Changes in weight gain, faecal oocyst count and packed cell volume of *Eimeria tenella*-infected broilers treated with a wild mushroom (*Ganoderma lucidum*) aqueous extract

A O Ogbe*, S E Atawodi†, P A Abdu‡, A Sannusi§ and A E Itodo¶

ABSTRACT
An experiment was conducted to study the effect of a wild *Ganoderma lucidum* aqueous extract in coccidial-infected broilers. At 6 weeks of age the birds were randomly allocated to 6 treatment groups of 20 Ross broilers each in wire cages. Groups A, B and C were infected with *Eimeria tenella* Houghton strain at the rate of 36 250 sporulated oocysts/m³ per bird. The remaining 3 groups D, E and F were uninfected controls. At 7 weeks the birds in group A were treated with *G. lucidum* aqueous extract and those in B with amprolium in drinking water *ad libitum* at the rate of 200 mg/ml each for 7 days consecutively. Body weight gain, feed intake, faecal oocyst output and some haematological parameters were monitored. The result showed that all the infected birds in groups A, B and C had clinical signs of weakness and reduced appetite on day 4 post-infection. By the 5th day post-infection their faeces became bloody and watery, and large numbers of *E. tenella* oocysts were present in the faeces. On day 3 after treatment the oocysts detected were considerably reduced in both treated groups A and B and slightly higher in the untreated group C. The faeces of the uninfected control groups were normal and free of coccidial oocysts. After treatment for 7 days no coccidial oocysts were found in faeces of the birds that had been treated. Infected, untreated birds showed a slight drop in feed intake and weight gain from 7 to 8 weeks of age. The final mean weight gain recorded in the treated groups A and B was comparable to that of the uninfected birds in the 3 control groups, while it was lower in the untreated group C. The feed to gain ratio was higher in C than in the other groups. A slight drop in packed cell volume was observed in groups A, B and C at 7 weeks of age, 1 week after infection. This study showed that treatment with *G. lucidum* results in a marked reduction in the number of *E. tenella* oocysts shed in the faeces, leading to improved weight gain and decreased weight loss. The results confirmed the virulence of the Houghton strain of *E. tenella* and the effectiveness of both amprolium and *G. lucidum* extract against *E. tenella*.

Keywords: broilers, *Eimeria tenella*, *Ganoderma lucidum*, oocyst count, packed cell volume, weight gain.


INTRODUCTION
Certain mushrooms have been reported to offer benefits that include fast growth, improved nutrition, resistance to and protection from pathogens. Proximate and phytochemical analysis has shown that they contain bioactive compounds that function as good feed supplements and medicines to treat certain parasitic diseases and to improve wound healing. Reproduction of the parasites results in the formation of unsporulated oocysts. The unsporulated oocysts are shed in the faeces, and can infect other susceptible birds by ingestion of contaminated litter, feed and water or via mechanical carriers such as poultry house equipment, clothing, footwear, insects, other animals, wild birds and dust.

The Houghton strain of *E. tenella* (H strain) was isolated in the United Kingdom in about 1949 from a field case of caecal coccidiosis in chickens and maintained at the Houghton Poultry Research Station (HPRS), Houghton, and thereafter at the Institute for Animal Health (IAH) at Compton, UK, and has at various times been provided to other institutions and groups carrying out coccidiosis research on fowls. The characteristics of the parasite include its ubiquitous presence in the field, high virulence, and ease of handling in the laboratory due to the high rate of replication, ease of recovery of oocysts from the blind-ended caecal pouches, excellent sporulation of the oocysts and robustness of the sporozoites. Houghton (H) strain of *E. tenella* is fully pathogenic. A dose of 20 000 *E. tenella* oocysts (H strain) was sufficient to cause substantial mortality and reduction in weight gain. A comparison of the H strain of *E. tenella* and various laboratory and field strains revealed no significant differences in pathogenicity. Young chicks of about 4 weeks of age are most susceptible and the infection is characterised by acute onset of the 1st clinical sign, namely bloody droppings resulting from the invasion of the caecal mucosal epithelium by 2nd generation merozoites. Early emphasis in the control of coccidiosis in poultry was placed on strict hygienic measures combined with preventive medication using sulphonamides such as sulphadimidine and sulphamethoxine, and other drugs as they became available, including amprolium (a thiamine analogue), diaveridine (a pyrimidine), ionophores...
(polyether antibiotics such as monensin, salinomycin and narasin), nitrofuran and furazolidone, robenidine (a guanidine derivative) and quinolones\textsuperscript{25}. Many poultry flocks currently receive preventive medication and curative treatment to control coccidiosis. Despite these control measures, coccidiosis continues to be a major constraint for efficient production of poultry in Nigeria, possibly owing to resistant strains and complexity of the parasites. Some drugs may kill the parasites (coccidiocides) but others only arrest the development of the parasites (coccidiostats).

In tropical Africa, particularly Nigeria, most species of wild mushroom (both edible and medicinal) are collected from the fields and marketed by women and children for human consumption and as medicines to improve health. \textit{Ganoderma lucidum}, for instance, is a well-known species of medicinal mushroom in Chinese medicine, where it is cultivated, processed and sold as dried and whole or as powders, capsules, or tablets to treat certain diseases of the respiratory and gastrointestinal tracts, and for immunomodulation in humans\textsuperscript{22-24}. The objective of this study was to determine whether the aqueous extract of wild \textit{G. lucidum} can reduce \textit{E. tenella} oocysts output in infected broilers, improve body weight gain and mitigate haematological changes that may occur.

### MATERIALS AND METHODS

#### Study site

The study was conducted at the Federal College of Animal Health and Production Technology, National Veterinary Research Institute in Vom, Plateau State, Nigeria.

#### Experimental birds

One hundred and twenty day-old Ross broilers were obtained on 15 August, 2006 from a hatchery in Jos, Plateau State, Nigeria. The birds were randomly distributed into 6 treatment groups (A–F) of 20 chicks each in wire cages, each measuring 80 × 100 cm.

#### Preparation of aqueous extract of \textit{Ganoderma lucidum}

Wild \textit{Ganoderma lucidum} with red open caps were harvested from wooden logs and tree stumps on farm land in Vom, Plateau State, Nigeria. They were washed in distilled water, sun-dried, ground to powder using a mortar and pestle and then blended using a Corona grinder (Landers & CIA, SA). The mushroom powder was again sun-dried for 3 h and the blood was immediately transferred into a set of sterile tubes containing anticoagulant, disodium-salt of ethylene diamine tetra-acetic acid (EDTA) for determination of packed cell volume (PCV). The blood samples were collected weekly between 08:30 and 10:30 at 2, 4, 6 and 8 weeks of age, and the tests were conducted within 2 h of collecting the blood.

#### Collection of faecal samples and laboratory examination

The faeces of the broilers were collected daily in polythene bags from day-old to 10 weeks of age for parasitological exami-
nation. *E. tenella* oocyst output was also measured and expressed per gram faeces using a McMaster counting chamber. Faeces from each group were thoroughly mixed in plastic bottles using a spatula. One gram of the faecal sample was placed in a sterile bottle and homogenised by mixing with 1 m of flotation sodium chloride (NaCl) salt solution to make a suspension that was then mixed with 9 m of the salt solution, sieved in gauze wire mesh or muslin, the solid matter discarded and the filtrate collected in clean sterile plastic tubes filled to the brim and a cover slip was placed on top taking care to exclude air bubbles. The bottles were allowed to stand upright for 15 min to enable coccidia oocysts to float to the cover slip before examination under a light microscope at ×10 and ×40 magnifications. A portion of the positive sample only was used to fill the McMaster counting chamber and allowed to stand for about 15 min to enable oocysts to float and settle at the top of the chamber to facilitate identification and counting of the oocysts under the microscope using a differential counter. Absolute numbers of coccidia oocysts counted per m of the solution were recorded.

### Statistical analysis

Feed:gain ratio per bird/group was determined. Duncan’s multiple range test was used to separate the means that were significantly different. Statistical analysis of variance was carried out.

### RESULTS

On day 4 post-infection (PI) all the birds in the infected groups A, B and C appeared dull and weak and had reduced appetite. By day 5 PI their faeces became bloody and watery and *Eimeria tenella* oocysts were detected in their faeces (Table 2), indicating that infection of the broilers with the *E. tenella* was successful. Prior to treatment of the birds the oocyst output was 30,000 oocysts/g faeces (group A), 28,000 oocysts/g faeces (group B), and 25,000 oocysts/g faeces (group C) (Fig. 1). The faeces of uninfected groups D, E, F were not bloody and were free of coccidial oocysts (Table 2). On the 3rd day after treatment the oocysts detected in the *Ganoderma lucidum*-treated group (A) had reduced significantly in number (2500 oocysts/g faeces) compared to the amprolium-treated group (B) (5900 oocysts/g faeces). Group C showed a significant increase in oocysts released (32,600 oocysts/g faeces) on the 3rd day after treatment of the birds the oocyst output was 30,000 oocysts/g faeces (group A), 28,000 oocysts/g faeces (group B), and 25,000 oocysts/g faeces (group C) (Fig. 1). The faeces of uninfected groups D, E, F were not bloody and were free of coccidial oocysts (Table 2). On the 3rd day after treatment the oocysts detected in the *Ganoderma lucidum*-treated group (A) had reduced significantly in number (2500 oocysts/g faeces) compared to the amprolium-treated group (B) (5900 oocysts/g faeces). Group C showed a significant increase in oocysts released (32,600 oocysts/g faeces)
at 7 weeks of age (1 week PI), and the birds showed slight drop in PCV in groups A (23.5 % ± 0.7 and higher (2.54 kg/bird) (Table 4). The total feed intake in untreated groups was also significantly higher (3.8) compared to all other groups (Table 3). The feed intake was lower (1.8 kg, each) after treatment at 9 weeks post-infection. There was a significant reduction in body weight gain in infected but untreated birds compared to those that were infected and treated, which showed improved weight gain. Their feed:gain ratio was also better (3.4) than the birds that were infected but not treated, which showed significantly lower (P < 0.05) weight gain.

### DISCUSSION

In this study, the broilers were successfully infected with H strain of *Eimeria tenella* demonstrated by the fact that all the infected birds (100 %) showed clinical signs of weakness, reduced appetite, and bloody diarrhoea, and oocysts were present in their faeces by the 5th day post-infection. There was a significant reduction (P < 0.05) in faecal oocyst output in birds that were treated with either aqueous extract of *G. lucidum* or amprolium. There was also a significant reduction in the weight gain of the infected but untreated birds compared to those that were infected and treated, which showed improved weight gain. Their feed:gain ratio was also better (3.4) than the birds that were infected but not treated, which had the highest (3.8) feed to gain ratio. In other earlier studies, a significant reduction in body weight occurred in broilers infected with a dose of 10 000 sporulated oocysts of *E. tenella*.

The high feed:gain ratio (3.8) observed in the infected, untreated birds provides evidence of depression of feed intake due to infection with *E. tenella*. The highest feed:gain value reported was 1.61 in broilers that were infected with *E. tenella* which resulted in significant reduction in the body weight%.

Table 3: Group mean feed intake (kg) of broilers infected with *Eimeria tenella* and then treated with *Ganoderma lucidum* aqueous extract and amprolium.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Age (week)</th>
<th>Group mean feed intake (kg/bird)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>At pre-infection</td>
<td>5</td>
<td>1.10</td>
</tr>
<tr>
<td>At infection time</td>
<td>6</td>
<td>1.68</td>
</tr>
<tr>
<td>Before treatment</td>
<td>7</td>
<td>1.63</td>
</tr>
<tr>
<td>Three days after the treatments</td>
<td>8</td>
<td>1.52</td>
</tr>
<tr>
<td>Seven days after the treatments</td>
<td>9</td>
<td>1.81</td>
</tr>
<tr>
<td>Total feed intake</td>
<td></td>
<td>7.74</td>
</tr>
</tbody>
</table>

* Asterisk indicates value is significantly lower (P < 0.05) than the others in the same row. A = infected/treated with *Ganoderma* extract, B = infected/treated with amprolium, C = infected/not treated, D = not infected/not treated, E = not infected/treated with *Ganoderma* extract, F = not infected/treated with amprolium.

Table 4: Group mean weight gain (kg/bird) of broilers infected with *Eimeria tenella* and then treated with *Ganoderma lucidum* aqueous extract and amprolium.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Age (week)</th>
<th>Group mean weight gain (kg/bird)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>Initial weight</td>
<td>1</td>
<td>0.21</td>
</tr>
<tr>
<td>At pre-infection</td>
<td>5</td>
<td>1.04</td>
</tr>
<tr>
<td>At infection time</td>
<td>6</td>
<td>2.30</td>
</tr>
<tr>
<td>Before treatment</td>
<td>7</td>
<td>2.26</td>
</tr>
<tr>
<td>Three days after the treatments</td>
<td>8</td>
<td>2.50</td>
</tr>
<tr>
<td>Seven days after the treatments</td>
<td>9</td>
<td>3.12</td>
</tr>
<tr>
<td>Mean weight gain</td>
<td></td>
<td>2.91</td>
</tr>
<tr>
<td>Feed to gain ratio</td>
<td></td>
<td>3.41</td>
</tr>
</tbody>
</table>

* Asterisks indicate values that are significantly different (P < 0.05) from the others in the same row. A = infected/treated with *Ganoderma* extract, B = infected/treated with amprolium, C = infected but not treated, D = not infected/not treated, E = not infected/treated with *Ganoderma* extract, F = not infected but treated with amprolium.

Fig. 1. By day 5 after treatment, the oocysts released in A and B had reduced to just 1 oocyst/g faeces and by day 7 the birds in these groups were negative, while group C continued to discharge *E. tenella* oocysts up to day 17 post-infection. The uninfected control groups D, E and F did not pass any oocysts during the entire period of the experiment. No mortality occurred in any of the groups before the end of the experiment, but the caeca of the infected broilers were severely haemorrhagic (Table 2).

The results further showed that at 7 to 8 weeks after infection with *E. tenella*, the feed intake of birds in all the infected groups dropped from 1.6 kg to 1.5 kg, but this was followed by a compensatory increase in feed intake in group A and B (1.8 kg, each) after treatment at 9 weeks (Table 3). The feed intake was lower (1.6 kg) in group C (infected but not treated) and the feed to gain ratio was significantly higher (3.8) compared to all the other groups, B (3.5), D (3.4), A (3.4), F (3.4), E (3.3) (Table 4). The total feed intake in group C was significantly lower (P < 0.05). The mean weight gain of the birds in group C was also significantly lower (2.54 kg/bird) (Table 4).

Haematological analysis showed a slight drop in PCV in groups A (23.5 % ± 0.7), B (28.0 % ± 2.8) and C (27.5 % ± 0.7) at 7 weeks of age (1 week PI), and the birds voided bloody diarrhoea between 4 and 6 days PI (Table 2). There was no bloody diarrhoea in groups D, E and F and the PCV of the birds in these groups remained higher (29.5 % ± 0.7 and higher).
E. tenella and so in the absence of further development the broilers improved in weight gain better than those that were infected but not treated. It is also possible that the extract and amprolium stimulate appetite, so the broilers that were treated with them ate more and improved in weight gain better than those not treated. The broilers that were not infected but treated with either amprolium or Ganoderma performed even better in terms of weight gain than those that were infected and treated. It appears that this wild mushroom contains compounds that are active against E. tenella. The mushroom was found to be non-toxic in animal toxicity studies and in humans, even when used at high therapeutic doses. Bioactive compounds or polysaccharides are known to play vital roles in enhancing health; they block colonisation of the intestine by pathogens, thereby improving their elimination from the body. Some biologically active compounds or organic acids, resins, and glycosides which include steroid and terpenoid saponins are known to have therapeutic uses against microbes and parasites. The mushrooms used in this study were found to contain these compounds.

Although the ranges of normal PCV values measured in these broilers were wide (23.5–32.5 %), the decline observed in Groups A, B and C may be attributed to coccidial infection. According to some authors, PCV may be sensitive to or affected by coccidiosis. A mean PCV of 19.0 % at 6 days post-inoculation was observed to be the minimum for survival in birds. In this experiment, there was no mortality and the minimum PCV recorded was 23.5 % post-inoculation. Fluctuations in the haematological values were reported by other authors. The variations may depend on the physiological state of the birds.

Other studies have shown that some mushrooms have polysaccharides that play a role in stimulating the activities of many interdependent cell types such as T and B-lymphocytes, macrophages, and natural killer (NK) cells, inducing production and secretion of cytokines and complement. Other mushrooms (e.g. F. vesca, Boletus and Lactarius spp.) have also been reported to prevent intestinal coccidiosis in poultry. The active principles and the mechanisms of action of these mushrooms have not been fully elucidated, and should be the subject of future studies.

CONCLUSIONS
This experiment confirmed that infection of broilers with Eimeria tenella causes bloody diarrhoea as a result of damage to the intestinal mucosa leading to depression of feed intake and loss of body weight. Treatments with either Ganoderma lucidum or amprolium resulted in amelioration of clinical signs of bloody diarrhoea and reduction of faecal oocyst count. It also improved feed intake and weight gain. The treatments did not adversely affect PCV in E. tenella-infected and non-infected broilers. The results confirmed the virulence of Houghton strain of E. tenella and the effectiveness of amprolium against E. tenella.

ACKNOWLEDGEMENTS
The authors wish to acknowledge and dedicate this work to the memory of our dear colleague, the late Dr L.O Mgbokwue who passed away in a ghastly motor accident. We also acknowledge the following for their assistance: Drs M.S Ahmed, A.O Olabode, Tai Cole, B.O Akanbi, Musa Usman, J. Kamani, J.K Tanko and Mr Samson Edokpolo, J.A Edache, D.S Gbise and Noel Dus, as well as Messrs B.O Oguntayo and T. Agnes, all of the National Veterinary Research Institute, Vom.

REFERENCES
3. Anon. 2007 Mushrooms may be active against fowl parasite. THTSDAY 11(4280): 36
11. Eisevo K A N, Saror D I 1992 A laboratory manual in veterinary clinical pathology (1st edn). Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria
26. Ogbe A O, Adeyefa C A O, Joshua R A 2003 Growth rate and haematological parameters of broiler chickens vaccinated with IBD (Gumboro) vaccines exposed to different...

Book review — Boekresensie

SOS dog – The purebred dog hobby re-examined

Johan and Edith Gallant


SOS Dog is an eye-opener for anyone involved in breeding, treating, showing or selling pure-bred dogs. The book is thought-provoking and easy to read, but its challenge of practices that are still widely considered to be acceptable in the field of dog breeding and showing is bound to cause some controversy in dog fancier circles. Veterinarians in particular are likely to find the book stimulating in terms of re-examining their role as canine health care professionals when it comes to treating the increasing number of hereditary and breeding problems encountered in purebred dogs. Anybody who has ever visited a dog show or participated in one will identify strongly with the introduction where the main author explains the basis of the book in honest, humorous terms.

Johan and Edith Gallant, from Pietermaritzburg, KwaZulu-Natal, put forward a very convincing case for a total re-examination of why people breed purebred dogs, how this is done and the effect it is having on the well-being of the dogs. The fact that they are highly experienced and respected breeders of show dogs, having bred and shown dogs first in Europe and then in South Africa for several decades, lends credibility to their writing. They contend that if dog breeding continues the way it has been going for the past century since breed standards were implemented, the purebred dog may reach its demise fairly soon.

In building their argument, the authors use anthropological, historical and ethological data to paint the picture of how the dog developed from its prehistoric ancestors to what it is today. The major impact of the formation of the British Kennel Club in the late 19th century is emphasised as this was when breed standards were formulated. The role of subjective interpretation of breed standards, the shift away from functional towards show-breeding and inbreeding resulting in decreasing genetic pools of purebred dogs is well described in the build-up to the main argument. In the chapter entitled ‘The Seven Capital Sins of Modern Dogdom’, the authors spell out clearly and courageously their view of how breeders today are actively destroying the purebred dog. As is the case in the rest of the book, this chapter is written in a factual, non-accusatory but unambiguous style which effectively points out the problems with dog breeding today and the way in which practices such as forced matings and ignoring the social and behavioural development needs of puppies do not contribute to the well-being of dogs.

The last chapter, ‘Closing Arguments’ is preceded by a fascinating chapter on ‘Genuine Dogs’ which makes the case that land races such as the African village dog, or AfriCanis, are authentic dogs, physically and behaviourally, as they have had to survive with minimal interference from humans and could very well be part of the solution to the problems portrayed in earlier chapters. This idea is developed further in the last chapter and other concrete recommendations for breeders and breeding organisations are made.

The book is written in an easy-to-read style and is well-referenced and scientifically credible. Important facts and comparisons are presented in boxes and tables, enhancing the user-friendliness of the book. The book is well illustrated with relevant photographs and reproductions. There is a comprehensive bibliography which clearly shows the degree of research that has gone into the publication. While each chapter covers a well-defined area making it easy to read chapters on their own, the book as a whole is well-structured, flowing effectively from chapter to chapter.

I thoroughly enjoyed reading this book and strongly recommend it to any veterinarian or veterinary student. It has certainly made me think, not only about the history of the purebred dog fancy but also about the role of the veterinary profession in these developments. I am sure that not all veterinarians will agree with everything in the book, but the debate on veterinary ethics and animal welfare that it will undoubtedly generate, will be valuable in forming opinions, formulating policies and implementing practices that affect the veterinary profession.

Q Sonntag
EduPet Veterinary Consulting
Moreleta Plaza
Pretoria
E-mail: quixi@mweb.co.za