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Effects Different Levels of Nanoparticles Chromium Picolinate Supplementation on Growth Performance, Mineral Retention, and Immune Responses in Broiler Chickens

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Abstract

This study was conducted to investigate the effects of different levels nanoparticles of chromium picolinate (NanoCrPic) on the performance, immune responses, mineral retention, and tissues accumulation of chickens. A total of 180 broilers were randomly allocated into 0 (control), 500 ppb (μg kg^{-1}) Cr and 3000 ppb Cr groups with 6 replicates (10 birds/pen) for a 35-day experiment, the Cr is nanoparticles of chromium picolinate (NanoCrPic). In addition, 36 birds were used for metabolic experimental investigation. The results of the experimentation indicated that there were no significant differences in average body weight gain between groups, but feed conversion ratio (FCR) in 3000 ppb group was better than control group during 1-21 days. The carcass yields slightly lower in 3000 ppb group than control ($p<0.1$). Retention ratio of Zn, Fe, Mn, Ca, and P were significantly ($p<0.05$) increased in the 500 ppb Cr group. The addition of NanoCrPic caused increased mineral concentrations, such as Cr, Ca and P in the subjects' livers. Furthermore, the addition of NanoCrPic significantly increased lymphocytes and decreased both heterophils and H/L ratio ($p<0.05$). The ND (Newcastle disease) antibody titer was not affected in the broilers. In conclusion, supplemental NanoCrPic improved the retention of Zn, Fe Ca, notably it increased the concentration of Cr and Ca in the liver, and also increased the number of lymphocytes in broiler chickens.

Keywords: nanoparticle, chromium, mineral retention, immune response, broilers

1. Introduction

Chromium is an essential mineral element for humans and domestic animals (Lukaski, 1999). Trivalent chromium (Cr (III)) is associated with the metabolism of carbohydrates, lipids, and proteins in animals, it also term the "glucose tolerance factor (GTF)", since chromium regulates the metabolic action of insulin (Schwarz & Mertz, 1957). Wang and Xu (2004) suggested that the absorption and utilization of Cr may be dependent on its status in the intestinal tract. However, different Cr (III) forms have diverse rates of absorption. Organic Cr (III) has greater biological availability (utilization ability of animal) than inorganic Cr (III) (NRC, 1997; Lukaski, 1999). Inorganic Cr (e.g. $CrCl_3$) is very low, in the range of 0.5-2% (Mertz, 1969); organic Cr (e.g., CrPic) is better, in the range of 10-25% (Seerley, 1993).

In poultry, supplemental dietary CrPic resulted in an increase in egg production and improvements in the feed conversion ratio (Sahin et al., 2001; Yildiz et al., 2004). Moreover, organic Cr supplementation, particularly at 1200 ppb, increased the performance criteria, egg quality, and serum insulin concentrations of Japanese quails (Sahin et al., 2002). CrPic supplementation did not affect the body weight, feed consumption, or feed conversion ratio of broilers during 1-21 days, but the mortality rate of broilers was reduced and breast meat yield improved with supplemental Cr at either 300 or 400 ppb (Hossain et al., 1998). However, Ward et al. (1993) reported that organic Cr supplementation at 200 and 400 ppb Cr did not affect weight gain, feed intake, feed conversion ratio, nitrogen retention, or muscle crude protein and ether extract content of broilers at three weeks of age.

Immunological function has been enhanced by Cr (III), and its effects seem more pronounced during stress (Borgs & Mallard, 1998). Increasing in immune responses by Cr supplementation has been observed in broilers (Luo et al., 1999). Notably, most poultry diets are basically composed of plant origin ingredients, corn-soybean base diet, which have usually low content of chromium (Giri et al., 1990).

Nanotechnology is a multidisciplinary scientific undertaking involving the nature of the polymer, zeta potential, and vehicle. Nanoparticles, which involves at least one dimension reduced to a nano-metric scale, exhibits new electrical, magnetic, mechanical, and biological properties (Gref et al., 1994), and have been identified as critical factors influencing particle uptake (Delie, 1998). Therefore, the new phenomena and properties of nanoparticles may have unique potential applications. In previous work, chromium nanoparticles have been shown to produce beneficial effects on growth performance, body composition, as well as increased tissue concentrations of Cr in selected muscles (Wang & Xu, 2004). They have also been shown to enhance Cr digestibility and absorption in rats when supplemented with 200 ppb NanoCr as well as exhibiting the ability to alter some blood metabolite concentrations (Zha et al., 2007; Lien et al., 2009). Wang and Xu (2004) found that the supplemented 200 ppb nanoparticles chromium were able to produce beneficial effects on carcass characteristics, pork quality, and individual skeletal muscle weight. Results showed an approximate two- to three-fold higher tissue chromium deposition in selected muscle and organs compared to the control group, which implicated higher absorption and bioavailability of nanoparticles chromium. The toxicity study indicated that NanoCrpic at 1000 ppb *in vivo* and at 300 ppb *in vitro* shows no signs of toxicity to rats (unpublished data).

It is well known that a mineral intake at high levels will have antagonist effects on the other minerals. The knowledge pertaining to this antagonist in Cr still is in its infant stage. Iron-binding proteins are involved in chromium binding, transport, and storage (Feng et al., 2003). $CaCO_3$ reduces Cr uptake and retention in rats (Seaborn & Stoecker, 1990). It has also been reported that in stressed animal losses of zinc, copper, iron, and manganese were reduced by supplemental chromium (Sahin & Sahin, 2002*a*; Schrauzer et al., 1986). On the other hand, Sahin and Sahin (2002) reported that the utilization of nitrogen and Ca, P, Zn, and Fe is improved with supplemental chromium and ascorbic acid.

Our hypothesis is that the nanoparticles chromium may also have an antagonist effect on other minerals utilization and excretion, and consequently influence the animal growth performance and immune function. Therefore, this study was designed to compare the effects of dietary supplementation of nanoparticles of chromium picolinate on growth performance, mineral retention, and the immune responses of broiler chickens.

2. Materials and Methods

2.1 Nanoparticles Size of Chromium Picolinate Determination

The powder NanoCrPic was supplied by the Industrial technology research institute of Taiwan (Shintsu, Taiwan). The NanoCrPic size was measured by TEM (Figure 1). The average particle size of NanoCrPic was 80.8± 2.7 nm.

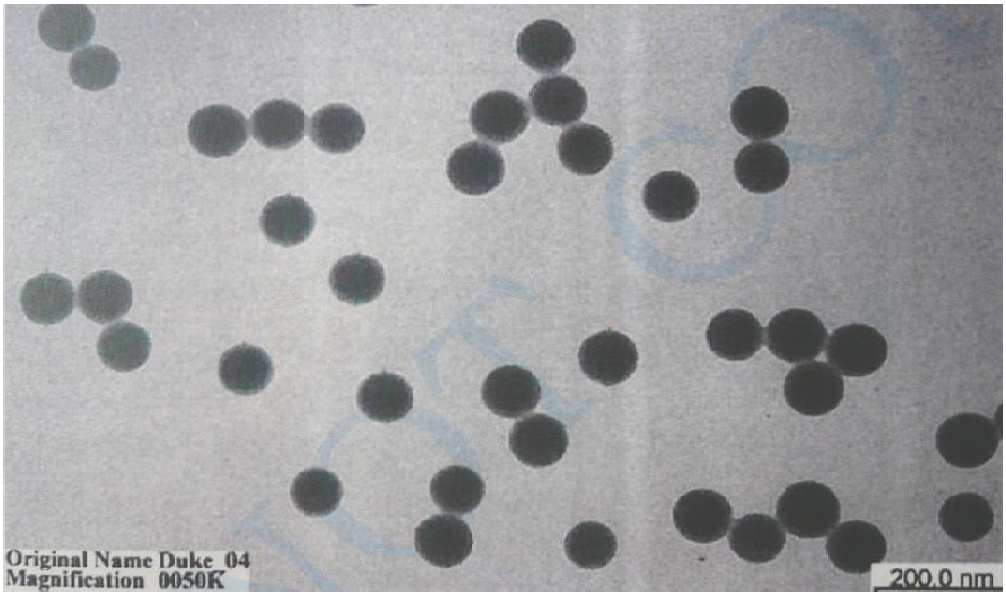

Figure 1. The image of nanoCr particles measured by TEM. Average particle size of NanoCrPic was 80.8±2.7 nm.

2.2 Experimental Design

Table 1. The basal diet composition of broiler chicken

Ingredients	starter (0-3wks)	grower (4-5wks)
	----------%----------	
Yellow Corn	41.59	47.81
Soybean meal	22.5	12.5
DDGS[1]	5.0	10.0
Full fat soybean meal	17.5	15.0
Soybean oil	3.0	2.0
Lard oil	2.0	2.0
Fish meal	2.5	2.5
Wheat middling	2.5	5.0
Dicalcium phosphate (18%)	1.2	1.1
Limestone (35%)	1.4	1.3
Sodium chloride	0.3	0.3
DL-Methionine	0.2	0.15
Vitamin premix[2]	0.15	0.13
Mineral premix[3]	0.15	0.13
Total	100	100
Calculated value		
Crude protein (%)	23.00	20.00
ME (MJ kg^{-1})	13.24	13.26
Calcium (%)	1	0.90
Available phosphorus (%)	0.45	0.35
Copper (mg)	8	6
Zinc (mg)	40	44
Analysis value		
Crude protein (%)	22.36	20.04
Chromium (ppb)	747.26	715.6

[1] DDGS : Distiller's dried grains with soluble.

[2] Vitamin premix supplied per kilogram contain: retinol 12,500 IU; cholecalciferol 25,000 ICU; DL-α tocopheryl acetate 2,000 IU; menadione 250 mg; thiamine 200 mg; pyridoxine 300 mg; cyanocobalamin 12 mg; pantothenate 120 mg; niacin 350 mg; biotin 200 μg; folic acid 100 mg.

[3] Mineral premix supplied per kilogram contain: Fe ($Fe_2(SO_4)_3$) 153 mg; Mn ($MnSO_4$) 200 mg; Cu ($CuSO_4$)17.64 mg; Zn (ZnO) 105.8 mg; Mg ($MgSO_4$) 25.3 mg; Co (Co SO_4) 0.4 mg; I (KI) 0.057 mg; Se (Na_2SeO_3) 0.25 mg.

One hundred and eighty (Arbor Acres) one day-old broiler chickens (equal numbers of each sex) were randomly divided into three groups with six replicates (10 birds/pen) (experiment unit). The three groups used in the present study consisted of the following: (1) basal diet (Table 1) (control group), was formulated based on NRC (1994); (2) basal diet supplemented with 500 ppb (μg kg^{-1}) Cr, i.e., nanoparticles of CrPic (NanoCrPic); (3) basal diet supplemented with 3000 ppb NanoCrPic for a 35 day trial with the trial period divided into two stages, namely, the starter period (0-3 weeks) and the growth period (4-5 weeks). Birds were floor-fed (width and length of each treatment: 160 cm×330 cm). All birds were provided with uniform floors, electric lamps, feeders and gutters.

Additionally, blood samples from 5 random birds per pen were taken for analysis at 14, 21 and 35 day intervals during the experiment. At the final of the experiment, 12 broilers (2 birds/pen) (pen is experiment unit) in each group were euthanized for carcass traits determine and liver samples were taken for mineral analysis. The average room temperature was 28.2±2.5°C and humidity was 71±5.5%. A continuous lighting program was provided during the experiment. The animals were reared according to the *Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching*, and this study was approved by the University's Animal Care and Use Committee.

One day old chicks were inoculated for Newcastle disease + infectious bronchitis (ND + IB). B1 strains were inoculated at seven days and the ND inactive vaccine was injected on the 28th day of the experiment.

2.3 Metabolic Trial

Thirty-six chickens were chosen (the average weight about 1.5 kg) at the day of 35, with 6 birds per group (three males and three females) in duplicates (2 birds/pen) (pen is the experiment unit) randomly assigned to three dietary groups. The individual cages were 90 cm x 60 cm x 60 cm with a plastic bag for collecting of total excreta. Birds were adapted for 7 days. The metabolic trial was lasting five days (on day 43-47). The total amount of food consumed (about 125 g day^{-1} $bird^{-1}$) and excreta voided from each chicken was recorded (dry matter basis) and sampling for components analysis. Mineral retention ratio was calculated using the following equation:

$$Mineral\ retention\ ratio = \frac{(WFI \times EF) - (WEV \times EE)}{(WFI \times EF)} \times 100$$

WFI=weight of feed intake (125 g)

EF=concentration of element in feed

WEV=weight of total excreta voided

EE=concentration of element in total excreta

2.4 Determination Traits

At 1, 21, and 35 days of age, individual body weight and feed consumed were recorded to calculate feed conversion ratios (feed/gain).

2.5 Basal Diet Components Analysis

Basal diet components of crude protein (CP: No. 7.015) were analyzed according the method of AOAC (2000). Gross energy using bomb calorimeter (Parr instrument company's, Illinois, USA) to measure.

2.6 Blood Traits Determination

Chickens were euthanized by severing the jugular vein; the blood sample (about 10 mL) was collected, after coagulation then centrifugation (1500×g, 15 min), the serum was obtained and stored at -40°C for hematological analysis.

Newcastle disease antibody titer (ND antibody titer) determined in duplicates were followed the method described by Giambrone (1981) with a hemagglutination inhibition (HI) procedure after the 35th day of the experiment.

Chickens blood was collected in a tube containing EDTA to prevent coagulation. Based on Campbell (1995), a small drop of blood was smeared on a slide and rapidly air dried. The smear slide was examined at 1000×magnification under oil immersion to count leucocytes up to a total of 100 cells, including basophils, eosinophils, lymphocytes, heterophils, and monocytes.

2.7 Chromium Analysis

Chromium analysis was base on the method of Anderson et al. (1985). Weighting 0.2 g of ground feed and excreta, and liver of each pen samples in duplicates were placed into crystal beakers, in which 10 mL of 70% nitric acid was added and rested for 8 h before beginning heat digestion (about 80°C) for 8 h. The samples were allowed to cool at which point the samples were diluted with 50 mL of de-ionized water. The filtered solution was kept in a polypolene bottle. Then, chromium was analyzed using a polarized Zeeman atomic absorption spectrometer (Hitachi, Japan) equipped with a graphite furnace, the analysis was done in duplicates.

2.8 Copper, Zinc, Manganese, and Iron Analysis

According to AOAC (2000) methodology (No. 2.109), the steps are as follows: Weighting 1 g of feed, excreta, and liver of each pen samples in duplicates were crushed under a crucible. Following this, samples were subjected to 550^{o}C for 5 h in a furnace to be converted to ash. The crucible was subjected to 10 mL 3 N HCl under a heating plate and heated until the solution became clear. It was then allowed to cool (filtration quantitative to 50 mL with 0.1 N HCl). An atomic absorption spectrometer (Perkin Elmer, Atomic Analyst 100, USA) was used to analyze the copper, zinc, manganese and iron contents.

2.9 Calcium and Phosphorous Analysis

According AOAC (2000) methodology (Ca: No. 7.096, P: No. 7.119), the steps are as follows: weighting 1 g of feed, excreta, and liver of each pen samples in duplicates were crushed under a crucible. Following this, samples were subjected to 550^{o}C for 5 h in a furnace to be converted to ash. The crucible was subjected to 10 mL 3 N HCl under a heating plate and heated until the solution became clear. It was then allowed to cool (filtration quantitative to 50 mL with 0.1 N HCl).

Calcium content analysis was undertaken by adding lanthanum 185.4 μL at 50,000 ppm to 6 mL samples solution. Then, an atomic absorption spectrometer (Perkin Elmer, Atomic Analyst 100, USA) was used for analysis.

For phosphorous determination, a 1 mL sample plus 1 mL Vanadium-molybenum acid and 3 mL de-ionized water (to a total of 5 mL) was left standing for 10 minutes and an automatic scanning sub-ray spectrometer (Beckman, DRR 640i, USA) was employed, with the wavelength set at 400 nm.

2.10 Statistical Analysis

The experiment data were subjected to statistical analysis using SAS software (version 9.1, SAS 1998). The general linear model procedure (GLM) was used based on the completely randomized design (CRD). Tukey's tests were adopted in the model to determine the P values among the variables. According to the following model, treatment (T) was the main effect.

$$Y=\mu+T_i+P_j+e_{ijk}$$

Where Y is the dependent variable, μ represents the mean, P is the pen (replicate, experiment unit) effect and e is the random residual error term. The level of significantly different was set at $p<0.05$.

3. Results and Discussion

3.1 The Effects of Different Levels of NanoCrpic on the Growth Performance of Broiler Chickens

The effects of dietary NanoCrPic supplementation on broiler chickens performance are shown in Table 2. This table demonstrates that a significant improvement in the feed conversion ratio (FCR) in 3000 ppb NanoCrPic group during 1-21 days of age was observed as compared to the control group ($p=0.05$). However, the average feed intake (AFI) was significantly decreased ($p<0.05$) by the 3000 ppb NanoCrPic supplementation in the period of 1-21 days and 22-35 days, as well as the 500 ppb groups in the period of 1-35 days. However, average body weight gain (ABWG) and body weight (BW) were insignificantly different with NanoCrPic supplementation.

Previous studies showed that the Cr supplementation at 0, 200, and 400 ppb had little or no effect on feed intake and feed efficiency in broiler chickens at six weeks of age (Motozono et al., 1998). Similarly, Anandhi et al. (2006) reported the same conclusions on the inclusion of chromium in their diet. Moreover, CrPic supplementation did not affect the body weight, feed consumption, or feed conversion ratio of broilers during 1-21 days, the mortality rate was reduced, and breast meat yield was improved with supplemental Cr at 300 or 400 ppb levels (Hossain et al., 1998). On the other hand, Sahin et al. (2002) reported that increased supplemental chromium (200, 400, 800, or 1200 ppb CrPic) resulted in an increase in body weight, feed intake, and feed efficiency in broilers reared under heat stress. In addition, Sahin et al. (2003) found the decrease in live weight gain and feed efficiency in broilers reared under heat stress was alleviated by dietary chromium and vitamin C supplementation.

The carcass characteristics of broiler chickens, namely carcass yield, percentage of dressing, relative liver, spleen, and thigh weight were not affected by the dietary groups (Table 3). Increased carcass yield in broilers has been reported for diets supplemented with CrPic (Sahin et al., 2002b 2003; Saikat et al., 2008). It is well known that Cr is involved in protein metabolism (Anderson, 1987). Also, Cr plays an important role as an integral component of the glucose tolerance factor (GTF), which potentiates the action of insulin and regulates glucose metabolism (Mertz, 1969). However, in this study no effects on carcass characteristics were observed during NanoCrPic supplementation, except that carcass weight was slightly lower in 3000 ppb group than control group ($p< 0.1$).

Table 2. The effect of different level of nano-chromium on performance of broilers

Age [days]	Supplemented dietary NanoCrPic (ppb)			SEM	P value
	0	500	3000		
	Body weight (g) (BW)				
1	34.00	34.00	34.00	0.33	0.58
21	749.0	710.0	688.0	16.0	0.11
35	1901	1851	1788	56.9	0.33
	Average body weight gain (g bird^{-1}) (ABWG)				
1-21	715.0	675.0	654.0	15.3	0.10
22-35	1152	1140	1100	27.5	0.62
1-35	1867	1817	1754	56.8	0.28
	Average feed intake (g bird^{-1}) (AFI)				
1-21	1113^{a}	1014^{ab}	939^{b}	12.3	0.01
22-35	2398	2279	2223	36.9	0.13
1-35	3503^{a}	3294^{b}	3162^{b}	42.3	0.01
	Feed conversion ratio [feed/gain] (FCR)				
1-21	1.56^{a}	1.50^{ab}	1.44^{b}	0.02	0.01
22-35	2.08	2.01	2.03	0.11	0.78
1-35	1.88	1.81	1.80	0.08	0.36

^{a,b} Means within the same row without the same superscripts differ significantly ($p<0.05$).

SEM: standard error of mean.

*n=6 (10 birds/pen).

Table 3. Effect of different level of nano-chromium on carcass characteristics and meat quality of 35 day-old broilers

Item	Supplemented dietary NanoCrPic (ppb)			SEM	P value
	0	500	3000		
Carcass weight (g)	1526	1488	1442	52.6	0.08
Dressing percentage (%)	80.3	80.4	80.4	0.55	0.97
Liver (g 100 g BW^{-1})	3.03	2.79	2.74	0.15	0.23
Spleen (g 100 g BW^{-1})	0.18	0.19	0.17	0.02	0.81
Thigh (g 100 g BW^{-1})	27.1	28.7	27.3	1.96	0.81

^{a,b} Means within the same row without the same superscripts different significantly ($p<0.05$);

SEM: standard error of mean;

*n = 6 (2 birds/pen).

3.2 The Effects of Different Levels of NanoCrPic on the Minerals Retention Ratio in Broiler Chickens

Chromium and other minerals, namely Cu, Zn, Fe, Mn, Ca and P retention ratio in the broilers are shown in Table 4. The mineral retention ratio of Cr ($p<0.0001$), Zn ($p<0.0001$), Fe ($p= 0.008$), Mn ($p=0.01$), Ca ($p<0.0001$) and P ($p=0.01$) were increased through NanoCrPic supplementation. However, retention of Cu was not affected by dietary treatments.

Amatya et al. (2004) reported that supplemental Cr had effect on the intake and retention of trace minerals (Cu, Zn, Fe, and Mn) in broilers. El-Husseiny and Creger (1981) also found that broilers reared under environmental stress had lower rates of Ca, Cu, Fe, K, Mg, Mn, Na, P, and Zn retention. Notably, stress increases chromium

mobilization from tissues and its excretion (Borel et al., 1984; Anderson, 1987). The retention of Ca and P among the Cr supplemented groups was varied; the 500 ppb group was better than the 3000 ppb group.

Table 4. The effect of different levels of nano-chromium on minerals retention ratio of broiler chickens

Items (%)	Supplemented dietary NanoCrPic (ppb)			SEM	P value
	0	500	3000		
Cr	3.72[b]	19.86[a]	24.03[a]	2.79	0.0001
Cu	24.77	32.01	28.42	3.03	0.83
Zn	51.48[b]	66.83[a]	67.42[a]	0.50	0.0001
Fe	51.88[b]	60.91[a]	57.72[a]	1.16	0.008
Mn	35.87[b]	48.69[a]	40.68[b]	1.12	0.01
Ca	42.59[c]	60.11[a]	48.84[b]	0.68	0.0001
P	24.12[b]	29.94[a]	26.25[b]	0.90	0.01

[a,b,c] Means within a row with no common superscripts are significantly different ($p<0.05$);

SEM: standard error of mean;

Mineral retention ratio = (intake - excreta) ÷ intake × 100%;

n = 6 (2 birds/pen).

3.3 The Effects of Different Levels of NanoCrPic on the Liver Minerals Retention in Broiler Chickens

The effects of dietary NanoCrPic supplementation on liver minerals are shown in Table 5. This table demonstrates that a significant accumulation of chromium in the liver ($p=0.0002$), as well as the phosphorus ($p<0.0001$) and calcium ($p<0.0001$) of NanoCrPic groups were observed as compared to the control group. Cu, Zn, Fe, and Mn were not affected by NanoCrPic supplementation.

Previous research studies indicated that Cr supplementation offers protection against stress-induced losses of Zn, Fe and Mn in liver and heart tissues (Schrauzer et al., 1986). Increased tissue concentrations of Cr, as observed in the present study, would have such phenomena. Amatya et al. (2004) reported that the concentration of copper, iron and zinc in the liver were increased while manganese was not affected by dietary chromium in broiler chickens. Increasing dietary chromium supplementation increased liver chromium and zinc concentrations, whereas copper concentrations decreased and liver iron concentrations were not affected in Japanese quails (Sahin et al., 2002). Those reports are in agreement with our results. The results of this study indicate that chromium is accumulated in liver.

Table 5. The effect of different level of nano-chromium on liver minerals content of broiler chickens

Items	Supplemented dietary NanoCrPic (ppb)			SEM	P value
	0	500	3000		
Cr (μg kg^{-1})	300[b]	563[a]	670[a]	36.3	0.0002
Cu (mg kg^{-1})	6.59	7.48	7.12	0.66	0.63
Zn (mg kg^{-1})	46.4	56.3	56.8	4.57	0.23
Fe (mg kg^{-1})	179	185	193	11.8	0.69
Mn (mg kg^{-1})	3.16	3.05	3.06	0.28	0.96
Ca (%)	0.015[b]	0.024[a]	0.025[a]	0.001	0.0001
P (%)	0.132[b]	0.221[a]	0.218[a]	0.005	0.0001

[a,b] Means within a row with no common superscripts are significantly different ($p<0.05$).

SEM: standard error of mean.

[*]n = 6 (2 birds/pen).

3.4 The Effects of Different Levels of NanoCrpic on Immune Responses in Broiler Chickens

The effects of supplemental dietary NanoCrPic on hematological parameters at the 35th day of the experiment is shown in Table 6. Statistical analysis revealed no significant difference in white blood cells (WBC) and WBC sub-groups, namely basophils, eosinophils, and monocytes. On the other hand, lymphocytes significantly

increased (p=0.0004) in chicken fed 3000 ppb NanoCrPic, whereas heterophils and heterophil to lymphocyte ratio were significantly decreased in both 500 and 3000 ppb NanoCrPic groups ($p<0.05$). The results were agreement with those of Zha et al. (2008) who found that dietary supplementation of 150, 300, and 450 ppb Cr from NanoCr enhanced the lymphoproliferative response in Sprague-Dawley rats; Toghyani et al. (2007) also reported increases in lymphocyte counts and decreases in heterophil to lymphocyte ratios in 1000 and 1500 ppb CrPic supplementation in heat-stressed chicks.

The number of heterophils increased in the blood of corticosterone (stress) fed chicks (Gross & Siegel, 1983). The heterophil to lymphocyte (H/L) ratio was used as an index of chronic stress (Bonier et al., 2007), based on the observation that increased glucocorticoid secretion may result of lymphocytopenia and a subsequent increase in heterophil numbers (Harmon, 1998). Moreover, the H/L ratio increases in response to a variety of stressors including malnutrition, water deprivation, and injury (Gross & Siegel, 1983; Vleck, 2000). Thus, the results of this study indicated that NanoCrPic supplementation exhibits an anti-stress function.

Table 6. The effect of different level of nano-chromium on concentration and subgroup ratio of white blood cells (WBCs) parameter in 35 days old broiler chickens

Hematological parameters	Supplemented dietary NanoCrPic (ppb)			SEM	P value
	0	500	3000		
WBC count (10^3 cells/mm^3)	27.6	24.8	25.7	1.46	0.42
WBC sub group (%)					
Basophils	12.5	11.0	12.2	0.84	0.43
Eosinophils	2.25	2.08	2.30	0.34	0.89
Lymphocytes	45.1^{b}	50.6^{b}	58.6^{a}	1.83	0.0004
Heterophils	27.5^{a}	23.9^{b}	23.8^{b}	0.53	0.002
Monocytes	7.92	9.00	9.50	1.28	0.68
H/L ratio	0.61^{a}	0.47^{b}	0.41^{c}	0.02	0.0001

[a, b] Means within the same row without the same superscripts differ significantly ($p<0.05$);

SEM: standard error of mean;

H/L: heterophils to lymphocyte ratio;

[*] n = 6 (5 birds/pen).

Dietary 3000 ppb NanoCrPic group had significant (p=0.01) effects on Newcastle disease antibody titers at 21 days; while at 28 and 35 days, no affects were resultant by NanoCrPic supplementation (Table 7). The results are in agreement with those of Toghyani et al. (2007) who reported that the antibody titers against Newcastle and Influenza virus tended to increase in broiler chickens receiving 1000 ppb and 1500 ppb CrPic. Elevated antibody titers against Newcastle disease were reported in broiler chicks with supplemental 2 or 10 mg/kg Cr, either in the form of $CrCl_3$ or Cr-yeast (Guo et al., 1999).

The role of chromium in the immune responses of mammals and chicken is well established (Burton et al., 1993; Lee et al., 2003). It has also been reported that chromium modulates the immune response through its effect on cytokine release (Wang et al., 1996).

Table 7. Effect of different level of nano-chromium on Newcastle disease antibody titer in broilers

Age (day)	Supplemented dietary NanoCrPic (ppb)			SEM	P value
	0	500	3000		
	ND[1] antibody titer ($\log_2$)				
21	2.72^{b}	2.85^{b}	3.54^{a}	0.18	0.01
28	3.43	3.87	3.64	0.16	0.18
35	3.87	3.95	3.98	0.17	0.87

[a,b] Means within a row with no common superscripts are significantly different ($p<0.05$).

SEM: standard error of mean.

[1] ND: Newcastle disease.

[*] n = 6 (5 birds/pen).

4. Conclusion

This study found that NanoCrPic supplementation in chickens can improve utilization of Zn, Fe, Ca and decrease the Zn, Fe, Ca content of excreta, showing increased minerals Cr, Ca concentration in the liver as well as in lymphocytes of broiler chickens.

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