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EFFECT OF ADDITION OF Ca, Cu, Fe, Zn AND LACTIC ACID TO DRINKING WATER ON CONTENT OF THESE ELEMENTS IN MUSCLES OF SLAUGHTER TURKEYS

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Abstract

The study was conducted on 300 turkey cocks type BUT-9, grown under optimal conditions for this kind of poultry. The feed was a typical commercial full-dose mix for turkeys. The birds were divided into 5 experimental groups. From the 3rd week of their life, they were given preparations with their drinking water. Group I was the control group, while drinking water for the test groups was enriched as follows: lactic acid (0.4%) for group II, $CuSO_4$ in the amount of 30 mg $Cu \cdot dm^{-3}$ H_2O for group III, $CuSO_4$ (30 mg $Cu \cdot dm^{-3}$) and 0.4% of lactic acid for group IV and $CuSO_4$ in the amount of 50 mg $Cu \cdot dm^{-3}$ for group V. Application of the preparations was terminated after 3 days, when symptoms of poisoning were observed in turkey cocks of groups II and IV, alongside increased mortality rate. Samples of drinking water were taken for analyses, and from each group 10 birds were selected for slaughter to take tissue samples (breast and leg muscles).

The objective of the experiments was to determine the concentration of Ca, Mg, Zn, Cu and Fe in the consumable tissues of turkey cocks.

The study showed that the highest rate of mortality of turkey cocks was observed in the group that was given lactic acid with their drinking water, and these turkeys had increased levels of Ca, Cu, Zn and Fe. In none of the groups, the content of Zn in the breast muscle exceeded 20 mg kg^{-1}, while the concentration of copper in all experimental groups (except the control) was above the level of 10 mg kg^{-1}. Increased doses of copper caused an increase in the concentration of magnesium in the leg muscles, while a combination of copper supplementation with lactic acid resulted in increased accumulation of Cu in both the breast and leg muscles.

Key words: turkeys, zinc, copper, lactic acid, breast and leg muscles.

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WP£YW DODATKU Ca, Cu, Fe I Zn ORAZ KWASU MLEKOWEGO DO WODY PITNEJ NA ZAWARTOŒÆ TYCH PIERWIASTKÓW W MIÊŒNIACH INDORÓW RZEŹNYCH

Abstrakt

Badania przeprowadzono na 300 indorach typu BUT-9, odchowywanych w optymalnych warunkach przewidzianych dla tego typu ptaków. Pasz¹ by³a typowa komercyjna mieszanka pe³noporcjowa dla indyków. Ptaki podzielone na 5 grup doœwiadczalnych, od 3. tygodnia ¿ycia otrzymywa³y preparaty do wody pitnej. Grupa I by³a grup¹ kontroln¹, grupa II otrzymywa³a do picia wodê z kwasem mlekowym (0,4%), grupa III otrzymywa³a do wody pitnej dodatek $CuSO_4$ w iloœci 30 mg $Cu \cdot dm^{-3}$ H_2O, grupa IV – dodatek $CuSO_4$ (30 mg $Cu \cdot dm^{-3}$) i 0,4% kwasu mlekowego, grupa V – dodatek $CuSO_4$ w iloœci 50 mg $Cu \cdot dm^{-3}$. Podawanie preparatów zakoñczono po 3 dniach, poniewa¿ w grupach II i IV zaobserwowano u ptaków objawy zatrucia i zwiêkszon¹ liczbê padniêæ. Pobrano próbki wody pitnej do analiz oraz wybrano z ka¿dej grupy po 10 ptaków do uboju, w celu pobrania prób z tkanek (miêsieñ piersiowy i miêsieñ udowy).

Celem badañ by³o okreœlenie kumulacji Ca, Mg, Zn, Cu i Fe w tkankach konsumpcyjnych indorów.

W badaniach stwierdzono, ¿e najwiêcej padniêæ indorów zaobserwowano w grupie otrzymuj¹cej dodatek kwasu mlekowego do wody pitnej, w której stwierdzono zwiêkszone iloœci Ca, Cu, Zn i Fe. W miêœniu piersiowym indorów ze wszystkich grup zawartoœæ Zn nie przekracza³a 20 mg kg^{-1}, natomiast kumulacja miedzi we wszystkich grupach doœwiadczalnych, oprócz kontrolnej, przekracza³a 10 mg kg^{-1}. Zwiêkszone dawki miedzi wp³ynê³y na podwy¿szenie koncentracji magnezu w miêœniu udowym, a suplementacja miedzi z kwasem mlekowym spowodowa³a wzrost kumulacji Cu w miêœniach piersiowym i udowym.

S³owa kluczowe: indyki, cynk, miedŸ, kwas mlekowy, miêsieñ piersiowy, miêsieñ udowy.

INTRODUCTION

Zinc is one of the trace elements that occur in organisms, mainly in the liver, kidneys and in the bones. The metabolism of zinc and of other elements is subject to hormonal regulation with participation of glycocorticoids, insulin and catecholamines. Also, zinc transformations are aided by interleukin-1, related with the immune system, inflammatory conditions and stress (MALINOWSKA 1993). Its most important function is participation maintaining the activity of numerous enzymes which determine the proper course of physiological processes. Zinc is the only element that is represented by at least one enzyme in all of the 6 main classes (KIDD et al. 1996, KORELEWSKI, ŒWI¥TKIEWICZ 2001). Absorption of zinc from feed varies between 10 and 40%, and takes place primarily in the proximal small intestine. When the zinc content in feed is low, its absorption in the intestines increases, while a high supply of zinc inhibits its absorption from the gastrointestinal system in poultry (NOY et al. 1994). Zinc absorbed in increased quantities accumulates in the bones, pancreas, liver, kidneys and intestines. When the tissue capacity is exhausted but the organism continues to receive zinc, its concentration in the plasma grows and first symptoms of zinc poisoning appear. As regards turkeys, these symptoms can appear at zinc levels of at least 2230 $mg \cdot kg^{-1}$ of feed (Kidd et al. 1996, Noy et al. 1994). The bio-availability of zinc depends on a number of factors, the presence of which may facilitate or inhibit its absorption. According to Noy et al. (1994) and Wedekind et al. (1992), the main inhibitors of zinc absorption include phytic acid, tannins, arginine, and such elements as Ca, Cd, Cu, Mg and P. The primary factor that determines the absorption of zinc is the water solubility of its compounds formed in the gastrointestinal tract. Ligands with smaller molecular weight, i.e. amino acids or organic acids, may promote the absorption of zinc.

Although turkeys do not need much copper (*ca* 2-4 $mg \cdot kg^{-1}$ of feed), this metal shapes the proper course of numerous processes and functions, i.e. the formation of cross-bonds in collagen, mineralisation of bones, biosynthesis of haem, oxidative phosphorylation, metabolism of glucose and cholesterol, immune functions, functioning of the central nervous system, and many others. Copper is frequently added in increased amounts to poultry feeds for prophylactic purposes, as a growth stimulator (Pesti, Bakalli 1996). In practice, however, copper deficit in turkeys appears frequently due to the presence of phytins and phosphates in feed, and because of antagonisms between certain elements, e.g. Zn and Cu (Pang, Applegate 2007). In respect of turkeys, copper deficit is especially dangerous, as it may be the cause of spontaneous rupture of arteries (Madej et al.1994). At present, with the currently binding prohibition on using antibiotics for prophylactic purposes in poultry, Cu appears to be a good substitute owing to its biological properties. However, copper should be applied at doses notably higher than the ones allowed by the current Polish poultry feeding standards (Standards of Poultry Feeding 2005).

Calcium appears in large amounts in birds because of its structural role in the bone structure. It also plays a highly important role in the regulation of numerous cellular functions. In an ionised form, it participates in numerous biochemical transformations in an organism that lead to a variety of effects manifested by contraction, secretion or proliferation. With a high supply of calcium in a diet and high calcium content in the organism, the absorption of this element is low and takes place mainly through passive transport from the intestinal lumen to the enterocyte space (Anderson 1991).

Apart from its structural function, magnesium in the organism constitutes the primary regulator of the cell growth cycle. The fundamental functions of magnesium include participation in the processes of synthesis and decomposition of high-energy compounds (ATP) and activation of most enzymes. The magnesium-adenosine-5'-triphosphate complex is a substrate for numerous enzymes participating in carbohydrate and fat transformations (Aikawa 1981).

In animals, most iron occurs in metabolically active compounds, in a form bound with protein complexes. As a component of haemoglobin, myoglobin and many enzymes, it performs fundamental functions in oxygen transport and storage, de-saturation of fatty acids, tyrosine iodination, biosynthesis of prostaglandins. The amount of absorbed iron is variable and depends for example on the needs of an organism, i.e. it is more intensive in states of deficiency and decreases when the state of organism's saturation with iron is attained (NAGURNA-STASIAK, LECHOWSKI 1994).

In industrial aviculture of turkeys, it is a frequent practice to add small amounts of lactic acid (usually 0.2%-0.3%) on silica carrier to feed or directly to drinking water. Lactic acid is a strong prebiotic; by lowering pH to the level of 5.0-6.8 in particular sections of the gastrointestinal tract, it creates optimum conditions for colonisation by beneficial bacteria, and facilitates resorption of mineral components. In a study by BYRD et al. (2001) and HIGGINS at al. (2007), it was demonstrated that lactic acid given to birds in their drinking water notably reduced the multiplication of *Salmonella* s. Enteritidis and s. Typhimurium in their gastrointestinal tract, and lowered their content in the excrements. Lactic acid is classified as a strong acid and is capable of destroying metal objects (it even etches stainless and acid-resistant steel), which is why it is difficult to apply in aviculture.

The objective of the study was to determine the concentration of Ca, Mg, Zn, Cu and Fe in the breast and leg muscles of turkey cocks given increased doses of Cu, Zn, Fe and Ca in drinking water, in combination with lactic acid.

MATERIAL AND METHODS

The study was conducted on medium-weight turkey cocks type BUT-9 maintained under conditions that complied with those recommended for birds of this type. On the 2^{nd} day of their life, the squealers were divided into 5 groups of ca 60 turkey chicks each that were fed typical full-dose mixes suitable for their age. An approximate content of basic mineral components in the full-dose feed mixes for turkeys, based on analyses performed by the manufacturer, is given in Table 1. From the 3^{rd} week of the birds' life, application of copper compounds and lactic acid was begun. Group I was the control group, while drinking water for the other groups was enriched as follows: lactic acid in the form of 0.4% solution for group II, $CuSO_4$ in the amount of 30 mg $Cu \cdot dm^{-3}$ H_2O for group III, combined $CuSO_4$ (30 mg $Cu \cdot dm^{-3}$) and 0.4% solution of lactic acid for group IV and $CuSO_4$ at the level of 50 mg $Cu \cdot dm^{-3}$ for group V. The day after the first application of the experimental preparations, and on subsequent days, an increase was observed in the rate of mortality of turkeys in groups II and IV (Table 1). As

Table 1

Content of basic mineral components in full-dose feed mixes for turkeys (manufacturer's data)

Full-dose feed mixes	Ca	Mg	K	P	Zn	Fe	Cu	Mn
	($g\ kg^{-1}$)				($mg\ kg^{-1}$)			
I (weeks 0-3)	14.21	2.85	4.57	7.34	84.34	48.94	20.47	115.62
II (weeks 4-6)	15.37	3.21	3.94	8.06	87.42	52.14	24.82	119.26

a result, after 3 days the application of the preparations was discontinued and samples of drinking water were taken for chemical analyses. From each group, 10 birds were selected for slaughter, to take tissue samples (breast and leg muscles). Since then, From the turkeys were kept in their cages but they were no longer given the experimental preparations. In groups II and IV some young turkeys observed to have of poisoning (they had no appetite and tended to assume immobile positions with their wings lowered). These signs receded gradually, but were still observable in the 4th week of the birds' life. In the tissue samples taken from the breast and leg muscles of 3-week old turkeys, determinations were made of the content of Zn, Cu, Ca, Mg, Fe and K with the AAS method after prior wet mineralisation in a mixture of HNO_3 and $HClO_4$ (5:1) and dilution with distilled water. Also the samples of drinking water taken from the tanks of each experimental group were subjected to chemical analysis for the content of the same mineral components as those determined in the tissue samples, except mineralisation. The results were subjected to statistical analysis using Statistica version 5 software, with the ANOVA test of single-factor analysis of variance, adopting 0.01 and 0.05 levels of significance.

RESULTS AND DISCUSSION

Table 3 presents the content of mineral components in the drinking water given to the turkeys. The results indicate that in groups II and III the drinking water contained increased levels of Zn, Cu, Fe, Ca and Mg. The increased concentration of copper in the water was in agreement with the assumptions of the biological experiment and corresponded to the amount of copper added to the drinking water in the amounts of 30 mg in groups III and IV, and 50 mg in group V. In the water supplemented with lactic acid, increased concentrations of Zn, Fe, Ca and Mg were observed. Acidification of drinking water with strong lactic acid caused increased dissolution of zinc (group II – 550 $mg \cdot dm^{-3}$, and group IV – 682 $mg \cdot dm^{-3}$) and iron (group II – 24.73 $mg \cdot dm^{-3}$, and group IV – 20.09 $mg \cdot dm^{-3}$) from the water supply system which was made of zinc-coated iron alloy. Increase in the concentration of Cu, Zn and Fe in drinking water for turkeys, however, should not be life threatening, as these concentrations were still significantly below the levels assumed to be toxic, i.e. 500 mg kg^{-1} of feed for copper, 2230 mg kg^{-1} of feed for zinc, and 2000 mg kg^{-1} of feed for iron (KIDD et al. 1996). There was also a 4-fold increase in the level of Ca and 2-fold increase in that of Mg with relation to the mains drinking water. In the groups of turkeys that were given lactic acid, lack of appetite was observed immediately the day after the first application, which was followed by apathy and, finally, increased mortality (Table 2). After detailed examination of the dead birds, performed by a veterinarian, all causes of death other than poisoning were excluded. Zinc should be taken into consideration among factors that might have caused the poisonings, as its content in the drinking water was the closest to the toxic level.

Table 2

Deaths of turkeys during 5-week aviculture

Group	Number of deaths					
	week 1	week 2	week 3	week 4	week 5	total
I	5	2	1	0	0	8 (13.3%)
II	4	0	4	5	0	13 (27.7%)
III	18	2	0	0	0	20 (33.3%)
IV	12	1	23	5	0	41 (68.3%)
V	7	1	2	0	1	11 (18.3%)

Table 1 presents the mortality rate of turkeys during the 5-week aviculture. The highest incidence of bird deaths was recorded in the first week of their life. Immediately after transport the squealers were weighed, earmarked and assigned to the particular experimental groups. After three weeks, the application of the experimental preparations was begun, which resulted in an increase of mortality among turkeys in groups supplemented with lactic acid. The highest mortality rate was recorded in group IV (23 birds), where lactic acid was applied in combination with $CuSO_4$. However, the increased level of copper fed to the turkeys in that group probably had no influence on absorption of zinc in the form of lactate. Increased mortality was also observed in group II (8 birds), where 0.4% lactic acid was applied. The effects of poisoning were still observable in the 4th week of the birds' life, when 5 deaths each were recorded in groups II and IV.

Based on the mean daily feed consumption by turkey cocks, on the content of mineral components in full-dose feed mixes (Table 1) and on the concentration of these elements in drinking water (Table 3), it is possible to make a theoretical calculation of the daily intake of Zn and Cu by the turkeys. Thus, it was calculated that in the 3rd week of their life, the turkeys absorbed the following amounts: group I (control) 7.57 mg Zn and 1.91 mg Cu, group II: 91.59 mg Zn and 1.93 mg Cu, group III: 7.74 mg Zn and

Table 3

Content of mineral components in drinking water for turkeys

Sample	Mineral components ($mg \cdot dm^{-3}$)					
	Ca	Mg	K	Zn	Cu	Fe
Tap water	65.10	7.98	1.85	5.38	0.01	0.003
H_2O+lactic acid	269.10	12.30	1.22	550.00	0.15	24.73
$H_2O+CuSO_4$ (30 mg $Cu \cdot dm^{-3}$ H_2O)	57.30	6.54	1.63	6.72	32.25	0.005
$H_2O+CuSO_4$+lactic acid	274.80	11.80	4.20	682.00	34.50	2009
$H_2O+CuSO_4$ (50 mg $Cu \cdot dm^{-3}$ H_2O)	62.18	8.32	1.73	5.28	51.03	0.003

6.91 mg Cu, group IV: 111.9 mg Zn and 7.23 mg Cu, group V: 7.56 mg Zn and 9.78 mg Cu. On this basis, we can conclude that the dose of 111.9 mg Zn absorbed by group IV turkeys during one day was enough to be toxic as it caused increased rate of mortality (Table 2).

Table 4 presents the content of mineral components in the breast muscle of turkeys that received increased amounts of copper (30 and 50 mg $Cu \cdot dm^{-3}$ H_2O), zinc (550 and 682 mg $Zn \cdot dm^{-3}$ H_2O) as well as calcium and iron in their drinking water. The concentration of Ca in the breast muscle determined in all the experimental groups was similar except group V, where under the effect of $CuSO_4$ in the higher dose (50 mg $Cu \cdot dm^{-3}$ H_2O) there appeared a decrease in the concentration of this element, statistically significant versus the other groups of turkeys. The minerals (Zn, Cu, Ca and Fe) contained in drinking water had no significant effect on the content of Mg in the breast muscle. However, it should be noticed that the highest concentrations of Mg were observed in group III (30 mg $Cu \cdot dm^{-3}$ H_2O), and the lowest in the control group. The concentration of zinc, in turn, was similar in all the experimental groups, even though it was probably zinc that caused the poisoning of turkeys in groups II and V. Similar observations were made by Bou et al. (2005) in their study on chickens, which did not show any effect of increased doses of Zn in the diet on the content of this element in the muscles. However, as the Zn concentration in the diet increased significantly in the 3rd week of chickens' life, Sandoval et al. (1998) observed a linear relation between the concentration of Zn in muscles and its level in the feed. In none of the experimental groups, the mean zinc content in the breast muscle was over 20 mg Zn kg^{-1}.

In all the experimental groups, the concentration of copper was higher with relation to the control, and the differences obtained were confirmed statistically. It was observed that the level of copper accumulation in the breast muscle depended on its concentration in drinking water given to the turkeys (Table 3). The same dependence was observed by Makarski et al.

Table 4

Concentration of mineral components in breast muscle of turkeys

Group	Mineral components ($mg \cdot kg^{-1}$)				
	Ca	Mg	Zn	Cu	Fe
I	38.17^{a}	395.28	16.75	7.76^{a}	23.57
	±12.85	±42.16	±4.26	±1.22	±12.43
II	43.80^{a}	385.06	15.94	18.13^{b}	17.97
	±15.70	±99.48	±5.85	±7.80	±5.36
III	40.42^{a}	471.81	15.83	15.03^{b}	18.45
	±16.49	±109.83	±1.10	±7.80	±4.93
IV	36.69^{a}	439.56	15.45	18.55^{b}	27.53
	±13.23	±30.03	±2.44	±7.77	±17.66
V	24.08^{b}	460.55	14.22	27.84^{b}	17.48
	±0.40	±21.00	±0.58	±0.81	±2.20

a, b – differences significant at $p < 0.05$

2004, MAKARSKI, ZADURA 2006. Noteworthy is the result obtained for group II of turkeys receiving lactic acid, where increased accumulation of copper was observed in the breast muscle of the birds in spite of its absence in drinking water. In this group, the concentration of copper was 18.13 $mg \cdot kg^{-1}$ and was higher than in the control group (7.76 $mg \cdot kg^{-1}$), as well as in group III, where copper concentration in the breast muscle was 15.03 $mg \cdot kg^{-1}$. We can suppose that copper contained in feed, after its transformation into lactate, is more easily resorbed from the alimentary tract compared to other chemical forms of copper. In other studies (PANG, APPLEGATE 2007), antagonism between Cu and Zn was observed. In our study, it was demonstrated that the concentration of copper in the breast muscle of turkeys being administered additives of copper and lactic acid considerably exceeded the level of 10 mg Cu kg^{-1}. It was only in the control group that the concentration of copper remained at a low level.

The concentration of magnesium in the breast muscle of turkeys from all the experimental groups was higher than in the control group, but the differences were not statistically significant. In the case of iron concentrations, its highest value was observed in turkeys from group IV, and the lowest in birds from group V. The differences were not statistically significant.

Table 5 presents the content of mineral components in the leg muscle of turkeys. It was found that the highest concentration of Ca (134.48 $mg \cdot kg^{-1}$) in this muscle appeared in birds from group IV. In contrast, the maximum dose of copper applied in the experiment (50 mg $Cu \cdot dm^{-3}$ H_2O) caused

Table 5

Concentration of mineral components in leg muscle of turkeys

Group	Mineral components ($mg \cdot kg^{-1}$)				
	Ca	Mg	Zn	Cu	Fe
I	94.38	324.74^b	38.21^a	8.64^a	21.62
	±18.62	±17.26	±2.71	±1.53	±3.65
II	85.87	$351.27a^b$	22.70^b	11.14^a	24.80
	±12.77	±20.48	±6.17	±0.59	±6.83
III	93.02	371.97^a	37.54^a	10.92^a	23.74
	±44.30	±23.12	±4.00	±0.80	±0.85
IV	134.48	324.10^b	33.00^a	20.55^b	21.33
	±46.39	±87.48	±9.10	±16.12	±5.63
V	55.74	362.60^a	30.53^a	11.60^a	27.55
	±4.50	±17.46	±2.34	±0.87	±1.12

a, b – differences significant at $p < 0.05$

a decrease in the concentration of Ca from 94.38 $mg \cdot kg^{-1}$ (control) to the level of 55.74 $mg \cdot kg^{-1}$. The differences, however, were not confirmed statistically. Analogous variation in the concentration of Ca under the effect of the experimental factors was observed in the breast muscle of the turkeys (Table 4), where it was statistically confirmed. It should be noticed that the concentrations of Ca in the leg muscle were considerably higher than in the breast muscle. In the study by LEACH at al. (1990), a relationship was found between the intestinal absorption of copper and the content of calcium in the gastrointestinal tract, and it appears that the results obtained in our study can be attributed to such interaction.

Increased doses of Zn in groups II and IV had no effect on the concentration of Ca in the leg muscle. In the experiment, a significant increase in the concentration of Mg was observed in groups III and V (371.97 $mg \cdot kg^{-1}$ and 362.60 $mg \cdot kg^{-1}$, respectively) versus the control group (324.74 $mg \cdot kg^{-1}$). In these groups, the turkeys were administered a supplement of $CuSO_4$ in the amounts of 30 and 50 mg $Cu \cdot dm^{-3}$ H_2O. Under the effect of the experimental factors, a significant decrease in the concentration of zinc in the leg muscle appeared. These results are of particular significance to the consumers, as they indicate that increased doses of zinc and copper did not cause increased accumulation on Zn in the leg muscle of the turkeys. A decrease in the Zn concentration under the effect of increased doses of Cu in feed appears to support antagonism between these two elements observed in numerous studies (NOY et al. 1994, WEDEKIND et al. 1992, SANDOVAL et al. 1998, PANG, APPLEGATE 2007). Similarly to the effects observed on the breast muscle, all the experimental factors caused an increase in the concentration of copper in the leg muscle. A particularly high concentration of copper was observed in group IV under the effect of $CuSO_4$ and lactic acid, which would indicate that the lactic acid supplement enhanced Cu resorption from the gastrointestinal tract of turkeys. It also appears that the phenomenon of increased Cu resorption may be strongly affected by the content of Ca in drinking water, which was confirmed by LEACH at al. (1990). The concentration of Cu in the leg muscle in particular groups of turkeys, except the control, exceeded the level of 10 mg Cu kg^{-1}, which may constitute a hazard to consumers. With respect to Fe, the experimental factors did not cause any significant changes in the concentration of this element in the leg muscle of the turkeys.

CONCLUSIONS

1. The highest incidence of deaths of turkeys occurred in groups administered 0.4% lactic acid and when the administration of lactic acid was combined with copper supplementation.

2. The study showed that a daily intake of zinc in the amount of 111.9 mg was toxic to young turkeys.

3. The concentration of Zn in the breast and leg muscles of turkeys from all the experimental groups was at a constant level, while the concentration of Cu increased considerably in groups administered copper and lactic acid supplements.

4. Lactic acid added to drinking water for turkeys, due to its chemical properties, may constitute a hazard to birds' health, especially when water supply pipes are made of metal.

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