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Effects of higher levels of chromium and copper on broiler health and performance during the peak tropical summer season

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ABSTRACT

The effects of higher levels of chromium alone, and in combination with copper, were investigated in broiler chicks divided into seven equal groups. Group G served as control, receiving no treatment. Groups A, B and F received chromium chloride at a rate of 2 g/kg and nicotinic acid 150 mg/kg, while groups C, D and E received chromium chloride 8 mg/kg and nicotinic acid 150 mg/kg. Broilers in groups A and C received copper sulfate at a rate of 200 mg/kg, while groups B and D received 400 mg/kg feed. Both live and carcass mass showed non-significant difference between treatment groups and control during the treatment period. However, during the withdrawal period these were higher ($P<0.05$) in chromium-fed birds (alone and in combination). Feed conversion ratio (FCR) was better in the treatment groups. Relative mass of liver to body mass during the first two weeks showed lower ($P<0.05$) values in birds fed low levels of chromium alone, or in combination with two levels of copper, and those fed high levels of both compounds, compared with control. At the third week the relative mass of liver to body mass was high in all treatment groups than in the control. Relative mass of lung to body mass showed a significant increase in birds fed higher levels of both chromium and copper during the early period, whereas it decreased significantly after the third week than in the control group. During the withdrawal period, relative mass of kidney, lung and heart to body mass were lower ($P<0.05$) in birds fed chromium alone. Relative heart mass to body mass during termination of treatment showed a significant relationship with relative liver (0.99, $P<0.05$) and lung (0.99, $P<0.05$) mass to body mass in the low chromium-fed group, while during the withdrawal period a significant relationship developed of relative heart mass with relative liver (0.99, $P<0.05$) and lung (0.78, $P<0.05$) mass to body mass in birds fed high chromium with high copper. Both gross and histological studies showed no change in any organ studied.

Key words: chromium, nicotinic acid, copper sulphate, broilers, organs mass, body mass, carcass mass, feed conversion ratio

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Introduction

Chromium has been reported to be an abundant element in the earth's crust and occurs in oxidation states ranging from Cr^{+2} to Cr^{+6}, but only trivalent and hexavalent forms are of biologic importance. In the biologic system there is very low chance of conversion of trivalent chromium to hexavalent chromium and it is the hexavalent chromium that crosses the cell membrane and becomes reduced to trivalent chromium, which is then involved in various cellular reactions (KLAASSEN et al., 1986). Studies have confirmed that chromium plays a critical role in carbohydrate and lipid metabolism both in mammals and birds, as it has been found to be an active component of glucose tolerance factor (GTF), which make the metabolic action of insulin more effective (SCHWARTZ and MERTZ, 1957). Chromium supplementation in diet has been related to increased protein deposition (SEERLEY, 1993; WARD et al., 1995), with decrease in muscle fat (WARD et al., 1995). It also affects body mass and feed conversion ratio at level 300 :g/kg feed (HOSSAIN et al., 1998). Its role in stress condition in animals and birds is more appreciated and in which it helps to reduce the negative influence of environmental and nutritional stress.

The use of copper at 200 mg/kg in white leghorn hens has shown a positive response, while levels of 400 mg/kg and above has shown a progressively negative response (CHIOU et al., 1997). A significant increase in serum aspartate aminotransferase (AST), lactate dehydrogenase (LDH) and creatine kinase (CK) with a copper level of 600 mg/kg has been reported, and at levels of 15 mg/kg increased broiler mass gain (KOH et al., 1996). In higher doses of more than 250 mg/kg feed, copper caused proventriculitis in broilers (WIDEMAN, 1996) and at 1477 ppm level it also produced oral ulcers as well as reduced feed intake and a drop in egg production in layers (GILBERT et al., 1996). There is considerable information available on the use of chromium in lower levels, but not at higher levels, and as both chromium and copper therefore affect growth the combined use at studied dose levels was planned to discover any exacerbated toxicity/effects on broiler mass and organs mass along with feed conversion ratio at weekly intervals. Correlation statistics were also computed to discover the relationship between various organs.

Materials and methods

A total of 175 day-old broiler chicks of both sex in equal proportion were purchased from a local market (High Tech). Chicks were randomly divided into seven equal experimental groups, A to G, and kept in wire cages under identical conditions of feeding and management. After one week of age all the groups were given experimental feed except group seven (G) which was kept as control and was given basal feed (Table 1). Basal feed was commercial feed (National Feed) purchased from a local market having metabolizable energy to 11.995 MJ/kg, with a composition as shown in Table 2. Experimental feed was also prepared by using the same feed mixed with various levels of compounds under study. The study was carried out for five weeks. At the end of each week five birds were slaughtered from each group, including the control, for various studies. All the experimental groups received treatment for the first four weeks, while during the 5th week all groups were given a basal diet to study the withdrawal effect. The experiment was conducted during the summer season with maximum temperature ranging from 40-45 EC and the lowest between 35-38 EC, which can be stated as a heat stress period.

It should be mentioned that chromium chloride was combined with nicotinic acid to make inorganic chromium organic, as chromium combines with nicotinic acid to form chromium polynicotininate. Hence, in the discussion the word chromium is used to indicate the organic form and no emphasis is placed on nicotinic acid.

Table 1. Broilers receiving various treatments during experimental trials through feed

Groups	Chromium chloride g/kg	Nicotinic acid mg/kg	Cooper sulphate mg/kg
A	2	150	200
B	2	150	400
C	8	150	200
D	8	150	400
E	8	150	---
F	2	150	---
G	---	---	---

Note: ME = 11.995 MJ/kg

Table 2. Chemical composition of basal feed

Chemical analysis	g/kg
Protein	200.0
Fat	45.0
Carbohydrate	420.0
Fiber	50.0
Lysine	12.0
Ash	55.0
Calcium	10.0
Phosphorus	5.0
Sodium	1.5
Methionine + Cystine	7 .0
Methionine	4.0

Note: ME = 11.995 MJ/kg

Weekly live, dressed carcass and organ mass of all the birds in each group were recorded by using an electric digital weighing scale; tissues were processed for histological studies. Daily feed consumption was recorded at around 9 *am* for each group to calculate feed conversion ratio. Relative organ mass-to-body -mass were calculated.

Gross and histological changes in various organs collected were recorded at the end of each week. Post-mortem was performed on dead birds; gross and histological changes were also recorded. Tissue sections stained with H and E and were viewed under a light microscope.

Data thus obtained were analysed by using the General Linear Model Procedure and means were compared by LSD on a personal computer by using SAS statistical software package (SAS User's Guide. Statistic. Version 6.21 Edition. Cary. North Carolina USA. SAS INSTITUTE, 1996). Pearson's correlation statistic was also computed to establish the relationship between various organs in different groups.

Results and discussion

The present study was designed to evaluate high levels of chromium and copper for beneficial and/or toxic effects in broilers. Previous studies on these compounds showed growth promoting, immunostimulating (CHANG et al., 1994; CHANG et al.,

Table 3. Comparison of means ± SD between groups of live and carcass mass at each week

Groups	Weeks				
	1st	2nd	3rd	4th	5th
Live mass (g)					
A	280.0 ± 18.95	450.80 ± 35.90	684.0 ± 37.84	972.0 ± 37.84	1230.0 * ± 68.20
B	291.0 ± 11.95	476.80 ± 19.80	775.0 ± 91.52	948.0 ± 48.10	1340.0 * ± 10.0
C	287.0 ± 11.81	445.67 ± 30.75	792.0 ± 29.44	1010.0 ± 40.0	1280.0 * ± 58.31
D	268.0 ± 9.96	431.0 ± 13.33	852.0 ± 40.0	1010.0 ± 142.65	1187.50 * ± 147.70
E	300.20 ± 17.30	497.40 ± 27.28	790.0 ± 73.14	950.0 ± 47.43	1366.67 * ± 72.65
F	309.0 ± 12.63	467.80 ± 20.56	718.33 ± 31.67	783.30 ± 148.14	1450.0 * ± 28.87
G (control)	309.33 ± 5.21	425.0 ± 43.68	650.0 ± 76.38	883.43 ± 16.67	1050.0 ± 50.0
Carcass mass (g)					
A	136.4 ± 11.03	238.2 ± 23.9	363.60 ± 27.14	465.4 ± 19.35	750.0 * ± 38.73
B	148.4 ± 4.83	256.4 ± 12.2	390.8 ± 39.93	522.00 ± 23.23	772.0 * ± 11.58
C	180.2 ± 26.38	247.0 ± 7.0	412.4 ± 15.83	535.30 ± 22.97	780.0 * ± 40.62
D	131.8 ± 6.05	239.6 ± 12.93	478.0 ± 11.28	520.6 ± 74.35	766.67 * ± 72.65
E	147.6 ± 8.93	244.0 ± 12.67	388.8 ± 35.04	502.6 ± 31.06	816.67 * ± 44.09
F	143.2 ± 4.00	276.8 ± 11.04	382.0 ± 14.18	342.67 ± 82.38	850.0 * ± 28.87
G (control)	146.67 ± 4.41	230.67 ± 24.06	350.67 ± 40.99	383.33 ± 56.74	633.33 ± 44.09

1995), and other useful effects, including reduction of fat and cholesterol in meat (SEERLEY, 1993). During the present study much higher levels of these compounds were used to evaluate their safety/toxicity, particularly during a heat stress period (summer season). The results obtained are presented and discussed under separate headings.

Table 4. Comparison of means ± SEM between groups of FCR at each week

Groups	Weeks				
	1st	2nd	3rd	4th	5th
FCR					
A	0.94	0.95	1.18	1.18	1.41
B	0.91	0.90	1.11	1.21	1.3
C	0.92	0.96	1.11	1.13	1.36
D	0.98	0.99	0.95	1.13	1.36
E	0.98	0.86	1.03	1.21	1.26
F	0.85	0.90	1.11	1.46	1.21
G (control)	0.85	1.01	1.25	1.29	1.66

Clinical signs. During the experiment no clinical signs were observed in broilers receiving chromium 2 and 8g and copper 200 and 400 mg/kg feed. This was similar to earlier studies made by O'DELL et al. (1962), POUPOULIS and JENSEN (1976) and STEVENSON and JACKSON (1981) who also observed no clinical signs in broilers fed copper sulfate through feed at lower levels than those used during the present study. STEEL and ROSEBROUGH (1981) also reported no clinical signs in broilers fed chromium chloride through feed. It can be inferred that these compounds at the studied levels (in combination and chromium alone) were not hazardous to broilers, as no behavioural alterations were observed.

Affect on mass. Both live and carcass mass were non-significantly influenced by feeding chromium alone and in combination with copper at experimental dose levels. However, during the withdrawal period both live and carcass mass were

Table 5. Comparison of means ± SEM between groups of proventriculus and gizzard mass index at each week

Groups	Weeks				
	1st	2nd	3rd	4th	5th
Proventriculus					
A	0.79 ± 0.15	0.76 ± 0.19	0.72 * ± 0.11	0.48 ± 0.13	0.42 ± 0.07
B	0.81 ± 0.08	0.76 A ± 0.07	0.63 ± 0.12	0.54 ± 0.11	0.42 ± 0.04
C	0.94 ± 0.11	0.77 ± 0.06	0.59 ± 0.09	0.43 ± 0.04	0.45 ± 0.05
D	1.01 ± 0.13	0.61 ± 0.07	0.48 ± 0.05	0.46 ± 0.11	0.42 ± 0.08
E	0.99 ± 0.14	0.61 ± 0.07	0.57 ± 0.05	0.53 ± 0.05	0.42 ± 0.03
F	0.99 ± 0.11	0.59 ± 0.11	0.61 ± 0.08	0.59 ± 0.11	0.50 ± 0.06
G (control)	0.89 ± 0.07	0.61 ± 0.23	0.57 ± 0.03	0.58 ± 0.05	0.48 ± 0.04
Gizzard mass					
A	3.40 ± 0.17	2.68 ± 0.38	2.38 ± 0.16	1.89 ± 0.09	1.99 ± 0.36
B	3.59 ± 0.59	2.67 ± 0.26	2.07 ± 0.08	2.19 ± 0.43	1.77 ± 0.24
C	3.32 ± 0.33	2.44 ± 0.12	2.04 ± 0.36	1.90 ± 0.27	1.81 ± 0.19
D	3.47 ± 0.27	2.55 ± 0.38	2.02 ± 0.20	1.88 ± 0.43	1.89 ± 0.13
E	3.53 ± 0.25	2.58 ± 0.26	1.88 ± 0.26	2.01 ± 0.13	2.02 ± 0.04
F	3.13 ± 0.12	2.50 ± 0.59	2.54 ± 1.11	1.97 ± 0.30	1.72 ± 0.06
G (control)	3.51 ± 0.18	2.90 ± 1.09	2.22 ± 0.21	1.79 ± 0.18	2.04 ± 0.48

* Indicate significant difference at P<0.05 from control

Table 6. Comparison of means ± SEM between groups of liver and kidney mass index at each week

Groups	Weeks				
	1st	2nd	3rd	4th	5th
Liver mass					
A	3.58 * ± 0.17	3.09 * ± 0.30	3.85 * ± 0.34	2.65 ± 0.49	2.57 ± 0.37
B	3.83 * ± 0.51	3.00 * ± 0.23	4.15 * ± 0.22	3.13 ± 0.58	2.54 ± 0.15
C	3.65 * ± 0.15	3.28 * ± 0.23	4.08 * ± 0.69	2.73 ± 0.42	2.40 ± 0.20
D	3.94 ± 0.29	3.58 ± 0.41	4.87 * ± 0.69	3.52 ± 0.84	2.79 ± 0.63
E	4.13 ± 0.46	3.43 ± 0.48	3.97 * ± 0.75	3.03 ± 0.32	2.25 ± 0.16
F	3.61 * ± 0.18	3.15 * ± 0.37	3.72 * ± 0.16	3.20 ± 0.86	2.35 ± 0.14
G (control)	4.36 ± 0.43	4.08 ± 0.93	2.79 ± 0.31	3.12 ± 0.47	2.54 ± 0.59
Kidney mass					
A	0.53 ± 0.08	0.60 ± 0.18	0.57 ± 0.15	0.71 ± 0.16	0.78 ± 0.23
B	0.52 ± 0.08	0.59 ± 0.09	0.58 ± 0.22	0.69 ± 0.07	0.77 ± 0.07
C	0.52 ± 0.04	0.63 ± 0.13	0.56 ± 0.07	0.76 ± 0.04	0.75 ± 0.18
D	0.59 ± 0.09	0.61 ± 0.06	0.49 ± 0.05	0.73 ± 0.37	0.88 ± 0.21
E	0.49 ± 0.07	0.59 ± 0.09	0.57 ± 0.17	0.78 ± 0.16	0.62 * ± 0.03
F	0.49 ± 0.02	0.57 ± 0.06	0.55 ± 0.04	1.03 ± 0.32	0.68 * ± 0.11
G (control)	0.50 ± 0.03	0.63 ± 0.09	0.65 ± 0.13	0.80 ± 0.02	0.97 ± 0.19

* Indicate significant difference at $P<0.05$ from control

Table 7. Comparison of means ± SEM between groups of lung and heart mass index at each week

Groups	Weeks				
	1st	2nd	3rd	4th	5th
Lung mass					
A	0.51 ± 0.09	0.68 ± 0.17	0.65 ± 0.13	0.72 ± 0.15	0.75 ± 0.15
B	0.49 ± 0.05	0.59 ± 0.03	0.61 ± 0.19	0.69 ± 0.12	0.72 ± 0.07
C	0.49 ± 0.11	0.68 ± 0.13	0.57 ± 0.07	0.65 ± 0.14	0.72 ± 0.11
D	0.59 * ± 0.11	0.67 ± 0.06	0.48 * ± 0.03	0.67 ± 0.29	0.75 ± 0.27
E	0.49 ± 0.04	0.53 ± 0.12	0.56 ± 0.19	0.72 ± 0.08	0.60 * ± 0.02
F	0.46 ± 0.04	0.53 ± 0.04	0.60 ± 0.04	0.87 ± 0.27	0.56 * ± 0.02
G (control)	0.45 ± 0.04	0.61 ± 0.11	0.74 ± 0.19	0.76 ± 0.01	0.89 ± 0.07
Heart mass					
A	0.96 ± 0.11	0.82 ± 0.19	0.63 ± 0.04	0.54 ± 0.09	0.48 ± 0.04
B	0.89 ± 0.21	0.80 ± 0.08	0.66 ± 0.10	0.58 ± 0.11	0.47 ± 0.04
C	1.10 ± 0.20	0.82 ± 0.01	0.55 ± 0.08	0.47 ± 0.04	0.42 ± 0.03
D	1.08 ± 0.18	0.64 ± 0.11	0.50 ± 0.06	0.51 ± 0.18	0.47 ± 0.01
E	1.09 ± 0.14	0.61 ± 0.14	0.55 ± 0.05	0.61 ± 0.04	0.47 ± 0.08
F	0.98 ± 0.14	0.63 ± 0.09	0.56 ± 0.08	0.66 ± 0.30	0.41 * ± 0.04
G (control)	1.08 ± 0.13	0.69 ± 0.18	0.59 ± 0.10	0.62 ± 0.05	0.54 ± 0.05

* Indicate significant difference at $P<0.05$ from control

higher ($P<0.05$) in birds fed chromium alone and in combination (Table 3). These results were in contrast to those of STEEL and ROSEBROUGH (1981) as they reported an increase in mass gain in turkey poults at a dose rate of 20 ppm of chromium chloride. Later, HOSSAIN et al. (1998) also observed an increase in carcass mass of broilers at levels of 300 g Cr per kg feed. LEIN et al. (1999) also observed improvement in feed consumption with an increase in mass gain in broilers supplemented with chromium 1600 and 3200 g/kg feed. POUPOULIS and JENSEN (1976) observed mass gain in broilers given copper sulfate through feed at 400-ppm. The use of copper at 200 mg/kg in white leghorn hens has shown a positive response, while at 400 mg/kg and above it has shown a progressively negative response (CHIOU et al., 1997). Previous studies and the present findings suggest that both chromium and copper cause an increase in live mass when given at lower levels, while when fed at much higher levels and in combination they had a non-significant effect. The significant increase in mass during the withdrawal period in treatment groups may be because these compounds have decreased in levels and thus have contributed in mass gain, as the cause of this effect is most probably the metabolic variation at different concentrations of these compounds. The results of feed conversion ratio suggested that these compounds were useful even at the experimental dose levels, as converting feed into body mass (Table 4) was in line with earlier findings in growing poultry (SAHIN et al., 2001). In the light of the present findings on mass gain and feed conversion, it can be said that even at higher levels these compounds had no deleterious effects. However, it is suggested that their effects may be investigated with alternating patterns of administration/ feeding and at various parameters, including the immune system.

Relative mass of organs to body mass. Relative organ mass at each week of the treatment trial for proventiculus, gizzard, liver, kidney, lung and heart were determined and compared (Tables 5-7). Relative mass of proventriculus and gizzard to body mass showed a non-significant difference between treatment groups and control, except at week three where relative proventricular mass to body mass was significantly higher ($P<0.05$) in birds fed lower levels of chromium and copper in combination (chromium 2g; copper 200 mg/kg feed) (Table 5). The change was a transient one and did not persist after continuous feeding of these compounds and may not be considered to be of significance. However, in higher doses of more than 250 mg/kg feed copper caused proventriculitis in broilers (WIDEMAN, 1996) but it was not observed during present study. This may be because of chromium, as it is the "Gold Standard" to normalize copper. In fact, both chromium and copper are antagonists and at the studied levels proved to negate the effects of each other. Relative mass of liver to body mass during the first two weeks showed low values in birds fed low levels of chromium alone, or in combination with two levels of copper, and those fed high levels of both compounds compared with control (Table 6). while at the third week, relative mass of liver to body mass was higher in all treatment groups than in the control. However, during the fourth week and the withdrawal period it became non-significant. This probably suggests metabolic reasons in these groups during the first three weeks rather any toxic effect, as no gross and histological changes were observed in liver of any group. Previously, copper at the studied level showed a toxic effect on liver (WIDEMAN et al., 1996; CHIOU et al., 1997) that was absent during the present study and may be due to antagonism between the two compounds. Relative mass of kidney to body mass showed a non-significant difference between treatment groups and control. However, during the withdrawal period it was lower ($P<0.05$) in birds fed chromium alone at both levels (Table 6). This suggests that liver and kidneys are involved in metabolism of Cr and might have different mechanisms to deal with Cr, thus contributing to the variation in relative mass to body mass of these organs at various concentration levels, which might have been the case during treatment and the withdrawal period. It can be stated here that the changes in mass of these organs were without any pathology, both at the gross and microscopic levels. ANDERSON et al. (1997) reported that supplemental Cr at 300 g/kg feed led to increased total Cr in kidney (1.1 vs 2.3 g) and liver (5.9 vs 8.8 g) which suggests that these organs are involved in Cr metabolism. Relative lung to body mass showed a significant increase ($P<0.05$) in birds fed higher levels of both chromium and copper during the early period, while it decreased significantly after the third week than in the control group (Table 7). The change is difficult to explain as to which mechanism worked in this organ. However, during the withdrawal period here again the change was significant in chromium-fed birds, with a decrease in mass as observed for other organs. However, it may be said that the change in mass may be due to circulatory changes in this organ without significant light microscopic alterations. A study in rats also showed no detectable differences on histological evaluation in liver and kidney of controls and those fed 100 mg/kg Cr as Cr chloride or picolinate (ANDERSON et al., 1997).

Relative heart to body mass showed a non-significant difference during the treatment period, although during the withdrawal period it was significantly lower ($P<0.05$) in birds fed a low level of chromium alone (Table 7). It can therefore be extrapolated that the decrease in heart mass could have contributed to circulatory changes in other organs as mentioned above and thus caused the decrease in mass of those organs, especially the lung. The decrease in relative heart mass to body mass itself might also be due to same reason: circulatory changes. Relative heart to body mass during termination of treatment, i.e., at the fourth week, showed a significant relationship to relative liver (0.99, $P<0.05$) and lung (0.99, $P<0.05$) mass to body mass in the low chromium-fed group. This relationship was the reverse and became non-significant, although it remained close to the significant level during the withdrawal period. However, in birds fed high chromium with high copper, a significant relationship developed of relative heart mass with relative liver (0.99, $P<0.05$) and lung (0.78, $P<0.05$) mass to body mass during the withdrawal period. These findings support the evidence of earlier changes in mass of these organs and may be a reason for the circulatory changes contributing in this effect.

Conclusions

It can be concluded from the present observations that chromium causes a decrease in relative mass of kidney, lung and heart to body mass without producing pathological alterations at gross and microscopic levels, and that these compounds have an antagonistic effect at the studied level.

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SAŽETAK

Istražen je učinak većih razina samog kroma i u kombinaciji s bakrom na zdravlje i proizvodnost tovnih pilića. Pilići u skupinama A, B i F bili su hranjeni hranom u koju je bio umiješan krom u obliku krom klorida u količini od 2 g/kg te nikotinska kiselina u količini od 150 mg/kg. C, D i E skupine bile su hranjene hranom u koju je primiješan krom klorid u količini od 8 mg/kg te nikotinska kiselina u količini od 150 mg/kg. Pilićima skupine A i C bio je primiješan bakreni sulfat u dozi od 200 mg/kg, dok je skupinama B i D primiješan bakreni sulfat u dozi od 400 mg/kg. Težine pilića pokusnih skupina nisu se znatno razlikovale u odnosu na kontrolu, iako su u razdoblju bez primjene bile više u ptica hranjenih kromom odnosno onih kromom i bakrenim sulfatom. Konverzija hrane bila je bolja u pokusnih skupina u odnosu na kontrolu. Relativna težina jetre u odnosu na težinu tijela tijekom prva dva tjedna bila je niža ($P<0,05$) u svim skupinama bez obzira na razinu kroma i bakra. U trećem tjednu relativna težina jetre je porasla u svim pokusnim skupinama u odnosu na kontrolnu. Relativna težina pluća u odnosu na ukupnu težinu bila je veća nakon primjene visokih razina kroma i bakra u ranom razdoblju. Ona se znatno smanjila u trećem tjednu. Tijekom razdoblja uskraćivanja relativna težina bubrega, pluća i srca u odnosu na tjelesnu težinu bila je niža ($P<0,005$) u ptica koje su dobivale samo krom. Relativna težina srca u odnosu na tjelesnu težinu pri kraju primjene odgovarala je relativnoj težini jetre (0,99, $P<0,05$) i pluća (0,99, $P<0,05$) ptica u skupini koja je dobivala manje kroma. U razdoblju bez primjene uočena je povezanost relativne težine srca s relativnom težinom jetre (0,99, $P<0,05$) i pluća (0,78, $P<0,05$) u ptica hranjenih visokim razinama kroma i bakra. Patološkoanatomskom i histološkom analizom nisu dokazane nikakve promjene.

Ključne riječi: krom, nikotinska kiselina, bakreni sulfat, tovni pilići, težina organa, tjelesna težina, konverzija hrane