EFFECT OF CHROMIUM PICOLINATE SUPPLEMENTATION ON GROWTH PERFORMANCE, SERUM BIOCHEMICACL PARAMETERS, AND IMMUNE STATUS OF BROILERS

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ABSTRACT

Two hundred and forty broiler chicks (one day old), were randomly divided into four equal groups, each group was subdivided to be in doublicate (30 chicks for each). Four levels of dietary chromium supplementation (0 control, 800, 1600, and 3200 µg /Kg) were provided in the form of chromium picolinate throughout the experimental period (6 weeks). The chicks were fed on starter diet (23% CP & 3200 Kcal ME/Kg) for the first 3 weeks and grower-finisher diet (20% CP & 3200 Kcal ME/Kg) through the next three weeks. Dody weight and feed intake were determined and feed conversion ratio was calculated. At the end of experiment, blood samples were collected for obtaining clear sera which were used for determination of the total proteins, albumin, globulin, glucose. triacylglycerol, high density lipoprotein (HDL), low density lipoprotein (LDL), phospholipids and non estrifled free fatty acids (NEFFAs). Also, liver function enzymes including aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma glutamyl transferase (GGT) were determined. In addition, serum samples were used for determination of haemagglutination inhibition tiler against Newcastle disease, γ globulin, IgG and IgM. At end of the experiment, five birds from each replicate were used for determination of careass traits and composition. The obtained results revealed that feeding high chromium levels (1600 and 3200 µg/Kg) significantly increased (P<0.05) the final body weight and average daily weight gain. However, chromium had no effect on feed conversion. The dietary supplements of chromium (1600, 3200 µg/Kg) significantly reduced the blood glucose, the tatal cholesterol level, LDL and NEFFAs. Also, chromium supplements (1600 and 3200 µg/Kg) increased serum phospholipids. HDL total proteins, albumin and globulin. The liver function tests (AST, ALT and GGT) indicated no differences between the different groups. High chromium supplementation

(1600 and 3200 μ g/Kg) increased the γ -globulin levels. Hacmagglutination inhibition antibody titer against Newcastle vaccine. IgG and IgM were increased with increasing the chromium level in the diets. The carcass yield did not affected by chromium supplementation (1600 and 3200 μ g/Kg). There were decrease in the abdaminal pad and increase in breast yield at the level of 1600 and 3200 μ g/Kg of chromium. The protein percent of carcass was increased and fat percent was decreased while the ash and moisture were not significantly affected.

INTRODUCTION

Trivalent chromium is involved in earbohydrate metabolism and recognized as active component of glucose tolerance factor (Rosebrough and Steele, 1981). It has been reported that chromium plays an important role in regulating glucose metabolism in human and laboratory animals (Mertz, 1993). Chromium facilitates interaction between insulin and insulin receptors (Mooradian and Morley, 1987), and involved in lipid, protein and nucleic acid metabolism (Okada et al., 1984; Ohba et al., 1986; Press et al., 1991; McCarty, 1991; Page, 1991; Lein et al., 1999). Cell proliferation and synthesis of regulatory protein form the basis of an effective immune response. It can be affected by dictary chromium as improved immune response has been observed when organic forms of chromium were supplemented to stressed feeder calves (Chang and Mowat, 1992; Moonsie-Shageer and Mowat, 1993) and to dairy cows (Burton et al., 1993).

Stresses and diseases both increased urinary exerction of chromium (Borel et al., 1984; Anderson et al., 1988) and may exacerbate a marginal chromium deficiency. Therefore, deficiency of trivalent chromium may occur in chicken under stress condition because of metabolic changes mediated by various stressors. Chromium deficiency may slow growth rate, impair glucose tolerance and ultimately lead to diabetes or coronary heart disease (Simonoff et al., 1984). So, chromium is recognized as an essential trace element for human and NRC (1989) has recommended an intake of 50 to 200 µg /Kg of trivalent chromium for the adult human. However, an appropriate recommendation of the chromium requirement of poultry has not been made (NRC, 1994), because of poor absorption of chromium chloride which was largely used as a chromium supplement in the early studies (Dowling et al., 1989; Lien et al., 1996). Several investigators have demonstrated that the effect of chromium chloride is insignificant as a dictary supplement (Li and Stoecker, 1996; Lien et al., 1996).

Chromium picolinate, an organic and low toxic form of trivalent chromium can stimulate insulin activity (Evans, 1989; Walker, 1993; Amoikon et al., 1995). Organic chromium im-

proved immune response and increased the chromium concentration in the kidney and spleen (Anderson and Kozlovsky, 1985; Anderson et al., 1993; Mooney and Chromwell 1997). Plant products contain a low content of chromium, implying that broilers may have deficiency of chromium because their diets mostly are of plant ingredients (Gibson 1989). Poultry required higher levels of chromium because β-cells of panereas of poultry are not being active as those of mammals and secreting a small amount of insulin (Lien et al., 1993; 1996; Ward et al., 1994). Therefore, the present study is to investigate the effects of dietary supplementation levels of chromium picolinate on the growth performance, scrum blochemical parameters and immune response.

MATERIALS AND METHODS

Two hundred and forty broiler chicks (one day old), were equally divided at random into four groups, each group was subdivided to be in doublicate (30 chicks for each). Four levels of dictary chromium supplementation (0 control, 800, 1600, and 3200 μ g Cr /Kg) were provided in the form of chromium picolinate. The broilers were raised for 6 weeks in a pen with natural ventillation. The chicks were fed on starter diet (23% CP & 3200 Keal ME/Kg) for the first 3 weeks and grower-fluisher diet (20% CP & 3200 Keal ME/Kg) through the next three weeks (table, 1).

The feed and water were provided ad-libitum. The birds were vaccinated against Newcastle disease virus using Hitchener and LaSota vaccines at 7 and 14 days of age, respectively. Body weight and feed intake were determined and the feed conversion ratio was calculated. At end of the experiment, blood samples were collected from the wing vein of 10 birds from each group (5 birds for each replicate). Blood samples were centrifuged for obtaining clear sera which were used for determination of total proteins (Cornel et al., 1949), albumin (Dournas, 1971), globulin (subtracting albumin from total proteins), glucose (Trinder, 1969), triacylglycerols (Young and Postaner, 1975), total cholesterol (Melattini, 1978), HDL (Clark et al. 1983), LDL (Friedwald et al., 1973), phospholipids (Zilversmit, and Davis, 1950) and NEFFAs (Duucombe, 1964). Liver function tests including AST, ALT (Reltman and Frankel, 1957) and GGT [Persijn and Vonderk, 1976) were determined. Regarding the immune response, the serum samples were used for determination of hacmagglutination inhibition titer against Newcastle disease (Anon, 1980), γ-globulin (Davis and Ornistein, 1964), IgG and IgM (Erhard et al., 1992).

At end of the experiment, five birds from each replicate were weighed, slaughtered and used for studying the careass traits and composition according to AOAC (1984). The obtained data were analyzed using one way ANOVA test (Senedecor and Cochran, 1989).

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Table (1): Physical and chemical composition of experimental diel

Ingredients	0-3 weeks (%)	3-6 weeks (%)
Ground yellow corn	53.9 6	63.82
Soybean meal(44%)	30.00	23.10
Fish meal (72%)	7.25	6.12
Soybean oil	5.63	4.00
Limestone	0.91	0.88
Dicalcium phosphate	1,72	1,53
Sodium chloride	0.30	0.30
Vit.& min. premix*	o.25	0.25
DL-Methionine	0.07	
Calculated value**		
ME (Kcal/Kg)	3200	3200
Crude protein %	23.0	20.0
Calcium %	1.00	0.90
Available phosphate %	0.45	0.40
Lysine %	1.33	1.12
Methionine %	0.50	0.39

Vitamin and mineral premix per 2.5 Kg supplied the following; Vitamin A, 3.5x10⁶ IU; Vitamin D 5x10⁵ IU; Vitamin E, 25000 IU; Vitamin K, 150 mg; riboflavin 1000 mg; pantothenic acid, 3000 mg; niacin, 7000 mg; choline, 3.5x10⁵mg; biotin, 50 mg; folic acid, 150 mg; vitamin B₁₂ 3.0 mg; thiamin, 500 mg; pyridoxine, 800 mg; Cu, 50g, Fe, 100g; Mn, 70 g; Zn, 50 g; iedine, 350 mg and selenium, 150 mg. The producer recommended 2.5 Kg premix/ ton diet).

[&]quot; Values are calculated according to feed composition table of NRC (1994).

RESULTS AND DISCUSSION

The effects of feeding the diets supplemented with different levels of chromium picolinate on broiler performance are presented in table 2. Feeding the diets supplemented with chromium picolinate at the level of 800 mg had no significance effect on body weight, feed intake and feed conversion, while the high chromium levels (1600 and 3200 μg /Kg) significantly increased (P<0.05) the final body weight and the average daily weight gain. Kim et al., (1996) reported that supplementation of 100 to 800 mg chromium picolinate /Kg diet did not affect growth performance of broflers. Also, previous investigations found no response in broiler growth performance when dictary supplements of 100 to 400 mg chromium picolinate /Kg were used (Lein et al., 1993; Ward et al., 1994; Mortozona, et al., 1998; Hegazi et al., 2001). Furthermore, Lein et al., (1999) reported that a dietary level of 800 µg /Kg chromium picolinate did not affect growth performance of brotler but higher levels of chromium improved the body weight and average weight gain. In mammalian studies, dictary supplements of 200 mg chromium pleolinate /Kg diet tended to enhance the growth rates of pigs and calves (Chang and Mowat, 1992; Moonsle-Shageer and Mowat, 1993; Lindemann et al., 1995). The difference between broilers and mammals in term of effectiveness of chromium supplementation might be attributed to that pancreatic β -cells in poultry not being are active as those in mammals (Lien et al., 1999). Increased weight gain could be due to chromium action via reduction of serum cortisol decreasing its catabolic effect (Brockman, 1986). In addition to chromium role in metabolic process, it has been reported that chromium affect nuclear protein synthesis (Okada et al., 1984). Also, the increased average datly weight gain in brotlers fed high chromium-diet may be due to increase daily feed intake, however, chromium did not affect feed conversion ratio (table, 2). Similar results were reported by Hossain et al., (1998) who found that organic chromium supplementation increased the body weight gain of broilers but did not affect feed converslon.

The effect of feeding diets supplemented with different levels of chromium on scrum biochemical parameters and liver function tests are shown in table, 3. The obtained results exhibited that the high dietary supplements of chromium (1600 and 3200 µg /Kg) reduced the blood glucose level. This may be through stimulation of the biological activity of insulin by increasing insulinsensitive cell receptors or binding activity (Anderson et al., 1991; Morris et al., 1993; Ward et al., 1994). Plasma insulin concentration was reported to be increased linearly, whereas corticosterone concentration was decreased as dictary chromium supplementation increased (Sahin et al., 2001). Cortisol promotes gluconeogenesis and reduces glucose utilization (Weekes, 1991). Insulin can stimulate glucose uptake and utilization by cells (Cupo and Donaldson, 1987). Related studies in human and other animals have indicated that a dietary supplement of chromi-

um reduced the serum glucose (Lefavi, et al., 1993; Mertz, 1993; Amolkon, et al., 1995; Lein et al., 1996; 1999).

The results of the present study (table, 3) indicated that chromium supplements (1600 and 3200 μ g /Kg) increased serum total proteins, albumin and globulin. These results are supported by **Hegazi et al.**, (2001) who found chromium picolinate increased serum total proteins. It has been reported that chromium had an effect on nuclear protein and RNA synthesis (**Okada et al.**, 1984; **Chang and Mowat**, 1992). This increase in protein level may be due to the anabolic effect of insulin.

The dictary chronium picolinate supplements (1600 and 3200 µg /Kg) significantly reduced (P<0.05) the serum levels of total cholesterol. LDL and NEFFAs while increased levels of both phospholipids and HDL. These findings may explain the role of chromium in decreasing the incidence of coronary heart disease in human (Simonoff et al., 1984). Similarly, McCarty (1991) found that the chromium increased the HDL level of human serum. Howard et al., (1993) indicated that insulin increased liver LDL receptors, so reducing the LDL level in serum. Lein et al., (1999) noticed that high chromium levels in diets of brotlers increased the serum phospholipids while both total cholesterol and triacylglycerol were not affected. On the other hand, Hegazi et al., (2001) found that chromium picolinate reduced the serum total lipids and total cholesterol. The chromium acts as activator of the biological activity of insulin which depresses adipocyte lipolysis by reducing the activity of cAMP and hormone sensitive lipase (Lambert and Jacquemin, 1979). Moreover, Pigs fed chromium picolinate supplemented ration had lower plasma NEFFAs concentration than control pigs (Matthews, et al., 2001). The liver function tests indicated no difference between the different groups in AST, ALT and GGT (table, 3). Gursoy (2000) mentioned that organic chromium is more available, safe and has less toxic effect.

The effects of chromium picolinate supplementation on the immune response of broilers are presented in table. 4. High chromium supplementation (1600 and 3200 µg /Kg) increased the regional levels. Also, haemagglutination inhibition antibody titer against Newcastle vaccine, IgG and IgM were increased with increasing the chromium level in the diet. Similarly, Chang and Mowat (1992) found that chromium picolinate supplementation increased total immunoglobulins and IgM. However, Moonsie-Shageer and Mowat (1993) reported that chromium supplementation had no effect on IgM and IgG2 levels but significantly increased IgG1. Improving the immune response may be due to the effect of chromium on reduction of cortisol, which has immunosuppressive effect and inhibiting production and activities of cytokines and antibodies (Kelly, 1988; Kegley and Spears, 1995). In addition, chromium supplementation in stressed animals can prevent urinary losses of zinc, fron, copper, manganese and selentum (Schrauzer et al., 1986; Anderson et al., 1988). The deficiencies of such minerals had been re-

ported to lower resistance to diseases (Bull, 1990).

Concerning the effect of chromium supplementation on the carcass traits (table, 5), it was found that carcass weight but not carcass yield was increased by chromium supplementation at the levels of 1600 and 3200 µg/Kg diet. Also, there was a decrease in the abdominal fat pad and an increase in hreast yield at the level of 1600 and 3200 µg/Kg of chromium supplementation. Moreover, the protein percent of carcass was increased and fat percent was decreased while the ash and moisture were not significantly different, Hossain et al., (1998) and Choet (1999) found that abdominal fat decreased and breast meat yield increased in broilers fed diets supplemented with chromium picolinate at keyels of 300 and 500ppb, respectively. Evans and Bowman (1992) and Kim et al., (1996) demonstrated that the increase in protein content of broiler carcass may be attributed to the effect of insulin on amino acid uptake. Additionally, Lein et al. (1999) mentioned that chromium increased the liver lipid accumulation so decreasing accumulation of fat in abdominal pad.

Collectively from the result of the present study, it could be concluded that chromium picolinate supplementation at a level of 1600 ppb for broiler diets has beneficial effects evidenced by improved growth performance and careass traits as well as improving the immune response. It also has beneficial consequences in terms of enhanced resistance to diseases by enhancing the effectiveness of vaccines.

Table (2): Effect of chromium picolinate supplementation on performance of broilers (Mean \pm SE)

ltes	Supplemental chromium (µg /Kg dlet)			
	Control (0)	800	1600	3200
Initial body weight	35.04 ± 0.19	35.18 ± 0.25	35.1 ± 0.28	34.8 <u>+</u> 0.21
Final body weight (g)	1920 ± 51.26 ^b	2165 ± 42.33 ^{ab}	2280 ± 46.51 ^a	2265 ± 35.29 ^a
Average daily gain (g)	44.88 ± 1.29 ^b	50.71 ± 1.12 ^{ab}	53,45 ± 0.85ª	53.1 ± 1.65a
Average daily feed intake (g)	86.17 <u>+</u> 3.26 ^b	94.83 ± 3.12 ^{ab}	101.2 ± 4.28ª	98.77 <u>+</u> 3.8ª
Feed conversion ratio	1.92 <u>+</u> 0.25	1.87 ± 0.19	1.89 ± 0.28	1.86 ± 0.25

ab Means in the same raw with no common superscript are significantly different (P<0.05).

Table (3): Effect of chromium picolinate supplementation on serum biochemical parameters (Mean \pm SE) of broiler chickens.

Blochemical parameters	Supplemental chromium (µg /Kg diet)			
	Control (0)	800	1600	3200
Glucose (mg/dl)	179.13 ± 2.1ª	166 ± 2.44 ^a	123.5 ± 1.78 ^b	124.17 ± 2.11 ^b
Total proteins (gm/dl)	5.70 ± 0.22 ^b	6.78 <u>+</u> 0.4 ^b	9.97 ± 0.38 ^a	11.04 ± 0.17ª
Albumin (gm/dl)	2.97 <u>+</u> 0.31 ^b	4.15 ± .35 ^b	6.25 ± 0.29 ^a	7.33 ± 0.32a
Globulin (gm/dl)	2.73 ± 0.28 ^b	2.63 ± 0.21b	3.72 ± 0.32ª	3.71 ± 0.25 ^a
Triacylglycerol (mg/dl)	30.51 ± 1.46	27.97 ± 1.45	28.4 ± 1.28	27.68 ± 1.53
Total cholesterol (mg/dl)	89.66 ± 2.84 ^a	79.23 ± 2.31b	76.19 ± 3.11 ^b	78.35 ± 1.9 ^b
Phospholipids (mg/dl)	98.69 <u>+</u> 2.56 ^b	103.81 ± 3.1 ^b	119.37 ± 2.3ª	121.23 ± 2.4a
HDL (mg/dl)	68.83 <u>+</u> 1,19 ^{ac}	74.15 ± 1.36b	82.92 ± 2.13 ^a	85.15 ± 1.98ª
LDL (mg/dl)	26.16 ± 0.85 ^a	21.03 ± 0.72 ^b	19.25 ± 0.53 ^b	15.09 ± 0.66°
NEFFA (mg/dl)	26.18 <u>+</u> 2.1a ^a	24 ± 1.60 ^a	14.8 ± 1.1 ^b	6.6 ± 0.23 ^c
ALT(U/L) (U/L)	40.21 <u>+</u> 2.45	41.33 ± 1.52	39.33 ± 1.29	42.09 ± 1.0 ⁵
AST(U/L)	20.43 ± 1.80	21.67 ± 0.69	23 ± 0.82	23.36 ± 0.98
GGT (U/L)	8.87 ± 0.63	9.12 ± 0.35	9.47 ± 0.27	9.41 ± 0.26

abc Means in the same raw with no common superscript are significantly different (P<0.05)

Table (4): Effect of chromium picolinate supplementation on immune response (Mean \pm SE) of broiler chickens.

lten	Supplemental chromium (µg /Kg diet)			
	Control (0)	800	1600	3200
HI titre* Y-Globulin (gm/dl) IgG (mg/dl) IgM (mg/dl)	$2.93 \pm 0.13^{\circ}$ $0.85 \pm 0.07^{\circ}$ $589.14 \pm 25.7^{\circ}$ $154.21 \pm 8.83^{\circ}$	4.37 ± 0.23^{b} $0.88 \pm .11 \text{ b}$ 597.59 ± 36.7^{b} 196.11 ± 6.79^{b}	7.49 ± 0.38^{a} 1.36 ± 0.08^{a} 845.64 ± 40.36^{a} 238.33 ± 12.8^{a}	7.41 ± 0.26^{a} 1.44 ± 0.09^{a} 838.58 ± 17.6^{a} 274.18 ± 17.6^{a}

abc Means in the same raw with πο common superscript are significantly different (P<0.05).

Table (5): Effect of chromium picolinate supplementation the carcass trials of broilers (Mean \pm SE)

Items	Supplemental chromlum (μg /Kg diet)			
	Control (0)	800	1600	3200
Carcass weight (gm)	1295 ± 39.02 ^b	1435 ± 32.14 ^{ab}	1519 ± 34.03a	1515 ± 26.16 ^a
Carcass yield (%)	67.44 ±3.29	66.28 ± 3.35	66.62 ± 2.94	66.88 ± 3.68
Abd. Fat pad (% of carcass)	3.84 ±0.34ª	3.61 ± 0.28 ^a	2.58 ± 0.30 ^b	2.62 ± 0.41 ^b
Breast yield (% of carcass)	20.1 <u>+</u> 1.96 ^b	20.7 ± 1.38 ab	21.73 ± 1.64 ^a	21.65 ± 0.97 ^a
Carcass composition				
Moisture %	63.21 ±4.40	62.35 ± 4.35	64.91 ± 3.81	63.46 ± 0.81
Protein %	18.23 ±1.74 ^b	18.69 <u>+</u> 1.82 ^{ab}	19,89 <u>+</u> 1.59 ^a	19.94 ± 1.63 ^a
Fat %	16.26 ±1.30 ^a	16. 3 2 ± 0.89 ^a	13.66 ± 1.12 ^b	14.06 ± 0.92
As h %	1.8 <u>+</u> 0.17	1.79 ± 0.31	1.82 ± 0.21	1.89 ± 0.30

ab Means in the same raw with no common superscript are significantly different (P<0.05).

^{*} HI values are log2 of the original liters.

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الملخص العربي

تأثير إضافة بيكلونات الكروم على النمو والقياسات الكيميائية الحيوية وخصائص الذبيحة والمستوى المناعى فى دجاج التسمين طارق إبراهيم محمد و جهاد رمضان السيد و قسم التغذية وأمراض مو التغذية، وقسم الكيميا ، الحيوية وكيميا ، التغذية و

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أجربت هذه الدراسة على مائتين وأربعين من كتاكيت التسمين (عمر يوم) قسمت إلى أربعة مجموعات متساوية وقسمت كل مجموعة إلى تحت مجموعتين متساويتين في كل منها ثلاثين كتكوتاً، وغذيت الكتاكيت على علاتق تحتوى على بيكلونات الكروم بنسب (صفر للمجموعة الضابطة ١٦٠٠، ١٦٠٠، ١٦٠٠ ميكروجرام / كجم) وتم تربيتها لمدة أسابيع وعين الرزن النهائي وكمية العلف المستهلك وكذلك معدل التحويل الغذائي. في نهاية التجربة أخذت عينات من الدم لعمل القياسات البيوكيميائية، وأيضاً تم ذبع عشرة طيور من كل مجموعة لدراسة مواصفات الذبيحة وتحليلها كيميائياً.

وقد أظهرت النتائج أن التغذية على المستويات العالبة (١٦٠، ٣٢،٠ ميكروجرام /كبجم) من بيكلونات الكروم أدت إلى حدوث زيادة معنوبة في الوزن النهائي وكذلك في متوسط الزيادة السرمية في الوزن، ولم يؤثر علي معدل التحريل الغذائي.

أما بالنسبة لتأثير الكروم على القياسات البيوكيميائية الحيوية في مصل الدم فقد أظهرت النتائج أن التغذية على المستويات العالية من الكروم (١٦٠٠، ٣٢٠٠ ميكروجرام /كجم) أدت إلى نقص معنوى في نسبة الجلوكوز والكلوسترول الكلى والليبوبروتينات منخفضة الكثافة وكذا نسبة الأحماض الدهنية الحرة وعلى النقيض أدت هذه المستويات العالبة إلى زيادة نسبة الفسفوليبيدات والبروتينات الكلية والألبيومين ونسبة الجلوبيولين، ولم تحدث تغيرات معنوية في إنزعات كفاءة الكبد (الأسبرتات ترانس أمينيز والجلوتامات ترانس أمينيز والجاماجلوتاميل ترانسفيريز).

وجد أن تأثير إضافة الكروم على الزبادة في وزن الذبيحة كان واضحاً حبث أنها زادت بزيادة كمية الكروم المضافة المجروجرام / كجم). وحدث نقص معنوى في نسبة دهون منطقة البطن وزيادة في عضلات منطقة

الصدر، ووجد أن هناك زيادة في نسبة البروتيئات ونقص في نسبة الدهون في الذبيحة ولكن لم يحدث تغير في نسبة الرطوية وكذا نسبة الأملاح.

كسا وجد أن زيادة الكروم المضاف (١٦٠٠ ، ١٦٠٠ ميكروجرام / كجم) أدى إلى زيادة نسبة الجاما جلوبيولين، بالإضافة إلى زيادة نسبة إختبار منع التجلط ضد التحصين لمرض النيوكاسل وكذا نسبة إختبار منع التجلط ضد التحصين لمرض النيوكاسل وكذا نسبة المستوى المناعى للدجاج.

وقد أوضعت النتائج عامة أن التغذية على المستويات العالية (٣٢٠٠.١٦٠٠ ميكروجرام / كجم) من بيكلونات الكروم أدت إلى زيادة معنوية في الوزن وأدت إلى إعتدال مستوى فياسات التمثيل الغذائي والمؤشرات البيوكيميائية كما أدى إلى تحسين المستوى المناعي.