

Effect of Different Sources of Supplemental Chromium on Performance and Immune Responses of Broiler Chicks

¹Amir Naghieh, ¹Majid Toghyani, ²Abbas Ali Gheisari, ¹Shahin Eghbal Saeed and ³Hadi Miranzadeh
¹Department of Animal Science, Islamic Azad University, Khorasgan (Esfahan) Branch, Esfahan, Iran
²Department of Animal Science, Esfahan Agriculture Research Center, Esfahan, Iran
³Jahad-e-Keshavarzi Educational Center, Esfahan, Iran

Abstract: This study was conducted to evaluate the effect of different sources of chromium supplementation on performance and immune responses of broiler chicks. Four hundred and thirty two broiler chicks (308 Ross) were randomly allocated to 9 treatments and 4 replicates in a completely randomized design. Treatments consisted of: Control group, Cr-chloride, Cr-yeast, Cr-nicotinate and Cr-methionine at two levels of 600 and 1200 $\mu\text{g kg}^{-1}$ Cr-supplementation. Feed intake, weight gain and feed conversion ratio measured in different periods. Antibody titers against Newcastle and Influenza virus were measured at 20 and 31 days and serum albumin to globulin ratio at 31 days. Results showed feed intake and body weight of broilers received 600 $\mu\text{g kg}^{-1}$ Cr-nicotinate were significantly increased ($p < 0.05$). Supplemental Cr had not significant effect on feed conversion ratio. Broilers were fed 1200 $\mu\text{g kg}^{-1}$ Cr-methionine and 600 $\mu\text{g kg}^{-1}$ Cr-chloride had the highest carcass yield and the lowest abdominal fat respectively ($p < 0.05$). Antibody titers against Newcastle and Influenza viruses were elevated in broilers received 1200 and 600 $\mu\text{g kg}^{-1}$ Cr-nicotinate, respectively. The results of this experiment showed between different Cr-sources were used in this study Cr-nicotinate improved feed intake, body weight and antibody titer in broiler chicks.

Key words: Broiler chicks, Cr-source, performance, antibody titer, carcass traits

INTRODUCTION

Trivalent Cr is an essential element that is required for the normal metabolism of carbohydrates, proteins and lipids in humans and laboratory animals (Anderson, 1997). Primary role of Cr in metabolism is to potentiate the action of insulin through its presence in an organometallic molecule called Glucose Tolerance Factor (GTF) (Vincent, 2000). Moreover, chromium is thought to be essential for activating certain enzymes and for stabilizing proteins and nucleic acids (Okada *et al.*, 1984). Overall, chromium deficiency can disrupt carbohydrate and protein metabolism, reduce insulin sensitivity in peripheral tissues, also impair growth rate.

The supplementation of Cr in the livestock diet is gaining importance since published reports have indicated that Cr-supplementation can improve animal metabolism and thus, enhance production performance and the composition of animal products (Spears, 1999). Dietary Cr-supplementation has been shown to positively affect growth rate and feed efficiency in growing poultry (Lien *et al.*, 1999; Sahin *et al.*, 2001; Kheiri and Toghyani, 2009). Supplemental Cr in diet of broiler has been observed to have a positive effect on meat quality

(Amatya *et al.*, 2004; Toghyani *et al.*, 2008) and carcass traits (Ward *et al.*, 1993; Debski *et al.*, 2004). Immunological function has been enhanced by trivalent Cr and its effects seem more pronounced during times of stress (Borgs and Mallard, 1998). Also benefits of Cr supplementation in broiler under heat stress on some immune responses have reported (Toghyani *et al.*, 2007).

Nevertheless, recommendations regarding the dietary inclusion level of Cr in diets of livestock including poultry are yet to be finalized (NRC, 1994). Organic Cr-compounds like Cr-nicotinic acid, Cr-picolinate or Cr-yeast complex may elicit better responses from livestock owing to the better absorbability of organic Cr (Underwood and Suttle, 1999) though contradictory reports are also available (Nam *et al.*, 1995).

This study was conducted to compare the effectiveness of inorganic and organic sources of Cr on performance and immune responses of broiler chicks.

MATERIALS AND METHODS

Four hundred and thirty two, one-day-old broiler chicks (308 Ross) were randomly allotted by body weight to nine treatments and 4 replicates pens of 12 chicks/pen

Table 1: Composition of the basal diets

Ingredients (%)	Starter	Grower	Finisher
Corn	55	58	64.1
Soybean meal (CP43%)	40	35.3	29.9
Soybean oil	1	3	2.63
¹ Dicalcium phosphate	1.94	1.73	1.63
Calcium carbonate	0.86	0.8	0.77
Salt	0.36	0.35	0.35
² Vitamin premix	0.25	0.25	0.25
³ Mineral premix	0.25	0.25	0.25
L-lysine	0.164	0.07	0
DL-methionine	0.316	0.24	0.15
Calculated composition			
Metabolizable energy (Kcal kg ⁻¹)	2825	3000	3050
Crude protein (%)	22.28	20.5	18.6
Calcium (%)	0.94	0.85	0.8
Available phosphorus (%)	0.47	0.42	0.4
Methionine + cysteine (%)	0.99	0.88	0.75
Lysine (%)	1.37	1.17	0.97
Cranalyzed (mg kg ⁻¹)	5.57	4.75	3.40

¹Dicalcium phosphate contains 25% Calcium and 16.4% phosphorus.

²Vitamin premix contains followings in 2.5 kg: vitamin A, 3,600,000 IU; vitamin D2, 800,000 IU; vitamin E, 7.2 g; vitamin K3, 0.8 g; vitamin B1, 0.71 g; vitamin B2, 2.64 g; niacin, 11.88 g; panthothenic acid, 3.92 g; vitamin B6, 1.176 g; vitamin B9, 0.4 g; vitamin B12, 6 mg; vitamin H2, 60 mg; choline chloride, 100 g. ³Mineral premix contains followings in 2.5 kg: manganese, 39.68 g; zinc, 33.88 g; iron, 20 g; copper, 4 g; iodine, 3.97 mg; selenium, 80 mg

Broiler chicks were housed in floor pens (length 120 cm × width 120 cm × height 80 cm) equipped with feeders and waterers. Chicks were maintained on a 24 h light and allowed ad libitum access to experimental diets and water.

In this study treatments consisted of the basal diet supplemented with 0 (control), Cr-chloride (contain 18% Cr), Cr-yeast (contain 160 mg Cr kg⁻¹), Cr-nicotinate (contain 12.25% Cr) and Cr-methionine (contain 1000 mg Cr kg⁻¹) at two levels of 600 and 1200 µg kg⁻¹ Cr-supplementation. The birds were fed a maize soybean meal starter diets until 11 days of age followed by growing diet from 11-29 and a finishing diet from 29-42 days. Ingredients and chemical composition of the starter, grower and finisher basal diets are shown in Table 1.

The basal diets were formulated to meet or exceed the nutrient requirements of broilers by the Ross manual catalogue (2002). Cr-contents were 5.57, 4.75 and 3.40 mg kg⁻¹ in starting, growing and finishing basal diets, respectively, as measured by atomic absorption spectrometer with a graphite furnace (Perkin-Elmer, AAnalyst 600, USA).

Feed intake, weight gain and feed conversion ratio measured in different periods and in overall growth period. At 20 and 31 days of age two birds were chosen from each replicate and blood samples were collected from the wing vein to determine antibody titers against Newcastle and Influenza virus. Also, concentration of total protein, globulin and albumin in serum were determined at 31 days.

At 42 days of age, two broilers from each pen were chosen, weighed, slaughtered and carcass yield and percent of liver, abdominal fat pad, pancreas and lymphoid organs (Buras of Fabericius and spleen) as a percentage of live weight were calculated.

The experiment data were analyzed using SAS statistical program (SAS, 1997). General linear model was used to analyze variance and significant differences ($p < 0.05$) among treatments mean were determined using Duncan's new multiple range test.

RESULTS AND DISCUSSION

Effects of Cr-supplementation on performance of broiler chicks are shown in Table 2. Dietary Cr-source and level had no significant effect on feed intake in finisher period but feed intake was significantly affected by Cr-supplementation in starter, grower and overall growth period ($p < 0.01$). Broilers were fed 600 µg kg⁻¹ Cr-nicotinate had the highest feed intake.

Cr is generally accepted as the active component in the Glucose Tolerance Factor (GTF), which increases the sensitivity of tissue receptors to insulin, resulting in increased glucose uptake by cells and increased oxidation of glucose (Jeebhoy *et al.*, 1977). It was hypothesized that increased glucose uptake, decreased blood glucose and increased appetite should increase feed intake. Lien *et al.* (1999) reported that 1600 and 3200 µg kg⁻¹ Cr-picolinate supplementation in broiler diets increased feed intake. Sahin *et al.* (2002) and Toghyani *et al.* (2006) observed an increase of the feed intake by supplementation diet of broiler under heat stress condition with Cr-picolinate. However, Anandhi *et al.* (2006) reported that supplementation with organic Cr in diet of broilers revealed no significant difference in feed intake.

Supplementation with different sources and levels of Cr had no significant effect on body weight at 10 and 42 days, but body weight was significantly affected by Cr-supplementation at 28 days ($p < 0.01$) and broilers received 600 µg kg⁻¹ Cr-nicotinate were the heavier than other treatments at 28 days.

Supplemental Cr improve amino acid uptake by tissues and muscle cells and increase protein retention and tend to increase body weight in some treatments. It seems that increasing in feed consumption in 600 µg kg⁻¹ Cr-nicotinate treatment tended to increase body weight in this group at 28 and 42 days.

Feed conversion ratio was significantly affected by different source and level of Cr only in starter period. In this period control group had the highest feed conversion ratio and 1200 µg kg⁻¹ Cr-chloride and nicotinate

Table 2: Effect of different sources of Cr on performance of broiler chicks

		Cr-chloride		Cr-nicotine		Cr-methionine		Cr-yeast		
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Parameters	Control	600	1200	600	1200	600	1200	600	1200	SEM
Feed intake (g day ⁻¹)										
0-11 days	25.1 ^{ab}	24.5 ^{ab}	25.1 ^{ab}	25.6 ^a	24.1 ^{ab}	24.4 ^{ab}	23.8 ^b	24.1 ^{ab}	24 ^{ab}	0.89
11-29 days	94 ^{abc}	90.5 ^{cd}	95.5 ^{ab}	97 ^a	92.3 ^{abcd}	94.3 ^{abc}	90.9 ^{bcd}	88.9 ^d	90.6 ^{bcd}	0.92
29-42 days	158	152.1	155.1	161	153.9	156.9	155	156	152.7	3.31
0-42 days	96.7 ^{abc}	93.5 ^{bc}	96.7 ^{abc}	99.7 ^a	95.1 ^{bc}	97.1 ^{ab}	94.7 ^{bc}	93.5 ^{bc}	92.9 ^c	1.45
Body weight (g)										
10 days	202.9 ^{ab}	204.7 ^{ab}	213.8 ^a	212 ^{ab}	206.6 ^{ab}	206.4 ^{ab}	203.5 ^{ab}	200.4 ^{ab}	199.3 ^b	6.64
28 days	1177 ^{abc}	1108.4 ^d	1188 ^{ab}	1226 ^a	1195 ^a	1176 ^{abc}	1163 ^{abcd}	1128 ^{bcd}	1116 ^{cd}	14.5
42 days	2148	2064	2108	2165	2092	2145	2097	2096	2057	54.6
Feed:gain (g:g)										
0-11 days	1.58 ^a	1.53 ^{ab}	1.48 ^b	1.52 ^{ab}	1.48 ^b	1.51 ^{ab}	1.50 ^{ab}	1.54 ^{ab}	1.55 ^{ab}	0.003
11-29 days	1.74	1.81	1.76	1.78	1.68	1.75	1.70	1.73	1.78	0.061
29-42 days	2.12	2.08	2.20	2.16	2.23	2.11	2.16	2.33	2.12	0.039
0-42 days	1.88	1.90	1.92	1.93	1.91	1.90	1.89	1.95	1.90	0.05

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

Table 3: Effect of different sources of Cr on the carcass traits of broilers

Carcass traits	Control	Cr-chloride		Cr-nicotine		Cr-methionine		Cr-yeast		SEM
		600	1200	600	1200	600	1200	600	1200	
Carcass	71.7 ^{ab}	71.4 ^{ab}	72.5 ^a	70.8 ^b	72 ^{ab}	71.8 ^{ab}	72.9 ^a	72.4 ^{ab}	72.1 ^{ab}	0.481
Abdominal fat	2.18 ^{abc}	1.74 ^c	2.03 ^{abc}	2.03 ^{abc}	2.03 ^{abc}	2.30 ^a	2.23 ^{ab}	2.01 ^{abc}	1.82 ^{bc}	0.141
Liver	2.13	2.07	2.02	2.01	2.20	2.01	2.12	2.23	2.18	0.06
Pancreas	0.232 ^{ab}	0.200 ^b	0.220 ^{ab}	0.226 ^{ab}	0.228 ^{ab}	0.252 ^a	0.215 ^{ab}	0.230 ^{ab}	0.210 ^{ab}	0.014

^{a,b}Means within the same row without common superscripts differ significantly (p<0.05), *: Percentage of live weight

Table 4: Effect of different sources of Cr on the lymphoid organs of broilers

Lymphoid organ	Control	Cr-chloride		Cr-nicotine		Cr-methionine		Cr-yeast		SEM
		600	1200	600	1200	600	1200	600	1200	
Spleen	0.115 ^b	0.147 ^a	0.133 ^{ab}	0.141 ^{ab}	0.133 ^{ab}	0.120 ^{ab}	0.133 ^{ab}	0.111 ^b	0.113 ^b	0.006
Bursa of fabricius	0.149 ^{ab}	0.137 ^{ab}	0.176 ^{ab}	0.176 ^{ab}	0.181 ^{ab}	0.131 ^{ab}	0.153 ^{ab}	0.186 ^a	0.118 ^b	0.003

^{a,b}Means within the same row without common superscripts differ significantly (p<0.05), *: Percentage of live weight

treatments had the lowest. It seems that role of the Cr on feed intake is greater than body weight gain in starter period and so all of the treatments had the higher feed conversion ratio than control group. Studies with different sources of Cr such as Cr-picolinate (Sahin *et al.*, 2002), Cr-chloride (Uyanik *et al.*, 2002; Ahmed *et al.*, 2005), Cr-yeast (Hossain *et al.*, 1998; Krolczewska *et al.*, 2004) showed improvement in feed conversion ratio but supplementation with Cr-picolinate in diet of heat stressed broiler chicks did not affect feed conversion ratio (Toghyani *et al.*, 2006).

Effect of supplemental Cr on the carcass traits of broilers as a percentage of live weight at 42 days are shown in Table 3. Carcass yield increased in broilers fed 1200 µg kg⁻¹ Cr-methionine and broiler received 600 µg kg⁻¹ Cr-chloride had the lowest abdominal fat. The liver weight was not affected significantly by Cr-source and level. Pancreas weight as a percentage of live weight was significantly increased in broilers fed 600 µg kg⁻¹ Cr-methionine.

It has been shown that Cr-supplementation causes significant changes in the chemical composition of animal carcasses (Lukaski, 1999). It is well known that Cr is

involved in protein metabolism (Anderson, 1987). In addition Cr has a role in nucleic acid metabolism as an increase in stimulation of amino acid incorporation into liver protein *in vitro* was observed (Ohba *et al.*, 1986). Sahin *et al.* (2002) and Toghyani *et al.* (2006) observed that supplemental Cr-picolinate resulted in an improvement in carcass yield. Hossain *et al.* (1998) and Debski *et al.* (2004) reported an increase in carcass yield by supplementation with Cr yeast in diet of broiler chicks. Gursay (2000) found that supplementation with organic Cr in diet of broiler caused to improvement in carcass yield but in other study supplementation with different levels of organic Cr in diet of broiler chicks had no significant effect on carcass yield (Anandhi *et al.*, 2006). Cr is essential for lipid metabolism and Cr-stimulates insulin action and insulin has an important role on lipid metabolism and so leading to reduction of abdominal fat pad in some treatments. In low levels of insulin, glucose converts to lipid and store in adipose tissues (Mertz, 1993). Broiler supplemented with Cr-picolinate (Ward *et al.*, 1993; Lien *et al.*, 1999; Shahin *et al.*, 2002; Toghyani *et al.*, 2006), Cr-yeast (Hossain *et al.*, 1998; Debski *et al.*, 2004) and organic Cr (Anandhi *et al.*, 2006; Gursay, 2000) exhibited a decrease in abdominal fat pad.

Table 5: Effect different sources of Cr on the albumin to globulin ratio and antibody titer against Newcastle and Influenza viruses in broilers

		Cr-chloride		Cr-nicotinate		Cr-methionine		Cr-yeast		
Parameters	Control	600	1200	600	1200	600	1200	600	1200	SEM
Albumin:Globulin										
31 days	2.15 ^b	3.40 ^{ab}	2.70 ^{ab}	2.96 ^{ab}	2.38 ^{ab}	3.19 ^a	2.20 ^{ab}	2.41 ^{ab}	3.35 ^{ab}	0.808
Newcastle titer (Log2)										
20 days	3 ^b	2.75 ^b	3 ^b	3.38 ^{ab}	4.57 ^a	3.14 ^b	3.75 ^{ab}	3.88 ^{ab}	3.75 ^{ab}	0.543
31 days	4	4	4	4.25	4	4.25	4.25	4.38	4	1.091
Influenza titer (Log2)										
20 days	3.50 ^{ab}	3.38 ^{ab}	3.25 ^{ab}	3.63 ^a	3.38 ^{ab}	3 ^{ab}	3.50 ^{ab}	2.63 ^b	3 ^{ab}	0.419
31 days	3.25	3.38	3.14	3.38	3.63	3.63	3.63	3.38	4	1.534

^{a,b}Means within the same row without common superscripts differ significantly ($p < 0.05$), *: Percentage of live weight

Significant differences in lymphoid organs of broilers as a percentage of live weight was found in broilers receiving Cr-supplement (Table 4). At 42 days broilers were fed 600 $\mu\text{g kg}^{-1}$ Cr-chloride and Cr-yeast showed the highest spleen and bursa of Fabricius as a percentage of live weight, respectively. In other research supplementation with different levels of Cr-picolinate in diet of heat-stressed broiler no effect on lymphoid organs were observed (Toghyani *et al.*, 2007).

Supplementation with Cr had significant effect on serum albumin to globulin ratio at 31 days and all of the treatments had the higher albumin to globulin ratio than control group (Table 5). Broilers were fed 600 $\mu\text{g kg}^{-1}$ Cr-chloride showed the highest albumin to globulin ratio. Dietary Cr-source and level had significant effect on antibody titer against Newcastle and Influenza viruses at 20 days but treatments were not significantly affected by supplemental Cr at 31 days (Table 5). Broilers received 1200 and 600 $\mu\text{g kg}^{-1}$ Cr-nicotinate had the highest antibody titer against Newcastle and Influenza viruses at 20 day, respectively.

Elevating in antibodies titer may be relate to Cr role on insulin and cortisol hormones. The immune function may be affected in association with insulin and cortisol activity, because of cortisol acting as a suppressor for antibodies (Borgs and Mallard, 1998). Lee *et al.* (2003) observed an improving in antibody titer against infectious bronchitis in broiler were consumed 400 $\mu\text{g kg}^{-1}$ Cr-picolinate. In other research, supplementation with different levels of Cr-picolinate specially at 1500 $\mu\text{g kg}^{-1}$ level in diet of heat-stressed broiler chicks tend to increasing antibody titer against Newcastle and Influenza viruses (Toghyani *et al.*, 2007).

CONCLUSION

The results of this experiment showed between different Cr-sources were used in this study Cr-nicotinate improved feed intake, body weight and antibody titer and Cr-methionine and chloride improved carcass traits in broiler chicks.

REFERENCES

- Ahmed, N., S. Haldar, M.C. Pakhira and T.K. Ghosh, 2005. Growth performances, nutrient utilization and carcass traits in broiler chickens fed with a normal and a low energy diet supplemented with inorganic chromium (as chromium chloride hexahydrate) and a combination of inorganic chromium and ascorbic acid. *J. Agric. Sci.*, 143: 427-439. DOI: 10.1017/S0021859605005617.
- Amatya, J.L., S. Haldar and T.K. Ghosh, 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., R. Mathivanan, K. Viswanathan and B. Mohan, 2006. Dietary inclusion of organic chromium on production and carcass characteristics of broilers. *Int. J. Poult. Sci.*, 5 (9): 880-884. <http://www.pjbs.org/ijps/fin682.pdf>.
- Anderson, R.A., 1987. Chromium: Trace Elements in Human and Animal Nutrition. Academic Press, New York, pp: 225-244.
- Anderson, R.A., 1997. Chromium as an essential nutrient for humans. *Regul. Toxicol. Pharmacol.*, 26: S35-S41. http://www.sciencedirect.com/science?_ob=PublicationURL&_tokey.
- Borgs, P. and B.A. Mallard, 1998. Immune-endocrine interactions in agricultural species: Chromium and its effect on health and performance. *Domestic Anim. Endocrinol.*, 15: 431-438. DOI: 10.1016/S0739-7240(98)00018-6.
- Debski, B., W. Zalewski, M.A. Gralak and T. Kosla, 2004. Chromium yeast supplementation of broilers in an industrial farming system. *J. Trace Element Med. Biol.*, 18: 47-51. DOI: 10.1016/j.jtemb.2004.02.003.
- Gursoy, U., 2000. Chromium in broiler diets. *Feed Int.*, pp: 24-26.
- Hossain, S.M., S.L. Barreto and C.G. Silva, 1998. Growth performance and carcass composition of broiler fed supplemental chromium from chromium yeast. *Anim. Feed Sci. Technol.*, 71: 217-228. DOI: 10.1016/S0377-8401(97)00160-0.

- Jeejeebhoy, K.N., R.C. Chu, E.B. Marliss, G.R. Greenberg and A. Bruce-Robertson, 1977. Chromium deficiency, glucose intolerance and neuropathy reversed by chromium supplementation in a patient receiving long term total parenteral nutrition. *Am. J. Clin. Nutr.*, 30: 531-538. <http://www.ajcn.org/cgi/content/abstract/30/4/531>.
- Kheiri, F. and M. Toghyani, 2009. Effect of different levels of inorganic chromium on performance and immunity of broiler chicks. *J. Anim. Vet. Adv.*, 8 (9): 1819-1823. <http://medwelljournals.com/fulltext/java/2009/1819-1823.pdf>.
- Kroliczewska, B., W. Zawadzki, Z. Dobrzanski and A. Kaczmarek-Oliwa, 2004. Change in selected serum parameters of broiler chicken fed supplemental chromium. *J. Anim. Physiol. Nutr.*, 88: 393-400. DOI: 10.1111/j.1439-0396.2004.00496.x.
- Lukaski, H.C., 1999. Chromium as a supplement. *Ann. Rev. Nutr.*, 19: 279-302. DOI: 10.1146/annurev.nutr.19.1.279.
- Lee, D.N., F.Y. Wu, Y.H. Cheng, R.S. Lin and P.C. Wu, 2003. Effect of dietary chromium picolinate supplementation on growth performance and immune responses of broilers. *Asian-Aust. J. Anim. Sci.*, 16: 227-233. <http://www.ajas.info/absView.asp>.
- Lien, T.F., Y.M. Homg and K.H. Yang, 1999. Performance, serum characteristics, carcass traits and lipid metabolism of broilers as affected by supplement of chromium picolinate. *Br. Poult. Sci.*, 40: 357-363. DOI: 10.1080/00071669987458.
- Mertz, W., 1993. Chromium in human nutrition: A review. *J. Nutr.*, 123: 626-633. <http://jn.nutrition.org/cgi/content/abstract/123/4/626>.
- Nam, K.T., H.Y. Lee, C.W. Kang and I.J. Yoo, 1995. The effects of dietary Chromium (Cr) in broiler chicks under heat stress. *Proceedings of the World Poultry Congress*, New Delhi, India, pp: 209-212.
- NRC (National Research Council), 1994. *Nutrient Requirements of Poultry*. 9th Rev. Edn. National Academy Press, Washington, DC. ISBN-13: 978-0-309-04892-7.
- Ohba, H., Y. Suzuki and H. Ohba, 1986. Enhancement of ribonucleic acid synthesis by chromium (III)-bound chromatin. *J. Inorg. Biochem.*, 27: 179-188. DOI: 10.1016/0162-0134(86)80059-9.
- Okada, S., H. Tsukada and H. Ohba, 1984. Enhancement of nucleic acid synthesis by chromium (III) in regenerating rat liver. *J. Inorg. Biochem.*, 21: 113-119. DOI: 10.1016/0162-0134(84)85044-8.
- Sahin, K., O. Kucuka, N. Sahin and O. Ozbey, 2001. Effects of dietary chromium picolinate supplementation on egg production, egg quality and serum concentrations of insulin, corticosterone and some metabolites of Japanese quails. *Nutr. Res.*, 21: 1315-1321. [http://www.nrjournal.com/article/S0271-5317\(01\)00330-X/abstract](http://www.nrjournal.com/article/S0271-5317(01)00330-X/abstract).
- Sahin, K., N. Sahin, M. Onderci, F. Gursu and G. Cikim, 2002. Optimal dietary concentration of chromium for alleviating the effect of heat stress on growth, carcass qualities and some serum metabolites of broiler chickens. *Biol. Trace Element Res.*, 89: 53-64.
- SAS Institute, 1997. *SAS Users Guide*. Version 6.12 review edition, SAS Institute Inc., Cary, NC.
- Spears, J.W., 1999. Reevaluation of the metabolic essentiality of the minerals. *Asian-Aust. J. Anim. Sci.*, 12: 1002-1008. <http://www.ajas.info/absView.asp>.
- Toghyani, M., M. Shivazad, A.A. Gheisari and S.H. Zarkesh, 2006. Performance, carcass traits and hematological parameters of heat-stressed broiler chicks in response to dietary levels of chromium picolinate. *Int. J. Poult. Sci.*, 5 (1): 65-69. <http://www.pjbs.org/ijps/ab535.htm>.
- Toghyani, M., S. Zarkesh, M. Shivazad and A. Gheisari, 2007. Immune responses of broiler chicks fed chromium picolinate in heat stress condition. *J. Poult. Sci.*, 44: 330-334. <http://www.scopus.com/scopus/inward/record.url?eid=2-s2.0-34547600483&partnerID=40>.
- Toghyani, M., A. Khodami and A.A. Gheisari, 2008. Effect of organic and inorganic chromium supplementation on meat quality of heat-stressed broiler chicks. *Am. J. Anim. Vet. Sci.*, 3 (2): 62-67. <http://www.scipub.org/fulltext/AJAV/AJAV3262-67.pdf>.
- Underwood, E.J. and N.F. Suttle, 1999. *The Mineral Nutrition of Livestock*. 3rd Ed. CAB International, Wallingford, UK, pp: 517-518. ISBN: 0-85199-128-9.
- Uyanik, F., A. Atasever, S. Ozdmar and F. Aydin, 2002. Effects of dietary chromium chloride supplementation on performance, some serum parameters and immune response in broilers. *Biol. Trace Element Res.*, 90: 99-115. <http://www.ovid.com/site/catalog/Journal/1697.jsp>.
- Vincent, J.B., 2000. The biochemistry of chromium. *J. Nutr.*, 130: 715-718. <http://jn.nutrition.org/cgi/content/abstract/130/4/715>.
- Ward, T.L., L.L. Southern and S.L. Boleman, 1993. Effect of dietary chromium picolinate on growth, nitrogen balance and body composition of growing broiler chicks. *Poult. Sci.*, (Suppl. 1): 37 (Abstr.). <http://www.poultscience.org/pab93toc.htm>.