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EFFECT OF DIETARY CHROMIUM SOURCE WITH OR WITHOUT PROBIOTIC ON PRODUCTIVE PERFORMANCE AND IMMUNE STATUS OF BROILER CHICKEN

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SUMMARY

An experiment was conducted using 360 one day old sexed male broiler breeder COBB chicks to investigate the effect of dietary Cr source (inorganic-organic) supplementation in broiler diets with or without addition of probiotics in drinking water on productive performance and immune status of broiler chicken.

Inorganic Cr source (chromium chloride) was supplemented to the basal diets of two groups, the first group drank water with 1ml/L probiotic and the second group drank water without probiotic. Organic Cr source (OCY) was supplemented to the basal diets of another two groups, one of them drank water with 1ml/L probiotic and the second group drank water without probiotic. Cr sources were supplemented at the same dose (4.0 mg Cr/kg diet). The experiment was extended for 6 weeks.

Body weight development, body weight gain, feed intake and FCR were calculated weekly. Serum GOT,GPT, uric acid and creatinine were determined. Lymphoid organs weight (bursa, spleen, thymus) were recorded.

Both Cr sources supplementation increased body weight gain of the birds significantly except chromium chloride with probiotic addition which showed non significant increase. Feed intake from either inorganic or organic Cr source was lower than the control group. FCR was improved by Cr supplementation in both sources, but inorganic Cr supplementation showed better FCR than organic Cr source. Over all results of HI test, skin response test, lymphoid organ weight showed that supplementation of Cr in both source

was stimulate the immune response of bird.

The current study indicated that dietary Cr supplementation (inorganic- organic) in broiler diet achieved maximal performance of broiler chicken. Inorganic and organic Cr supplementation stimulates immune response of the birds in various degrees.

INTRODUCTION

Chromium (Cr) has been considered an essential trace element for animals many years ago(NRC, 1980). It is involved in various metabolisms inside the animal body, carbohydrate(Matthews et al, 1997), protein (Evans and Bowman, 1992), lipid (Lien et al, 1999), nucleic acid(Okada et al, 1982).

Many researchers indicated that dietary chromium supplementation in broiler diets improve chickens performance (Mohamed and Afifi 2001, El- Kaiaty et al,2005, Kroliczewska et al,2005and Toghyani et al,2006). In contrary (Bee et al, 1999, Uyanik et al, 2002and Debski et al, 2004) noticed that chromium supplementation did not affect body weight gain of broilers.

Immune response against ND was increased when chicks adminstred chromium (El-Kaiaty et al, 2005 and Ghoraba,2005).Anderson et al. (1996) concluded that chromium incorporation into animal tissues is highly dependent upon the form. Mowat. (1997), stated that bioavailability of organic Cr is around 25-30% and inorganic chromium form poor availability when the two general forms of chromium have been used in diet supplementation. Lukaski (1999) concluded that the inorganic and organic chromium forms seem to have various availability.

Therefore, it was of interest to compare the performance of broiler chickens raised on dietary supplementation of organic vs inorganic form. It was found that probiotics enhance mineral absorption (Kralik et al, 2004).

The main objective of the present study is to investigate the effect of dietary Cr source (organic and inorganic) supplementation in broiler diets with or without addition of probiotics on broiler performance as well as the immune status of the birds.

MATERIALS AND METHODS

The current study was conducted at the Dept. of Nutrition and Clinical Nutrition, Faculty of Veterinary Medicine, Cairo University. A total of 360 one day-old- male broiler breeder Cobb chicks were fed on the starter diet from one-day-old until second weeks of age, grower diet from second week of age until fourth weeks old and finisher diet from fourth weeks old until the end of the study. The study was conducted for 6 weeks of age. The basal diet was formulated to meet the breeding catalogue recommendations (Table 1).

Table (1) Composition and calculated analysis of the basal diets.

Ingredient	Starterdiet%	Grower diet%	Finisher diet%
Ground yellow corn	50.9	60.4	61.3
Soy bean meal (44% CP)	40.50	27.27	26.00
Corn gluten meal (60%)		5.0	4.0
Vegetable oil	4.5	3.0	4.7
Ground lime stone	1.65	1.65	1.55
Mono-calcium phosphate	1.45	1.45	1.35
Vitamin and mineral premix*	0.25	0.25	0.25
salt	0.35	0.35	0.35
Sodium bicarbonate	0.20	0.20	0.15
DL-Methionine (99%)	0.200	0.175	0.172
lysine		0.255	0.178
Calculated analysis			
ME kcal/kg	2992.24	3082.07	3187.03
CP%	22	20	19
EE%	7.00	5.78	7.46
CF%	4.13	3.48	3.39
Ca%	1.00	0.91	0.93
Available P%	0.5	0.45	0.42
Lysine%	1.3	1.1	1.1
Methionine%	0.56	0.55	0.53
Methionine + cystine %	0.92	0.89	0.84

* Premix of MUVCO company its Vitamin and mineral content/ 2.5kg premix (Muvco premix): vitA 12000.000 IU, vitD$_3$ 2000.000IU, vitE 10gm, vitK1gm, vitB$_1$ 1000mg, vitB$_2$ 5gm, vitB$_6$ 1.5 gm, pantothenic acid 10gm, vitB$_{12}$ 10mg, niacin 30gm, folic acid 1000mg, biotin 50mg, choline chloride 600gm, iron 30gm, manganese 60gm, copper 4gm, iodine 300mg, cobalt 100mg, selenium 100 mg and zinc 50gm.

The birds were vaccinated against ND at 7, 21, 35 day old and against Gomboro at 12,24 day old by eye drop of live vaccine in the both. While, the birds were vaccinated S/C injection against avian influenza virus at 7 day old with killed vaccine (H5N2).

At the begging of the experiment, the birds were randomly divided into six equally groups . The six experimental groups were designed as follow (table2); group (1) kept for control without any supplementation in feed or water. Group(2) without any supplementation in feed but probiotic was added in drinking water at 1ml/L. Group (3,4) were supplemented with 4.0 mg Cr/kg diet chromium chloride (inorganic Cr source) and with 1ml/ L probiotic addition in drinking water for group (4) only. Group (5,6) were supplemented with 4.0 mg Cr/kg diet chromium yeast (OCY) as organic Cr source and with 1ml/L probiotic addition in drinking water for group (6) and without probiotic addition in drinking water for group (5).

Table (2): the experimental design

Group	Diet	Experimental supplementations		
		Probiotic Drinking water ml/L	Chromium forms	
			inorganic	organic
			***Chromium Chloride mg/kg	****Chromium Yeast mg/kg
1(control)	basal diet	----	-----	-----
2	basal diet	1.0	-----	-----
3	basal diet	----	20 ➢	-----
4	basal diet	1.0	20 ➢	-----
5	basal diet	----	-----	4000 ➢
6	basal diet	1.0	-----	4000 ➢

***Chromium Chloride (19.5% chromium).
****Chromium Yeast (OCY) (1000mg/kg chromium).
➢ Furnish 4.0 mg Cr/kg diet.

Body weight development, body weight gain, feed intake and feed conversion ratio were calculated weekly. At the end of the experiment lymphoid organs (thymus, bursa and spleen) weight were recorded.

Blood samples collected at 42 day of age to obtain serum for determination of Serum Glutamic Oxal Acetic Transaminase (SGOT) (Reitman and Frankel, 1957) , Serum Glutamic-Pyruvic Transaminase (SGPT) (Reitman and Frankel, 1957), Serum Uric Acid (Barham and Trinder, 1972) and Serum Creatinine(Larsen, 1972).

Five birds from each group were subjected to skin response test at 40th day of age according to Uyanik et al. (2002) by inoculated intra dermal in the right wattle with 0.1 ml of 1.2 mg protein / 2 ml phytohemagglutenin (PHA) suspension in sterile distilled water. The left wattle was inoculated with sterile distilled water as a control. The thickness of the wattle was measured with calaberator just before and at 6,12,18 and 24 hr after injection. Investigation of HI for ND It was done by micro technique according to (Takatasy, 1955).

Obtained data were calculated and statistically analyzed according to Wayne, 1998 by SPSS 11 version for Windows. The differences between groups were determined with variance analysis (one-way analysis of variance [ANOVA] using the probability level of 0.05 for the rejection of the null hypothesis. Significant differences among means were determined by the Student-newman-kuels test. Among means were deter-

mined by the Student-newman-kuels test.

RESULTS AND DISCUSSION

1-Body weight development

The present data of body weight development (table3) indicated that body weight of the birds received Cr in both sources (inorganic/ organic) increased significantly comparable to control except that group which fed inorganic Cr source with addition of probiotic in drinking water which showed non significant increase. Kroliczewska et al., (2004) reported that the Cr supplementation increased growth performance parameters of broiler. El- Kaiaty et al., (2005) and Kroliczewska et al., (2004) reported that Cr yeast in broiler diets lead to significant improvement in body weight. However, the best body weight development achieved in group fed on inorganic Cr source (chromium chloride) without addition of brobiotic. Rosebrough and Steele (1981) concluded that dietary supplementation of Cr chloride improved body weight of turkey poults. Mohamed and Afifi., (2001), Debski et al (2004) and Eren and Baspinar (2004) indicated that birds received Cr chloride in the diet had significant increase in body weight.

2- Body weight gain

Table (4) indicated that all the Cr treated groups in both source had higher body weight gain as compared to control group. Whereas the highest body weight gain was recorded in inorganic Cr source (Cr chloride) without addition of probiotic supplemented group. This finding is agreed with Debski et al (2004). Body weight improvement achieved due to Cr supplementation may be attributed to the role of chromium in carbohydrate, lipid, protein and nucleic acid metabolic functions (Anderson and Kozlovsky, 1985). Okada et al., (1983) suggested a biological function of chromium in nucleic acid metabolism Ribonucleic acid synthesis in mouse liver was significantly increased by trivalent chromium, in the presence of DNA or chromatin. These effects were also present when the DNA or chromatin was first complexes with chromium prior to incubation. Chromium promotes glucose metabolism, enhances glycogenesis from glucose and accelerates glucose transport (Rosebrough and Steele, 1981). Chromium is also a cofactor of insulin, promoting insulin activity (McCarty,1991). Insulin can also stimulate anabolism and inhibit catabolism (Lien et al., 1999).

3- Feed intake

The current results of total feed intake as compared to control (table4) indicated that total feed intake of broiler chicken fed on Cr supplemented diets in both source was lower than control group. Motozono et al.,(1998) noticed that feed intake tended to decrease in the all of Cr supplemented diets groups and yeast Cr (400 ppb) tended to have a greater negative effect on feed intake than Cr picolinate. Also Uyanik et al. (2002) concluded that the chromium chloride at 20 mg / kg

reduced feed consumption by 18.57%.

4- Feed conversion ratio

The obtained FCR figures (table 4) revealed that their were marked improvement in FCR in all Cr treated groups as compared with control. The finding confirm the work of Mohamed and Afifi (2001) and Uyanik et al (2002).

Regarding the Cr sources supplementation, inorganic Cr (chromium chloride) supplemented group showed better FCR than those obtained from organic Cr (Cr yeast) supplemented group. These results are closely in agreement with Ghoraba (2005).

General FCR improvement in all experimental group attributed to high gain and low feed intake recorded in the current work comparable to control group.

5- Immune response

A- HI against ND

Table (5) revealed that HI titer of chicken received diet supplemented with organic Cr (chromium yeast) was significantly increase when compared with those of other groups. Guo et al (1999) concluded that Cr yeast increased serum antibody titer to Newcastle disease virus.

Inorganic Cr (chromium chloride) showed non significant increase in HI titer. Mohamed and Afifi (2001) concluded that dietary supplementation of chromium chloride significant increase heamagglutinine antibody titer at 21 and 28 days post immunization with sheep red blood cells.

B- Skin response against phyto heam agglutinie (PHA)

Data in table (6) showed that all the experimental groups were reacted positively at 6 hr post injection . the highest response were recorded in Cr supplemented groups except that one which fed on inorganic chromium with probiotic. Uyanik et al (2002) concluded that high response to PHA was recorded in broiler chicken fed on chromium supplemented diets.

C- Lymphoid organ weight

Lymphoid organs (bursa, thymus, spleen) weight present in table (7). There is non significant difference between bursa weight of the treated groups and the control while bursa weight of chromium chloride with probiotic group showed significant decrease as compared to control. This finding are disagree with Guo et al. (1999) who found that fowls given diets with chromium chloride sources had a higher fabricius bursa weight than those given diets with yeast chromium.

Spleen weight not affected by any dietary treatment. While thymus weight showed a significant increase in group fed on chromium chloride supplementation.

This results is agree with Mohamed and Afifi (2001) who indicated that the thymus weight

where significantly increased in Cr supplemented group

The mechanism by which chromium enhances the immune system may be attributed to reduced serum cortisol levels which known to suppress the immune system, Mohamed and Afifi,2001.

6- Liver and kidney function tests

Table (8) revealed that Cr supplementation in both sources have no effect on GOT, GPT, uric acid or creatinine. The finding indicated that the Cr supplementation in both source has no deleterious effect either on liver or kidney function Mohamed and Afifi,2001.

Table (3): Effect of chromium (organic or inorganic) supplementation with or without probiotic on weekly body weight development of broiler chicken (g)

Group / Age	Control	Probiotic 1ml/L drinking water	Chromium Chloride 4mg Cr/kg diet	Chromium Chloride 4mg Cr/kg diet + Probiotic 1ml/L drinking water	Chromium Yeast 4mg Cr/kg diet	Chromium Yeast 4mg Cr/kg diet + Probiotic 1ml/L drinking water
Initial	40 ±.0.53 ^{a}	40 ±.0.53 ^{a}	40 ±0.52 ^{a}	40 ±0.50 ^{a}	40 ±0.51 ^{a}	40 ±0.51 ^{a}
1 week	155± 2.06 ^{a}	158± 2.04 ^{a}	156±2.19 ^{a}	150±1.86 ^{a}	150±2.21 ^{a}	150±1.72 ^{a}
2 week	324±4.95 ^{cd}	352±4.25 ^{ab}	314±3.88 ^{d}	299±3.65 ^{e}	339±4.44 ^{bc}	349±4.8 ^{ab}
3 week	600±10.45 ^{c}	659±7.71 ^{a}	625±6.42 ^{abc}	617±7 ^{bc}	627±8.96 ^{abc}	595±10.36 ^{c}
4 week	1242±14 ^{ab}	1239±11.3 ^{ab}	1278±11.39 ^{ab}	1246±11.27 ^{ab}	1285±17.96 ^{ab}	1304±18.49 ^{a}
5 week	1688±45.7 ^{bc}	1795±18.29 ^{a}	1821±14.09 ^{a}	1738±13.54 ^{abc}	1763±22.63 ^{ab}	1754±22.27 ^{abc}
6 week	2223±59.88 ^{cd}	2271±21.98 ^{bcd}	2428±17.47 ^{a}	2266±22.7 ^{bcd}	2356±35.85 ^{ab}	2376±31.64 ^{ab}

Values are means ± SE of the mean.

Values in the same raw with different superscripts are significantly different at P≤0.05

Table (4): Effect of chromium (organic or inorganic) supplementation with or without probiotic on body weight development, body weight gain, feed intake and FCR of broiler chicken

	Control	Probiotic 1ml/L drinking water	Chromium Chloride 4mg Cr/kg diet	Chromium Chloride 4mg Cr/kg diet + Probiotic 1ml/L drinking water	Chromium Yeast 4mg Cr/kg diet	Chromium Yeast 4mg Cr/kg diet + Probiotic 1ml/L drinking water
Initial wt (g)	40 ±.0.53^{a}	40 ±.0.53^{a}	40 ±0.52^{a}	40 ±0.50^{a}	40 ±0.51^{a}	40 ±0.51^{a}
Final wt (g)	2223±59.88^{cd}	2271±21.98^{bcd}	2428±17.47^{a}	2266±22.7^{bcd}	2356±35.85^{ab}	2376±31.64^{ab}
Weight gain(g)	2183	2231	2388	2226	2316	2336
Total feed intake (g)	4066	3783	3738	3657	3871	3649
FCR	1.86	1.69	1.56	1.64	1.67	1.56

Values are means ± SE of the mean.

Values in the same raw with different superscripts are significantly different at P≤0.05

Table (5): Effect of chromium (organic or inorganic) supplementation with or without probiotic on heamagglutination inhibition (HI) titer against ND virus vaccination of broiler chicken

Group / Age	Control	Probiotic 1ml/L drinking water	Chromium Chloride 4mg Cr/kg diet	Chromium Chloride 4mg Cr/kg diet + Probiotic 1ml/L drinking water	Chromium Yeast 4mg Cr/kg diet	Chromium Yeast 4mg Cr/kg diet + Probiotic 1ml/L drinking water
2 w	3±0.32^{a}	1.4±0.245^{bc}	1±0.32^{c}	1.4±0.245^{bc}	2.6±0.245^{ab}	1.8±0.37^{bc}
4 w	2.8±0.49^{a}	2.6±1.21^{a}	3.8±0.8^{a}	2.8±0.92^{a}	3±0.837^{a}	4.4±0.4^{a}
6 w	3±0.45^{bc}	5±0.95^{ab}	3.8±0.37^{bc}	3.6±0.245^{bc}	5.8±0.58^{a}	3.4±0.245^{bc}

Values are means ± SE of the mean.

Values in the same raw with different superscripts are significantly different at P≤0.05

Table (6): Effect of chromium (organic or inorganic) supplementation with or without probiotic on the skin response (cm) to thickness of broiler chicken

Group / Time	Control		Probiotic 1ml/L drinking water		Chromium Chloride 4mg Cr/kg diet		Chromium Chloride 4mg Cr/kg diet + Probiotic 1ml/L drinking water		Chromium Yeast 4mg Cr/kg diet		Chromium Yeast 4mg Cr/kg diet + Probiotic 1ml/L drinking water	
	R	L	R	L	R	L	R	L	R	L	R	L
0hr	0.128 ±0.007	0.131 ±0.005	0.118 ±0.005	0.124 ±0.005	0.147 ±0.007	0.152 ±0.005	0.136 ±0.007	0.1461 ±0.0086	0.144 ±0.01	0.145 ±0.008	0.143 ±0.003	0.151 ±0.002
6hr	0.21 ±0.01	0.138 ±0.007	0.212 ±0.017	0.134 ±0.007	0.228 ±0.02	0.157 ±0.003	0.206 ±0.004	0.146 ±0.008	0.204 ±0.015	0.145 ±0.008	0.202 ±0.014	0.158 ±0.004
12hr	0.179 ±0.01	0.138 ±0.007	0.188 ±0.007	0.124 ±0.005	0.209 ±0.011	0.157 ±0.003	0.196 ±0.009	0.146 ±0.008	0.19 ±0.014	0.143 ±0.01	0.196 ±0.009	0.151 ±0.002
18hr	0.2 ±0.01	0.138 ±0.007	0.204 ±0.013	0.124 ±0.005	0.214 ±0.008	0.152 ±0.005	0.196 ±0.014	0.146 ±.009	0.204 ±0.02	0.145 ±0.008	0.202 ±0.004	0.147 ±0.002
24hr	0.224 ±0.006	0.138 ±0.007	0.188 ±0.006	0.124 ±0.005	0.222 ±0.007	0.152 ±0.005	0.2 ±0.008	0.146 ±0.009	0.22 ±0.007	0.145 ±0.008	0.218 ±0.007	0.147 ±0.002

Values are means ± SE of the mean.

Table (7): Effect of chromium (organic or inorganic) supplementation with or without probiotic on the lymphoid organ weight of broiler chicken(g)

Group / lymphoid organ weight	Control	Probiotic 1ml/L drinking water	Chromium Chloride 4mg Cr/kg diet	Chromium Chloride 4mg Cr/kg diet + Probiotic 1ml/L drinking water	Chromium Yeast 4mg Cr/kg diet	Chromium Yeast 4mg Cr/kg diet + Probiotic 1ml/L drinking water
bursa	1.77±0.4 ^{b}	1.92±0.2 ^{b}	1.31±0.3 ^{bc}	0.856±0.1 ^{c}	1.12±0.3 ^{bc}	1.17±0.2 ^{bc}
spleen	2.9 ^{a} ±0.7 ^{ab}	2.8±0.7 ^{abc}	3.61±1.2 ^{a}	2.68±0.4 ^{bc}	2.19±0.5 ^{c}	2.75±0.7 ^{bc}
thymus	9.16±2.3 ^{bc}	10.66±3.1 ^{bc}	13.99±2.5 ^{a}	7.98±0.6 ^{c}	10.53±1.3 ^{bc}	11.3±2.1 ^{ab}

Values are means ± SE of the mean.

Values in the same raw with different superscripts are significantly different at P≤0.05

Table (8): Effect of chromium (organic or inorganic) supplemetation with or without probiotic on some liver enzymes and kidney function of the broiler chicks

Group / Age		Control	Probiotic 1ml/L drinking water	Chromium Chloride 4mg Cr/kg diet	Chromium Chloride 4mg Cr/kg diet + Probiotic 1ml/L drinking water	Chromium Yeast 4mg Cr/kg diet	Chromium Yeast 4mg Cr/kg diet + Probiotic 1ml/L drinking water	
GPT (unit/ml)	6 w	16.63±2.67	15.62±0.05	15.54±1.9	14.6±1.45	16.87±2.6	14.86±2.6	ns
GOT (unit/ml)	6 w	56.8±8.5	63.09±3.95	54.24±2.4	56.75±4.04	55.3±5.6	54.17±2.2	ns
Uric acid (mg/dl)	6 w	4.82±0.77	4.7±1.05	5.4±0.95	5.62±1.15	5.01±1.17	5.19±1.50	ns
Creatinine (mg/dl)	6 w	2.42±0.54	2.5±.0.42	2.43±0.46	2.32±0.79	2.38±0.43	2.43±0.71	ns

Values are means ± SE of the mean.

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تاثير إضافة الأملاح العضوية و الغير عضوية للكروميوم مع او بدون البروبيوتيك على الكفاءة الانتاجية و الحالة المناعية لدجاج التسمين

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استخدم فى التجربة عدد ٣٦٠ كتكوت عمر يوم (ذكور امهات كوب) و ذلك للوقوف على مدى تاثير اضافة مصدر الكروميوم (عضوى وغير عضوى) فى علائق الدواجن مع او بدون اضافة البروبيوتيك فى مياه الشرب على الكفاءة الإنتاجية و المناعية لدجاج التسمين.

اضيف الكروميوم الغير عضوى (كروميوم كلوريد) فى العليقة الاساسية لمجموعتين الاولى مع اضافة البروبيوتيك فى الماء بمعدل ١ملل/لتر ماء و الثانية بدون اضافة البروبيوتبك فى الماء و تم اضافة الكروميوم العضوى فى علائق المجموعات الاخرى احدهما مع اضافة البروبيوتيك فى الماء و الاخرى بدون اضافة البروبيوتيك فى الماء وكان معدل اضافة الكروميوم ٤مجم/ كجم عليقة و استمرت التجربة لمدة ٦ اسابيع.

حسبت الزيادة فى وزن للطيور و معدل الزيادة فى الوزن الحى و استهلاك العلف و معامل التحويل الغذائى اسبوعيا. كما تم تحليل كل من creatinine،GOT,GPT, uric acid فى الامصال. وكذلك تم وزن الاعضاء الليمفاوية المناعية (غدة فابريش ، الطحال، الغدة التيموثية).

لوحظ تحسن معنوي لمعدل الزيادة فى الوزن ماعدا المجموعات التى اضيف لها البروبيوتيك فى الماء. وايضا انخفاض فى استهلاك العلف و تحسن فى معامل التحويل الغذائى مع اضافة الكروميوم العضوى و الغير عضوى و بالاخص عند اضافة الكروميوم الغير عضوى حيث انه اعطى معامل تحويل غذائى افضل، وأظهرت نتائج HI, skin response test, lymphoid organ weight ان مصدرى الكروميوم يحفزا المناعة لدى الطيور.

خلصت هذه الدراسة إلي ان اضافة الكروميوم العضوى و الغير عضوى فى علائق دجاج التسمين يحسن اداء الطيور و يؤدى الى تحسن الحالة المناعية لديها بدرجات مختلفة.