Copper metabolism in goat–kid relationship at supplementation of inorganic and organic forms of copper

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ABSTRACT: The aim of the experiment was to compare the effect of inorganic and organic forms of copper (Cu) supplementation on Cu status of goats and their kids, colostrum and milk composition and quality, and on the Cu concentrations in amniotic fluids and fetal membranes. The experiment involved 22 clinically healthy pregnant goats with similar mean Cu concentration in blood serum. Goats were divided into 3 groups: E1, E2, and C. Basal feed ration differed only in Cu form and concentration in a grain mixture. The goats of experimental groups E1 and E2 received the supplement of Cu sulfate and Cu chelate, respectively. Control group C was without Cu supplementation. Blood samples from goats and kids were collected on the day of parturition (day 0; in kids before colostrum intake) and on days 2, 7, and 21 postpartum. On the same days the kids were weighed. Colostrum or milk samples were collected on days 0, 5, and 30. During delivery, also samples of amniotic fluids and fetal membranes were collected. Both forms of Cu supplementation resulted in higher average concentration of Cu (compared to control group) in blood serum of goats (19.5 ± 1.7 and 18.5 ± 2.5 vs. 15.2 ± 4.4 μmol/l, respectively) and blood serum of kids (6.7 ± 0.8 and 6.0 ± 0.5 vs. 6.0 ± 1.0 μmol/l, respectively). Significant differences in Cu serum concentration on the day of parturition in goats were observed in group E1 compared to control ($P < 0.05$) and also in kids of group E1 compared to group E2 and control ($P < 0.05$). The Cu concentration in the blood of kids on the day of parturition was significantly ($P < 0.01$) lower compared to that in maternal blood. Percentages of Cu concentration in the blood of kids in groups E1, E2, and C were 34, 33, and 39% of that in maternal blood. The results of Cu concentration in blood serum of goats on days 2, 7, and 21 were without significant differences between groups. Significantly higher Cu serum concentrations ($P < 0.05$) were observed in kids on day 2 in group E1 compared to control group and also on day 21 in group E1 compared to group E2. Colostrum Cu concentration was significantly ($P < 0.05$) higher in group E1 (10.6 ± 3.3 μmol/l) compared to group E2 (7.1 ± 1.5 μmol/l). There were no significant differences observed in Cu concentration in amniotic fluids and fetal membranes. The kids on both forms of Cu supplementation (on day 0 in group E1 and on days 2, 7, and 21 in group E2) had significantly ($P < 0.05$) higher average weight than the kids from control group. Our results are suggesting that the inorganic form of Cu (copper sulfate) is more efficient than organic (copper chelate) in influencing the Cu metabolism in goat–kid relationship and that Cu supplementing has a positive effect on the weight of kids.

Keywords: trace elements; organic copper; inorganic copper; ruminants; colostrum; milk; fetal membranes; amniotic fluid; weight gains

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INTRODUCTION

The importance of trace elements for physiological function is well known. Since the 1970s, it has been known that the supplementation of trace elements with parenteral nutrition is required in order to avoid the clinical manifestations of their deficiencies (Wong, 2012). Copper (Cu) is an essential component of several enzymes called cupro-enzymes (Cummins et al., 2008), and participates in the hemoglobin synthesis. The positive effect of Cu supplementation on weight gains and immunity in kids was described by Solaiman et al. (2007). Copper also has an important role in fat metabolism (Engle et al., 2000; Datta et al., 2007). The essential importance of Cu for the quality of hair and skin is also well known. Sheep affected by Cu deficiency produced low-quality wool fibres, as described by Zhang et al. (2008). As a consequence of Cu deficiency the depigmentation is also described (Zhang et al., 2009). The positive impact of some Cu forms on reducing the incidence of gastrointestinal nematodes was mentioned (Burke et al., 2010; Miller et al., 2011). Authors Ozkul et al. (2012) describe relationship between significantly decreased serum Cu levels and the swayback disease in goats. Copper deficiency in goats is also associated with osteoporosis, dwarfism, and anemia (Buck et al., 2012). Most studies related to Cu supplementation in ruminants mention positive effect of Cu on production rate (Ward and Spears, 1997; Engle and Spears, 2000) or digestion (Essig et al., 1972; Saxena and Ranjhan, 1978; Zhang et al., 2009), but the results are not identical.

Literary sources indicate the transfer of Cu through the placenta to the bovine fetus; however, serum Cu concentrations in neonatal calves are significantly lower compared to those in their mothers (Pavlata et al., 2004). It does not mean that the similar situation in trace element metabolism exists in goats, because there are significant differences in the trace elements metabolism among the cattle and goats (Misurova et al., 2009; Pavlata et al., 2012).

Views on the availability of Cu to the body when administered in various forms and concentrations are not identical (Spears, 1996; Cheng et al., 2008). In the study of Datta et al. (2007) in kids fed inorganic (Cu sulfate) and organic (Cu proteinate) form of Cu it was observed that body weight increased linearly with increased dose of Cu. This study revealed better digestibility of Cu proteinate and the kids showed greater gains in body weight when compared to kids fed Cu in inorganic form. There are many studies (e.g. Du et al., 1996; Datta et al., 2007) stating that chelate compounds of amino acids and peptides can increase the absorption of trace minerals, which has an enhancing effect on the growth and overall health.

The objective of the present paper was to describe the Cu metabolism in mother–kid relationship in goats, evaluation of the effects of organic and inorganic Cu supplementation on the Cu status of the mother and goat organisms, and evaluation of its influence on Cu concentration in blood serum, colostrum, milk, fetal membranes and fluids, and on weight gains of kids.

MATERIAL AND METHODS

The experiment was conducted on 22 clinically healthy pregnant goats of the White Short-haired breed at the Ruminant Clinic, Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences Brno. The experiment started 2 months before the expected parturition. The goats were divided into 3 groups: E1 (n = 8), E2 (n = 6), and C (n = 8) with average Cu concentration in blood serum 16.6–16.9 µmol/l. The individual groups of animals were kept in shared boxes with straw litter. The basal feed ration of the groups differed only in the form of Cu supplemented in a granulated grain mixture. The amount of the feed ration and the nutrient composition corresponded with category and stage of reproduction of the experimental animals. The goats were in the same stage of pregnancy and lactation during the experiment. They were fed twice a day a supplementary diet (granulated grain mixture) at a rate of 300 g per animal and day and they had *ad libitum* access to hay, drinking water, and to salt lick (NaCl). Goats after parturition were fed an additional 0.15–0.5 kg of oat. The supplementary diet composition was as follows: barley 30%, wheat 20%, lucerne meal 18%, unpeeled sunflower extracted meal 10%, wheat bran 10%, corn 5%, malt sprouts 5%, dicalcium phosphate 1.1%, sodium chloride 0.7%, and calcium carbonate 0.2%. The basic composition was identical in all groups while the contents of supplemented Cu differed between the individual trial groups.

The goats of experimental groups E1 and E2 received a supplement of 30 mg Cu per kg of dry matter of grain mixture in the form of Cu sulfate ($CuSO_4$) – group E1 and Cu chelate (Bioplex Cu (10% copper); Alltech, Lexington, USA) – group E2, respectively. Bioplex are minerals linked to a mixture of mono-, di-, and tripeptide to yield 10% organic mineral chelates. Control group (C) was without Cu supplement (basal concentration of Cu in grain mixture was 10 mg/kg dry matter). The appropriate amount of supplementary diet (*n*-times 150 g per one feeding) was added to the individual groups into the common feeders that were of the size that enabled all animals to eat at the same time.

Blood samples from forty-one goats and kids in total (group C n = 16, group E1 n = 16, group E2 n = 9) were collected on the day of parturition (day 0; in kids before colostrum intake) and on days 2, 7, and 21 postpartum (pp) from *vena jugularis*. The kids were individually weighed on the same days using digital weighing scale. The colostrum and milk samples were collected on days 0, 5, and 30 pp by hand milking. During delivery, samples of amniotic and allantoic fluid (puncture and aspiration) and fetal membranes (cutting after a release) were also collected.

Samples of biological materials for determination of Cu concentration were frozen and analyzed subsequently (Cu in blood serum was determined directly by flame AAS on Atomic Adsorption Spectrophotometer; colostrum, milk, fetal membranes, and fluids Cu concentrations were determined by flame AAS after microwave digestion of samples (Pechová et al., 2005)). The specific gravity of colostrum was detected by colostrometer, composition of colostrum/milk (protein content, lactose, fat, and dry matter) were analyzed according to FT-NIR method (Navrátilová et al., 2006) from fresh samples.

The data were statistically analyzed by the F-test to evaluate the variance of the individual sets and according to the results Student's t-test was used for sets with equal/non equal variances for results between experimental groups. MS Excel 2010 software was used for the evaluations.

RESULTS

Both forms of Cu supplementation (groups E1 and E2) resulted in most cases in higher average concentration of Cu compared to control group C (Table 1). In the control group of goats the mean blood serum Cu concentration of 15.2 ± 4.4 μmol/l was observed on the day of parturition. In both experimental groups, Cu concentrations were higher, but only in group E1 it was significant (P < 0.05). When evaluating the results of specified concentrations of Cu in serum of goats in the next sampling (on days 2, 7, and 21), there is still a tendency towards higher concentrations of Cu in goats of both experimental groups compared to control group but the differences are not significant. The results for kids from the same sampling days showed significantly higher concentration of Cu on day 2 in group E1 (compared to control) and also on day 21 in group E1 compared to E2 group. In kids of C group (n = 16), average Cu concentration in blood serum on the day of birth was 6.0 ± 1.0 μmol/l, while kids of mothers supplemented with $CuSO_4$ (E1, n = 16) showed Cu level of 6.7 ± 0.8 μmol/l and Cu concentration in kids of mothers receiving Cu-chelate (E2, n = 9) was 6.0 ± 0.5 μmol/l. Significant (P < 0.05) difference was found between C and E1 groups and between E1 and E2 groups (Table 1).

Table 1. Copper concentrations in blood serum (mean ± standard deviation) of goats and kids on days 0, 2, 7, and 21 postpartum

Day	Group	n		Cu concentrations (μmol/l)	
		goats	kids	goats	kids
0	C	8	16	15.2 ± 4.4^a	6.0 ± 1.0^a
	E1	8	16	19.5 ± 1.7^b	6.7 ± 0.8^b
	E2	6	9	18.5 ± 2.5	6.0 ± 0.5^a
2	C	8	16	16.9 ± 4.9	7.9 ± 1.1^a
	E1	8	16	20.7 ± 1.6	9.3 ± 1.2^b
	E2	6	9	19.2 ± 2.5	9.0 ± 1.4^b
7	C	8	16	15.6 ± 6.5	14.3 ± 2.4
	E1	8	16	20.5 ± 3.9	15.2 ± 2.4
	E2	6	9	21.2 ± 2.5	16.4 ± 2.8
21	C	8	16	16.5 ± 4.6	12.4 ± 1.0
	E1	8	16	20.2 ± 2.5	13.1 ± 1.3^a
	E2	6	9	20.0 ± 1.6	11.5 ± 1.5^b

C = control group, E1 = goats supplemented with Cu sulfate, E2 = goats supplemented with Cu chelate

$^{a:b}P$ < 0.05 comparison of results among the individual groups (mothers and their kids) in individual days

When comparing the mean serum concentration of Cu in the blood of kids and their mothers on the day of birth, it is obvious that the concentration of Cu in blood serum in kids was significantly (P < 0.01) lower than in their mothers and reached the follow-

Table 2. Colostrum composition (mean ± standard deviation) in experimental groups of goats

Day	Group	Cu (μmol/l)	Protein (%)	Lactose (%)	Dry matter (%)	Fat (%)	Colostrum density (kg/m^3)
	C	8.3 ± 4.8	12.0 ± 2.5	3.9 ± 0.6	23.9 ± 4.0	6.6 ± 2.1	1049.9 ± 6.2
0	E1	10.6 ± 3.3^a	13.8 ± 3.9	3.4 ± 0.9	25.9 ± 4.5	6.6 ± 1.6	1054.0 ± 10.4
	E2	7.1 ± 1.5^b	10.2 ± 2.4	4.1 ± 0.5	21.8 ± 3.3	5.8 ± 1.3	1044.2 ± 4.2
	C	6.8 ± 2.8	3.1 ± 0.3	4.7 ± 0,5	14.0 ± 2.9	6.2 ± 2.5	
5	E1	9.2 ± 1.5	3.2 ± 0.2	4.8 ± 0.6	14.7 ± 2.4	6.4 ± 2.6	
	E2	7.7 ± 1.9	3.0 ± 0.2	4.6 ± 0.3	13.6 ± 1.7	5.3 ± 1.9	
	C	5.7 ± 1.0	2.9 ± 0.2	4.6 ± 0.4	11.7 ± 1.1	3.3 ± 0.7	
30	E1	6.7 ± 0.7	2.9 ± 0.3	4.4 ± 0.3	12.5 ± 1.9	3.6 ± 1.0	
	E2	6.5 ± 0.2	2.8 ± 0.4	4.4 ± 0.3	12.1 ± 1.0	3.5 ± 0.4	

C = control (n = 8), E1 = goats supplemented with Cu sulfate (n = 8), E2 = goats supplemented with Cu chelate (n = 6)
$^{a:b}P$ < 0.05 comparison of results among the individual groups in individual days

ing percentage in the individual groups: C – 39%, E1 – 34%, and E2 – 33% of the mother's Cu concentration. There are evidently very important dynamic changes in the Cu concentration in blood serum of kids during early postpartum period (Table 1). On the days following after parturition the rapid increase of mean Cu concentration was observed in blood serum in kids of both experimental groups. On day 2 it increased from 45 to 47%, on day 7 from 74 to 92%, and on day 21 from 56 to 65% of the mother's Cu concentration in serum.

The mean Cu concentrations in colostrum were 8.3 ± 4.8 μmol/l in the control group, 10.6 ± 3.3 μmol/l in group E1, and 7.1 ± 1.5 μmol/l in group E2. The colostral Cu concentration in E1 group was significantly (P < 0.05) higher when compared with the E2 group. Other parameters of colostrum composition did not show any significant differences (Table 2).

No significant differences in the Cu concentration in placenta and amniotic fluid were found between groups (Table 3). The table shows that in amniotic and allantoic fluids the concentration of Cu was very low and the differences between these fluids were minimal (in amniotic fluid 0.9 to 1.3 μmol/l and in allantoic fluid 1.0–1.1 μmol/l). Concentration of Cu in fetal membranes was very low, too (mean concentration of Cu between groups was 8.2–9.1 μg/kg of fresh tissue) and it was not affected by Cu supplementation.

In our study Cu supplementation caused the increase in weight of kids (Table 4). When comparing the mean weight of the kids on day 0, there was significantly higher mean weight in E1 group compared to control. When comparing the mean

Table 3. Copper content (mean ± standard deviation) in fetal fluids (μmol/l) and placenta (μg/kg fresh tissue) in individals groups of goats

Group	Amnion	Allantois	Placenta
C (n = 8)	1.0 ± 0.3	1.0 ± 0.1	9.1 ± 2.1
E1 (n = 8)	0.9 ± 0.3	1.1 ± 0.2	8.5 ± 2.0
E2 (n = 6)	1.3 ± 0.5	1.0 ± 0.1	8.2 ± 2.2

C = control, E1 = copper sulfate supplement, E2 = copper chelate supplement

Table 4. Weight of goat kids (kg) (mean ± standard deviation) in experimental groups

Day	Group	Weight
	C	3.5 ± 0.5^a
0	E1	3.8 ± 0.5^b
	E2	3.9 ± 0.6
	C	4.0 ± 0.5^a
2	E1	4.2 ± 0.5
	E2	4.4 ± 0.5^b
	C	5.3 ± 0.6^a
7	E1	5.5 ± 0.6
	E2	5.9 ± 0.7^b
	C	8.0 ± 1.1^a
21	E1	8.7 ± 0.8
	E2	9.5 ± 1.3^b

C = control (n = 16), E1 = goats supplemented with Cu sulfate (n = 16), E2 = goats supplemented with Cu chelate (n = 9)
$^{a:b}P$ < 0.05 comparison of results among the individual groups in individual days

weight of the kids on days 2, 7, and 21, there was significantly higher mean weight in group E2.

DISCUSSION

Although many literary sources state that trace elements bound in organic form appear to be more accessible for organisms in comparison to inorganic form, our results in this study and also other studies of trace mineral metabolism in goats are not necessarily supporting this statement. Pechova et al. (2009) described that the efficiency of different organic and inorganic forms of zinc supplemented in goats was similar. Pavlata et al. (2011) found that the effects of supplementation with selenite and the lactate-protein selenium complex are similar with regard to selenium status but that the increase in glutathione peroxidase (GSH-Px) activity occurred much faster with selenite, which therefore appears to be a more biologically available form of selenium for creation of biologically active selenoproteins. In the study on pregnant goats Pavlata et al. (2012) found that all the above forms of selenium (sodium selenite, lactate-protein selenium complex, selenium proteinate, and selenium yeasts) were similarly utilized and transferred into the fetus in the goats. The results of the study by Panev et al. (2013) demonstrate that the actual intake of both organic and inorganic selenium is reflected in selenium concentration in ruminal fluid and ruminal biomass and, similarly, in selenium content and GSH-Px activity in blood. The form of supplemented selenium did not have a significant effect on ruminal fermentation parameters.

The above findings are clearly showing the need for more studies to improve the knowledge in this field. It is reported that the advantage of organically bound Cu is its greater solubility and ability to better withstand negative interactions with the antagonists (Brown and Zeringue, 1994). Panev et al. (2013) however suggested possible negative interaction between the intake of organically bound selenium and the concentration of Cu in blood of sheep. Zeng and Botnen (2004) described that selenite and selenocysteine can cause cell cycle arrest via distinct mechanisms, and suggest that Cu may interact with selenite extracellularly, which represents the basis of antagonism between copper and selenite. Wang et al. (2010) described interaction between selenomethionine and copper ions.

Some authors (Nockels et al., 1993; Rabiansky et al., 1999) in their studies pointed to the Cu-lysine, which seemed to be more accessible for organism of ruminants compared to Cu sulfate. This was also confirmed by the authors Coffey et al. (1994) and Apgar et al. (1995). In the study of Datta et al. (2007) organic form of Cu was clearly a better form of supplementation when compared to inorganic form such as Cu sulfate. However, in our study Cu sulfate seems to be more effective to increase Cu in colostrum and blood of goats and their kids in comparison to the organic form of Cu. Eren et al. (2013) in their study that was conducted to evaluate the accumulation of Cu and Zn in serum, hair, and faeces of goats fed diets supplemented with organic Cu and Zn at levels by 25% lower than recommendations. Treatment diets were supplemented with Cu and Zn chelates whereas control diet was supplemented with Cu and Zn sulfate. At the end of the experiment in both supplemented groups Cu and Zn levels in serum and in hair samples were found higher than the mean values at the beginning of the experiment but the difference between the mean values was not statistically significant. The mean Cu and Zn levels in faeces were significantly lower in the treatment group. Although organic Cu and Zn were given to the goats at low doses, the study confirmed that this amount gave similar results as inorganic Cu and Zn.

Blood Cu concentrations may be significantly influenced by the age of goats (Bhooshan et al., 2010). In this study, Cu concentration was low at birth and then increased with the age and reached the highest level at 11–12 months of age. In the study by Bhooshan and Kumar (2011) Cu concentration showed increasing tendency from the third month of gestation and reached significantly higher level in the mid of the fifth month of gestation. After that it decreased and remained low up to the first week of lactation. Our study shows increasing blood concentrations of Cu in kids of all groups during the first 21 days after birth while the Cu values in mothers did not show an increasing tendency. Physiological concentration of Cu in blood plasma of kids on the day of birth reached only 33–39% of the values in blood plasma of their mothers. In the early postpartum period, however, these concentrations were rising rapidly and were doubled in a week time. This should be taken into account when interpreting results of blood Cu concentration in kids in early postpartum period.

The positive effect of Cu supplementation on weight gains in kids (Solaiman et al., 2007) was confirmed also in our study. In kids of both experimental groups increased body weight gains were observed compared to control group in which they were the lowest. Statistically significant weight gains were observed in the group fed organic form of Cu compared to control group. Similar results were obtained in the study by Datta et al. (2007). It is possible that the number of kids born to one mother could have the influence, because in group E2 the average number of kids born was 1.5 per mother, whereas in group E1 and control group it was 2.0 per mother. It could also affect the weight gains among the groups.

Our results are suggesting that the inorganic form of Cu (Cu sulfate) was more efficient than organic (Cu chelate) in influencing the Cu metabolism in goat–kid relationship and that Cu supplementation has a positive effect on the weight of kids.

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