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INFLUENCE OF GRADED LEVELS OF DIETARY COPPER SULPHATE ON PERFORMANCE AND IMMUNE RESPONSE OF BROILER CHICKS TO NEWCASTLE DISEASE VACCINE

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ABSTRACT

***A total of one hundred and twenty (Arbor Acre plus) day old broiler chicks were used to study the effect of graded levels of dietary copper sulphate on performance and immunological response to Newcastle Disease Vaccine. At one week of age, the birds were randomly divided into three groups, (A, B and C) of forty chicks each. Groups A and B were respectively fed diets supplemented with copper sulphate at 240 mg\kg and 120 mg\kg of feed while group C was the control fed normal unsupplemented starter mash. These diets were fed to the various groups till five weeks of age when they were changed to broiler finisher with same levels of copper supplementation as in the starter stage until eight weeks of age when the study terminated. The various diets were fed ad-libitum and water given free-choice to the birds. The mean live-weights of the various groups were determined weekly. All the chicks were also routinely vaccinated with Newcastle disease virus vaccine at week four. At twelve days of age, six chicks per group were randomly selected and 5mls of blood collected from each for the determination of packed cell volume and serology using the haemagglutination inhibition test. There was an initial copper concentration dependent suppression of growth ($p<0.05$) in the two groups that received copper sulphate supplemented diets. However, after the third week the copper supplemented groups had overtaken the control ($p<0.05$), with the two supplemented groups A and B having comparable weights. At the end of the study (week 8), group A had significantly higher ($P<0.05$) mean live-weight relative to B and C while B was significantly ($P<0.05$) higher relative to C. The significantly higher packed cell volume recorded for the supplemented group was copper concentration dependent. The antibody titre to the Newcastle disease virus vaccine also showed a copper concentration dependency. In conclusion, the results suggest that copper sulphate at the concentration used in this study may be added to the diet of broilers as both growth promoter and immune modulator*.

Keywords: Broiler chicks, copper sulphate, live-weight, PCV, antibody titre.

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INTRODUCTION

Currently, there is a growing global demand and utilization of animal source food (ASF) like meat, milk and egg, occasioned by improved earnings and urbanization that have resulted in changes in traditional

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eating habits [1]. There is also increased awareness of dietary qualities of these products as well as increased demand due to growth in human population. Sustaining this increased appetite will require not only improvement in the total number of animals but also in the unit productivity shown in feed efficiency, egg lay and milk production.

As a means of improving productivity, increased feed efficiency per animal, reduction in disease incidence, addition of sub therapeutic doses of antibiotics to feed have been widely used to improve the productivity of animals [2]. However, due to recent concerns over the development of resistance by animal and human pathogenic bacteria to some conventional antibiotics, their uses as feed additives have become suspect. The situation has called for alternative compounds with antimicrobial and health enhancing properties that will not compromise the health of the consumers. One of such alternative compound is copper.

Over the years, copper has been added to poultry diets in excess of its nutritional needs as an antimicrobial and growth promoter [3]. Earlier studies on the use of this element in poultry feed showed that copper acts in an antibiotic fashion by influencing gut microbial growth. It has also been shown that copper alone or in conjunction with zinc play critical roles in immune-modulation in livestock and poultry [4,5]. Both compounds (Cu and Zn) are also required for growth and development [6,7]. Besides being essential for the development and maintenance of the immune system, the deficiency of copper negatively impacts on growth rate, causes anaemia as well as reproductive failure in animals [8,9].

Animals fed copper-deficient diets had reduced antibody forming cell responses [10] resulting in increased susceptibility to infections [11,12]. The growth promoting effect of dietary copper has been attributed to its antimicrobial action [13] and the enhancement of synthesis and retention by stimulating hormone and growth factors in broiler chickens [14,15]

Presently, there exists an urgent and challenging need to source feed additives that will not only stimulate the immune function as a measure to promote resistance and resilience to pathogenic microbes, suppress bacteria responsible for mild but unrecognizable infections, depress formation of toxins, reduce nutrient use by bacteria and finally enhance nutrient absorption with decreased immune stress that could cause a shift in protein synthesis in animals [16]. Moreover, it has been highlighted that nutrient requirements applied today are those based on researches conducted about four decades ago for stocks of indigenous temperate genotype [17].

Since the possibility of disease challenge is always present in today's poultry operations, the bird's metabolism and immune system are constantly adjusting to the stress and therefore, nutrient requirement may need to be adjusted at certain periods in the life of the bird. Moreover, accelerated growth is usually accompanied by impaired development and function of the immune system. Thus, it is the objective of this study to investigate the effect of dietary supplementation with graded levels of copper sulphate on production and the immune response of broiler chicks to Newcastle disease virus vaccine.

MATERIALS AND METHODS

Experimental Animals and their Management

A total of 120 day old Abor Acre Plus broiler chicks were used for the study. All the necessary hygienic/biosecurity measures were taken prior to the arrival of the chicks to the Poultry Development Unit of the Department of Animal Health and Production, University of Nigeria, Nsukka. Prior to the procurement of the chicks, the brooding pen was swept clean and disinfected while the drinkers and feeders were also washed adequately and disinfected. Newspaper sheets were laid on the floor. On arrival, the chicks were kept in a warm brooder pen. They were intra-ocularly vaccinated against Newcastle disease using New castle disease vaccine Lasota strain on procurement. At week 3 of age, they were all vaccinated against Infectious Bursal Disease (IBD) using the same route. All the vaccines were procured

from the National Veterinary Research Institute Vom, Plateau State, Nigeria and the manufacturer's instructions utilized in both reconstitution and dispensing. All the birds in each dietary group were closely monitored for clinical signs of ill health and feed consumption. At week four of age, all the chicks in the various dietary groups were again vaccinated intra-ocularly using the Lasota$^{(R)}$ strain of Newcastle disease vaccine.

The chicks were housed together for the first seven days during which they were fed broiler starter mash (Top Feeds Nig. LTD, Proximate composition Table 1). Water was provided ad libitum while warmth was provided using kerosene stove and a hoover.

At day seven post hatch, the chicks were randomly divided into three equal groups A, B and C, (n=40) and housed in three separate pens each measuring 1.5m^2. The brooders were started on three diets based on the addition and levels/concentration of copper sulphate in the commercial broiler starter and finisher diets (Top Feeds, Nig. LTD, proximate analysis, Table 1). From day 7 post hatch till the end of the study groups A and B chicks were respectively fed diets containing 240 mg\kg and 120 mg/kg of copper sulphate. Group C chicks remained on the broiler starter mash devoid of supplementary copper sulphate.

Table 1. Declared proximate composition of the starter and finisher diets (Top Feeds Nig. Ltd)

Ingredient type	Broiler starter (%)	Broiler finisher (%)
Crude protein	22.00	18.00
Metabolizable energy (Kcal.)	2900	2900
Crude fibre	5	5
Fats	6	6
Methionine	0.55	0.34
Lysine	1.20	0.85
Available phosphorus	0.45	0.40

The chicks in each dietary group were maintained ad libitum on their respective broiler starter diets for four weeks. Thereafter, their diets were changed to broiler finisher but with their respective additions or absence of copper sulphate. This diet was fed for another three weeks during which the chicks attained eight weeks of age post hatch when study was terminated.

Determination of Growth Performance

At day seven post hatch, when the random groupings were carried out all the chicks in every dietary group were weighed with a weighing balance to determine their live-weights and mean group live-weights. This exercise was repeated weekly till third week of study to be able to assess the growth performance of the various dietary groups. Thereafter, from week four of age, six birds were randomly selected at weekly intervals and the group average live-weights determined accordingly. This was repeated weekly till week eight of age when the study was terminated. The various means and the standard deviations of mean weight of each dietary group were calculated.

Blood Collection and Processing

On day twelve post hatch 5mls of blood was collected from six randomly selected birds in each group. These were used to determine the baseline values for the PCV and antibody titre levels respectively. At week seven of age, and twelve days post vaccination with Newcastle disease vaccine (Lasota^R), 5mls of blood was collected from each chick via the jugular or wing vein using a sterile syringe and needle from six birds randomly selected per dietary group. One milliliter (1ml) of the blood was dispensed into anticoagulant (ethyl diamine tetra-acetic acid, EDTA) containing bottles for the determination of packed

cell volume using standard procedures [18]. The remaining 4mls were dispensed into sterile bottles devoid of EDTA to enable coagulation, serum formation and recovery. The sera were harvested after clotting and kept in clean Eppendorf tubes and stored in at 4°C for use in serological studies.

Haemagglutination Inhibition Test (HIT)

The serological technique employed in the study was the haemagglutination inhibition test (HIT) [19]. Determination of antibody titer in the serum samples on day 12 post hatch and 14 days post Lasota vaccination was carried out using ND Lasota^{R} as the antigen [20]. The geometric titer (GMT) was determined as described by Villegas and Purchase [21].

Statistics

Statistical analysis was conducted using SPSS version 15 for Windows. Data on body weight (Bwt), was analyzed by repeated measure analysis of variance (ANOVA) in general linear model (GLIM). The student t test was used to compare differences between means of the PCV and the antibody titres. The acceptable level of significance was $P<0.05$.

RESULTS

Growth Performance

The changes in mean live-weights as a measure of growth rate are as shown in Table 2. The control group had an initial growth advantage over those supplemented with copper sulphate. There was a positive correlation between levels of supplementation and growth suppression, thus, between weeks I and 2 of supplementation, group A had significantly ($P<0.05$), lower mean live-weight relative to group, B while the respective values in both groups A and B were significantly ($p<0.05$) lower relative to the value of the control. However, at week 3 post commencement of supplementation, groups A and B had comparable live-weights while both groups had significantly ($P<0.05$) higher mean live-weights relative to the control. On weeks four and five, copper sulphate supplemented groups still showed superior growth relative to control. The moderately supplemented group B chicks had higher mean live-weight on week four but by week five group A had mean live-weight significantly higher relative to group B ($p<0.05$). However, groups A and B had superior weight relative to control ($p<0.05$).

Table 2. Changes in live weight of birds in the various dietary groups (gm)

Weeks post hatch	Dietary groups		
	Group A (240 mg/kg CuSO4)	Group B (120 mg/kg CuSO4)	Group C (Control; No CuSO4)
0	140.0 ± 0.2^{a}	140.0 ± 0.2^{a}	140.0 ± 0.3^{a}
1	350.0 ± 0.6^{b}	330.0 ± 0.6^{a}	360.0 ± 0.9^{c}
2	726.0 ± 0.7^{b}	708.0 ± 1.1^{a}	745.0 ± 0.7^{c}
3	1170.0 ± 0.4^{b}	1180.0 ± 1.0^{c}	1140.0 ± 0.6^{a}
4	1697.0 ± 4.0^{b}	1860.0 ± 0.6^{c}	1640.0 ± 1.0^{a}
5	2680.0 ± 0.1^{c}	2540.0 ± 2.0^{b}	2240.0 ± 1.6^{a}

^{abc}Means in a row with different superscripts are significantly different ($p<0.05$).

Packed Cell Volume

The PCV mean values of three groups at day 12 post hatch were 29%, 27% and 28% percent respectively for groups A, B, and C. The values of the PCV for the various dietary groups determined at the end of the study period were 31%, 28% and 26% for groups A, B, and C respectively. Thus, the PCV of group A

was significantly ($p<0.05$) higher relative to groups B and C while B was significantly ($p<0.05$) higher relative to group C. However, all the values are within normal range for clinically healthy chicken.

Serological Analyses

The result of the serological analysis of the blood samples taken at day 12 post hatch of the chicks were 6.0, 6.4, and 6.0 titres respectively for groups A, B and C respectively. The result of the serological analyses to determine the immune response to Newcastle disease vaccine by the various dietary groups gave the titre values of 9.4, 6.6 and 5.8 for groups A, B, and C respectively. Group A had a significantly higher titre level relative to groups B and C while group B had significantly ($p<0.05$) higher titre relative to C.

DISCUSSION

The results of this study demonstrated that dietary supplementation with copper sulphate promotes growth in broiler chicks. There was an initial growth suppression observed among the copper supplemented groups. This suppression in growth was copper concentration-dependent and was believed to be due to the initial reaction of the bird's gastro-intestinal tract to copper sulphate; a reaction that also involved reduced feed intake [23, 24]. Previous studies have reported that high levels of copper in the diet could cause injury to duodenal villi with interference in feed digestion and absorption [25] or interfere negatively with both giozzard and liver functions [26, 27, 28]. Jensen et al. [29]) had also reported oral lesions including gizzard damage in birds given more than 169mg\kg of feed.

The lineal concentration dependent reaction to copper supplementation in this study was transient with an accompanying reduction in feed intake which was not significant. Both the reduced feed intake and growth suppression were short lived and by the third week the supplemented groups had comparable mean live weights which were significantly higher than the control. The enhanced growth was more pronounced in group B which could indicate earlier resolution of the GI reaction as a result of lower damage arising from lower concentration of copper in the diet of that group. In their study, Pesti and Bakalli [3] demonstrated that dietary supplementation with copper had no growth promoting effect within the first three weeks of life but between days 22 and 42. Moreover, Samana et al. [30] reported that copper supplementation at 150 mg\kg of diet gave a significantly higher growth promoting effect than 250 mg\ kg. This agrees with our finding especially with respect to the period of supplementation which in their case was within the first twenty one days post hatch.

Generally, our result showed that the positive effect of copper on weight gain and feed efficiency was most evident during the finishing period. This is in agreement with previous studies that reported positive gain in live-weight in broilers as from 35 days post hatch [3, 15, 16, 28, 31, 32, 33]. It has been shown that copper improves feed metabolizability with increased dietary energy level as was the case during the finishing period [34]. This may be responsible for the late manifestation of the observed lineal and progressive significant increase in live-weight with higher dietary copper concentration.

Copper significantly raised the PCV of the broiler chicken as earlier reported ([22,23]. According to [23] copper plays a role as a co-factor in haematogenesis. It is one of the most critical elements in livestock because it is necessary for haemoglobin formation, iron absorption from the GI-tract and iron mobilization from the tissue stores [35]. Within the level of our design, the effect on PCV followed a concentration gradient with higher copper concentrations producing higher PCV which agrees with the established role of copper.

Dietary copper supplementation in form of copper sulphate significantly increased anti body titre to NDV. Earlier reports showed that copper plays an important role in immune-competence, including the development and maintenance of the immune system [8; 12; 36] and that copper deficiency results in reduced immune-competence shown by reduced antibody response including increased susceptibility to

infections [10, 11]. Moreover, Hosseini et al. [37] had also shown that antibody titres to NDV was elevated by increasing dietary copper supplementation at 30, 70 and 105 mg/kg of diet respectively. Specifically, Arshami et al. [38] showed that copper supplementation increased the titre levels of TIg and Igy as well as the relative weights of lymphoid organs like the bursar of Fabricius and thymus.

In conclusion, therefore, copper sulphate at the two dietary levels used in this study, appears to be a good growth promoter especially during the finishing period of broiler chicks. However, there is need for further studies to determine the critical inclusion ratio of copper in feed for optimal performance and also to explore the role of copper in immune-competence in broiler chick as a check against poultry pathogens.

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