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THE EFFECT OF COPPER AND ZINC CONCENTRATIONS IN FEED AND WATER ON THEIR DISTRIBUTION IN BEEF CATTLE TISSUES**

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Abstract: An investigation of the effect of copper (Cu) and zinc (Zn) concentrations on their distribution in beef cattle tissues was conducted using feed trial with the following four nutrition treatments applied. At the end of the trial, samples of the muscular tissue, liver and kidneys of slaughtered cattle were taken from 10 head of cattle of the I group, 30 head of the II and III groups and 15 head of the IV group.

The analyses were made by using the spectroscopic AAS method. Investigation results showed that all groups of cattle were given water with Cu and Zn contents below maximum permissible concentrations (MPC).

The content of Cu and Zn in the tissues of the beef cattle in our experiment directly depended on their content in the concentrated feed. Copper is deposited in the liver the most, and Zn in the muscular tissue (excepting the II group of cattle). Correlation dependence of Cu and Zn content in the tissues from content in DM is complete (total), except in the case of Zn content in kidney, where is above mentioned correlation very strong (0.795).

Key words: AAS, copper, zinc, beef cattle muscular tissue, kidney, liver,

Introduction

Copper and zinc belong to a group of micro elements which are very important for animal physiology. Copper is an integral part of the “a“ cytochrome. It is also present in blood plasma as ceruloplasmin, a copper-protein complex (*Holmerg C.G. and Laurell, C.B.* 1948). Copper is required for absorption in the cell, heart function, bone formation, connective tissue development, as well as for myelinization of the spinal cord (*McDowell,L.R.* 1992).

Baker and Ammermman (1995) showed that in beef cattle Cu is significantly better absorbed if supplemented in the form of aminoacid chelates. In his review, *O'Dell* (1984) also noted the potential for carbohydrate source to affect copper absorption. This is attributed to phytate as well as oxalate concentrations in the diet.

Zinc plays an important role in the enzyme system, in the synthesis of the ribonucleic acid, which points to its role in the development of germ and somatic cells. Zn easily binds to insulin. According to *Wedekind et al.* (1994), a higher calcium level in a ration contributes to better zinc utilizaiton, if it occurs as zinc-methionine in the feed. *Hahn and Baker* (1993) suggest that relative bioavailability for Zn-oxide is only 55% compared to 110-116% for Zn-methionine, when Zn-sulfate availability is 100%. *H. Hakan Aydin et al*. (2001) may be stated that the mechanisms for cellular uptake and accumulation related to these elements are different in hepatic and renal tissues. Metallothionein, a low molecular weight protein with a high cysteine content and a high affinity for Zn, Cd and Cu, is suggested to play an important role in the concentration of these elements in the liver and kidney.

There are many factors that could affect an animal's response to trace mineral supplementation such as the duration and concentration of trace mineral supplementation, physiological status of an animal, the absence or presence of dietary antagonists, environmental factors, and the influence of stress on trace mineral metabolism (*Baker et al*. 2000). Five areas deserve attention when discussing potential factors that may affect the trace mineral requirements of ruminants: breed, gestational status, stress, trace mineral antagonists, and age. High levels of dietary zinc and iron depress copper absorption and tend to increase the requirements - 100 ppm Zn reduce liver copper storage.

Nockels et al. (1993) reported that copper and zinc retention was decreased in steers injected with ACTH (a stressor), in conjunction with feed and water restriction. Stress in the form of an infection (IBRV), a metabolic disorder (ketosis), or deprivation of feed and (or) water can increase copper and zinc depletion from the animal. Researchers in Northern Europe described this wasting disease like deficient in cobalt, iron, and copper - animals having diarrhea, loss of appetite, and anemia. Cattle can die from copper poisoning - nausea, vomiting, salivation, abdominal pain, convulsions, paralysis. The usual cause is improperly formulated supplements or diets (*McDowell, L.R.*. 1992).

The highest concentrations of zinc were found in the following order: pancreas, liver, pituitary gland, kidney, and adrenal gland (*McDowell, L.R.* 1992). Taking into consideration the aforementioned data and the fact that currently, and in the future, beef is and will be increasingly used in human nutrition, we considered it important to determine the way of distribution of different Cu and Zn amounts intaken by feed and water within the tissues of beef cattle.

Material and methods

Investigations of the effect of Cu and Zn concentrations in feed and water on their distribution in beef cattle tissues (muscular tissue, liver and kidney) were performed using 4 groups of beef cattle in 4 facilities in the region of Kraljevo, applying the following treatments:

- I group of beef cattle was fed meadow hay and ground maize, without Cu and Zn supplements (the control);
- II group of beef cattle was fed meadow hay, ground maize (whole plant with corncob) and complete feed (CF) with 5-10 mg/kg Cu and 20-50 mg/kg Zn;
- III group of beef cattle was fed meadow hay, ground maize (whole plant) and CF with 20-25 mg/kg Cu and 80-100 mg/kg Zn;
- IV group of beef cattle was fed meadow hay and CF with 70-80 mg/kg Cu and 100-120 mg/kg Zn.

All groups of beef cattle were given water ad libitum from troughs connected to the local water supply system. The beef cattle were housed in boxes on a slatted floor, free (loose). I group of beef cattle was fed ground maize as concentrated feed in the amount of 5.5 kg daily, supplemented with 4 kg of meadow hay. Each of the other groups of cattle were fed 5.5 kg CF with adequate Cu and Zn amounts and 2 kg of meadow hay, and in the winter period, the II and III group of beef cattle were also fed 4.5 kg of silage daily. The beef cattle from the I and IV group were estimated to have drunk 38 l of water each, and those of the II and III groups 36 l each. The feeding of all groups of beef cattle was performed twice (in the morning and in the evening).

The average body weight of all trial cattle was 300-350 kg at the beginning of the trial. The average body weight of cattle at slaughter was 428 kg, 533 kg, 539 kg and 520 kg for the I, II, III and IV group of cattle, respectively. At each change of concentrated and forage feed (approximately once a month) water and feed sampling was performed and Cu and Zn content was determined.

Water samples were prepared for the analysis in such a way that a sample of 500 ml water was acidified by adding concentrated nitric acid (about 2% concentration). The water was then vaporized, the dry residue was dissolved in several ml of nitrohydrochloric acid and the solution was transferred into a 50 ml measuring vessel through a quantitative filter paper. CF samples were first homogenized by grinding in a "Cyclotec" 1092 Tecator grinding mill. The powdered sample thus obtained was measured to about 2-3 g (± 0.0001 g) sample, burned on a hot-plate and heated in a torrefying furnace at a temperature of 550 ^{0}C. The ash obtained was dissolved in HCl (1:1) and quantitatively transferrred by redistilled water into 50 ml containers. Ground maize and hay were prepared in the same way. 7 samples of water, hay, ground maize, silage and CF for each group of beef cattle were treated.

The muscular tissue, liver and kidneys were sampled at the slaughtering line. The muscular tissue was taken from the neck (from trapezoidal muscles, without suet). The samples were first homogenized using a meat mixer and then measured to 5-10 g (± 0.0001) test samples and treated as in the case of CF.

In all samples investigated the content of Cu and Zn was determined by using the AAS method (Perkin Elmer, model 3300/96), *AOAC* (1990), and statistically worked out.

Results and discussion

The drinking water must satisfy criteria prescribed by the *Regulations on the Hygienic Drinking Water Safety* (the Official Gazette of the FRY 42/98). Under normal conditions, the water should contain 2.0 mg/l Cu and 3.0 mg/l Zn. The beef cattle of all trial groups were supplied with water containing concentrations of the elements investigated which were lower than permitted by the Regulations.

The concentrated feed for the I (control) group was ground maize, so these results show the Cu and Zn content in the maize from the region of Kraljevo. An average Cu and Zn content in the ground maize – concentrated feed for the I group was 7.86 mg/kg, and 24.01 mg/kg, respectively (Table 1. and 2.). Previous results served as a basis for determining the Cu and Zn amounts to be supplemented to ground maize when preparing CF for trial groups. The second, third and fourth trial group of beef cattle gained 7.53 mg/kg, 21.30 and 75.51 mg/kg on an average, respectively. An average Zn amount supplied to the II, III and IV group of cattle through feed was 40.34 mg/kg, 88.78 mg/kg and 115.61 mg/kg, respectively. Previous investigations of Cu and Zn contents in CF for beef cattle showed that the content of Cu was 12.35 mg/kg and that of Zn – 86.22 mg/kg (*Vukasinović et al,* 1997).

The current *Regulations on the Quality and Other Animal Feed Requirement*s (the Official Gazette of the FRY 20/2000 and 38/2001), prescribe the lowest Cu and Zn amounts being 5.0 mg/kg and 20.0 mg/kg, respectively.

Table 1. Cu content in concentrated feed of all trial groups of beef cattle, mg/kg

	Experimental group			
	I	II	III	IV
Xsr	7.86	7.53	21.30	75.51
Sd	0.85	1.50	2.96	3.88
Cv	10.75	19.90	13.88	5.14
Iv	6.23-9.01	5.04-8.80	17.46-24.90	70.39-81.52

Table 2. Zn content in concentrated feed of all trial groups of beef cattle, mg/kg

	Experimental group			
	I	II	III	IV
Xsr	24.01	40.34	88.87	115.61
Sd	5.96	11.63	24.22	7.98
Cv	24.84	28.82	27.25	6.90
Iv	14.71-27.98	27.86-56.79	71.51-140.15	99.48-123.25

Average values of Cu and Zn content in the examined samples of the meadow hay consumed by all groups of beef cattle were uniform, ranging from 6.75-9.01 mg/kg and from 12.55-16.85 mg/kg, respectively. The content of dry matter (DM) was determined for all feeds, and the daily intake of Cu and Zn in diet DM was calculated according to their amounts in the feeds and their content in the water. The control and II group of beef cattle were recorded to have a similar Cu intake during the day (69.623 and 82.668

mg/kg), whereas the III and IV group were supplied with considerably higher Cu amounts (183.286 and 472.394 mg/kg).

Daily Zn intake ranged from 192.414 mg/kg as recorded for the control to 719.847 mg/kg as registered for the IV group.

Table 3. Average content of Cu determined in the muscular tissue of the trial cattle, mg/kg

	Experimental group			
	I	II	III	IV
$\overline{x}$	3.08	3.17	4.86	7.84
Sd	0.62	0.49	1.67	5.46
Cv	20.24	15.56	34.37	69.76
Iv	1.99-4.00	2.25-3.94	3.59-12.98	2.64-16.90

Table 4. Average content of Zn determined in the muscular tissue of the trial cattle, mg/kg

	Experimental group			
	I	II	III	IV
$\overline{x}$	22.77	40.92	47.67	93.19
Sd	18.45	11.03	4.49	40.10
Cv	81.00	26.95	19.92	43.03
Iv	8.99-70.46	24.47-62.43	25.58-64.66	24.17-160.11

The Cu amounts determined in the muscular tissue of the beef cattle of the I and that of the II trial group were very close. The content of Cu and Zn in the muscular tissue of the II trial group of cattle was by only 2.92% and 79.95%, respectively, higher than that of the I group (Table 3. and 4.). The content of Cu in the muscular tissue of the cattle of the III trial group was higher by 57.79% and the content of Zn by 109.35% compared to the content in the muscular tissue of the cattle in the I, control group. The muscular tissue of the IV group of trial cattle contained 143.86% more Cu and 310.53% more Zn compared to the content in the muscular tissue of the I group of cattle. As the contents of Cu and Zn in water, hay and silage were similar in all trial groups and significantly different in concentrated feed.

This is not a simple correlation, because differences in the Cu content in the concentrated feed as compared to the control were as follows: 4.20% lower, 170.99% higher and 860.99% higher Cu amounts were recorded in the II, III and IV group of cattle, respectively. Our study confirmed that copper intake by feed and water was accumulated in the liver of beef cattle and in case of copper-deficient nutrition, it was withdrawn from the depot being distributed to all tissues and circulation.

Table 5. Contents of Cu in the liver of the experimental feed cattle, mg/kg

	Experimental group			
	I	II	III	IV
$\overline{x}$	22.28	62.15	81.21	120.00
Sd	12.34	42.54	34.32	52.89
Cv	55.40	68.44	42.27	44.08
Iv	12.26-49.71	14.59-189.15	42.16-175.80	36.81-200.31

Table 6. Contents of Zn in the liver of the experimental feed cattle, mg/kg

	Experimental group			
	I	II	III	IV
$\overline{x}$	30.72	31.63	53.13	106.79
Sd	6.62	43.39	10.63	60.91
Cv	21.79	143.51	20.01	57.04
Iv	20.77-35.84	12.37-34.63	35.20-80.27	38.49-232.88

Table 7. Contents of Cu in the kidneys of the experimental feed cattle, mg/kg

	Experimental group			
	I	II	III	IV
$\overline{x}$	5.73	6.61	6.66	14.46
Sd	0.91	1.33	0.77	11.39
Cv	15.73	20.12	11.55	78.71
Iv	4.78-7.67	3.45-10.12	4.70-7.99	4.41-37.60

Table 8. Contents of Zn in the kidneys of the experimental feed cattle, mg/kg

	Experimental group			
	I	II	III	IV
$\overline{x}$	13.28	14.92	15.72	57.10
Sd	4.62	1.68	1.51	22.06
Cv	30.21	11.26	9.62	38.63
Iv	10.52-15.62	10.59-17.98	13.04-19.84	25.14-122.00

Table 9. Analysis of variance

Cu and Zn in tissues	Sum of square ss	df	Means of square	SS mistake	df mistake	MS mistake	F
Musc. tissue - Cu	243.60	3	81.20	509.00	81	6.28	12.92**
Liver - Cu	64245.15	3	21415.05	127128.70	81	1569.49	13.64**
Kidney - Cu	787.64	3	262.55	1890.50	81	23.339	11.25**
Musc. Tissue - Zn	38020.37	3	12673.46	31981.10	81	394.83	32.10**
Liver - Zn	73620.11	3	24540.04	56750.50	81	700.62	35.03**
Kidney - Zn	21903.16	3	7301.05	7155.20	81	88.34	82.65**

Table values: $F_{0.05} = 2.74$ and $F_{0.01}=4.08$

Table 10. Significance of differences in Cu content in muscular tissue of beef cattle between experimental groups (T-test)

Cu	$t_{I, II}$	$t_{I, III}$	$t_{I, IV}$	$t_{II, III}$	$t_{II, IV}$	$t_{III, IV}$
Musc. tissue	0.098^{ns}	1.25^{ns}	4.65*	2.57^{ns}	5.89**	3.80*
Liver	2.76^{ns}	4.07*	6.04**	1.86^{ns}	4.62*	3.10*
Kidney	0.48^{ns}	0.50^{ns}	4.41*	0.04^{ns}	5.14**	5.11**

Table 11. Significance of differences in Zn content in muscular tissue of beef cattle between experimental groups (T-test)

Zn	$t_{I, II}$	$t_{I, III}$	$t_{I, IV}$	$t_{II, III}$	$t_{II, IV}$	$t_{III, IV}$
Musc. tissue	2.50^{ns}	3.43*	8.67**	1.32^{ns}	8.32**	7.25**
Liver	0.16^{ns}	2.38^{ns}	7.09**	3.14*	8.98**	6.41**
Kidney	0.48^{ns}	0.71^{ns}	11.42**	0.33^{ns}	10.99**	13.92**

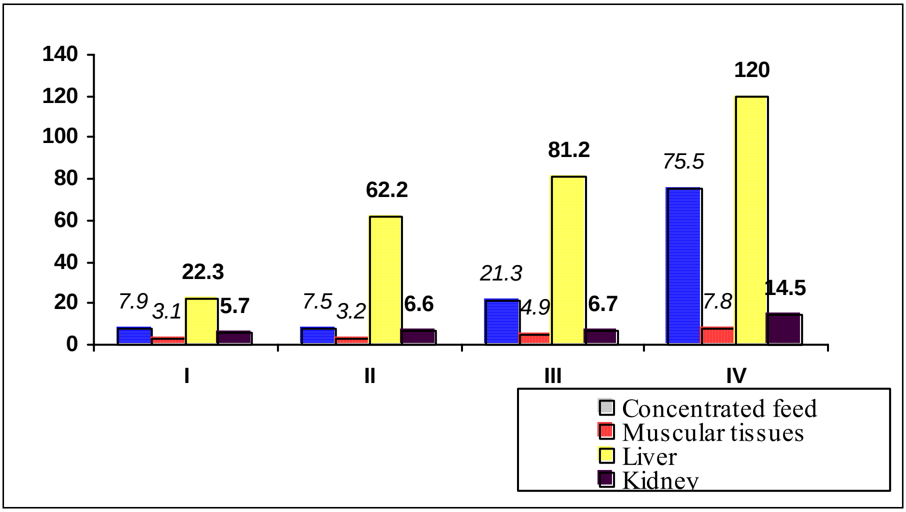
Table 12. Correlation dependence of Cu and Zn content in the tissues from content in DM

Tissues	Coefficient of correlation	
	Cu	Zn
Muscular tissue	0.995	0.916
Liver	0.925	0.913
Kidney	0.975	0.795

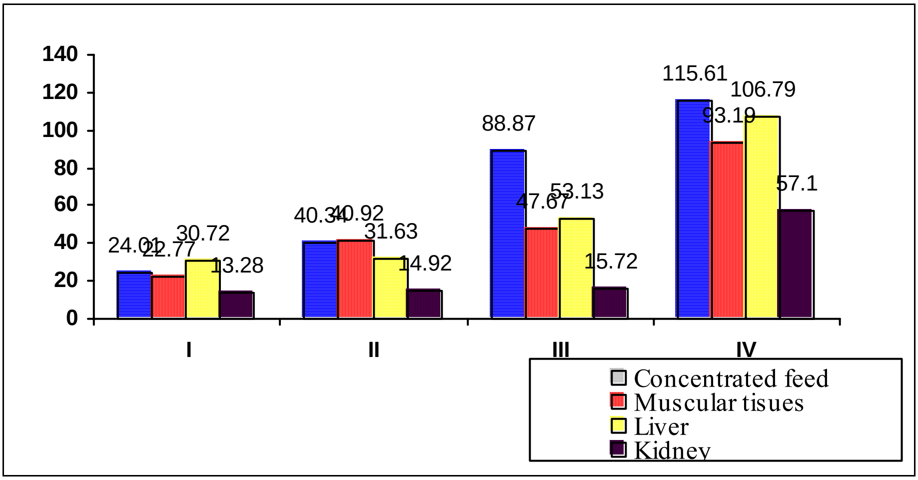
The F test was used to examine differences in an average content of Cu and Zn in the muscular tissue, liver and kidneys between the groups (Table 9). In all cases was determined very significant diferences. In order to determine differences between the groups, individual testing between the groups was done by T-test (Table 10. and 11.): between I and II, I and III, I and IV, II and III, II and IV and III and IV groups. Differences in Cu content in muscular tissue was significant between I and IV and III and IV group, between I and IV it was very significant. Examination of differences in Cu contents in liver (Table 5. and 6.) by T test confirmed statistically significant differences between I and III, II and IV and III and IV group, between I and IV very significant differences. Differences in Cu content in kidney of experimental beef cattle (Table 7. and. 8) between I and IV, II and IV and III and IV group were statistically significant (Graph 1). In the case of content of Zn in muscular tissue differences was statistically significant between I and III group, while between I and IV, II and IV and III and IV groups were very significant. Examination of differences of Zn content in liver confirmed that there were statistically significant differences between II and III group, very significant between I and IV, II and IV and III and IV group. Differences in Zn content in kidney of experimental beef cattle between I and IV group were statistically significant, but very significant between II and IV and III and IV experimental groups (Graph 2).

Correlation dependence of Cu and Zn content in the tissues from content in DM was presented in table 12: correlation dependence of Cu and Zn content in the tissues from content in DM is complete, except in the case of Zn content in kidney, where is above mentioned correlation very strong (0.795).

The values for the Cu content obtained in our investigations were considerably lower than those obtained by *Šević et al* (1989) in their research of trace elements sampled from 8 sites in the region of Kraljevo. The Cu content in hay, according to the mentioned authors, ranged from 13.34 – 20.20 mg/kg. Content of Cu in hay in the region of Požega varied from 10.54 – 14.47 mg/kg. Lower values of Cu content (5.88 mg/kg) were recorded in their investigations of Cu content in 17 meadow hay samples, and the Zn content determined was similar to our results (23.36 mg/kg DM of hay).



Graph 1: Content of Cu in concentrated feed and tissues of experimental feed cattle, mg/kg



Graph 2: Content of Zn in concentrated feed and tissues of experimental feed cattle, mg/kg

The values of Cu content in the muscular tissue of the trial cattle in our investigation were considerably higher than those determined by *Rogowski et al* (1981) - 100 mg/100g and *Radović et al.* (1986) - 0.79-1.74 mg/kg.

Rogowski et al. (1981) showed that the muscular tissue of cattle was rich in zinc and contained 4.2 mg/100 g, which was at the concentration level for the trial cattle of the II trial group in our investigations and considerably above the amount determined in the muscular tissue of cattle from the control group (22.77 mg/kg).

Falandysz J. et al., 1989 was determined the content of copper and zinc, in muscle tissue of 59, liver of 55 and in the kidneys of 56 cattle, slaughtered in the northern region of Poland, in 1985. The mean values obtained related to wet weight (mg/kg) for the muscle tissue of cattle were: 0.98 **Cu**; 23 **Zn**. In the liver of cattle the content was: 15 Cu and 35 Zn.

According to previous research (*Costa Nisk*, 1996), Zn is relatively non-toxic to animals and has a depressing effect if the amount intaken exceeds 700 mg/kg DM and it depends on Cu concentrations. High Cu amount in the ration, according to these authors, increases its level in the blood, liver, kidneys and bones, and particularly in the spleen, hair and lungs. This Zn accumulation in cattle spleen was not confirmed in our previous paper, because 87.05 mg/kg of Zn was determined in the liver and only 21.73 mg/kg in the spleen. (*Vukašinović and Rajić*, 1989).

Content of Cu and Zn in the liver and kidney in the I and II group of cattle are in accordance with the results of authors (*Radović et al.,* 1986) and (*Radović and Saičević*, 1990), who reported that Cu and Zn content in the cattle liver ranged from 19.54-50.64 mg/kg and from 14.82-37.30 mg/kg, and Zn content in cattle kidney to range from 8.82-17.94 mg/kg. Our results on the content of Cu and Zn in the liver in the III and IV groups and kidney of the IV group of cattle were far higher, which was the result of increased intake of these elements through concentrated feed. *Van Ulsen* (1971) indicate that liver and kidneys of warmblooded animals normally contain 80.00-135.00 mg/kg Zn.

Conclusion

The content of Cu and Zn in the tissues of the beef cattle in our experiment directly depended on their content in the concentrated feed. Copper is deposited in the liver the most, and Zn in the muscular tissue (excepting the II group of cattle). Correlation dependence of Cu and Zn content in the tissues from content in DM is complete (total), except in the case of Zn content in kidney, where is above mentioned correlation very strong (0.795).

UTICAJ KONCENTRACIJE BAKRA I CINKA U HRANI I VODI NA NJIHOVU ZASTUPLJENOST U TKIVIMA JUNADI U TOVU

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Rezime

Ispitivanje uticaja koncentracije bakra (Cu) i cinka (Zn) na njihovu zastupljenost u tkivima junadi u tovu obavljeno je primenom hranidbenog ogleda u kome su primenjena 4 tretmana ishrane. Na kraju ispitivanja, uzeti su uzorci mišićnog tkiva, jetre i bubrega od zaklanih životinja I grupe (10 grla), 30 iz II i III grupe i 15 iz IV grupe. Analize su obavljene primenom spektroskopske AAS metode.

Rezultati ispitivanja pokazali su da su sve grupe junadi dobijala vodu koja je sadržavala Cu i Zn ispod maksimalno dozvoljene količine (MDK).

Sadržaj Cu i Zn u tkivima tovne junadi u našem eksperimentu direktno zavisi od njihovog sadržaja u koncentrovanoj hrani. Bakar se uglavnom deponuje u jetri, a zink u mišićnom tkivu (izuzev životinja u II oglednoj grupi).

Utvrdili smo da je korelaciona zavisnost sadržaja Cu i Zn u mišićnom tkivu, jetri i bubregu od sadržaja u SM potpuna, osim u slučaju sadržaja Zn u bubregu, gde je zavisnost vrlo jaka (0.795).

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