = slight recovery.
Table 3: Number of oocysts passed out per gramme of faeces

<table>
<thead>
<tr>
<th>Days post infection</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
<th>T5</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>9th day</td>
<td>12.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.67&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.67&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.67&lt;sup&gt;d&lt;/sup&gt;</td>
<td>13.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.36</td>
</tr>
<tr>
<td>16th day</td>
<td>34.67&lt;sup&gt;b&lt;/sup&gt;</td>
<td>33.33&lt;sup&gt;c&lt;/sup&gt;</td>
<td>25.33&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.33&lt;sup&gt;d&lt;/sup&gt;</td>
<td>59.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.04</td>
</tr>
<tr>
<td>23rd day</td>
<td>34.33&lt;sup&gt;b&lt;/sup&gt;</td>
<td>33.33&lt;sup&gt;c&lt;/sup&gt;</td>
<td>26&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.67&lt;sup&gt;d&lt;/sup&gt;</td>
<td>62.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.60</td>
</tr>
<tr>
<td>30th day</td>
<td>25.67&lt;sup&gt;b&lt;/sup&gt;</td>
<td>27&lt;sup&gt;b&lt;/sup&gt;</td>
<td>25.67&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.33&lt;sup&gt;c&lt;/sup&gt;</td>
<td>58.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.92</td>
</tr>
</tbody>
</table>

SEM: Standard error of the mean  
<sup>a</sup>b<sup>c</sup>: Means within each row with different superscripts are significantly different (P<0.05)

Intestinal damage is done which would have affected the digestive tract adversely and consequently reduce feed intake.

**Feecal droppings/Morbidity**

The feaces dropped by treatment 4 chicks had normal consistency but only diarrhoeic on the fourth day (Table 2). The other groups experienced normal consistency of the feaces for the first three days. The blood-stained diarrhoea observed in groups 1, 2 and 5 was in agreement with the findings of Soulsby<sup>12</sup> who noted that the disruption of the second generation schizonts and the overlying *epithelium* releases the merozoites into the lumen of the caecum and when large numbers of second generation *schizonts* do this, a massive haemorrhage into the Caecal lumen may be evidenced at about the ninety-sixth hour of infection. This may be assumed that the birds in groups 1, 2 and 5 had the ingested oocysts developing into the second-generation schizonts causing the epithelial destruction and the resultant haemorrhagic feaces dropped by the birds. The period of highest morbidity as exemplified by diarrhoea and huddling might also partly be due to pawpaw extract as observed by soulsby<sup>13</sup> who reported that aqueous extracts increased the number of wet feaces and the movement of the intestinal contents. There was significant difference among the Treatments (P<0.05) with Treatment 5 having the highest number of oocyst count and treatment 4 the lowest (Table 3). There was a dose dependent response among the leaf extract treated groups. This could have been due to the rate at which the administered drugs could disturb the development of the sporozoites so those small amounts of the ingested sporulated oocysts develop into mature coccidiano.

Table 4: Average body weight (in grams) at different periods

<table>
<thead>
<tr>
<th>Age (in weeks)</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
<th>T5</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>107&lt;sup&gt;a&lt;/sup&gt;</td>
<td>120&lt;sup&gt;a&lt;/sup&gt;</td>
<td>134&lt;sup&gt;a&lt;/sup&gt;</td>
<td>130&lt;sup&gt;a&lt;/sup&gt;</td>
<td>127&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.74</td>
</tr>
<tr>
<td>6</td>
<td>151&lt;sup&gt;b&lt;/sup&gt;</td>
<td>170&lt;sup&gt;b&lt;/sup&gt;</td>
<td>191&lt;sup&gt;b&lt;/sup&gt;</td>
<td>192&lt;sup&gt;b&lt;/sup&gt;</td>
<td>171&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.62</td>
</tr>
<tr>
<td>8</td>
<td>193&lt;sup&gt;c&lt;/sup&gt;</td>
<td>216&lt;sup&gt;c&lt;/sup&gt;</td>
<td>238&lt;sup&gt;c&lt;/sup&gt;</td>
<td>249&lt;sup&gt;c&lt;/sup&gt;</td>
<td>210&lt;sup&gt;c&lt;/sup&gt;</td>
<td>10.08</td>
</tr>
<tr>
<td>10</td>
<td>225&lt;sup&gt;d&lt;/sup&gt;</td>
<td>248&lt;sup&gt;d&lt;/sup&gt;</td>
<td>272&lt;sup&gt;d&lt;/sup&gt;</td>
<td>299&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>13.00</td>
</tr>
<tr>
<td>12</td>
<td>254&lt;sup&gt;e&lt;/sup&gt;</td>
<td>279&lt;sup&gt;e&lt;/sup&gt;</td>
<td>305&lt;sup&gt;e&lt;/sup&gt;</td>
<td>346&lt;sup&gt;e&lt;/sup&gt;</td>
<td>276&lt;sup&gt;e&lt;/sup&gt;</td>
<td>15.74</td>
</tr>
<tr>
<td>14</td>
<td>283&lt;sup&gt;f&lt;/sup&gt;</td>
<td>306&lt;sup&gt;f&lt;/sup&gt;</td>
<td>338&lt;sup&gt;f&lt;/sup&gt;</td>
<td>387&lt;sup&gt;f&lt;/sup&gt;</td>
<td>293&lt;sup&gt;f&lt;/sup&gt;</td>
<td>18.76</td>
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<tr>
<td>16</td>
<td>308&lt;sup&gt;g&lt;/sup&gt;</td>
<td>333&lt;sup&gt;g&lt;/sup&gt;</td>
<td>364&lt;sup&gt;g&lt;/sup&gt;</td>
<td>426&lt;sup&gt;g&lt;/sup&gt;</td>
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<tr>
<td>TWG</td>
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<td>213&lt;sup&gt;h&lt;/sup&gt;</td>
<td>230&lt;sup&gt;h&lt;/sup&gt;</td>
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<td>187&lt;sup&gt;h&lt;/sup&gt;</td>
<td>3.26</td>
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</tbody>
</table>

SEM: Standard error of the mean.  
<sup>a</sup>b<sup>c</sup>: Means within each row with different superscripts are significantly different(P<0.05)  
TWG: Total weight gain.
Table 5: Percentage mortality of the experimental chicks

<table>
<thead>
<tr>
<th>Days Post - infection</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
<th>T5</th>
<th>SEM</th>
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<tr>
<td>2nd day</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4th day</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6th day</td>
<td>0b</td>
<td>0b</td>
<td>0b</td>
<td>0b</td>
<td>0b</td>
<td>45a</td>
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<td>8th day</td>
<td>8.33b</td>
<td>0c</td>
<td>0c</td>
<td>0c</td>
<td>0c</td>
<td>40a</td>
</tr>
<tr>
<td>10th day</td>
<td>3.33b</td>
<td>6.67a</td>
<td>0c</td>
<td>0c</td>
<td>0c</td>
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<td>3.33c</td>
<td>3.33c</td>
<td>0a</td>
<td>0a</td>
<td>1.70</td>
</tr>
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<td>14th day</td>
<td>0a</td>
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<td>0a</td>
<td>0a</td>
<td>0.33</td>
</tr>
<tr>
<td>Total</td>
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<td>13.33c</td>
<td>10.0d</td>
<td>0a</td>
<td>95a</td>
<td>3.41</td>
</tr>
</tbody>
</table>

T : Treatment

Average Weight Gain

There were significant differences (P < 0.05) between the means of the body weight gained weekly by the chickens. The total weight gain on the average was highest for chickens on Treatment 4 and a decrease in weight gain from Treatment 3, Treatment 2, Treatment 1 and Treatment 5 respectively. The difference could be adduced to differences in feed intake especially between the sulphur treated birds and the others with the resultant weight loss and the difference in the extent of tissue damage. This is in conformity with the observation of McIlroy that infections can result in clinical disease with a variable degree of intestinal lesions resulting in reduced weight gain and impaired feed conversion.

Mortality

Table 5 shows percent mortality of the experimental chickens. Percent mortality was highest in the untreated birds (Treatment 5) and least for Treatment 4 (sulpha drug treated chicks) (P<0.05). It was noted by McIlroy that the economic effects of coccidiosis in broiler breeders can be dramatic, causing severe lesions in the intestines, haemorrhage and death.

Conclusion

From the results of the experiment, it can be concluded that crude pawpaw leaf extract has activity against coccidian organism in a dose dependent manner. Though the sulphur drug gave a better result using the parameter considered, the extract at 4ml/kg can be recommended for treatment of coccidiosis in remote areas with no access to standard drugs.

References


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RESISTANCE OF ABIGAR, GURAGHE, HORRO AND SHEKO BREEDS OF CATTLE TO TICK INFESTATION IN GHIBE – TOLLEY VALLEY

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RESISTANCE DES RACES BOVINES ABIGAR, GURAGHE, HORRO ET SHEKO A L’INFESTATION PAR LES TIQUES DANS LA VALLEE DE GHIBE TOLLEY

Résumé

Les collectes de tiques, à un mois d’intervalles pendant dix mois, sur la moitié du corps des génisses appartenant à quatre races locales (Abigar, Guraghe, Horro et Sheko) infestées naturellement, ont révélé que les races Horro et Guraghe abritaient beaucoup moins de tiques (en moyenne 12 ± 1,7 /mois) que les races Sheko (en moyenne 16 ± 4,1 /mois) et Abigar (15 ± 3,4 /mois) (P< 0,001). Les nombres de tiques A. variegatum recueillies ont également montré de nettes différences entre les races Sheko et Horro (P<0,05) avec la plus lourde charge de tiques chez les Sheko et la plus faible chez les Horro. Il s’est avéré que la charge de tiques chez tous les animaux dépendrait surtout de la saison puis de la race. Lors de l’évaluation du niveau de réaction de l’IgG aux tiques larvaires A. variegatum chez les quatre races avec le test ELISA indirect, on a observé un niveau très élevé d’IgG contre A. variegatum chez la race Horro par rapport aux autres races (P<0,05). Le niveau de l’IgG chez la race Horro était élevé durant la forte infestation, tandis que chez la race Sheko, le niveau de l’IgG était plus faible pendant la forte infestation. L’étude dans son ensemble montre que la race Horro est relativement résistante, ce qui pourrait être dû à une plus forte réaction de l’IgG dans le sérum comparé à la race Sheko, dont le faible niveau de l’IgG serait responsable de la forte charge de tiques.

Mots-clés : Tique, races bovines, résistance, anticorps IgG, Ethiopie.

Summary

Half-body tick collections, carried out on monthly intervals for ten months on naturally infested heifers belonging to four indigenous breeds (Abigar, Guraghe, Horro and Sheko) showed that the Horro and Guraghe breeds harboured significantly lower tick burden (mean count per month 12 ± 1.7) per month than the Sheko (mean count

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16 ± 4.1) and Abigar (15 ± 3.4) breeds (P < 0.001). Counts of *A. variegatum* ticks also showed significant differences between the Sheko and Horro breeds (P < 0.05), with the highest burden in Sheko and the lowest burden in Horro breed. The tick burden in all the animals was found to be mainly affected by season followed by breed. Upon assessment of the magnitude of IgG response to larval *A. variegatum* ticks in the four breeds by indirect ELISA, significantly highest level of IgG against *A. variegatum* was found in Horro breed than the rest of the breeds (P < 0.05). The magnitude of IgG in the Horro breed was high during high infestation, while in the Sheko breed the level of IgG was lower during high infestation. The overall study shows that the Horro breed is relatively resistant, which might be a result of the higher IgG response in the serum compared to the Sheko breed, in which the low level of IgG might be responsible for consistently high tick burden.

**Keywords:** Tick, cattle breeds, resistance, IgG antibodies, Ethiopia.

**Introduction**

Ticks are important ectoparasites throughout the world, especially in tropical and sub-tropical regions. The widely used control method has been the application of acaricides. However, due to the emergence of resistant tick strains to the most common and widely used acaricides and the limited access by many livestock keepers in developing countries due to cost considerations, development of alternative methods of tick control including integrated control approaches are essential. The use of host resistance to tick infestation deserves examination as a control method that would be directed against the target species with virtually no other impact on the host and the environment. In order to perform this, a thorough understanding of bovine immune responsiveness to tick infestation is an important prerequisite.

Host resistance to ticks is the inherent ability of a host, once primed, to mount an immune response to components of the saliva of feeding ticks, debilitating or killing them. Bovine resistance to *Ixodid* tick infestation was first reported by Johnson and Bancroft and the situation has been reviewed by various authors. Since then, bovine resistance has been reported to consist of innate and acquired components. Innate resistance is associated with genetic factors, since *Bos indicus* cattle are reported to be more innately resistant to tick infestation than *Bos taurus* cattle. The existence of breeds of cattle more resistant to ticks than others is well established for the *Ixodid* tick, *Boophilus microplus*. Evidence of resistance to two- and three-host ticks has been rarely shown. Acquired humoral and cell-mediated responses have also been observed to have partial protective role to tick infestations in both laboratory animals and cattle, with IgG production and delayed type hypersensitivity reaction being the major outcomes. Nevertheless, the immunological mechanisms underlying resistance or susceptibility of various cattle breeds to tick infestation are not fully characterised. To this end, it is important to understand the role of host immune responses in decreasing and maintaining the number of infesting ticks as well as the incidence of tick-borne pathogens in various cattle breeds.
The existence of a resistance potential of indigenous Ethiopian cattle to naturally infesting ticks has been implied by comparing the indigenous breeds with their crosses. Information on the level of resistance by a range of breeds of Ethiopian cattle to ticks is an important step in facilitating the development of control strategies by using host resistance as a complementary approach to chemical control. In the present study, we compared the tick burden in four indigenous breeds: Abigar, Gurage, Horro and Sheko, and we determined the magnitude of IgG specific for the tick Amblyomma variegatum antigens in each breed.

Materials and methods

Study area

The study was conducted at the Ghibe Valley at a location in Gullele/Tolley area located between latitude 8°15' N and longitude 37°30'E and 230 km south west of Addis Ababa (Fig. 1). The size of the study site is about 2000 hectares, with altitude ranging 1300 and 1400 meters above sea level. The area is part of the Omo river system’s tsetse belt, with medium to high tsetse density and trypanosomosis risk. The climate of the area consists of short rainy season in March-April and main rainy season from late May to September. The

![Figure 1. Location of the study area](image-url)
average annual rainfall during the study period ranged from 0-250mm. and annual minimum temperatures ranged from 12-16°C (National Metrology Agency). Vegetation consists mainly of bush and savannah with gallery forest along the drainage lines. Plenty of growth was observed during the wet season but during the dry season, vegetation becomes sparse. The dominant grass, Hyparrhenia spp., acacia and broad leaved deciduous plants are the major flora. Kudu, bushbuck, warthog, colobus and porcupine were some of the wild fauna observed.

**Study animals**

A total of 228 cattle, consisting of four indigenous breeds were brought to the study area in the year 2000 from four different locations of Ethiopia namely: Abigar, from Gambella area; Gurage from the Gurage zone; Horro from Wollega area and Sheko from Sheka zone. Information on the characterization of the breeds was found from Domestic Animals Genetic Resources Information System (DAGRIS)\textsuperscript{18}. The cattle grazed in a mixed herd controlled by three herdsman and were naturally exposed to tick infestation. At night all cattle were housed, the animals of each breed being held in a different pen. A total of 60 randomly selected heifers, 15 of which from each breed were involved in the present study. The Ghibe River was the source of water for the animals.

Disease management was effected mainly by regular vaccinations against infectious diseases eg: - anthrax, black leg, pasteurellosis, foot and mouth disease and through chemotherapy in the case of trypanosomosis and other infections or diseases as required. All the animals were ear- tagged for permanent identification. Acaricides were not applied as a matter of routine but strategically only when there was high burden of tick infestation and it was applied to all animals.

**Collection of feeding ticks from cattle**

From January to October 2002, regular half body tick collections were carried out from the sample heifers for seven days at monthly intervals on alternative body sides. The collection was done on eight anatomical sites; head, ear, dewlap, abdomen, hoof, tail and ano-vulval areas according to the procedure by Kaiser\textsuperscript{17} and placed in separate universal bottles containing 70% ethyl alcohol. Identification of ticks was subsequently done at National Animal Health Research Centre (NAHRC), Sebata, using light stereoscopic microscope employing standard keys of Hoogstraal\textsuperscript{18}, Morel\textsuperscript{19} and Matthysse and Colobo\textsuperscript{20}. Identified ticks were counted and recorded by species. The count from half body of each animal was doubled so as to give the total number of ticks per animal, assuming equal number of infesting ticks on both sides of the animal.

**Test serum collection**

Blood sample from each animal was collected in April 2002 by jugular venopuncture. The samples were then transported to the NAHRC laboratory at ambient temperature and were allowed to stand overnight and centrifuged for ten minutes at 20,000 rpm. Serum from each sample was separated and kept in sterile, labelled epindoroff tubes at 4°C overnight and then stored at -20°C until use.

**Control sera collection**

Blood was drawn from ten calves not previously exposed to ticks. The calves belonged to the International Livestock Research Institute’s herd at the Debre Zeit station. The serum was collected as above and was stored until use as negative control.
Serum antibody levels against Amblyomma variegatum larval antigens

Antigen preparation
Antigen from A. variegatum larvae was prepared according to the method described by Fivaz et al. 21. Approximately 1000 unfed, laboratory bred larvae were brought from NAHRC laboratory and washed by centrifugation in 100% ethyl alcohol followed by a wash in PBS, pH 7.2. The larvae were then macerated in a tissue grinder to which was added 3ml of PBS. The suspension was centrifuged at 10,000g for 1 hour at 4°C. The supernatant was then drawn out and divided into 0.5ml aliquots. The protein content of the larval antigen was determined by Lowry assay of protein concentration determination using BSA (bovine serum albumin) as a standard 22.

The absorbance of the test sample antigen was plotted as a function of BSA concentration and the best-fit line was calculated. Using the average absorbance for the three samples, the concentration was read from the plot. The aliquots of the antigens were stored at -20°C until use in the assay.

Determination of IgG levels
Optimal dilutions for antigen, serum and conjugate were initially determined by chequerboard titration 23. The optimal dilution was found to be 1:40 in PBS (PH 7.2 ± 0.2) for the antigen, 1:40 in 3% skimmed milk for the test and control sera and 1:20,000 in PBS-T for the conjugate. The test was performed according to the procedure of indirect ELISA for T. parva described by Katende et al. 24. ninety six (96)-well flat-bottomed polystyrene microtitre plates (Nunc Immulon, Denmark) were coated overnight at 4°C with 100 µl/well of 340µg/ml crude larval homogenate diluted appropriately. The plates were emptied by flicking the contents into a sink followed by washing manually with PBS-T once. Blocking was performed by adding 300 µl/well of 0.5% BSA in PBS and incubating the plates for 30 minutes at 37°C. The plates were washed three times with PBS-T, flicked and blotted on paper towel. 100 µl of diluted test sera were added in duplicate wells and the control sera (strong positive, weak positive, negative and conjugate controls), in 4 replicates and then incubated for 40 min at 37°C. The plates were washed four times as described above and immediately refilled with wash buffer and left for 10 minutes to be soaked at room temperature. After soaking, the wash buffer in the wells were flicked and blotted as before. Appropriately diluted Horseradish peroxidase (HRP)-conjugated rabbit Anti-bovine IgG (FAO/IAEA), in PBS-T was then added to all wells (100 µl/well) and incubated for 40 min at 37°C. Following incubation, plates were washed four times and soaked as described above. The HRP activities were detected by adding 150 µl/well of equal volumes of substrate (H₂O₂) and chromogen (TMB) and incubating the wells at 37°C for 15 minutes. The reaction was stopped by adding 100 µl/well of 1M H₃PO₄.

The enzyme activities were measured spectrophotometrically at 450nm in ELISA reader connected to a personal computer and the readings were transferred to the PC in a program called ProComm for Windows (version 2.4.2). The absorbance values were used as a measure of IgG levels directly.

Data analysis
The presence or absence of significant difference in the overall tick burden and the burden of A. variegatum between the breeds was analysed by employing GLM (General linear model) univariate analysis at P< 0.001 and 0.05 levels of significance respectively. The mean level of anti A. variegatum IgG in each breed of cattle was obtained from the
absorbance values and comparison was done using One Way Analysis of Variance (ANOVA) followed by Student-Neuman-Keuls test on SPSS programme. The effect of breed, season and the interaction of the two on the different stages tick burden was analysed by employing SAS Version 6 statistical software.

Results

Comparison of tick burden in the four breeds

Twelve tick species that belong to the genera *Amblyomma*, *Boophilus*, *Rhipicephalus*, *Hyalomma* and *Haemaphysalis* were collected from the experimental animals at different collections (Table 1). The mean ± S.E counts of the overall ticks indicated that the tick burden in Sheko breed was the highest of all (mean tick count per month 16 ± 4.1), followed by Abigar (mean tick count 15 ± 3.4), Gurge (mean tick count 14 ± 2.6) and Horro (mean tick count 12 ± 1.7), in decreasing order of tick burden (Fig. 2). Upon comparing the means on log-transformed counts, the Horro breed harboured significantly the lowest burden (P< 0.001) and Sheko and Abigar breeds harboured the highest tick burdens (P< 0.001). The tick burden of the Gurage was intermediate (P< 0.001). Significant differences were also found in the burden of *Amblyomma variegatum* ticks between Horro and Sheko breeds (P< 0.05) with mean ± SE count of 9 ± 3.4 and 20 ± 5.6 ticks respectively while in Abigar and Gurage breeds, intermediate burden of *A. variegatum* was found with mean and SD of 16 ± 3.2 ticks per month (Fig. 3).

The general pattern of abundance of all the ticks over the study period shows that ticks were abundant from January to May (dry season), when temperature was higher and rainfall lower and abundance was low from June to October, when rainfall was higher (wet season) (Fig. 5). The effect of season is found to be highly significant for variations in tick burden of all stages in all the breeds (Table 2).

![Figure 2](image-url)  
**Figure 2.** Mean count of the total ticks in each breed of the animals over the study period (Jan Oct 2002)
Comparison of serum IgG against Amblyomma variegatum tick species

The mean values of IgG level measurements in the four breeds of cattle showed the level of serum IgG that reacts against the crude homogenates of A. variegatum to be the highest in Horro than the rest of the breeds (Fig. 4) (P<0.05). No significant difference was found between Abigar, Gurage and Sheko breeds with respect to IgG concentration in the serum.

<table>
<thead>
<tr>
<th>Tick species</th>
<th>Abigar</th>
<th>Gurage</th>
<th>Horro</th>
<th>Sheko</th>
<th>Total Count (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amblyomma variegatum</td>
<td>3690</td>
<td>3717</td>
<td>1814</td>
<td>4956</td>
<td>14163 (39.51)</td>
</tr>
<tr>
<td>A. cohaerens</td>
<td>1060</td>
<td>878</td>
<td>531</td>
<td>1344</td>
<td>3765 (10.50)</td>
</tr>
<tr>
<td>Boophilus decoloratus</td>
<td>4974</td>
<td>2920</td>
<td>1632</td>
<td>4432</td>
<td>14016 (39.10)</td>
</tr>
<tr>
<td>Rhipicephalus evertsi evertsi</td>
<td>675</td>
<td>268</td>
<td>576</td>
<td>903</td>
<td>2395 (6.68)</td>
</tr>
<tr>
<td>R. praetextatus</td>
<td>352</td>
<td>374</td>
<td>159</td>
<td>528</td>
<td>1388 (3.87)</td>
</tr>
<tr>
<td>R. pravus</td>
<td>18</td>
<td>7</td>
<td>6</td>
<td>2</td>
<td>33 (0.09)</td>
</tr>
<tr>
<td>R. bergeoni</td>
<td>4</td>
<td>13</td>
<td>4</td>
<td>5</td>
<td>26 (0.07)</td>
</tr>
<tr>
<td>R. lunulatus</td>
<td>7</td>
<td>2</td>
<td>10</td>
<td>2</td>
<td>21 (0.06)</td>
</tr>
<tr>
<td>R. muhsame</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3 (0.01)</td>
</tr>
<tr>
<td>Hyalomma marginatum rufipes</td>
<td>13</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>25 (0.07)</td>
</tr>
<tr>
<td>Haemaphysalis aciculifer</td>
<td>2</td>
<td>4</td>
<td>0</td>
<td>4</td>
<td>10 (0.03)</td>
</tr>
<tr>
<td>Total</td>
<td>10798</td>
<td>8187</td>
<td>4736</td>
<td>12180</td>
<td>35845 (100.00)</td>
</tr>
</tbody>
</table>

Table 1. Total tick counts on the four cattle breeds between January and October 2002 (expressed as ticks collected for 15 animals per breed)
Table 2. Least square means (and standard errors) of tick counts by breed and season

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Adult male</th>
<th>Adult female</th>
<th>Larvae</th>
<th>Nymph</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>34.5±1.91</td>
<td>25.29±1.86</td>
<td>25.58±2.85</td>
<td>57.59±2.94</td>
<td>142.64±6.84</td>
</tr>
<tr>
<td>C.V. (%)</td>
<td>145.42</td>
<td>190.38</td>
<td>283.95</td>
<td>152.50</td>
<td>137.56</td>
</tr>
<tr>
<td>Breed</td>
<td>***</td>
<td>***</td>
<td>NS</td>
<td>NS</td>
<td>***</td>
</tr>
<tr>
<td>Abigar</td>
<td>42.71±3.82a</td>
<td>35.95±3.72a</td>
<td>27.39±2.56</td>
<td>64.20±5.88</td>
<td>170.22±13.88a</td>
</tr>
<tr>
<td>Guraghe</td>
<td>32.24±3.82a</td>
<td>22.34±3.72a</td>
<td>19.52±2.56</td>
<td>56.61±5.88</td>
<td>129.51±13.88a</td>
</tr>
<tr>
<td>Horro</td>
<td>18.42±3.82a</td>
<td>13.04±3.72a</td>
<td>20.70±2.56</td>
<td>49.01±5.88</td>
<td>101.14±13.88a</td>
</tr>
<tr>
<td>Sheko</td>
<td>44.76±3.82a</td>
<td>29.84±3.72ab</td>
<td>34.69±2.56</td>
<td>60.33±5.88</td>
<td>169.65±13.88a</td>
</tr>
<tr>
<td>Season</td>
<td>***</td>
<td>***</td>
<td>NS</td>
<td>NS</td>
<td>***</td>
</tr>
<tr>
<td>Dry</td>
<td>49.35±2.70</td>
<td>37.58±2.63</td>
<td>45.44±4.03</td>
<td>107.77±4.16</td>
<td>239.80±9.67</td>
</tr>
<tr>
<td>Wet</td>
<td>19.71±2.70</td>
<td>13.00±2.63</td>
<td>5.71±4.03</td>
<td>7.4±1.16</td>
<td>45.47±9.67</td>
</tr>
</tbody>
</table>

Interaction

<table>
<thead>
<tr>
<th>Breed x Season</th>
<th>Adult male</th>
<th>Adult female</th>
<th>Larvae</th>
<th>Nymph</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abigar x Dry</td>
<td>64.03±5.41</td>
<td>58.23±5.26a</td>
<td>51.00±8.05</td>
<td>121.15±8.32</td>
<td>294.40±19.35a</td>
</tr>
<tr>
<td>Abigar x Wet</td>
<td>21.39±5.41</td>
<td>13.67±5.26b</td>
<td>3.77±6.05</td>
<td>7.25±8.32</td>
<td>46.04±19.35b</td>
</tr>
<tr>
<td>Guraghe x Dry</td>
<td>45.12±5.41</td>
<td>30.16±5.26c</td>
<td>32.72±8.05</td>
<td>106.45±8.32</td>
<td>213.12±19.35c</td>
</tr>
<tr>
<td>Guraghe x Wet</td>
<td>19.36±5.41</td>
<td>14.52±5.26d</td>
<td>6.32±8.05</td>
<td>7.17±8.32</td>
<td>45.91±19.35d</td>
</tr>
<tr>
<td>Horro x Dry</td>
<td>25.97±5.41</td>
<td>18.59±5.26e</td>
<td>35.87±8.05</td>
<td>91.59±8.32</td>
<td>171.85±19.35e</td>
</tr>
<tr>
<td>Horro x Wet</td>
<td>10.88±5.41</td>
<td>7.51±5.26f</td>
<td>5.53±6.05</td>
<td>6.43±8.32</td>
<td>30.44±19.35f</td>
</tr>
<tr>
<td>Sheko x Dry</td>
<td>62.30±5.41</td>
<td>43.36±5.26g</td>
<td>62.17±8.05</td>
<td>111.91±8.32</td>
<td>279.84±19.35g</td>
</tr>
<tr>
<td>Sheko x Wet</td>
<td>27.23±5.41</td>
<td>16.32±5.26h</td>
<td>7.21±8.05</td>
<td>8.76±8.32</td>
<td>59.49±19.35h</td>
</tr>
</tbody>
</table>

*P<0.05; **P<0.01; ***P<0.001; ****P<0.0001; NS, not significant (P>0.05)

Figure 3 Mean counts of Amblyomma variegatum ticks in the four breeds of cattle over the study period (Jan - Oct 2002)
Figure 4. Concentration of IgG antibodies in the four breeds of cattle against *A. variegatum* crude larval homogenates

Figure 5. Pattern of abundance of tick species on the sample herd with respect to rainfall and minimum temperature in Ghibe/ Tolley station over the study period (Jan - Oct 2002)

**Discussion**

Field collections of ticks from the four breeds showed that the most common species of ticks were *Amblyomma variegatum*, *A. cohaerens*, *Boophilus decoloratus* and *Rhipicephalus evertsi evertsi*. The burden of these species was lower in Horro than the other three breeds. This result was supported by previous work, where Horro cattle breed were found to harbour less tick numbers than Boran and their crosses with various exotic breeds and thus be more
resistant\textsuperscript{10}. Animals develop resistance to ticks after natural infestation or after immunization with tick-derived antigens. Both antibody and cell-mediated immunity are involved but the degree of either immune response may depend on the host and the tick species\textsuperscript{25}. The present study revealed that the different breeds exhibited different magnitudes of antibody responses to tick infestations, which showed that the host immune response plays a partial protective role in acquisition of resistance. Assessment of the magnitude of humoral immune responses also revealed that the level of antibody responses to \textit{A. variegatum} was significantly higher in Horro breed compared to the other breeds. In April the population of \textit{A. variegatum} was high on the animals. Nevertheless, individual animals of the Horro breed were observed to maintain low burden and had high concentration of IgG. In contrast individual Sheko animals harboured significantly higher burden of \textit{A. variegatum} and lower IgG concentration. The production of antibody in response to tick infestation has been reported in a number of tick host associations. Barriga\textsuperscript{26} observed that substances extracted from tick salivary glands stimulate antibody response in the host, suggesting the contribution of humoral immunity to tick resistance. Rechav\textsuperscript{27} demonstrated the presence of negative correlation between mean weight of engorged female ticks and the level of serum gamma globulins in the host, suggesting the role of antibodies in bovine resistance to ticks. In contrast to our finding, studies on comparison of parasite burdens between N’ Dama and Zebu cattle showed no significant difference in antibody levels to adult and nymphal \textit{A. variegatum} antigens while positive correlation was observed between the level of \textit{eosinophilis} and resistance to tick infestation\textsuperscript{28}. Inverse relationship between resistance to \textit{L. ricinus} infestation and serum IgG levels in sheep was also observed, suggesting the shift of immune response to Th2 type for acquisition of resistance\textsuperscript{29}.

The reliability of homogenates from unfed ticks as antigen was reported by Smith \textit{et al.}\textsuperscript{25}. Regarding specificity of the responses in different stages of ticks, Jongejan \textit{et al.}\textsuperscript{30} showed that in rabbits, successive infestation by nymphs of \textit{R. appendiculatus} resulted in reduction in mean engorgement weights of adult \textit{R. appendiculatus} ticks, suggesting that immunity to infestation induced by feeding ticks of one stage can confer resistance to other tick instars. Furthermore, Fivaz \textit{et al.}\textsuperscript{21} demonstrated a significant reduction in engorgement weight of nymphal stages of \textit{R. zambeziensis} by repeated infestation of rabbits with the adult ticks.

\textbf{Conclusion}

The results of this study suggest that among the four indigenous cattle breeds, the Horro breed shows resistance to tick infestations and the possible factors for the exhibited resistance include host immunological factors, specifically antibodies. Future studies on the determination of level of resistance of cattle to tick infestation should include other immunological parameters such as level of \textit{eosinophilis} and test for the presence of delayed type hypersensitivity reactions on feeding sites of the ticks, since these factors have been considered to be related with host resistance to ticks as well as other parasites. The effect of immunological factors on fertility of female ticks should also be assessed in terms of difference in
engorgement weight, number of eggs produced and their hatchability. Moreover, long term population dynamics study of the present tick species should be carried out in order to have a consistent rank of tick burden in the individual animals. It would also help to achieve selection of individuals with factors for tick resistance.

Acknowledgements

We thank Dr. Workneh Ayalew and Dr. Markos Tibbo for comments on the manuscript and statistical analysis using SAS. We would also like to thank the EARO-ILRI Trypanotolerance project for providing the necessary facilities to carry out this research and Dr. Azage Tegene, manager of ILRI Debre Zeit research station for his permission to collect serum from immunologically naive calves.

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EVALUATION OF SELENIUM SUPPLEMENTATION ALONE, A COMBINATION OF SELENIUM AND VITAMIN E AND COMBINATION OF SELENIUM AND LOW DOSE DIMINAZINE ACETURATE IN TRYPANOSOMA BRUCEI INFECTED RATS

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EVALUATION DE LA COMPLEMENTATION DE SELENIUM SEUL, D'UNE COMBINAISON DE SELENIUM ET DE VITAMINE E ET D'UNE COMBINAISON DE SELENIUM ET D'UNE FAIBLE DOSE D'ACETURATE DE DIMINAZENE CHEZ LES RATS INFECTES PAR TRYPANOSOMA BRUCEI

Résumé

L'effet de la complémentation de sélénium seul, des combinaisons de sélénium / vitamine E ou sélénium / faible dose d'acéturate de diminazène (bérenil) a été étudié chez les rats infectés par Trypanosoma brucei brucei. L'effet de la complémentation de sélénium et des combinaisons a été évalué à l'aide des paramètres ci-après : la parasitémie, les changements de poids vif, la température, l'hématocrite, le dénombrement de globules rouges, le dénombrement total de leucocytes et la numération leucocytaire différentielle. Le sélénium seul et sa combinaison avec la vitamine E n'ont pas éliminé l'infection ; et lorsqu'il était combiné avec une faible dose de bérenil, il n'a pas pu prolonger l'intervalle de rechute. La complémentation de sélénium n'a pas beaucoup augmenté les valeurs de l'hématocrite et des globules rouges (P>0,05). Les combinaisons sélénium / vitamine E et bérenil / sélénium ont considérablement accru la numération de leucocytes (P<0,05). La complémentation de sélénium n'a pas réduit la température rectale des rats complémentés. L'infection n'a pas apporté de changement majeur (P=0,05) au poids des rats nourris de sélénium. Les leucocytes neutrophiles et les éosinophiles se sont accrus, mais les lymphocytes et les monocytes ont énormément augmenté (P<0,05). On peut donc conclure que le sélénium seul et les combinaisons sélénium / vitamine E ou sélénium/ faible dose de bérenil ont un effet bénéfique pour le traitement de la trypanosomose.

Mots-clés: Trypanosoma brucei, bérenil, sélénium, vitamine E, hématologie, rats.

Summary

The effect of selenium supplementation alone and a combination of selenium and Vitamin E or low dose diminazene aceturate (berenil) was investigated in Trypanosoma brucei brucei infected rats. The effect of the selenium supplementation and its combinations were assessed using the parasitaemia, changes in body weight, temperature, packed cell volume (PCV), red blood cell (RBC) count, total leucocyte count and differential leucocyte count. Selenium alone and its combination with Vitamin E could not clear the infection and when it was combined with low dose berenil, it could not prolong the relapse interval. Selenium supplementation could not improve PCV and RBC values significantly (P>0.05). Combination of selenium and vitamin E and combination berenil and selenium improved the WBC counts significantly (P<0.05). Supplementation with selenium did not reduce the rectal temperature of the supplemented rats. The infection did not cause any significant (P=0.05) change in weight of rats fed selenium. The neutrophils and the eosinophils showed increase, but lymphocytes and monocytes increased significantly (P<0.05). It could therefore be concluded that selenium alone and selenium in combination with vitamin E or with low dose berenil has some beneficial effect in management of trypanosomosis.

Key-words: Trypanosoma brucei, berenil, selenium, vitamin E, haematology, rats.

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Introduction

Trypanosome infection has been associated with oxidative stress caused by an increased production of pro-oxidants. Pathology of oxidative stress is caused by aggregation of key biomolecules within cell membranes and in other macromolecules like nucleic acid, as well as protein and carbohydrate moieties. They are highly susceptible to radical reaction. Infection with T. b. brucei has been reported to produce nitric oxide dependent and nitric oxide independent mechanism, and increased nitric oxide production in velvet monkey infected with T. b. gambiense.

Trypanosomes are also known to deplete tissue antioxidants. Nyden reported depletion of tissue ascorbic acid in acute phase of T. brucei infection in rats. Iheidoa and Anwa also reported a decrease in liver ceratinoid concentration following T brucei infection in rats. The depletion in systemic antioxidants thus undercuts the defense system against radicals thereby strengthening the belief that oxidative damage of membranes plays a significant role in cellular injury in trypanosome infection.

Trypanosome infection has been shown to cause immunosuppression of the host. It has been shown to cause depression in the host ability to respond to various antigens and mitogens and in secondary antibody / humoral responses to immunization. Immunosuppression is thought to be responsible for hosts’ inability to clear trypanosomes after administration of trypanocidal drugs.

Due to consequences of trypanosomosis, such as immunosuppression and cellular damage, it may be necessary to investigate the use of immunostimulants and antioxidants in enhancing the performance of trypanosome infected animals. Selenium, a micronutrient, has been shown to possess immunostimulatory and antioxidative qualities. Vitamin E is an antioxidant and immunostimulant.

This study is therefore aimed to evaluate the effect of selenium supplementation alone and combination of selenium with Vitamin E or selenium with low dose of diminazine aceturate in the course of T. brucei infection in rats.

Materials and methods

Thirty-six adult albino rats were used for the study. The rats were kept in wire bottomed rat cages and were given feed and water ad libitum.

Trypanosoma brucei isolated from a dog was used for the experiment. The specie of the trypanosomes was identified by wet mount and Giemsa stained thin blood smear preparation. Each experimental rat was given 1.54 x 105 trypanosomes in 0.5 ml saline diluted blood, intraperitoneally.

Diminazene Aceturate (Berenil®, Hoechst, Ireland) was used. The diminazene aceturate was given intraperitoneally at dose rate of 3.5mg per kg body weight.

Selenium, as Sodium Selenite and Vitamin E were manufactured by Biorganics Nigeria Limited, Ikeja-Lagos, Nigeria. The selenium and vitamin E were given through drinking water at a dose rate of 10 microgram per kg body weight and 12.5mg per rat respectively.

The rats were divided into 6 groups of 6 rats each and were kept in cages A, B, C, D, E and F. The groups were treated as follows;

Group A: Infected with T. brucei and treated with selenium for 2 weeks from day 7 to day 14 post infection (PI).

Group B: Infected and treated with Diminazene aceturate on day 7 PI.
Group C: Infected and treated with Diminazene aceturate on day 7 and selenium from day 7 to day 21 PI. Group D: Infected and treated with selenium and vitamin E from day 7 to day 21 PI. Group E: Infected but not treated (positive control) Group F: Not infected and not untreated. (negative control).

Rectal temperature, PCV, RBC count, total WBC count, differential WBC count, parasitaemia, body weight and survivability were monitored. The parameters were measured on day 0 and subsequently at 7 days intervals post infection. 1ml of blood was collected from each rat through the medial canthus of the eye in EDTA bottle. Detection of parasites using the microhaematocrit buffy coat method. The Packed cell volume, Red blood cell count and Total white blood cell count were determined as described by coles. The body weight was determined by use of the simple weighing balance. The differential WBC count was determined using the Leishmann stained slide, while the rectal temperature was obtained by inserting a clinical thermometer into the rectum and allowing it to stand for two minutes.

The statistical analysis was done using the Analysis of variance (ANOVA) method. Means were compared using the Least Significant Difference (LSD) method.

**Result**

After treatment (Table 1), a period of parasitaemia was only observed in Diminazene aceturate (group A) and Diminazene aceturate and Selenium (group B) treated groups. Relapse, however, occurred in both groups by day 21-post infection (PI). The Selenium only and

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<th>Exptl. period (days)</th>
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* Day treatment commenced
Figure 1: Weekly mean packed cell volume (%) of *Trypanosoma brucei* infected rat groups treated with (a) Selenium only, (b) Diminazene aceturate only, (c) Diminazene aceturate and Selenium, (d) selenium and Vitamin E, (e) Infected untreated, and (f) Control (uninfected untreated).

Figure 2: Weekly mean erythrocyte counts of *Trypanosoma brucei* infected rat groups treated with (a) Selenium only, (b) Diminazene aceturate only, (c) Diminazene aceturate and Selenium, (d) selenium and Vitamin E, (e) Infected untreated, and (f) Control (uninfected untreated).
Selenium + Vitamin E treatments could not clear the parasitaemia. The rats started to die on day 14 PI. The Selenium treated group lost a rat on day 14 PI. The Selenium and Vitamin E group and the infected untreated group lost two rats each also on day 14 PI. By day 35 PI, all the rats is the Selenium only treated group and the infected untreated group had all died. The Diminazene aceturate only, Diminazene aceturate and Selenium, and Selenium and Vitamin E treated groups lost 3, 2 and 5 rats respectively by day 35 PI.

The PCV of uninfected group was significantly (P<0.05) higher than infected groups on days 7 and 14 PI (fig 1). Following commencement treatment on day 7 PI, the PCV of the uninfected untreated groups was similar to those of the treated groups by day 21 PI with the exception of Selenium +Vitamin E group. Subsequently, the PCV of all the treated groups were similar and differed from the uninfected untreated group except for Diminazene aceturate and Selenium group and Selenium only group on days 28 and 35 PI respectively.

There was a marked fall in RBC counts in the infected rats before treatment (fig. 2). The mean RBC of infected rats showed a significant (P<0.05) reduction by day 7 PI when compared with the uninfected untreated group. Unlike the groups treated with Selenium only and Selenium + Vitamin E, there was a significant (P<0.05) improvement in the RBC count of rats treated with Diminazene aceturate only and Diminazene aceturate and Selenium group between days 21 to 35 PI.

The mean WBC counts was significantly (P<0.05) increased by day 7

Figure 3: Weekly mean total leukocyte counts of *Trypanosoma brucei* infected rat groups treated with (a) Selenium only, (b) Diminazene aceturate only, (c) Diminazene aceturate and Selenium, (d) selenium and Vitamin E, (e) Infected untreated, and (f) Control (uninfected untreated)
PI in the Selenium only group (fig; 3). Following treatment, the mean leucocyte count was significantly (P<0.05) higher by days 14 and 21 respectively in the Selenium and Vitamin E and Diminazene aceturate and Selenium groups.

There was a rise in temperature following infection (fig 4). However, after treatment, the Diminazene aceturate treated groups had their temperature similar to the uninfected control group by days 14 and 28 PI. The infection did not cause any significant change in the body weight (fig 5).

Experimental infection of rats with *T. brucei* caused a rise in mean lymphocyte count by day 7 PI. The uninfected untreated group was significantly (P<0.05) lower than the Diminazene aceturate only, Diminazene aceturate and Selenium and infected untreated groups by day 7 PI and with Selenium only treated group on day 21 PI. Infection of rats with *T. brucei* did not produce a significant increase in the mean neutrophil count. Also, the infection did not produce a significant change in the mean eosinophil count at days 7 and 14 PI, but on day 21 PI there was a significant (P<0.05) reduction in eosinophil count of infected untreated group. Monocyte count showed that there was no significant difference between the infected groups and the uninfected control. The infected untreated control however maintained a progressive increase in the mean monocyte count as the disease progressed.

**Discussion**

The parasitaemia was cleared following treatment with a diminazene aceturate whereas Selenium only and Selenium + Vitamin E treatment could not clear the

![Mean temperature (°C) vs. Experimental period (days)](image)

**Figure 4:** Mean weekly temperature of *Trypanosoma brucei* infected rat groups treated with (a) Selenium only, (b) Diminazene aceturate only, (c) Diminazene aceturate and Selenium, (d) selenium and Vitamin E, (e) Infected untreated, and (f) Control (uninfected untreated)
parasites. The short period of aparasitaemia could be attributed to the low dose of drug used (Berenil at dose rate of 3.5mg/kg Bdw). Relapse occurred in the trypanocide treated groups. Relapse after treatment has been reported by earlier workers\textsuperscript{21,22}. Relapse is usually considered to indicate resistance to the drug under test at the dose rate employed\textsuperscript{23}. Also Selenium supplementation failed to extend the period of aparasitaemia in diminazene aceturate and Selenium group. This and the failure of Selenium only and Selenium + Vitamin E groups to clear the parasites may be due to antigenic variation exhibited by \textit{T. brucei}\textsuperscript{24,25}. Donelson\textsuperscript{25} reported that trypanosomes evade immune response continually switching from the expression of one variant surface glycoprotein (VSG) on their surface to the expression of another immunologically distinct VSG.

Besides antigenic variation, the supplementation with Selenium and Vitamin E seven days PI could not have provided adequate time for the body immune system to be fully stimulated. Muray et al\textsuperscript{26} reported that administration of an immunostimulant before infection with \textit{T. brucei} or \textit{T. congolense} led to an increase in survival time of mice Selenium is known to be a major component of glutathione peroxidase and needs to be incorporated during haemopoiesis\textsuperscript{27,28}. The selenoenzyme, glutathione peroxidase, is present in cells and in plasma and removes hydrogen peroxides and free hydroperoxides\textsuperscript{29}. The
antioxidative effect of Selenium alone and in combination with Vitamin E and the trypanocid did not produce any significant effect on the parasitaemia.

The prolonged survival rate in the supplemented groups over the infected untreated group can be attributed to the protective effect of Selenium on the immune system of the rats. Destowitz and Barnwell reported that Selenium supplementation during malarial vaccination conferred significantly higher survival rate when mice were challenged with Plasmodium berghei.

Packed cell volume (PCV) and total red blood cell (RBC) count were used to assess anaemia. The mean PCV and total RBC counts dropped following infection in all the infected groups. The fall in mean PCV and RBC count were in agreement with earlier reports that trypanosomosis caused anaemia. The decline however improved following short period of a parasitaemia created by trypanocidal treatment on day 7 PI. The Selenium supplemented groups showed improvement in the PCV and RBC value (though not significant) over the infected but untreated group. The improvement may be attributed to the antioxidative and immunostimulatory effects of Selenium and Vitamin E. Selenium protects cells from peroxide damage thereby maintaining their cellular integrity. It also has its ability to enhance the immune state. Sherman and Halliquist reported that peroxidative haemolysis of the RBC was prevented by supplementation with antioxidant in the diet.

The infection did not cause significant change in leucocyte count. This does not agree with most reports that trypanosomosis cause leucopenia. However, Anosa and Kaneko reported leucocytosis in T. brucei infected deer mice. Also, Ndoutamia reported a rise in WBC in Sahelian goat infected with T. congolense. Edward et al did not observe any uniform pattern of leucocyte change which in agreement with the result of this experiment appears to support.

The rise in temperature following infection agree with other reports that trypanosomosis leads to rise in temperature. The increase in temperature has been attributed to trypanosome activity. The infection of rats did not result in weight loss contrary to the reports of other workers. The inability of the infection to affect the weight may be due to the acute course of the infection ran in these rats.

The infection also led to marked increase in lymphocyte count. Most researchers have reported decreased lymphocyte count in trypanosome infection. Increased numbers of lymphocytes have been associated with T. brucei infection of highly tolerant deer mice and with human trypanosomosis. The high lymphocyte count in treated group in this experiment when compared with the infected untreated and uninfected untreated groups could be attributed to the effect of Selenium. Selenium deficiency has been found to inhibit proliferation of T and B lymphocyte and response to mitogens and also in cytodestruction of T lymphocyte and natural killer lymphocyte. The infection did not produce any significant change in the neutrophil count. Neutropenia has been reported in the early acute or sub-acute phase of trypanosome infection but neutrophilia was recorded in the highly tolerant mice. Wintrobe attributed neutropenia to toxic depression of bone marrow and trapping of neutrophils in the spleen as part of the hypersplenism syndrome.

The eosinophil count was decreased with the infected rats being significantly (P<0.05) lower on day 21 PI. Eosinopenia
has been reported in several trypanosome infections\textsuperscript{36,37,42}. The eosinopenia is presumably due to depression of the granulocyte precursor in the bone marrow by trypanosome toxins.

The infection of rats with \textit{T.b.brucel} produced a significant increase in monocyte count. The result is in agreement with other reports that monocytosis is a consistent finding in trypanosomiasis\textsuperscript{36,37,43}. This has been linked with marked proliferation of macrophages in the tissue of infected animals, including the spleen, lymph nodes, kidney, testis, heart, and brain\textsuperscript{37}.

The supplementation with selenium alone and in combination with Vitamin E or low dose diminazene aceturate appear to have some beneficial effect on the course of \textit{T. brucel} infection.

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EVALUATION OF LIMITING AMINO ACIDS IN TILAPIA DIETS BASED ON SUNFLOWER CAKE

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EVALUATION DE LA REDUCTION DES ACIDES AMINES DANS LES ALIMENTS DE TILAPIA À BASE DE TOURTEAU DE TOUNESOL

Résumé

L'objet de l'étude était de déterminer les effets de la complémentation des aliments à base de tourteau de tournesol avec des acides aminés (lysine, méthionine et thréonine) sur la performance du tilapia *O. niloticus*. Chacun des trois acides aminés était complémenté, soit seul, soit dans diverses combinaisons, à l'aliment de base dans lequel le tourteau de tournesol à fibre réduite fournissait 80% de la protéine alimentaire. Toutes les rations expérimentales étaient isoénergétiques et elles étaient servies aux groupes de poisson de trois manières (poids initial : 24 + 0,59g) pendant 39 jours.

Le poisson nourri de ration de base (sans complémentation) avait un taux de croissance spécifique (% par jour) de 1,94 comparé à 2,05 ; 1,96 et 2,05 pour les poissons nourris d'aliments complémentés avec de la lysine, de la méthionine ou de la thréonine respectivement.

Les poissons nourris de rations de base complémentées avec diverses combinaisons de lysine / méthionine, lysine / thréonine, méthionine / thréonine avaient des taux de croissance de 2,17 ; 2,14 et 1,97 respectivement.

Les poissons nourris de ration-témoin (dans laquelle la farine de poisson et la farine de soja fournissaient la majeure partie de la protéine alimentaire) avaient le taux de croissance le plus élevé (2,28% par jour), tandis que ceux nourris de ration complémentée avec la combinaison des trois acides aminés avaient un taux de croissance de 2,08% par jour. Il y avait une tendance à l'amélioration du taux de conversion alimentaire avec la complémentation avec la lysine ou la thréonine seule, ou avec la lysine combinée avec la méthionine ou la thréonine.

Mots-clés : Lysine, méthionine, thréonine, tourteau de tournesol.
Summary

The objective of the study was to determine the effects of supplementing diets based on sunflower cake with amino acids lysine, methionine and threonine on the performance of tilapia *O. niloticus*. Each of the three amino acids was supplemented either sole or in various combinations to a basal diet in which a fibre-reduced sunflower cake provided 80% of the dietary protein. All experimental diets were isonitrogenous and isoenergetic and were fed to triplicate groups of fish (initial weight of 24 + 0.59 g) for 39 days.

Fish fed on the basal diet (without supplementation) had a specific growth rate (% per day) of 1.94 compared to 2.05, 1.96 and 2.05 of those fed on diets supplemented with either of lysine, methionine or threonine respectively.

Fish fed basal diets supplemented with combinations of lysine- methionine, lysine - threonine and methionine- threonine had growth rates of 2.17, 2.14 and 1.97% respectively.

Those fed the positive control diet (in which herring fishmeal and soybean meal provided most of the dietary protein) had the highest growth rate (2.28% per day), while those supplemented with the combination of the three amino acids had a growth rate of 2.08%. There was a trend for improved feed conversion ratio with supplementation of lysine, and threonine alone, or lysine combined with either methionine or threonine.

Key-words: Lysine, methionine, threonine, sunflower cake.

Introduction

Fishmeal has been the most widely used protein source for many cultured fish species\(^1\). Due to its high cost, many attempts have been made to reduce its proportion in fish diets\(^2\, 3\, 4\). Many protein sources have been tested as complete or partial replacements for fishmeal. Jackson *et al.* demonstrated that certain plant protein sources could be used to meet much of the protein requirements of tilapia (*O. mossambicus*). In their study, one of the limiting factors when high dietary inclusion levels of plant proteins were tested related to a deficiency of certain essential amino acids, particularly lysine and methionine. Information on limiting amino acids in plant protein sources for tilapia species is inadequate, making it difficult to use free amino acids to supplement comparatively poor protein sources.

Sunflower seeds are important sources of edible oils and protein for inclusion in animal feeds. The meal resulting from the oil extraction process is a valuable source of protein, and has been tested in many animal diets. In fish, studies on supplementation of diets based on sunflower meal with crystalline amino acids have yielded conflicting results. In the European eel, (*Anguilla anguilla*), Hinguer *et al.* noted that the inclusion of sunflower meal as the only source of dietary protein resulted in poor growth, and that growth could be improved when sunflower meal was mixed with fishmeal or supplemented with essential amino acids. Contrary to these findings, Sanz *et al.*\(^7\) found no improvement in dietary protein utilization of rainbow trout when diets that contained 39% sunflower meal were supplemented with amino acids lysine,
leucine, and methionine.

The objectives of this experiment were to determine the most limiting amino acids in diets based on fibre-reduced sunflower cake fed to *O. niloticus* and to evaluate the effect of supplementing these diets with the foregoing limiting amino acids on fish performance.

**Materials and Methods**

Hybrid sunflower seeds (Kenya Fedha) were purchased from a commercial trader (Rift Valley Products, Nakuru, Kenya). The seeds were partly dehulled using a manually-operated Cecoco dehuller which incorporated a dehuller and a sorting machine and the oil was extracted using a laboratory Komet screw press.

A basal diet was supplemented with the synthetic amino acids, L-lysine HCL, DL-methionine, and L-threonine sole or in various combinations as shown in table 1.

The basal diet was formulated with herring meal and sunflower cake as main sources of protein (table2), while soybean meal replaced sunflower cake in the positive control diet.

All the diets were mixed using a small laboratory mixer and made into 3 mm dry pellets using an Ottevanger pelleting machine. Ingredients that made up less than 2% of the diet were premixed with fish meal before they were added into the mixer. All the diets were stored at room temperature (18 °C) until used.

**Experimental procedure**

Sex-reversed tilapia (*O. niloticus*) male fingerlings of mean initial weight 23.9 ± 0.6 g were purchased from the Sagana Government Fisheries Farm in Kenya and used for this experiment. They were acclimated to experimental conditions for a period of two weeks before the onset of the trial.

They were then weighed in groups of 16 fish that were selected at random, and allocated to experimental circular tanks each with a diameter of one metre and filled with water to a depth of 0.5 metres.

Each diet was fed to triplicate groups of sixteen (16) fish in a completely randomized experimental design, with a total of 48 fish per treatment. Water temperatures and dissolved oxygen concentrations in the tanks were maintained between 25°C and 28°C and above 5.5 mg L-1, respectively. Water was completely exchanged in each tank every 48 hours or when the dissolved

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<th>Table 1: Experimental design</th>
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<td><strong>Diets</strong></td>
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<tr>
<td><strong>Amino acids</strong></td>
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<td>Lysine</td>
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<td>Methionine</td>
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<td>Threonine</td>
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oxygen concentration in the tanks fell below 5 mg L⁻¹. Fish were fed to satiation 3 times daily. Feed intake for each group of fish was recorded daily, while water temperatures were taken 3 times a day. Fish were weighed individually at the beginning of the trial and 39 days later. They were starved for a period of 24 hours before each weighing.

**Data collection and analytical procedures**

The following parameters were used to assess fish growth and performance: absolute weights, weight gain, specific growth rate, feed intake, and feed conversion ratio.

Specific growth rate (% per day) was calculated as: 100*(ln final wt (g) − ln initial wt (g))/number of experimental days. Feed conversion ratio was calculated as dry feed ingested (g)/wet weight gain (g). Feed intake was computed as total feed consumed divided by the number of fish in each tank.

**Chemical analyses**

All ingredients and feed samples were ground using a Wiley mill with a 1mm sieve, and subsequently stored in sealed containers. Standard procedures were used to determine the concentrations of the various proximate constituents. For amino acids analysis, performic acid oxidation was performed prior to hydrolysis, to oxidize cystine and methionine to cysteic acid and methionine sulfone, respectively. Sodium metabisulfite was added to neutralize the performic acid. Amino acids were released

**Table 2: Ingredient compositions and chemical analyses of the experimental diets (g kg⁻¹; air-dry basis).**

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<th>9</th>
<th>Control</th>
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<td>Threonine</td>
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<td>1000</td>
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<td>1000</td>
<td>1000</td>
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</tr>
</tbody>
</table>

**Chemical analysis DM basis**

| Dry matter (g/kg)                  | 927   | 921   | 921   | 920   | 921   | 921   | 921   | 921   | 921   | 908      |
| Protein (g/kg)                     | 530   | 531   | 534   | 534   | 531   | 532   | 533   | 533   | 531   | 399      |
| Crude fat (g/kg)                   | 196   | 202   | 200   | 199   | 204   | 203   | 201   | 205   | 47    | 15       |
| Crude fibre (g/kg)                 | 90    | 90    | 90    | 90    | 90    | 90    | 90    | 90    | 90    | 90       |
| Calcium (g/kg)                     | 12    | 12    | 12    | 13    | 13    | 13    | 13    | 13    | 13    | 13       |
| Phosphorus (g/kg)                  | 16    | 16    | 17    | 16    | 17    | 17    | 16    | 16    | 15    | 15       |

¹Vitamin A, 6000 IU; Vitamin D₃, 600 IU; Vitamin E, 100 mg; Vitamin K₃, 3 mg; Vitamin B₁, 10 mg; Vitamin B₂, 20 mg; niacin, 150 mg; D-pantothenic acid, 50 mg; Vitamin B₆, 10 mg; Vitamin B₁₂, 0.03 mg; folic acid, 4 mg; biotin, 0.8 mg; choline, 600 mg; Vitamin C, 600 mg; inositol, 300 mg; manganese, 192 mg; iron 51.2 mg; copper, 6.4 mg; zinc, 57.6 mg; selenium, 0.15 mg; traces of cobalt and iodine.

²All values were determined by analysis except for DE, which was estimated from published data.

In calculating the DE contents of the diets, apparent digestibility coefficients for energy in the ingredients were estimated as follows: soybean meal 70%¹, low-fibre and high-fibre sunflower cakes, 44% and 30% respectively¹. Digestible energy contents of herring meal and wheat flour were taken as 16.8 and 17.5 MJ/kg DM¹², corn starchy, 11.34 MJ/kg DM (for channel catfish), and corn oil as 34.02 MJ/kg.¹²
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<tr>
<th>Amino acids</th>
<th>Asp</th>
<th>Glu</th>
<th>Ser</th>
<th>His</th>
<th>Gly</th>
<th>Thr</th>
<th>Cyst</th>
<th>Met</th>
<th>Arg</th>
<th>Val</th>
<th>5Phen</th>
<th>Ileu</th>
<th>Leu</th>
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<td>61.0</td>
<td>12.5</td>
<td>5.6</td>
<td>15.6</td>
<td>10.5</td>
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<td>20.7</td>
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<td>12.4</td>
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<td>11.7</td>
<td>9.9</td>
<td>6.1</td>
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<td>2 (+ Lys)</td>
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<td>55.3</td>
<td>12.2</td>
<td>6.8</td>
<td>16.7</td>
<td>10.5</td>
<td>6.8</td>
<td>8.0</td>
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<td>11.1</td>
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<td>3 (+ Meth)</td>
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<td>61.7</td>
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<td>7.2</td>
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<td>12.8</td>
<td>20.0</td>
<td>11.8</td>
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<td>4 (+ Thr)</td>
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<td>61.4</td>
<td>12.8</td>
<td>7.0</td>
<td>17.1</td>
<td>13.6</td>
<td>6.8</td>
<td>7.6</td>
<td>22.2</td>
<td>14.5</td>
<td>13.5</td>
<td>12.2</td>
<td>18.4</td>
<td>11.8</td>
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<td></td>
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<tr>
<td>5 (+ Lys &amp; Meth)</td>
<td>29.1</td>
<td>60.4</td>
<td>12.1</td>
<td>6.9</td>
<td>15.8</td>
<td>11.3</td>
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<td>10.3</td>
<td>22.1</td>
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<td>12.9</td>
<td>10.9</td>
<td>17.7</td>
<td>17.0</td>
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<td></td>
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<tr>
<td>6 (+ Lys &amp; Thr)</td>
<td>28.4</td>
<td>58.6</td>
<td>12.2</td>
<td>6.9</td>
<td>16.7</td>
<td>13.1</td>
<td>6.8</td>
<td>8.0</td>
<td>22.0</td>
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<td>13.3</td>
<td>11.3</td>
<td>17.6</td>
<td>17.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 (+Meth &amp; Thr)</td>
<td>28.6</td>
<td>58.3</td>
<td>12.2</td>
<td>7.2</td>
<td>17.3</td>
<td>13.6</td>
<td>6.7</td>
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<td>22.4</td>
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<td>13.4</td>
<td>11.5</td>
<td>19.1</td>
<td>15.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 (+Lys, Meth &amp; thr)</td>
<td>29.0</td>
<td>58.0</td>
<td>12.7</td>
<td>6.8</td>
<td>17.2</td>
<td>13.8</td>
<td>7.2</td>
<td>12.9</td>
<td>22.2</td>
<td>16.7</td>
<td>13.4</td>
<td>10.5</td>
<td>17.0</td>
<td>16.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9 (Positive control)</td>
<td>33.7</td>
<td>57.4</td>
<td>14.3</td>
<td>7.6</td>
<td>16.8</td>
<td>14.0</td>
<td>6.8</td>
<td>10.4</td>
<td>22.7</td>
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<td>9.9</td>
<td>16.3</td>
<td>17.1</td>
<td>9.1</td>
<td>4.7</td>
</tr>
</tbody>
</table>

* Tilapia requirement (g/kg of dry diet) - - - 5.2 - 11.2 - 8.0 12.6 8.4 11.3 9.3 10.2 15.4 - 3.0

* For diets containing 280 g/kg dietary protein

* The stated requirement for methionine is for diets containing at least 1.5 g/kg of cysteine.

* Tyrosine should be at least 5 g/kg of the dry diet

Table 3: Performance of fish (absolute weights, weight gains, specific growth rates (SGR), feed intakes and feed conversion ratios in relation to the diets

<table>
<thead>
<tr>
<th>Diet</th>
<th>Initial wt (g/fish)</th>
<th>Final wt (g/fish)</th>
<th>SGR (% per day)</th>
<th>Feed intake (g/fish)</th>
<th>FCR (feed/gain)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diet 1 (Basal)</td>
<td>23.7</td>
<td>50.4</td>
<td>0.03</td>
<td>30.1</td>
<td>1.94b</td>
</tr>
<tr>
<td>Diet 2 (+L)</td>
<td>29.4</td>
<td>56.4</td>
<td>0.06</td>
<td>32.6</td>
<td>1.96b</td>
</tr>
<tr>
<td>Diet 3 (+Met)</td>
<td>23.3</td>
<td>53.3</td>
<td>0.07</td>
<td>30.4</td>
<td>1.80b</td>
</tr>
<tr>
<td>Diet 4 (+Thr)</td>
<td>23.3</td>
<td>53.3</td>
<td>0.07</td>
<td>30.4</td>
<td>1.80b</td>
</tr>
<tr>
<td>Diet 5 (+L &amp; Met)</td>
<td>23.3</td>
<td>53.3</td>
<td>0.07</td>
<td>30.4</td>
<td>1.80b</td>
</tr>
<tr>
<td>Diet 6 (+L &amp; Thr)</td>
<td>23.3</td>
<td>53.3</td>
<td>0.07</td>
<td>30.4</td>
<td>1.80b</td>
</tr>
<tr>
<td>Diet 7 (+L, Met &amp; Thr)</td>
<td>23.3</td>
<td>53.3</td>
<td>0.07</td>
<td>30.4</td>
<td>1.80b</td>
</tr>
<tr>
<td>Diet 8 (Control)</td>
<td>23.3</td>
<td>53.3</td>
<td>0.07</td>
<td>30.4</td>
<td>1.80b</td>
</tr>
</tbody>
</table>

Means (n = 3) in a column that do not have a superscript or share a common superscript letter are not significantly different (P > 0.05).

from protein by hydrolysis with 6 N HCl. Individual amino acids were quantified using a HPLC\textsuperscript{15}. Tyrosine was destroyed by oxidation and tryptophan by hydrolysis, and hence their concentrations were determined using a different procedure.

**Statistical analyses**

Data on absolute weight, weight gain, specific growth rates, feed intake and feed utilization were subjected to analysis using PROC GLM of the SAS\textsuperscript{16}. An analysis of covariance was done using the initial weights as the covariate. The covariate was not significant in any of the parameters. Treatment means were compared using Tukey’s multiple range test with the level of significance set at P < 0.05.

**Results**

**Chemical composition of the diets**

The chemical compositions of the diets are presented in Table 2. The digestible energy contents of the diets based on sunflower cake were approximately 12.39 MJ per kilogram, while the control diet contained 13.36 MJ/kg of digestible energy per kilogram of the diet.

The determined protein contents in the diets were similar in each case. The crude protein contents of the diets were approximately 330 g/kg (DM basis) while the protein to energy ratio was 26.9 g protein/MJ of energy in diets based on sunflower cake and 25.48 g/MJ in the control diet.

The dietary contents of lysine, methionine and lysine expressed as grams per kg of the dry diet and the stipulated requirements for these amino acids in tilapia are presented in Table 3. The stated requirements are for diets containing at least
280 grams of protein per kilogram of the diet.

**Fish performance**

Data on fish performance are presented in Table 4. No mortality was observed during the experimental period. The specific growth rate for fish fed the positive control diet (diet 9) was higher than for fish fed the other diets. The differences, however, were only significant (P < 0.05) relative to those fed the basal diet alone, and diets 3 (basal diet + methionine) and diet 7 (basal diet + methionine and threonine).

Fish fed the positive control diet had an average growth rate of 2.28% per day, while those fed on the basal diet had a mean growth rate of 1.94% per day. Supplementing the basal diet with lysine and methionine (diet 5) improved growth rate by approximately 12%, while the addition of lysine and threonine (diet 6) improved the rate of growth by 10.3%. Supplementing the basal diet with methionine alone (diet 3) or with threonine (diet 7) improved fish growth marginally (1% and 1.5% respectively).

There were no significant differences in feed intake between fish fed the various diets showing that diets containing high levels of sunflower cake were acceptable to the fish. There was however a trend for improved feed conversion ratios for the fish fed the diets supplemented with amino acids compared to those fed the basal diet.

**Discussion**

All the diets met the stated requirements for energy and protein. Due to the low digestible energy content of sunflower cake, corn oil was used to increase the energy level in the diets. This resulted in the crude fat levels in the diets based on sunflower cake being higher than in the control diet (Table 2).

The basal diet met or exceeded the requirements for most of the essential amino acids except for lysine, as well as methionine (total sulphur amino acid requirements were met) and threonine which were deficient. The stipulated requirements for these amino acids in tilapia are shown in Table 3.

Lysine has been identified as the first limiting amino acid in sunflower cake. Besides its effect on growth, it has been shown to chemically enhance feed intake together with glutamic, aspartic, citric and malic acids. In the current experiment, there was no evidence of higher feed intake in fish fed diets supplemented with lysine, but there was a trend for improved FCR values in fish fed diets where lysine was added alone (diet 2) or together with methionine (diet 5), threonine (diet 6) or both methionine and threonine (diet 8). FCR values for fish fed these diets were not significantly different from that of the control fish (P > 0.05).

The methionine level in the basal diet was marginally lower than that recommended by Santiago and Lovell, but it was higher than the value reported by Jackson and Capper and Jackson et al. The lack of response was presumably because a methionine level of 7.5 g per kilogram of the diet in the basal diet was adequate for the fish, especially in the presence of the high levels of cystine.

Threonine added alone or together with lysine improved growth rate and FCR of tilapia, but the values attained were not significantly different from those obtained for fish fed the basal diet. There was little response in weight gain or FCR in fish fed diets supplemented with both threonine and methionine when the two amino acids were added together, suggesting that they were
not the first limiting amino acids. The stipulated requirement for threonine in tilapia is 11.2 g/kg of the diet. It is also plausible that a threonine level of 10.5 g/kg in the basal diet was adequate for the fish at that stage of growth.

In all the performance indicators tested (Specific growth rates, weight gains and FCR's), the best trends were noted for fish fed diets in which the basal diet was supplemented with lysine alone or combined with methionine and threonine. This is an indication that lysine was indeed the limiting amino acid in the diets based on the fibre-reduced cake. It should be noted that the experiment was done over a short period of time. It is possible that a more definitive response would have been noted if the experiment had been done over a longer period.

Acknowledgements

The authors wish to acknowledge financial support from the Rockefeller Foundation and the Canadian International Development agency (CIDA). We also wish to thank the University of Nairobi and University of British Columbia for use of their facilities, Sagana fish farm in Kenya for providing the fingerlings, and Gary Sedgwick for his help in amino acid analyses and Patrick Charagu for helping in data analyses.

References


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SHORT COMMUNICATION

SEROPREVALENCE OF BRUCELLA ABORTUS INFECTION IN THE CROSSBRED DAIRY CATTLE IN TIGRAY REGION, NORTHERN ETHIOPIA

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¹Faculty of Veterinary Sciences, Mekelle University, P.O.Box 231, Mekelle, Ethiopia
²Faculty of Veterinary Medicine, Addis Ababa University, P.O.Box 34, DebreZeit, Ethiopia

Bovine brucellosis is found worldwide and it is still one of the most important disease problems in developing countries¹². In Africa, the disease is widespread and high prevalences have been reported in many countries³. In Ethiopia, different researchers have established the growing importance of bovine brucellosis in the intensively managed crossbred dairy cattle found in the urban and peri-urban areas of the country⁴,⁵,⁶,⁷. On the other hand, information on the epidemiology of brucellosis in animals and humans is scant in Tigray Region. The present paper reports the seroprevalence of bovine brucellosis in the crossbred dairy cattle of Tigray region of Ethiopia.

The study area comprised Tigray region of Ethiopia situated between 12° 15' N and 14° 57' N latitude and 36° 27' E and 39° 59' E longitude⁸ having population of 11,162 crossbred animals with 50% or more exotic blood level. A cross-sectional study was conducted from September 2004 to March 2005 using serological tests, Rose Bengal Plate Test (RBPT) and Complement Fixation Test (CFT). A structured questionnaire was also administered in person to 112 randomly selected farm-owners using two-stage cluster sampling⁹ to record the attributes of sampled animal and the type of farm and husbandry characteristics which are believed to influence individual-animal and herd-level seropositivity to brucellosis.

Out of the 1,135 serum samples tested, 4 (0.35%) were proved to be positive by RBPT from which 3 (0.26%) samples were positive reactors with a titre of 1:320 when retested with CFT. Moreover, in this study the overall herd-level prevalence was 2.68%, however, within-herd prevalence varied from

Table 1. The effect of risk factors on individual-animal seropositivity to bovine brucellosis

<table>
<thead>
<tr>
<th>Variables</th>
<th>N</th>
<th>Number (%) positives</th>
<th>Univariate Logistic Regression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
</tr>
<tr>
<td>Age (year)</td>
<td></td>
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<tr>
<td>0.6 - 3</td>
<td>496</td>
<td>1 (0.20%)</td>
<td></td>
</tr>
<tr>
<td>&gt; 3</td>
<td>639</td>
<td>2 (0.31%)</td>
<td></td>
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<tr>
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<td></td>
<td></td>
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<tr>
<td>1 - 20</td>
<td>884</td>
<td>2 (0.30%)</td>
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</tr>
<tr>
<td>21 - 50</td>
<td>164</td>
<td>1 (0.61%)</td>
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<tr>
<td>&gt; 50</td>
<td>307</td>
<td>0 (0.00%)</td>
<td></td>
</tr>
</tbody>
</table>

N= number of observations

*Corresponding author e-mail: gebretsadik.b@yahoo.com
0% - 33% based on CFT. The results revealed age and herd size factors had no significant effect (p>0.05) for brucellosis seropositivity (Table 1). On the other hand, an apparently higher seroprevalence (0.31%) was observed in animals greater than 3 years of age than those 0.6 - 3 years of age (0.20%). A higher prevalence was observed in farms that kept between 21 - 50 animals (1.56%) followed by farms that had 1 - 20 (0.30%) animals and no Brucella antibodies were detected in farms with greater than 50 animals. Moreover, the highest prevalence was observed in animals with single parturition status (0.53%) followed by multiple parturitions (0.39%) and none in animals with no parturition status. Furthermore, the results of Fisher’s Exact test revealed that individual animal seropositivity to brucellosis was independently associated (p<0.01) with history of previous abortions but not with stillbirths (p>0.05).

Among the 112 intensive farms investigated in this study, 4 (3.6%) farms had at least one reactor using RBPT and 3 (2.8%) by CFT. Within-herd prevalence varied from absence of reactor animals in the herd to presence of one reactor out of 3 animals (33.3%). Seven farm-level risk factors considered in the univariate analysis had no significant effect (p>0.05) on seropositivity to brucellosis (Table 2). The questionnaire results showed that the

<table>
<thead>
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<th>Variables</th>
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<th>Number and (%) positives</th>
<th>Univariate Logistic Regression</th>
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<td>P-value 95% CI</td>
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<td>2 (10.67%)</td>
<td>0.102 0.6-105.4</td>
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<td>21 - 50</td>
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<td></td>
</tr>
<tr>
<td>&gt; 50</td>
<td>3</td>
<td>0 (0.00%)</td>
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<tr>
<td>Livestock species intermix</td>
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<tr>
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<td>Presence</td>
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<td>2 (4.44%)</td>
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<tr>
<td>Intact</td>
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<td>Loose</td>
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<td>26</td>
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<td>Natural</td>
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<td>2 (1.92%)</td>
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<td>Good</td>
<td>6</td>
<td>1 (16.67%)</td>
<td>0.073 0.8 - 134.9</td>
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<td></td>
</tr>
<tr>
<td>No</td>
<td>105</td>
<td>2 (1.90%)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>7</td>
<td>1 (14.29%)</td>
<td>0.097 0.7 - 108.6</td>
</tr>
</tbody>
</table>

N= number of observations, *=farms practicing less than three hygienic measures (separation of cows during parturition, proper disposal of fetal membranes and/or aborted fetus, cleaning and disinfection of stables and proper waste and manure disposal)
management and hygienic measures practiced in the visited farms were poor. This was evidenced by the absence of maternity pen or separation of cows during parturition, lack of proper disposal of aborted fetus and/or fetal membranes and poor farm hygienic status.

The 0.26% seroprevalence of bovine brucellosis in the crossbred animals was very low in comparison to 8.1-39% reported by other workers in Ethiopia\textsuperscript{4,6,10}. However, the present finding is in agreement with the reports of few workers\textsuperscript{11,12}. The low seroprevalence of \textit{brucellosis} in the present study could be associated with the relatively young age and small herd size of the crossbred dairy system in the region. The low seroprevalence of brucellosis in this study probably could shadow the strength of associations between the farm or management related risk factors and brucellosis seroprevalence. However, the influences of management related risk factors and characteristics of the population for occurrence of infection in a herd are reported to have an important role\textsuperscript{13,14}.

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SHORT COMMUNICATION

TUBERCULOUS LYMPHADENITIS CAUSED BY MYCOBACTERIUM AVIUM IN TWO CROSS-BREED DAIRY COWS

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Tuberculosis caused by the avian bacillus, Mycobacterium avium, is not a major problem in mammals but it can cause some difficulties in interpreting the tuberculin reaction as the avian tubercle bacillus reacts positively to tuberculin1. The M. avium complex (MAC) comprises a group of opportunistic pathogens of animals and human which include M. avium subsp. avium, M. avium subsp. silvaticum, and M. avium subsp. paratuberculosis2. The aim of this study was to report the detection of M. avium in two crossbred cows in Sudan.

A six-years-old crossbred cow (local Kenanna vs. Holstein Friesian) at south Khartoum state had external nodular lesions at the prescapular and the precrural (femoral) areas. The infection in this case simulated the endemic bovine farcy caused by M. farcinogenes. The cow died later and postmortem was not possible. The second case, a three-years-old cow at Wad Medani, Gezira state, was under observation for 6 months. The lesions were typical of those

Figure 1. A three-year-old crossbred cow with tuberculous lymphadenitis caused by Mycobacterium avium strain SD115. Note the multiple nodular abscesses on the head and neck.

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of farcy-infected cattle, and resembling the first case at El Bagair. The cow was examined and monitored during the six months period. The lesions were restricted to the head and neck, notably in the area of the parotid, retropharyngeal and submandibular lymph nodes (Fig. 1). The cow was slaughtered just before death and a postmortem examination took place.

Smears from the superficial lymph nodes of the two cows revealed the presence of acid-fast bacilli. Growth of the suspected mycobacteria was obtained after three weeks incubation at 37°C on Lowenstein Jensen (LJ) slants. The isolated strains SD114 and SD115 had morphological characteristics of the slow-growing, non-photochromogenic mycobacteria. The colonies were smooth, cream-yellow in colour and slightly rose from the surface of the medium. The colonies numbered 3 to 6 per medium bottle.

Histopathological examination of sections prepared from the infected lymph nodes and subcutaneous nodules from the two cows revealed severe granulomatous lesions. There were central foci of caseation, cavitations and necrosis, surrounded by an inflammatory zone clearly marked by numerous cells and fibrous tissues (Fig. 2). Thin layer chromatographic analysis (TLC) of mycolic acids extracted from the colonies of the two strains revealed alpha-mycolates, keto-mycolates, omega-carboxymycolates and wax esters. This pattern is characteristic for *M. avium*, thus it allowed an early tentative identification of the strains as members of the *M. avium*. The analysis of mycolic acids proved to be useful diagnostic method following Ziehl Neelsen (ZN) staining as it allowed *M. bovis* and M. farcinogenes to be eliminated early in this study. These

![Figure 2. Histopathological section from a retropharyngeal lymph node of three-years-old crossbred cow caused by Mycobacterium avium strain SD115. Note the granulomatous reactions with central zones of caseation, cavitations, and necrosis and infiltration of inflammatory cells and fibrous tissues.](image-url)
have been frequently reported from cattle in Sudan.

The identity of the isolated mycobacteria were both confirmed as members of the *M. avium* complex when the characteristic PCR amplification products of IS1245 (427 bp) were demonstrated on gel electrophoresis. The PCR was done according to Guerrero et al. The host range for IS1245 among M. avium complex (MAC) group was shown to be limited to *M. avium*. This unique property allowed the confirmation of the identity of our isolates as *M. avium* by the detection of the amplified IS1245 on agarose gel electrophoresis. Experimentation using IS1245 system in this case was performed after a negative IS6110 results. We performed IS6110 as a confirmatory test for the commonly encountered *M. bovis*. *M. bovis* is one of the *M. tuberculosis* complex and the major cause of the bovine tuberculosis. *M. bovis* and *M. farrinogenes* may produce similar external tuberculous inflammatory lesions as well as pulmonary lesions.

To our knowledge no *M. avium* infection had been reported among local breeds in Sudan. The Friesian cattle and their crosses with local Kenanna and Butana breeds have been found to be more susceptible than the local breed to many endemic tropical diseases and infections which are subclinical or mild in their counterpart local breeds.

Acknowledgements

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SHORT COMMUNICATION

BOVINE MASTITIS CAUSED BY NOCARDIA FARCINICA

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During the last 40 years mastitis becomes one of the major problems that faces dairy industry in Sudan as in many other countries, given the fact that many herd owners shifted to increasing milk productivity by selecting high yielding local breeds or importing foreign breeds namely the Friesian breed. Many infective agents have been implicated as causal agents, but the common causes are Staphylococcus aureus, Streptococcus agalactiae and E. coli.¹ Miscellaneous causes of mastitis in bovines such as nocardiae have long been described¹, 2, 3, 4, 5, 6. In African Countries mastitis due to nocardiae is not common, or is been passed udiagnosed or misedaignosed. In Sudan there are few reports of bovine mastitis caused by nocardiae⁷, 8, 9. The aim of this study was to examine the role of nocardiae in the etiology of mastitis in cattle and to determine some epidemiologic, clinical, pathological and bacteriological aspects in selected dairy farms from around Khartoum state. Eight thousand 8000 cross-bred cattle (Friesian x Kênanna) belonging to 275 herds from around Khartoum State were examined for the presence of mastitis. Epidemiological and clinical examination with especial attention to udder was done. Milk samples (5-10 ml) from each cow suspected to have mastitis was collected and transported following standard methods¹⁰.

Out of 8000 dairy cows examined in the present study, 84 (0.01%) were found positive for mastitis, 14 of the 84 samples (14.3%) revealed the presence of nocardiae which had been isolated on Tryptic Soya agar (TSA; Difco) when incubated aerobically at 37°C for up to 7 days. Twelve (12) isolates were identified as N. farcinica in accordance with the standard description of nocardiae¹¹. The identity of the remaining 2 actinomycetes has not being determined.

Clinical and pathological changes observed among the examined dairy cows infected with N. farcinica are summarized in Figure 1. Infection rate of mastitis due to N. farcinica mastitis increased with age, the studied cases showed highr rates during the 4th and 5th calving. In each calving infection increase with advancement of lactation weeks and it was noticed to reach the highest rate between week 3-5.

Analysis of mycolic acids from isolates on thin layer chromatography 12 showed that 12 of the 14 strains revealed mycolic acids (nocardio-mycolates) that co-
chromatographed with those of the reference *N. farcinica.*

Isolation of chromosomal DNA and PCR amplification of the 16S rDNA gene were carried out following the methods by Chun and Goodfellow and obtained sequence was analyzed using PHYDIT (Version 3.1, J. Chun) and TREECON (Version 3.1b, Yves van de Peer) programs. Five of the 12 isolates showed a 16S rDNA gene similarity of 100% with that of reference *N. farcinica* (Fig. 2). The latter results confirm that mastitis isolates belong to *N. farcinica.*

Two healthy lactating cows were used for experimental mastitis infections. The left front quarters of the two cows were used as controls which were infused with sterile saline solution. The remaining quarters were each given 1 ml (approximately 35000 cfu/ml) of clinical isolates of *N. farcinica.* Following intramammary infusion, clinical data were recorded daily, appropriate bacteriological and pathological specimens and data were taken, notably cultural and microscopic examination. The first cow showed all clinical signs of peracute mastitis notably fever (40.4°C), microscopically the disease was manifested by granulomatous reaction and the presence of branching filamentous acid fast bacilli which were demonstrated on smears made from infected milk sample. This cow was found dead on the morning of the eighth day following continuous progressive deterioration of her general and udder condition. The second cow showed acute clinical mastitis notably fever (40.8°C) with moderate swelling and hardness of the two right quarters. Slightly visible altered secretions were shed by the three infected quarters. The cow continued to be apparently healthy with good appetite, but mastitis did not responded to treatment with Neomastipra (Hipra, S.A., Spain) and continued to shed acid fast bacteria and high WBC for two months.

In the present study mastitis caused by nocardiae represents 14.3% from 84 mastitis milk samples. These findings emphasizes the increasing role of noccardiae in causing mastitis. Although

![Figure 1. Clinical and pathological changes in udders of dairy cows suffering from mastitis caused by *Nocardia farcinica.*](image)
there is a considerable number of studies on bovine mastitis in Sudan, but few reports of mastitis caused by nocardiae have been published including bovine\(^8\)\(^9\) and caprine\(^14\). The extent and effect of nocardia mastitis in Sudan and many tropical countries and determination of risk factors and effect of animal beddings on the occurrence of nocardiosis needed to be evaluated to promote future control efforts.

**Acknowledgements**

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**References**


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SHORT COMMUNICATION

ABATTOIR SURVEY OF SLAUGHTERED PREGNANT GOATS IN ZAMBIA

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Small ruminants are often considered to be very important for their contribution to the development of rural zones and people in sub-Saharan Africa (SSA)^1,2,3. Wastage of conceptus through the slaughter of pregnant females is probably one of the most destructive practices affecting goat production in SSA^4. In Zambia the proportion of slaughtered pregnant females are not known. Country specific information on the slaughter of pregnant does is important in order to have a clear understanding of the potential impact of slaughter of pregnant does on goat reproduction in Zambia and SSA. In order to assess the extent to which pregnant does are slaughtered in Zambia, an abattoir survey was conducted in the capital city Lusaka.

Source of reproductive organs

The goat uteri were obtained from a local informal goat abattoir located in the outskirts of the capital city. The abattoir is located near a livestock market where small livestock such as goats, pigs, chickens and a few sheep are sold. The majority of the goats sold at the market are slaughtered at the nearby abattoir. The majority of the goats originate from the southern and western parts of Zambia where the highest proportion of the goats in Zambia are reared^5.

Sample collection

Collection of the reproductive organs was done between October and December, 2006 everyday except Sundays. During the period October to December, there is generally food scarcity and hunger in Zambian villages. This period was therefore selected as an appropriate period to conduct the study since most subsistent farmers were more willing to sell their goats.

All the goats slaughtered during the study period were recorded. Uterine horns from all slaughtered does were obtained at the time of slaughter. The uterine horns were stored in a cooler box containing ice packs and transported to the school of veterinary medicine about 4 kilometres from the abattoir.

At the laboratory, the uterine horns were opened and inspected for the presence or absence of foetuses. The crown rump lengths (CRLs) of the foetuses were measured as estimates of their gestational ages^6.

The results of the proportions of female goats, male goats, and pregnant goats slaughtered are shown in table 1. 811 goats were slaughtered during the study period. Out of the total slaughtered goats 432 or 53.3% were female goats and 272 or 63% of the slaughtered female goats were pregnant. Results of the proportions of male

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Table 1. Proportions of goats slaughtered at an abattoir in Lusaka, Zambia.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>n</th>
<th>Proportion(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total goats slaughtered</td>
<td>811</td>
<td>100</td>
</tr>
<tr>
<td>Female goats slaughtered</td>
<td>432</td>
<td>53.3</td>
</tr>
<tr>
<td>Male goats slaughtered</td>
<td>379</td>
<td>46.7</td>
</tr>
<tr>
<td>Pregnant females slaughtered</td>
<td>272</td>
<td>63.0</td>
</tr>
</tbody>
</table>

and female foetuses and the stages of gestation are shown in table 2. 172 or 63.2% of the foetuses were female and 100 or 36.8% were male. 60% or 163 foetuses were classified as having been in their advanced stages of gestation while 40% or 109 were in their early stages of gestation.

Results in this study showed that more (53.3%) female goats were slaughtered than males (46.7%) during the period October to December, 2006. A high proportion (63%) of the slaughtered females were pregnant does and that 60% of the pregnant does were in their advanced stages of pregnancy as indicated by the CRLs. There were more female foetuses than males.

The proportions of slaughtered pregnant goats can vary depending on the country and season during which uteri are examined in different SSA countries. Similar work has been reported in some parts of SSA. In an abattoir survey of sheep and goats in the Gambia, 60% of the 1248 female goats slaughtered at an abattoir over a period of 1 year were pregnant. Both the sample size and the duration of study in that survey were different from the present study.

Reproductive losses are widely recognised as the most important constraint to increased small ruminant production and fetal waste due to slaughter of pregnant does is undoubtedly one of the major contributors to reproductive losses in goats in SSA. It is possible that in most small ruminant production systems of SSA, early pregnancy diagnosis may not be possible. However, there can be no doubt that slaughter of pregnant goats in their advanced stages of gestation has some motives other than ignorance. It may be that farmers sell

Table 2. Proportions of foetuses recorded at an abattoir in Lusaka, Zambia.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>n</th>
<th>Proportion(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male foetuses</td>
<td>100</td>
<td>36.8</td>
</tr>
<tr>
<td>Female foetuses</td>
<td>172</td>
<td>63.2</td>
</tr>
<tr>
<td>Foetuses beyond 3 months gestation</td>
<td>163</td>
<td>60.0</td>
</tr>
<tr>
<td>Foetuses below 3 months gestation</td>
<td>109</td>
<td>40.0</td>
</tr>
</tbody>
</table>
pregnant females because they look heavier and consequently sell at better prices than others. It may also be that because of financial resource limitations, females are indiscriminately sold for slaughter in time of crisis. It may further be that farmers wait for well-known poor producing females to be pregnant and so acquire apparent good condition before they sell them. Whatever the reason, it is most likely that many pregnancies are diagnosed before females are put to death. The evidence in this study and others suggest that the proportion of slaughtered pregnant females is highest when these are in their third to fifth months of gestation.

The slaughter of pregnant does may have future negative repercussions on the reproductive potentials of the Zambian rural goats. Moreover, the high number of female fetuses recorded at the abattoir means that an opportunity for the future female breeding stock to contribute to the goat reproductive efficiency in Zambian goats is lost.

There is need for studies into why pregnant female goats are sold and slaughtered so that losses due to the process can be prevented or minimized. Pregnancy diagnoses need to be performed at the abattoir prior to the slaughter of goat females. Training must also be provided to farmers on simple methods of pregnancy detection.

Acknowledgements

We are thankful to the Head of Department of the Department of Biomedical Sciences for facilitating transportation to and from the goat abattoirs. We also extend our gratitude to Mr Enos Phiri and Mr. Moses Nkonde for their technical assistance and the goat abattoir attendants at the Lusaka Goat Abattoir for their cooperation.

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