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EFFECTS OF CHROMIUM ON THE GROWTH AND IMMUNOLOGICAL RESPONSE OF BROILER CHICKENS

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Abstract
The objective of the study was to determine the effects of varying levels of chromium picolinate (CrPic) on the growth performance and mineral retention of chickens during a 35-day experiment. 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). Thirty-six birds were then subjected to the metabolic experiment. The results of the study revealed 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 yielded slightly lower than control after being given high concentrations of chromium picolinate. Retention ratio of Zn, Fe, Mn, Ca, and P were significantly ($p<0.05$) increased in the 500 ppb Cr group. The addition of CrPic caused the mineral concentrations in the livers to increase. It also decreased the H/L and heterophils ratio and increased lymphocytes. The chickens did not exhibit the Newcastle disease antibody titer. However, the added CrPic improved the retention of zinc, Fe Ca, and Cr in the liver.
Keywords: picolinate, chromium, mineral retention, immune response, broilers

Introduction

Chromium is a mineral element that is essential for humans and animals. It helps in the digestion of carbohydrates and helps in the production of proteins. Wang and Xu (2004) suggested that the function of chromium in the intestine is dependent on its status. However, different forms of chromium can provide different absorption rates. Organic Cr (III) has greater biological availability (utilization ability of animal) than inorganic Cr (III) (NRC, 1997; Lukaski, 1999). Inorganic and organic chromium can be different in terms of their concentration. In poultry, supplemental dietary chromium can improve the feed conversion ratio (Sahin et al., 2001; Yildiz et al., 2004).

Inorganic chromium supplementation with a concentration of 1200 ppb was associated with higher performance criteria and better serum insulin concentrations in Japanese quails (Sahin et al., 2002). It also improved the breast meat yield and reduced the mortality rate of the animals (Hossain et al., 1998). Ward et al. (1993) stated that organic chromium supplementation at 200 and 400 ppb did not affect the weight gain, feed intake, or the feed conversion ratio of young broilers (three weeks of age).

Cr has been known to improve the immune function of chickens (Luo et al., 1999). Most poultry diets are basically composed of plant origin ingredients, corn-soybeanbase diet, which have usually low content of chromium (Giri et al., 1990). It can also be observed that increasing levels of Cr can trigger a more effective response during stressful situations (Borgs & Mallard, 1998). It is widely known that a mineral's concentration can affect the effects of other minerals. One particular mineral that can affect the uptake and retention of chromium is CaCO3 (Seaborn and Stoecker, 1990). 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).

Studies also revealed that chromium can reduce the losses of various minerals such as copper, iron, manganese, and zinc in stressed animals Sahin & Sahin, 2002; Schrauzer et al., 1986). These findings suggest that supplemental chromium can improve the utilization of these minerals. It is hypothesized that the chromium can also have an antagonist effect on the minerals' excretion and growth performance. This study was designed to investigate the effects of chromium on the growth and immunological response of broiler chickens.

Methods

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

DDGS : Distiller's dried grains with soluble.

Vitamin PREMIX contains: retinol 12,500 IU, cholecalciferol 25,000 ICU, menadione 250 mg, DL-α tocopheryl acetate 2,000 IU; pyridoxine 300 mg, pantothenate 120 mg, niacin 350 mg; biotin 200 μg; thiamine 200 mg and folic acid 100 mg. Premixes for mineral mixtures contain Fe (Fe2(SO4)3) 153 mg; Mn (MnSO4) 200 mg; Cu (CuSO4)17.64 mg; Zn (ZnO) 105.8 mg; Mg (MgSO4) 25.3 mg; Co (Co SO4) 0.4 mg; I (KI) 0.057 mg; Se (Na2SeO3) 0.25 mg. A hundred and eighty day-old broiler chickens were randomly split into three groups. The three groups were respectively placed on a (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). All birds were floor-fed with uniform floors. They were provided with electric lamps and feeders. The blood samples of the participating birds were analyzed at various intervals (14, 21 and 35 days) during the experiment. The liver and carcass traits of the chickens 12 chickens (2 birds/pen) (pen is experiment unit) in each group were also determined. The conditions of the chickens were ideal for the study. The humidity and temperature were also ideal for the experiment. The average room temperature was 28.2±2.5oC and humidity was 71±5.5%. A continuous lighting program was provided during the experiment. The animals were kept in accordance with the University's animal care and use guidelines. A day old chicks were inoculated with Newcastle disease + infectious bronchitis (ND + IB). The B1 strains were inoculated for seven days. ND inactive vaccine was injected on the 28th day of the experiment. Thirty-six chickens with average weight 1.5kg were chosen for the experiment, with each group containing six birds. The animals were placed in three different dietary groups and were observed to collect total excreta. The metabolic trial lasted for seven days. The animals were also adapted to live for seven days. The data collected during the study revealed that the animals consumed about 125g of food a day. The mineral retention ratio was also calculated using the formula:

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

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

At 1 to 21, and up to 35 days of age, the individual's weight and feed consumption were recorded to determine the feed conversion ratios. The Basal Diet components of crude protein were analyzed using the AOAC (2000) method. Chickens were euthanized by cutting the jugular vein off. The blood samples were collected after centrifugation (1500×g, 15 min) to obtain a serum for analysis. A method similar to that used by Giambrone in 1981 was used to determine the Newcastle disease antibody titers. The chicken's blood was collected using a tube containing EDTA. The sample was then air dried and subjected to 1000xmagnification to count the cells.

The method used for chromium analysis was based on the study of Anderson et al. (1985). For the analysis, the samples were placed into crystal beakers with a weight of about 0.2 g of ground feed and excreta. The liver and nitric acid were then 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 chromium was then filtered using a polypolene bottle. It was then analyzed using a polarized atomic absorption spectrometer.

The following steps were established by AOAC (2000): 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 550oC 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. Also, the following steps were established in 2000 by AOAC. They involved crushing the samples weighing 1 g of feed, excreta, and liver into a crucible. The samples were then subjected to a temperature of 550 degrees Celsius for 5 h to convert them to ash. Then, the calcium content analysis was performed by adding lanthanum 185.4 L at 50,000 ppm to a 6 mL sample solution. For phosphorous determination, the samples were left standing for 10 minutes while they were analyzed using a sub-ray spectrometer. The data was then analyzed using SAS software. The statistical procedure was performed based on the general linear model. The model was then used to determine the P values for the various parameters.

$$Y=\mu+Ti+Pj+eijk$$

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 $p<0.05$.

Results and Discussion

The Effects of Different Levels of Crpic on the Growth Performance of Chickens

Table 2. The effect of different level of chromium on performance of chickens

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).

The effects of CrPic supplementation on the performance of broiler chickens were shown in Table 2. Compared to the control group ($p=0.05$), the table shows that the group's feed conversion ratio (FCR) in 3000 ppb CrPic improved significantly during the 1-21 days of age. In addition, the average feed intake of the chickens was decreased by 3000 ppb of CrPic during the study period. The effects of the supplement were were insignificantly different on the average body weight and average size of the chickens.

Studies have shown that Cr supplementation at zero, 200 and 400 ppb did not affect the feed intake and feed efficiency of broiler chickens at six weeks of age (Motozono et al., 1998). 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, studies conducted by Sahin et al. (2003) showed that increasing the consumption of vitamin C and chromium reduced the feed efficiency and live weight gain of broiler chickens.

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

Means within the same row without the same superscripts different significantly ($p<0.05$); SEM: standard error of mean; *n = 6 (2 birds/pen).

Although the effects of CrPic on various carcass characteristics were not affected by the dietary groups (Table 3), Sahin et al., (2003) and Saikat et al., (2008) reported that diets with the same nutrients boosted the chickens' carcass yield. It is widely known that Cr is involved in the regulation of protein metabolism and the action of the glucose tolerance factor. In this study, the effects of CrPic on the carcass characteristics were not observed except that carcass weight was slightly lower in 3000 ppb group than control group ($p< 0.1$).

The Effects of Different Levels of CrPic on the Minerals Retention Ratio in Chickens

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).

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 effects on the retention of trace minerals and intake in broilers. Also, exposure to environmental stress increases the chromium excretion from tissues.

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

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).

The effects of dietary CrPic on liver minerals are demonstrated in Table 5. This table shows 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 CrPic groups were observed as compared to the control group. Cu, Zn, Fe, and Mn were not affected by CrPic supplementation. Studies on the effects of Cr supplementation on the liver and heart tissues indicated that it can protect against the effects of stress on these organs (Schrauzer et al., 1986). Increased tissue concentrations of Cr, as observed in the present study, would have such phenomena. In addition, increasing dietary chromium concentrations boosted zinc and liver chromium concentrations. The results of this study support the notion that liver minerals are accumulated in the body.

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

Table 6. The effect of different level of 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).

At 35 days, the effects of CrPic on hematological factors were studied. Although the effects of the supplement on different sub-groups of white blood cells were not significant, lymphocytes exhibited a significant increase in response to CrPic. Statistical analysis in Table 6 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 CrPic, whereas heterophils and heterophil to lymphocyte ratio were significantly decreased in both 500 and 3000 ppb CrPic groups ($p<0.05$).

Zha et al. (2008) studied the effects of CrPic on the lymphoproliferative response of Sprague-Dawley rats. Zha et al. (2008) found that dietary supplementation of 150, 300, and 450 ppb Cr from NanoCr enhanced the lymphoproliferative response in Sprague-Dawley rats; In addition, Toghyani et al. (2007) showed that CrPic supplementation led to a decrease in the number of lymphoproliferative cells in heat-stressed chicks.

The number of heterophils in the blood of corticosterone fed chicks increased during episodes of chronic stress. The prevalence of these factors was then computed as an index of chronic stress. The stress response associated with various stress factors such as water deprivation and malnutrition was also exhibited by the study. Thus, the results of this study indicated that CrPic supplementation exhibits an anti-stress function.

Table 7. Effect of different level of 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).

The effects of ingesting 3000 ppb CrPic on the levels of antibodies against Newcastle disease were significant at 21 days. At 28 and 35 days, no effects were observed on the levels of antibodies against the virus as shown in Table 7. Toghyani et al. (2007) reported that the levels of antibodies against Influenza virus and Newcastle disease were higher in chickens that received 1500 and 1000 ppb CrPic. Guo et al., (1999), an immunological response against Newcastle disease was observed in broiler chicks that had 2 or 10 mg Cr.

Conclusion

This study conducted on broiler chickens revealed that NanoCrPic supplementation can improve the utilization of zinc, Fe, and Ca in the liver and decrease the Ca content in excreta.

References

A.O.A.C. (2000). *Official Methods of Analysis* (17th ed). Association of Official Analytical Chemist. Virginia, U.S.A.

Amatya, J. L., Haldart, S., & Ghosh, T. K. (2004). Effects of chromium supplementation from inorganic and organic sources on nutrient utilization, mineral metabolism and meat quality in broiler chickens exposed to natural heat stress. *Anim. Sci.*, *79*, 241-253.

Anandhi, M., Mathivanan, R., Viswanathan, K., & Mohan, B. (2006). Dietary inclusion of organic chromium on production and carcass characteristics of broilers. *Int. J. Poult. Sci., 5*, 880-884. http://dx.doi.org/10.3923/ijps.2006.880.884

Anderson, R. A. (1987). *Chromium: Trace Elements in Human and Animal nutrition* (pp. 225-244). New York: Academic Press.

Anderson, R. A., & Kozlovsky, A. S. (1985). Chromium intake, absorption and excretion of subjects consuming self-selected diets. *Am. J. Clin. Nutr., 41*, 1177-1183.

Bonier, F., Martin, P. R., Sheldon, K. S., Jensen, J. P., Foltz, S. L., & Wing Field, J. C. (2007). Sex-specific consequences of life in the city. *Behav. Ecology, 18*, 121-129. http://dx.doi.org/10.1093/beheco/arl050

Borel, J. S., Majeres, T. C., Polansky, M., Moser, P. B., & Anderson, R. A. (1984). Chromium intake and urinary chromium excretion of trauma patients. *Biolog. Trace Elem. Res.* (Abstr.).

Borgs, P., & Mallard, B. A. (1998). Immune-endocrine interactions in agricultural species: Chromium and its effect on health and performance. *Domes. Anim. Endoc., 15*, 431-438. http://dx.doi.org/10.1016/S0739-7240(98)00018-6

Burton, J. L., Mallard, B. A., & Mowat, D. N. (1993). Effects of supplemental chromium on immune responses of periparturient and early lactation dairy cows. *J. Anim. Sci., 71*, 1532-1539.

Campbell, T. W. (1995). *Avian Hematology and Cytology* (p. 108). Ames: IowaState University Press.

Delie, F. (1998). Evaluation of nano-and microparticle uptake by the gastrointestinal tract. *Adv. Drug Deliv. Rev., 34*, 221-233. http://dx.doi.org/10.1016/S0169-409X(98)00041-6

El-Husseiny, O., & Creger, C. R. (1981). Effect of ambient temperature on mineral retention and balance of the broiler chicks. *Poult. Sci., 60*, 1651. (Abstr.).

Feng, W., Li, B., Liu, J., Chai, Z., Zhang, P., Gao, Y., & Zhao, J. (2003). Study of chromium containing proteins in sub cellular fractions of rat liver by enriched stable isotopic tracer technique and gel filtration chromatography. *Analyt. Bioanalyt. Chem., 375*, 363-368.

Giambrone, J. J. (1981) Laboratory evaluation of the immune response of young broiler chickens vaccinated against *Newcastle* disease under field condition. *Poult. Sci.*, 60, 1204-1208. http://dx.doi.org/10.3382/ps.0601204

Giri, J., Usha, K., & Sunita, T. (1990). Evaluation of the selenium and chromium content of plant foods. *Plant foods Human Nut., 40*, 49-59.

Gref, R., Minamitake, Y. M., Peracchia, T., Trubetskoy, V., Torchilin, V., & Langer, R. (1994). Biodegradable long-circulating polymeric nanospheres. *Science, 263*, 1600-1603. http://dx.doi.org/10.1126/science.8128245

Gross, W. B., & Siegel, H. S. (1983). Evaluation of the heterophil/lymphocyte ratio as a measure of stress in chickens. *Avian Dis., 27*, 972-979. http://dx.doi.org/10.2307/1590198

Guo, Y. L., Luo, X. G., Hao, Z. L., Liu, B., Chen, J. L., Gao, F. S., & Yu, S. X. (1999). Effect of chromium on growth performance, serum biochemical traits, immune function and carcass quality of broiler chickens. *Sci. Agri. Sinica, 32*, 79-86.

Harmon, B. G. (1998). Avian heterophils in inflammation and disease resistance. *Poult. Sci., 77*, 972-977.

Hossain, S. M., Barreto, S. L., & Silva, C. G. (1998). Growth performance and carcass composition of broilers fed supplemental chromium from chromium yeast. *Anim. Feed Sci. Technol., 71*, 217-228. http://dx.doi.org/10.1016/S0377-8401(97)00160-0

Lee, D. N., Wu, F. Y., Cheng, Y. H., Lin, R. S., & Wu, P. C. (2003). Effect of dietary chromium picolinate supplementation on growth performance and immune responses of broilers. *Asian-Aust. J. Anim. Sci., 16*, 227-233.

Lien, T. F., Yeh, H. S., Lu, F. Y., & Fu, C. M. (2009). Nanoparticles of chromium picolinate enhance chromium digestibility and absorption. *J. Sci. Food Agric., 89*, 1164-1167. http://dx.doi.org/10.1002/jsfa.3569

Lukaski, H. C. (1999). Chromium as a supplement. *Annual Rev. Nutr., 19*, 279-302. http://dx.doi.org/10.1146/annurev.nutr.19.1.279

Luo, X., Guo, Y. L., Liu, B., Hao, Z. L., Chen, J. L., Gao, F. S., & Yu, S. X. (1999). Effect of dietary chromium on growth, serum biochemical traits and immune responses of broiler chicks during 0-3 weeks of age. *Acta veterin. Et Zootech. Sinica*, 30, 481-489.

Mertz, W. (1969). Chromium occurrence and function in biological systems. *Physiol. Rev., 49*, 163-239.

Motozono, Y., Hatano, K., Sugawara, N., & Ishibashi, T. (1998). Effects of dietary chromium picolinate on growth, carcass quality and serum lipids of female broilers. *Anim. Sci. Technol., 69*, 659-665.

National Research Council. (1994). Nutrient requirements of poultry, ninth revised edition. Washington, D.C.: National Academy Press.

Sahin, K., & Sahin, N. (2002). Effects of chromium picolinate and ascorbic acid dietary supplementation on nitrogen and mineral excretion of laying hens reared in low ambient temperature (7°C). *Acta Veterin. Brno, 71*, 183-189. http://dx.doi.org/10.2754/avb200271020183

Sahin, K., Kucuk, O., & Sahin, N. (2001). Effects of dietary chromium picolinate supplementation on performance and plasma concentrations of insulin and corticosterone in laying hens under low ambient temperature. *J. Anim. Physiol. A. Anim. Nutr., 85*, 142-147. http://dx.doi.org/10.1046/j.1439-0396.2001.00314.x

Sahin, K., Sahin, N., & Kucuk, O. (2002). Effects of dietary chromium picolinate supplementation on serum and tissue mineral contents of laying Japanese quails. *J. Trace Elem. Exper. Med., 15*, 163-169. http://dx.doi.org/10.1002/jtra.10013

Sahin, K., Sahin, N., & Kucuk, O. (2003). Effects of chromium, and ascorbic acid supplementation on growth, carcass traits, serum metabolites and antioxidant status of broiler chickens reared at a high ambient temperature. *Nutr. Res., 23*, 225-238. http://dx.doi.org/10.1016/S0271-5317(02)00513-4

Saikat, S., Haldar, S., Bahadur, V., & Ghosh, T. K. (2008). Chromium picolinate can ameliorate the negative effects of heat stress and enhance performance, carcass and meat traits in broiler chickens by reducing the circulatory cortisol level. *J. Sci. Food Agri., 88*, 787-796. http://dx.doi.org/10.1002/jsfa.3146

Schrauzer, G. N., Shrestha, K. P., Molenaar, T. B., & Mead, S. (1986). Effects of chromium supplementation on food energy utilization and the trace-element composition in the liver and heart of glucose-exposed young mice. *Biolog. Trace Elem. Res.* (Abstr.).

Schwarz, K., & Mertz, W. (1957). A glucose tolerance factor and its differentiation from factor 3. *Arch. Biochem. Biophys., 72*, 515-518. http://dx.doi.org/10.1016/0003-9861(57)90228-X

Seaborn, C. D., & Stoecker, B. J. (1990). Effects of antacid or ascorbic acid on tissue accumulation and urinary excretion of ^{51}chromium. *Nutr. Res., 10*, 1401-1407. http://dx.doi.org/10.1016/S0271-5317(05)80132-0

Seerley, R. W. (1993). Organic chromium and manganese in human nutrition: important possibilities for manipulating lean meat deposition in animals. *Proceeding of Alltech's 9th Annual Symposium* (pp. 41-51). KY, USA: Nicholasville.

Statistical Analysis System Institute Inc. (1998). *SAS/STAT User's guide: statistics*, Version 6.06, Cary, NC, USA: SAS Institute Inc.

Toghyani, M., Zarkesh, S., Shivazad, M., & Gheisari, A. (2007). Immune responses of broiler chicks fed chromium picolinate in heat stress condition. *J. Poult. Sci., 44*, 330-334. http://dx.doi.org/10.2141/jpsa.44.330

Vleck, C. M. (2000). Comparison of corticosterone and heterophil to lymphocyte ratios as indicators of stress in free-living birds. In: A. Dawson (ed.). Perspectives in avian endocrinology. India: Narosa Publ.

Wang, J. L., Tsukayama, D. T., Wicklund, B. H., & Gustilo, R. B. (1996). Inhibition of T and B cell proliferation by titanium, cobalt and chromium: Role of IL-2 and IL-6. *J. Biomed. Materi. Res., 32*, 655-661. http://dx.doi.org/10.1002/(SICI)1097-4636(199612)32:4<655::AID-JBM19>3.0.CO;2-C

Wang, M. Q., & Xu, Z. R. (2004). Effect of chromium nano-particle on growth performance, carcass characteristics, pork quality and tissue chromium in finishing pigs. *Asian-Aust. J. Anim. Sci., 17*, 1118-1122.

Ward, T. L., Southern, L. L., & Boleman, S. L. (1993). Effect of dietary chromium picolinate on growth, nitrogen balance and body composition of growing broiler chicks. *Poult. Sci., 72*, 37-43.

Yildiz, A., Parlat, S., & Yazgan, O. (2004). The effects of organic chromium supplementation on production traits and some serum parameters of laying hens. *Revue de Méd. Véterin., 12*, 642-646.

Zha, L., Zeng, J., Sun, S., Deng, H., Luo, H., & Li, W. (2008). Chromium (III) nanoparticles affect hormone and immune responses in heat-stressed rats. *Biolog. Trace Elem. Res., 129*, 157-169. (Abstr.). http://dx.doi.org/10.1007/s12011-008-8282-9

Zha, L.Y., Xu, Z. R., Wang, M. Q., & Gu, L. Y. (2007). Effects of chromium nanoparticles dosage on growth, body composition, serum hormones and tissue chromium in Sprague-Dawley rats. *J. Zhejiang University Sci., B*(8), 323-330. http://dx.doi.org/10.1631/jzus.2007.B0323