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EFFECT OF DIETARY COPPER SULPHATE SUPPLEMENTATION ON COCKERELS INFECTED WITH MIXED *Eimeria tenella* AND *Eimeria maxima*

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ABSTRACT

The effects of feed supplementation with copper sulphate at the rate of 240 mg/kg feed was investigated in cockerels infected with a mixture of Eimeria tenella and Eimeria maxima. A total of forty cockerels were divided into four groups (A, B, C and D) of ten birds each. From day old all the experimental groups were fed the same feed (Growers' mash) but the feed of Groups C and D was supplemented with copper sulphate for 23 days before the commencement of experimental infection at 6 weeks of age. The birds in Groups B (plane feed) and D (Copper supplemented feed) were each infected with 400,000 sporulated oocysts of a mixture of Eimeria tenella (80%) and Eimeria maxima (20%) while Groups A (Plane feed) and C (Copper supplemented feed) remained uninfected. The infection produced a severe clinical disease in both infected groups with varying degrees of clinical signs that included the voiding of watery brown faeces, weakness, ruffled feathers and reduced appetite. The severity of the manifestations progressed with the disease and was generally more severe in the non-supplemented/infected group all of which died by day 16 post infection. There was no significant ($p > 0.05$) difference in live weight between infected and non infected groups or between supplemented and non-supplemented groups. The prepatent period of the infection was 4 days. Thereafter, oocysts counts rose sharply in both infected groups. In general, oocyst count was significantly ($p < 0.05$) higher in the non-supplemented/infected group and had an earlier peak (day 8 post infection) than in the copper sulphate supplemented/infected group (day 12 post infection). However, there was no significant ($p > 0.05$) difference in the packed cell volume of either the two infected or uninfected groups. The results of this study suggest that copper sulphate supplementation of the diet of cockerels infected with Eimeria tenella and Eimeria maxima reduced the number and peak of oocyst output and ameliorated the clinical manifestations of the disease.

Keywords: **Copper suphate, supplementation, feed, coccidiosis, cockerels**

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INTRODUCTION

Poultry are kept throughout the world but their economic significance varies considerably from country to country. As a result of their adaptability to intensive management, poultry production in many countries has become increasingly organized, specialized and integrated into an industry of major national and often international importance [1]. The value of an industry to the economic and social well-being of a community is often reflected in the attention paid to factors which may adversely affect the industry. One such factor affecting the poultry industry is disease, which can have devastating effects particularly in intensive production systems [1,2,3]. Avian coccidiosis due to *Eimeria* species is one of the most important diseases affecting the poultry industry worldwide. It causes great economic loss in the poultry industry due to high rate of morbidity and mortality, reduced growth rate, feed conversion efficiency and egg production [4,5].

In the poultry, *Eimeria* species are intracellular in the intestinal cells where they cause tissue damage and interfere with food digestion and nutrient absorption, causing dehydration and blood loss [4]. The tissue damage can also expose the birds to secondary bacterial infections including those due to *Clostridium* and *Salmonella* species [6] Other diseases such as Marek's Disease and Infectious Bursal Disease that suppress the bird's immune system may act in synergy with and exacerbate coccidiosis to produce a more severe problem [7]. Sick birds lose weight, and are depressed, huddle together, with ruffled feathers and pass watery diarrhoeic faeces that may be bloody [8]. Mortality can range from mild to severe, depending on the species of *Eimeria* involved. All ages of poultry are susceptible to the infection, but the disease is usually self-limiting and resolves itself at about 6-8 weeks of age [9]. The disease is frequently subclinical and the flock may only show poor weight gain or feed conversion at the end of grow-out [4,10,11].

Avian coccidiosis due to *Eimeria* is caused by several species including *Eimeria acervulina*, *E. brunette*, *E. hagani*, *E. maxima*, *E. mitis*, *E. necatrix*, *E. praecox* and *E. tenella* [4,7,8]. The disease is usually manifested as caecal or intestinal form depending on the species involved [4,7]. Caecal coccidiosis, caused by *Eimeria tenella* usually occurs as an acute disease, in young birds aged about 4 weeks, characterized by diarrhoea and massive caecal haemorrhages. On the other hand, intestinal coccidiosis is caused by several other species of *Eimeria* that occur at various locations along the intestinal tract of the bird [4,7,8]. Intestinal coccidiosis runs a more chronic course and affects mostly older birds. In general, *Eimeria tenella* and *E. necatrix* are the most pathogenic and important species in domestic poultry.

Reproduction in *Eimeria* species is slowed by in-feed anticoccidial medication, low stocking density and low environmental humidity. Coccidiosis can also be controlled or prevented through good sanitation, litter management and use of anti-coccidiosis vaccines [9,11]. Anticoccidial medications act to either kill or stop the development of the parasite and help the birds to build immunity to the parasite [10,12]. It has also been noted that feed supplementation with some vitamins and minerals play an important role in coccidial development in chickens. Supplementation with these vitamins and minerals will either suppress or promote the complete development and pathogenic effects of *Eimeria* species [4].

Copper sulphate is an antifungal agent commonly added to proprietary poultry feeds to inhibit mould growth but the mineral is also necessary for normal metabolism [13,14,15,16]. This trace element which tends to conserve vitamin A in feed [17], is also involved in haemoglobin synthesis and immune responses and its deficiency results in nutritional anaemia in both man and animals [13,18,19,20,21,22,23]. Following natural infection with *Eimeria* species, chickens develop parasite-specific antibodies in both the circulation and mucosal secretions [2]. Vitamin A plays a significant role in the differentiation of epithelial cells and thus helps in maintaining the integrity of mucosal surfaces, it also affects both humoral and cell mediated immune responses against coccidiosis [23,24].

This study was therefore designed to determine the effect of copper sulphate supplementation on the pathogenesis, oocyst output and packed cell volume of cockerel chickens infected with mixed *Eimeria tenella* and *Eimeria maxima* infections.

MATERIALS AND METHODS

Animals and Housing

Forty cockerels were used in the study. They were obtained at day old from a local hatchery in Nsukka Local Government Area, Enugu State, Nigeria. The chicks were placed in a brooding pen and given intra-ocular vaccination against Newcastle disease the next day. They were brooded for 3 weeks during which the temperature of the brooding pen was gradually reduced from 37ºC to 27ºC. Feed (Growers mash; Top Feeds Nigeria Limited) and water were provided *ad libitum*.

Parasites

Oocysts of *Eimeria tenella* and *E. maxim* used in the study were isolated from a local chicken naturally infected with the parasites. The chickens were purchased from Ikpa, a local market in Nsukka, humanely sacrificed and routinely eviscerated. Thereafter, the gastrointestinal tract was opened and the contents of the caecum collected into a large plastic container. The caecal contents were washed several times with water through the process of sedimentation to remove colouring matter and large faecal particulate materials. The final sediment was reconstituted with 2% potassium dichromate solution, transferred into Petri dishes and allowed one week for sporulation to take place [8].

The sporulated oocysts were inoculated into coccidia-free, 4 weeks-old broiler chickens. The birds were monitored for patency of the infection which was confirmed by the development of clinical symptoms of coccidiosis and the presence of *Eimeria* oocysts in the faeces (including the passing of brownish faeces, inappetence, ruffled feathers, droopiness). The faeces of the infected birds were collected and processed to isolate the oocysts which were then routinely sporulated and stored in 2% potassium dichromate solution until used [8]. The parasites were identified as a mixture of *Eimeria tenella* (80%) and *E. maxima* (20%) based on the morphological characteristics and sporulation times of the oocysts as well as the clinical and pathological manifestations in the infected birds [8]. The infective oocyst dose used in the study was based on the results of previous studies [25,26].

Feed Supplement

The Copper sulphate used as feed supplement in this experiment were kindly supplied by Professor P. A. Nnadi of the Department of Animal Health and Production, Faculty of Veterinary Medicine, University of Nigeria, Nsukka. The copper sulphate was added to the feed of the supplemented groups at 240 mg/kg of feed.

Experimental Design and Procedure

The experimental chickens were randomly allocated into 4 groups of 10 chickens each. The groupings were as follows:

Group A:	Non-supplemented/uninfected control
Group B:	Non-supplemented/infected
Group C:	Supplemented/uninfected
Group D:	Supplemented/infected

Prior to experimental infection, the birds were fed plane (non supplemented) feed for 19 days before the supplementation of their feed. They were inoculated with the parasites 23 days following the commencement of feed supplementation. The birds were infected through the oral route using a syringe and needle with blunt tip to introduce the infective oocyst dose (400,000 sporulated oocysts/bird) into the oral cavity [25,26].

Parameters Evaluated

Clinical observations

The experimental birds were monitored daily for clinical signs that are suggestive of coccidiosis. The live weight of the birds in each experimental group was determined every four days using a triple beam balance.

Faecal Examination for Oocysts

The presence of oocysts in the faeces of the birds was determined by the floatation technique using saturated solution of sodium chloride as the floating medium. Following patency of the infection, oocyst counts were determined by the modified McMaster technique [27].

Packed cell volume

Packed cell volume (PCV) of the birds was determined by the microhaematocrit method [28] using blood collected from the wing vein of the birds.

Statistical Analysis

The data obtained from the study were summarized as percentages and means ± standard errors of means. The differences between the mean PCV and live weights were analyzed using analysis of variance (ANOVA) with probability values less than or equal to 0.05 ($p < 0.05$) being regarded as significant [29].

RESULTS

Clinical Observations

Four days post infection, most of the birds in the infected (non-supplemented/infected and supplemented/infected) groups started showing varying degrees of clinical symptoms of coccidiosis which included the voiding of watery brown faeces, weakness, ruffled feathers and reduced appetite. The severity of the manifestations progressed with the disease. The clinical symptoms were generally more severe in the non-supplemented/infected group than in the supplemented/infected group. Eleven days post- infection, some of the birds were on their way to recovery while 6 and 3 birds died in groups B (non-supplemented/infected) and D (supplemented/infected) respectively by day 12 post infection (Table 1). The study was terminated by day 16 post infection.

All the experimental groups gained weight during the study without any significant ($P > 0.05$) difference between infected and non infected groups or between supplemented and non-supplemented groups (Fig. 1).

Oocyst Counts

Oocysts were first detected in the faeces of all the infected bird groups by day 4 post infection. Oocyst count in group B (non-supplemented/infected) rose sharply to attain a peak (252,000 0pg) by day 8 before reducing sharply to the lowest (38,000 opg) level on day 16 post infection (Fig. 2). In group D (supplemented/infected), oocyst output rose gradually from day 4 to a peak on day 12 (132,200 opg) before being reduced to lower value (49,900 opg) on day 16. In general, oocyst count was significantly ($p < 0.05$) higher in the non-supplemented/infected group in which it attained an earlier peak than in the copper sulphate supplemented/infected group.

Packed Cell Volume

A steady PCV was maintained in the uninfected groups (A and C) throughout the period of observation (Fig. 3). In the non-supplemented/infected group (D), the PCV was reduced by day 4, rose gradually till day 12 and remained around that value to the end of the study. The PCV in both infected groups (cupper supplemented and non supplemented) became generally reduced and reached their lowest values on days 4 (non copper sulphate supplemented group) and 8 (copper sulphate supplemented group) following

infection. Thereafter, they showed slight recovery that was maintained to the end of the study. There was no significant ($p > 0.05$) difference between the PCV of the two infected groups (Group D, supplemented/infected and Group B, non-supplemented/infected) as well as between the two uninfected (Groups A and C) groups.

Table 1. Mortality pattern of cockerels infected with mixed *Eimeria* species and fed non supplemented and copper sulphate supplemented diets and their controls.

Group	No. in group	Mortality	
		No. (%) dead	Time post infection (days)
A (non-supplemented/uninfected)	10	0	NA
B (non-supplemented/infected)	10	6 (60)	12, 16
C (supplemented/uninfected)	10	0	NA
D (supplemented/infected)	10	3 (30)	12

NA = Not applicable

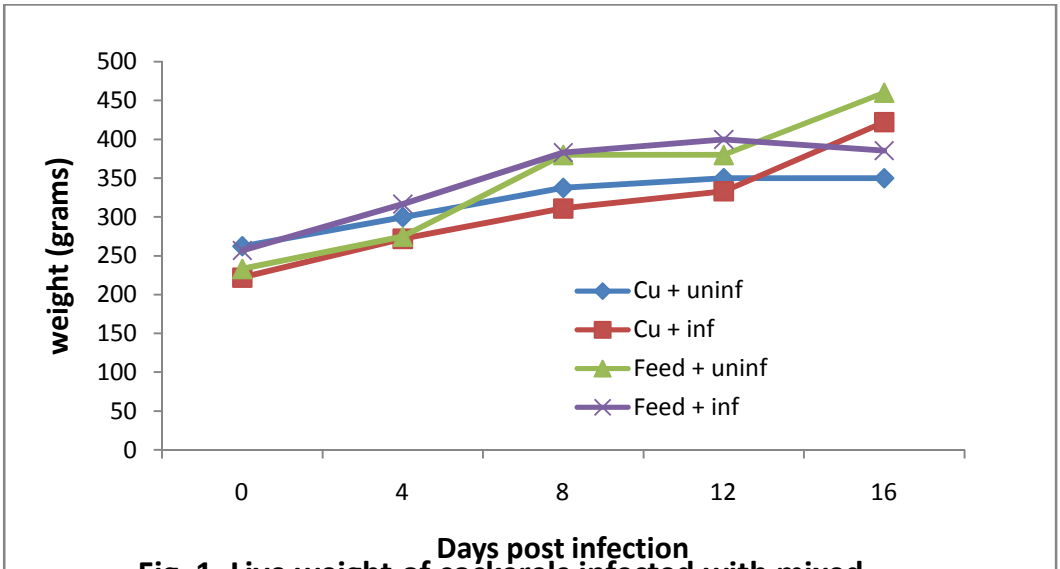

Fig. 1. Live weight of cockerels infected with mixed *Eimeria* species and fed non supplemented or copper sulphate supplemented diets and their controls.

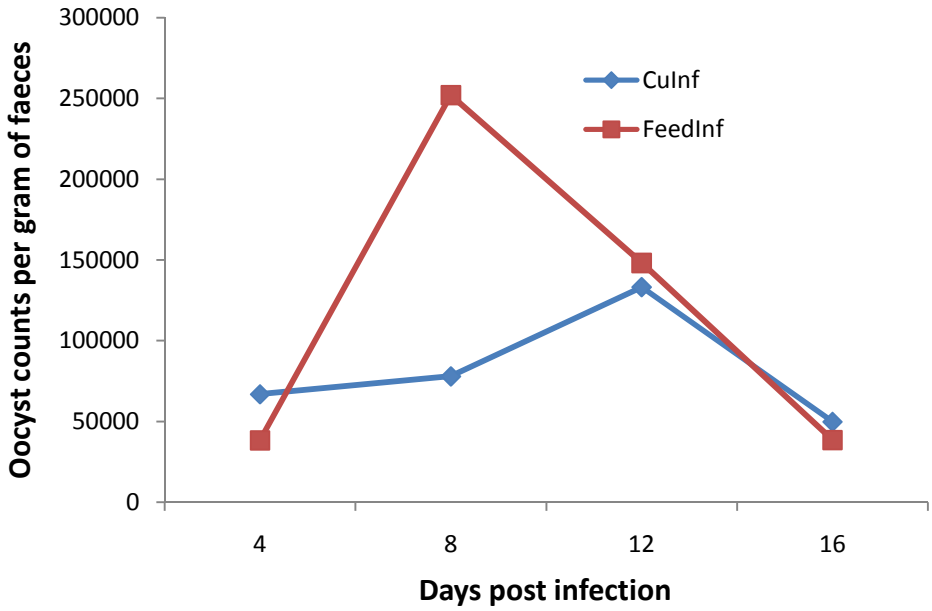

Fig. 2. Faecal oocyst counts of cockerels infected with mixed *Eimeria* species and fed non supplemented or copper sulphate supplemented diets and their controls.

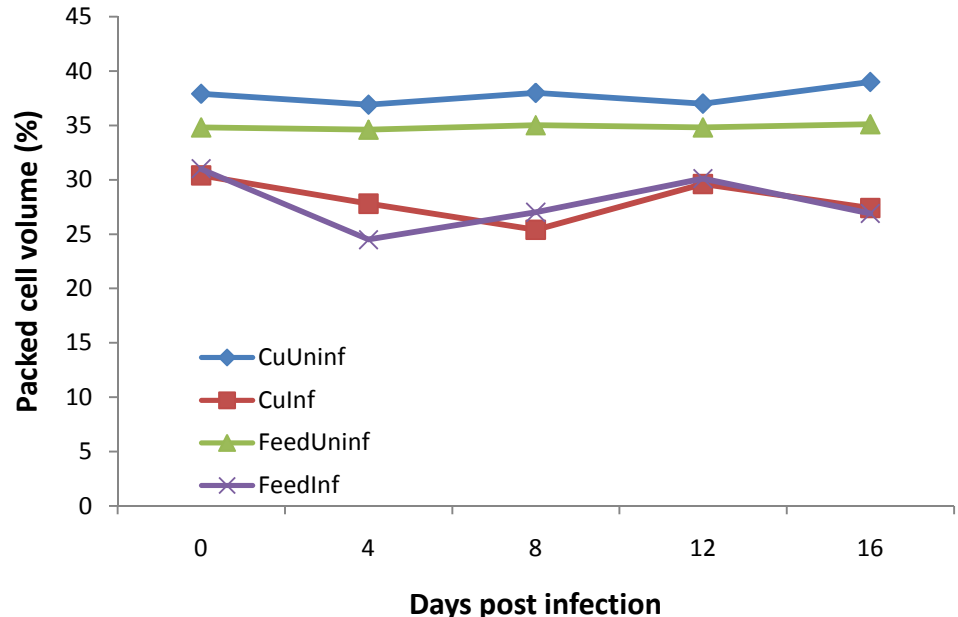

Fig. 3. Packed cell volume of cockerels infected with mixed *Eimeria* species and fed non supplemented or copper sulphate supplemented diets and their controls.

DISCUSSION

The *Eimeria* species (*Eimeria tenella* and *Eimeria maxima*) used in the study produced a moderate to severe clinical disease in the cockerel chickens. The clinical manifestations, which included ruffled feathers, drooping, depression and bloody diarrhea, were generally similar to those in previous reports [25, 26] from broilers given 120,000 oocysts each. Although cockerels are thought to be generally less susceptible to coccidiosis than broilers [8], the disease appeared to be more severe with greater mortality in the present study than in the broilers [25, 26]. The greater severity of the present study may probably be due to the fact that these birds received a higher dose of oocysts (400 sporulated oocysts/bird) and that field strain of the parasites was used in the infection. In addition, the results suggest that copper supplementation reduced the clinical severity of the infection since less number of birds in the supplemented group died compared to the non-supplemented group. It is known that *Eimeria* infections usually elicit parasite-specific antibodies in both the circulation and mucosal secretions [2]. These effects and the known involvement of copper in haemoglobin synthesis and immune responses [13,18,19,20,21,22] may have been responsible for the amelioration of the clinical severity of the disease in the cockerels fed the copper sulfate supplemented diet in comparison to those fed the non supplemented diet.

Weight loss and poor feed conversion ratio are common symptoms of avian coccidiosis [30]. In the present study, copper sulphate supplementation did not significantly ($p > 0.05$) affect the live weight of the birds as all the experimental groups of birds gained weight irrespective of supplementation during the study. A similar observation was reported by Nwosu *et al* [25,26] who noted that all the experimental broilers gained weight during their study irrespective of infection.

Oocysts output was significantly ($p < 0.05$) higher in the non supplemented /infected group than the group fed copper sulphate supplemented diet. This observation indicates that copper sulphate supplementation could reduce oocyst output by infected birds thereby also reducing the level of environmental contamination with oocysts. In addition, copper supplementation also reduced peak oocyst output while extending the peak time of oocyst production to day 12 instead of day 8 noted in the non-supplemented group. These findings may be attributed to the immunomodulatory and haematological effects of copper in the infected birds [31].

Furthermore, copper sulphate supplementation at the level used in this study did not produce any significant changes in the PCV of the infected birds. The PCV of both infected and uninfected groups were similar irrespective of copper sulphate supplementation. This finding is similar to the reports of Ezeh *et al*. [26] in broilers infected with 120,000 oocysts/bird.

CONCLUSION

The results of this study suggest that dietary copper supplementation in cockerels could reduce the number and peak of oocyst production as well as ameliorate the clinical severity of coccidiosis in the cockerels.

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