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EFFECT OF CHROMIUM PROPIONATE ON THE HUMORAL IMMUNE RESPONSE AND PERFORMANCE OF BROILERS VACCINATED AGAINST NEWCASTLE DISEASE IN THE TROPICS

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ABSTRACT

Chromium (Cr) has been considered as an essential micronutrient for humans and animals by nutritionists. The primary role of Cr in metabolism is in enhancing the glucose uptake by the cells. Its supplementation reduces the negative effects of environmental stress. Newcastle Disease (ND) virus potentially infects most species of birds, and for susceptible poultry it is highly contagious and usually fatal. Humoral immune responses indicated that total antibody titers to ND vaccines were much higher in groups I, III, and V chicks that received Chromium Propionate (Cr-Prop) when compared to groups II and IV that did not received Cr-Prop. Humoral immune responses of group I (2.1) was higher than group II (1.9) while that in group III (2.3) was higher than group IV (1.3) and group V had (2.5). Cr-Prop supplementation significantly ($p < 0.05$) increased body weights of the broilers. The body weight in group I (3.23Kg) was significantly ($p < 0.05$) higher than those in group II (2.55Kg), while, those in group III (3.13Kg) were higher than those in groups IV (2.62Kg) and V (3.29 Kg). The weekly mean body weight gains of the treated groups I, III and V at the end of the study were significantly ($p \geq 0.05$) higher than those of untreated groups II and IV. The feed conversion ratio revealed variable significant ($p \geq 0.05$) differences which did not reflect dietary inclusion of organic Cr-Prop. Cr-Prop supplementation improved the immune response to ND vaccine and the performance of broiler such as live body weight, body weight gain, and feed intake and feed conversion ratio in broilers.

Key words: Chromium propionate·Newcastle disease·Immune response·Broiler performance· Vaccination.

INTRODUCTION

Chromium (Cr) has been considered as an essential micronutrient for humans and animals by nutritionists (Odgaard and Greaves, 2001). Trivalent Cr (Cr^{3+}) is the most stable oxidation state in which chromium is found in living organisms and is considered to be the safest form of Cr (Lindeman, 1996). The primary role of Cr in metabolism is in enhancing the glucose uptake by the cells (Davis *et al.*, 1996). Cr also activates certain enzymes and stabilizes proteins and nucleic acids (Anderson, 1994). Cr supplementation reduces the negative effects of environmental stress (Mowat 1994; Lien *et al.,* 1999; Sahin *et al.*, 2002). Supplemental dietary Cr is recommended by National Research Council (NRC) (1997) for animals undergoing environmental stress. Even though Cr is not currently considered as an essential trace mineral for poultry, research data provide evidence that suggests a nutritional and physiological role for Cr as a micronutrient (Sands and Smith, 1999). The beneficial effects of Cr can be observed more efficiently under environmental, dietary, and hormonal stresses.

Newcastle disease (ND) results from infections with virulent Newcastle disease viruses (NDV), having intracerebral pathogenicity indices (ICPI) of ≥ 0.7 in day-old chickens (*Gallus gallus*) and/or having multiple basic amino acids (at least three arginine (R) or lysine (K) residues) at the C-terminus of the fusion protein cleavage site, starting at position 113, along with a phenylalanine at position 117 (OIE, 2009). NDV potentially infects most species of birds, and for susceptible poultry it is highly contagious and usually fatal (Alexander, 1998). This disease occurs on at least six of the seven continents of the world and is enzootic in many countries. In rural Africa, the predominant chicken production systems (backyard farms) are based on indigenous domestic fowl (*Gallus gallus domesticus*), and ND is rated as the most devastating disease in these farms (Kitalyi, 1998). NDV, the avian paramyxovirus serotype 1 (APMV-1), a member of the *Avulavirus* genus within the *Paramyxoviridae* family is the causal agent of a fatal respiratory and neurological disease that can result in 100% morbidity and mortality in chicken flocks (Alexander, 2003). Control of ND primarily consists of vaccination of flocks and culling of infected or likely infected birds. Current vaccine strategies can be effective in controlling serious illness and death in infected birds, but virus replication and shedding may still occur, albeit at a reduced level (Van Boven *et al.,* 2008). At present, vaccination programs for NDV include the use of lentogenic strains either inactivated (killed) or attenuated (live) in order to induce a good protective immunity while producing minimal adverse effects in birds. Both vaccines have their advantages and disadvantages, which have been reviewed (Bermudez and Stewart-Brown, 2003; Senne *et al.,* 2004). The objectives of this study were to determine the effects of Cr-Prop on the performance and the humoral response of broilers vaccinated against Newcastle disease.

MATERIALS AND METHODS

Experimental birds and housing arrangements: The experiment was conducted at Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka during the period from September to November 2011. Experimental birds were raised from day-old to 9 weeks of age. Seventy five (75) Anak 2000 broilers were placed in 5 floor pens (15 chicks per pen with adequate space/chick). Water and feed were supplied *ad libitum*. The lighting regimen was continuous, with 24h of light daily, throughout the brooding periods. A completely randomized experimental design was used to assign the chicks to their respective groups.

The pens were illuminated at night and early morning throughout the experimental period. The chicks were allotted at random to 5groups. Each experimental group consisted of five replicates of five birds each and the supplementation was continued for 35 days. Basal feed was commercial feed planned to meet the nutrient requirement of broilers as recommended by NRC (1994). 400µg chromium (Cr) per kg diet from Chromium Propionate (Cr-Prop) was added to feed given to groups I, III and V.

Chicks in groups I, II, III were vaccinated with ND vaccine I/O on day 1, and ND vaccine La Sota on day 14 of age while groups I, II, III and IV were vaccinated with IBD vaccine on day 7 of age, and repeated on day 21 of age according to vaccination programme adopted by Southeastern Agricultural zone of Nigeria.

Serology

Serology and immune responses: On weekly basis, ten birds from each group were chosen at random and blood samples were collected from the brachial vein. Serum samples were separated by centrifugation at 3000g for 15 min and the harvested sera stored at – 40^0C until used.

Haemagglutination Inhibition Test for ND: The HI titer was determined using standard procedures (Beard, 1989). Briefly, twofold serial dilution of serum, after inactivated at 56^0C for 30min, was made in a 96-well, V-shaped bottom microtitre plate containing 50µl of PBS in all wells and then 50µl of NDV antigen (4 HA units) were added into all wells. The antigen–serum mixture was incubated at 37^0C for 30 min. Then, 50µl of 1% chicken erythrocytes suspension were added into each well and re-incubated for 30min. A positive serum, a negative serum, erythrocytes and antigens were also included as controls. The highest dilutions of serum causing complete inhibition were considered the endpoints. The geometric mean titers were expressed as reciprocal log2 values of the highest dilutions that displayed HI as described by Villegas and Purchase (1989).

Growth Performance: Weekly live body weights were recorded individually for each chick and the average live body weights were calculated for each replicate and treatment during the nine-week experimental period. Cumulative weekly body weight and weekly body weight gains were calculated for each chick, replicates and treatments. Feed consumption was recorded weekly for each replicate. Feed conversion ratio was also calculated.

The experiment was conducted according to Completely Randomized Design (CRD) and data thus collected were analyzed using analysis of variance technique as described by Steel *et al*. (1997). The comparison of means was made using Duncan multiple range (Duncan, 1955).

RESULTS

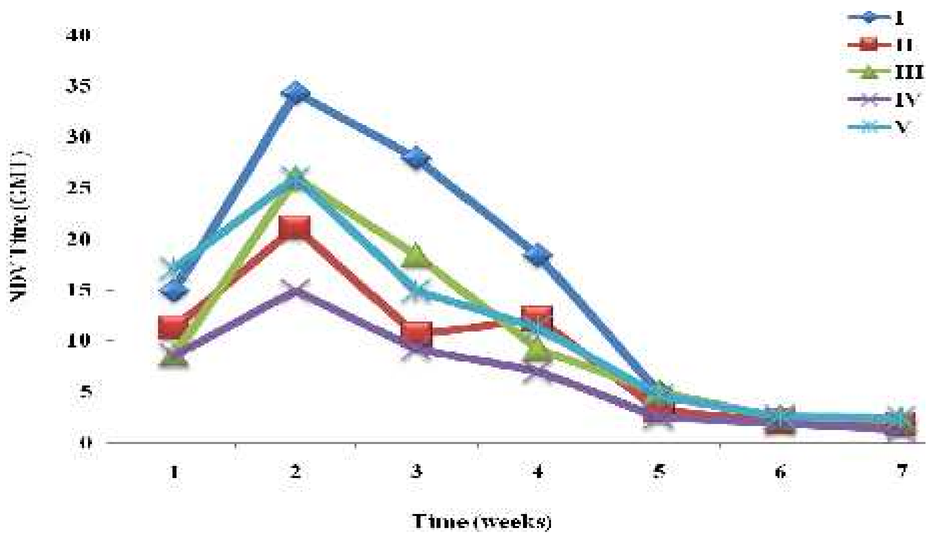
Haemagglutination Inhibition test: The results of the humoral immune responses are presented in Fig. 1 in geometric mean titers (GMT) expressed as Log_2 of the reciprocal values. These results indicated that total antibody titers against Newcastle disease vaccines were much higher in birds in groups I, III, and V chicks that received Cr-Prop when compared to those in groups II and IV without Cr-Prop. At the end of the study, the GMT of the treated and vaccinated groups I and V were 2.1 and 2.5 respectively while the untreated but vaccinated group II was 1.9 and unvaccinated but treated group III had 2.3 GMT. The untreated and unvaccinated group IV had 1.3 GMT. It is very significant to note that there is an appreciable difference between the GMT of group III and the group IV birds; suggesting that Cr-Prop supplementation could sustain maternal antibody of treated chicks.

Body Weight (Kg): The effects of Cr-Prop supplementation on growth performance of broiler chicks were determined on the basis of live body weight, weight gain and feed intake feed conversion ratio. The body weights at nineth week of age were 3.23, 2.55, 3.13, 2.62 and 3.29 Kg for groups I, II, III, IV and V respectively. Data in (Fig. 2) illustrated that Cr-Prop supplementation was associated with significant ($p < 0.05$) increase in body weights in the Cr-Prop supplemented groups I, III, and V as compared to non supplement groups II and IV.

Non significant difference ($p > 0.05$) in body weights was observed between non supplement groups II and IV.

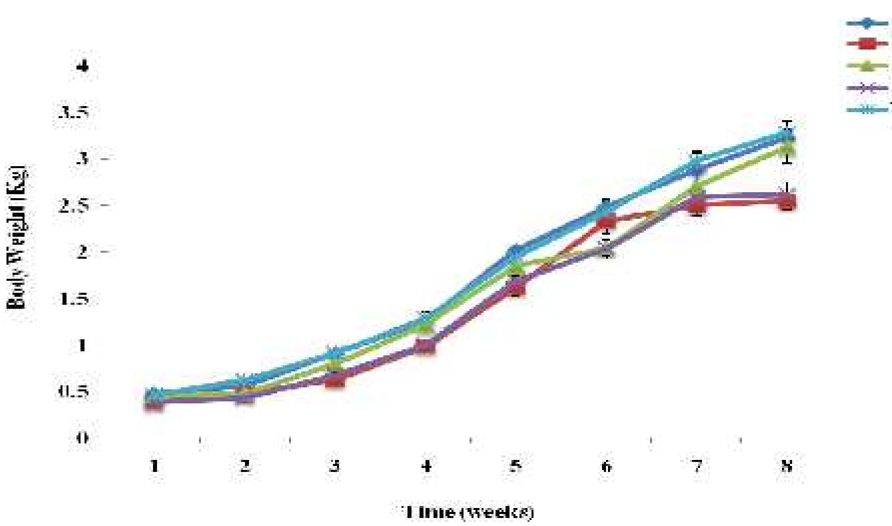
Feed Intake: The result illustrated in fig. 3 reveals non significant ($p > 0.05$) differences in feed consumption among the treated groups I, III, V and the untreated groups II and IV with values ranging from 620g – 857g in the treated groups and 522g - 702g in the untreated groups in the first week of feeding; but variable significant differences ($p \leq 0.05$) that ranged from 2057g – 3173g in the treated group, and 2002g - 2975g in the untreated groups in the last week of the experiment (fig. 3).

The results revealed non significant ($p > 0.05$) difference in weekly mean body weight gain in in birds in the treated groups I,III and V at the end of the study which were significantly ($p \geq 0.05$) higher than those in the untreated groups II and IV. The mean body weight gain in birds at the end of the study were 5405, 1550, 4975, 2265 and 5685g for groups I, II, III, IV and V respectively. The feed conversion ratio was significantly ($p \geq 0.05$) different among the groups and did not reflect dietary inclusion of organic Cr-Prop. The mean feed conversion ratio for groups I, II, III, IV and V at the end of the experiment were 3.31, 2.30, 9.16, 6.83 and 2.50 respectively.



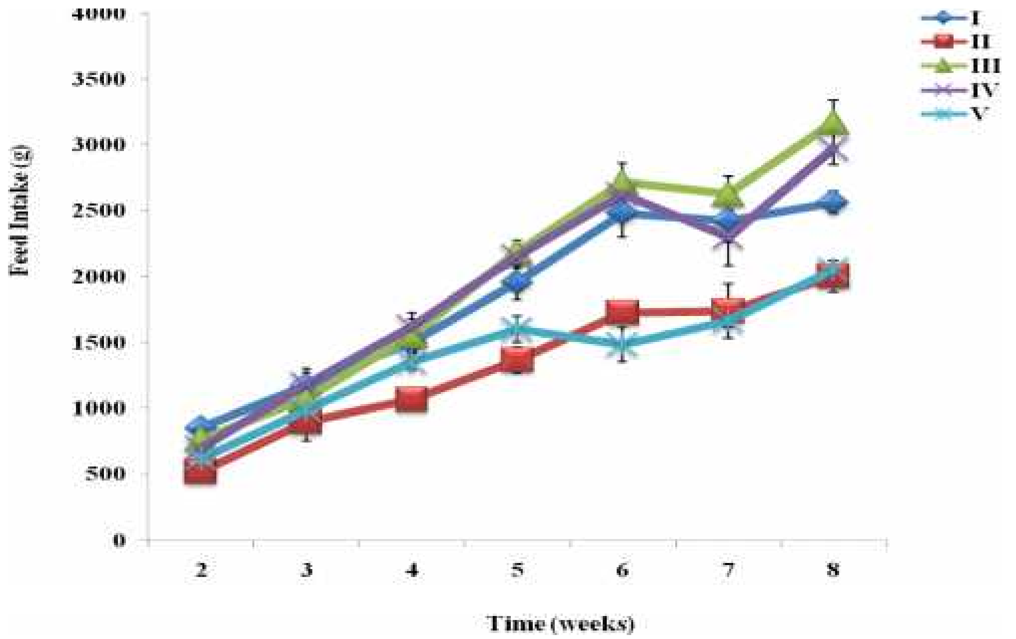
Group I = treated, ND vaccine vaccinated, IBD vaccinated. Group II = untreated, ND vaccine vaccinated, IBD vaccinated
Group III = treated, ND vaccine vaccinated, IBD vaccinated. Group IV = untreated, ND unvaccinated, IBD vaccinated
Group V = treated, ND unvaccinated, IBD unvaccinated.

Fig. 1. Effect of Chromium Propionate supplementation on immune responses GMT of broilers to NDV- La Sota.



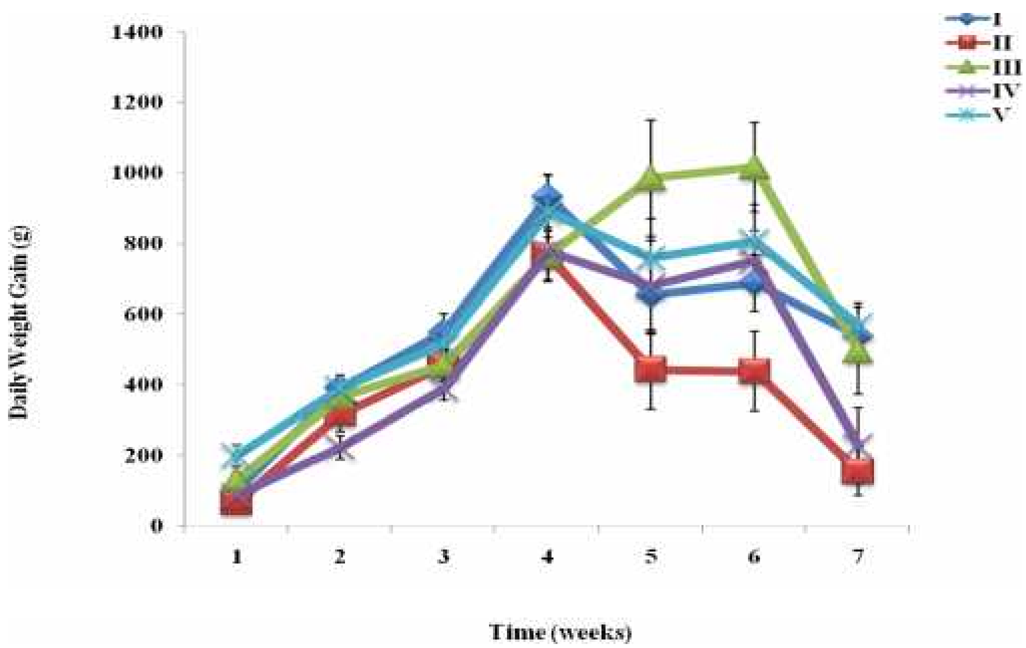
Group I = treated, ND vaccine vaccinated, IBD vaccinated Group II = untreated, ND vaccine vaccinated, IBD vaccinated
Group III = treated, ND vaccine vaccinated, IBD vaccinated Group IV = untreated, ND unvaccinated, IBD vaccinated
Group V = treated, ND unvaccinated, IBD unvaccinated

Fig. 2. Effect of Cr-Prop supplementation on body weights of broilers (Kg).



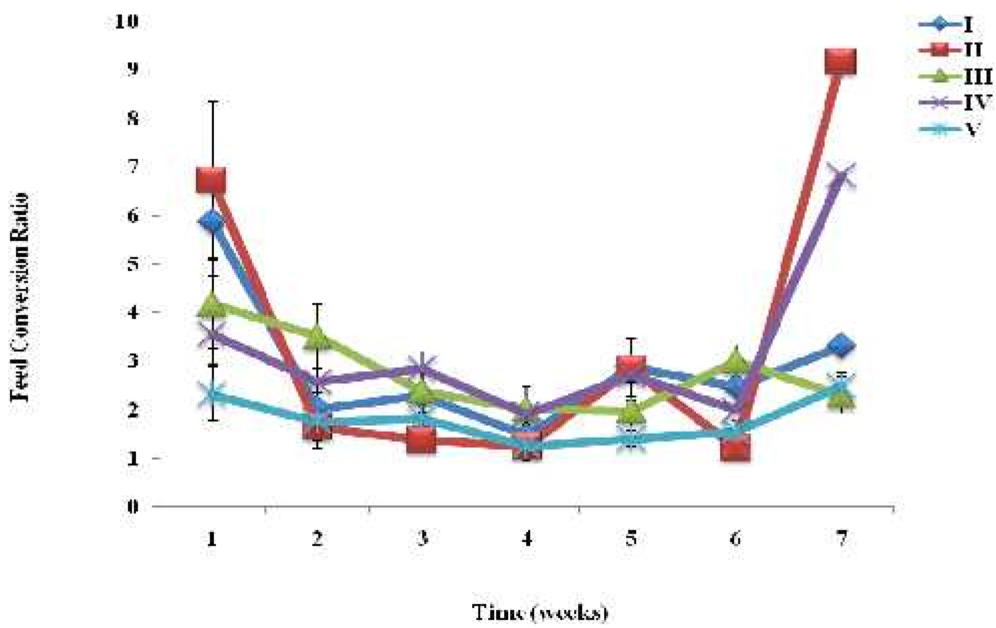
Group I = treated, ND vaccine vaccinated, IBD vaccinated
Group II = untreated, ND vaccine vaccinated, IBD vaccinated
Group III = treated, ND vaccine vaccinated, IBD vaccinated
Group IV = untreated, ND unvaccinated, IBD vaccinated
Group V = treated, ND unvaccinated, IBD unvaccinated

Fig. 3. Effect of Cr-Prop supplementation on daily feed intake of broilers (g).



Group I = treated, ND vaccine vaccinated, IBD vaccinated
Group II = untreated, ND vaccine vaccinated, IBD vaccinated
Group III = treated, ND vaccine vaccinated, IBD vaccinated
Group IV = untreated, ND unvaccinated, IBD vaccinated
Group V= treated, ND unvaccinated, IBD unvaccinated

Fig. 4. Effect of Cr-Prop supplementation on the daily body weight gain of broilers (g).



Group I = treated, ND vaccine vaccinated, IBD vaccinated
Group II = untreated, ND vaccine vaccinated, IBD vaccinated
Group III = treated, ND vaccine vaccinated, IBD vaccinated
Group IV = untreated, ND unvaccinated, IBD vaccinated
Group V = treated, ND unvaccinated, IBD unvaccinated

Fig. 5. Effect of Cr-Prop supplementation on feed conversion ratio of broilers.

DISCUSSION

The present study revealed that supplemental Cr-Prop increased antibody titer against ND vaccine. That supplemental Cr modulates immune response in mammals and birds has been substantiated reported by a number of authors (Lee *et al.*, 2003; Farshid and Majid 2009). The results of this study agree with those of Cao *et al*. (2004) who reported increased total antibody titers in broilers fed Cr supplemented feed. Similar results have also been reported by El-Hommosany (2008) who demonstrated that total antibody and IgG titers against sheep red blood cells (SRBCs) were significantly higher in quail chicks treated with supplemental Cr at low and middle doses (125 and 250 mg/kg feed respectively).

The mechanism by which dietary Cr influences the immune system has not been clearly understood substantiated. Wang *et al.* (1996) reported that Cr modulates immune response through its effects on Cytokine release. Cytokines are small molecules that transport information among cells. Cytokines together with their receptors play a vital role as central regulators of the immune system by affecting the activities of other cells. (Callard *et al*. 1999; Davison 2003).

Very few works have been documented on the effects of Cr-Prop in broilers. Jackson *et al.* (2008) reported improved feed efficiency and decreased mortality but no effect on carcass traits. Most work on Cr in broilers has been with the inorganic compound with varying results. Elevated antibody titers against ND (Guo *et al.,* 1999), infectious bronchitis (Lee *et al.,* 2003) and enhanced expression of IFN-c mRNA (Bhagat *et al.,* 2008) have been reported. The significance of Cr producing the same effect lies in the fact of its solubility and bioavailability. Inorganic Cr compounds are poorly absorbed in humans and animals. Absorption ranges from 0.4 to 3% or less, regardless of dose and dietary Cr status (Anderson *et al*., 1983). According to Mowat (1997), the bioavailability of organic Cr is around 25-30%. It is important to note that the varying or inconsistent results on immune response to supplemental Cr have been suggested to be due to differences in Cr forms, dosages, routes of administration and even species (Farshid and Majid 2009). Although intensive researches have shown the effects of Cr treatment on various performance characteristics and the challenge lies in drawing up a consistent conclusion on the element. In spite of this however, Cr is still considered an essential trace element. This has lead to a considerable research interest in the utilization of Cr in animal feed. In this field there is emerging evidence to suggest that pigs and poultry may have dietary requirement for Cr that exceed that found in a corn-soybean diet (Rao, *et al.,* 2012).

This research study revealed that Cr-Prop supplementation improved the performance of the broiler chickens with respect to mean weekly live-weight, mean daily live-weight gain, mean daily feed intake and feed conversion ratio. This is in agreement with the observations of Nam *et al.* (1995) and Amatya *et al.* (2004) who reported improved performance of broilers by feed supplementation with Cr Chloride. Lien *et al.* (1999) reported that performance of broilers given Cr picolinate supplementation in a broiler diet improved live weight gain. Kim *et al.* (1996) also observed increased weight gain in broilers given feed supplemented with Cr picolinate. These results are consistent with previous studies (Chen *et al.,* 2001; Eren and Baspnar 2004; and Kroliczewska *et al.,* 2004); reported that Cr supplementation to various poultry diets improved body weight, body weight gain and feed conversion ratio.

The improved liveweight, liveweight gain, feed intake and feed conversion ratio better utilization of the nutrients and improved immune response to ND vaccine in the Cr supplemented birds suggested beneficial effect of supplementing poultry diets with exogenous Cr sources.

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