

## Effect of inorganic and organic zinc supplementation on coccidial infections in goat kids

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### Abstract

The aim of this study was to identify the effect of zinc-enriched diet fed to goats and their kids on the number of *Coccidia* oocysts shed by kids, on clinical signs of coccidiosis, weight gains, and kids' blood plasma concentration of zinc. A total of 22 goat kids were divided into 4 groups of 5 or 6 animals. Goats and kids from the control group did not receive any additional zinc, the second group was supplemented with inorganic zinc (zinc oxide), the third group was given zinc lactate, and the fourth group received chelated zinc. Samples of kids' faeces were taken weekly from 3 to 9 weeks of their age (a total of 7 samples were taken). Samples of faeces were examined by a quantitative method to detect the number of oocysts. Kids were weighed weekly and their blood was taken in order to determine zinc concentrations in blood plasma. Animals from the group supplemented with zinc chelate and zinc lactate shed a significantly ( $p \leq 0.05$ ) lower number of oocysts (13.4% and 11.9%, respectively) compared to the number of oocyst shed by control and zinc oxide supplemented groups (25% and 49.7%, respectively). Shedding of oocysts was not accompanied by clinical symptoms of coccidiosis in any of the groups. Kids supplemented with zinc chelate showed significantly highest weight gains and blood plasma concentration of zinc ( $p \leq 0.05$ ) as compared to control and inorganic zinc supplemented groups. Organic zinc is to be recommended to be used as a prophylaxis against coccidiosis in goat kids.

*Eimeria*, coccidiosis, weight gain, zinc chelate, zinc lactate, zinc oxide

Infections caused by *Eimeria* coccidia belong to the most significant protozoal infections. In goat kids, six specific *Eimeria* species were detected, namely *E. arloingi*, *E. ninakohlyakimovae*, *E. christenseni*, *E. hirci*, *E. caprina* and *E. alievi*. Their prevalence is considered to be global. In Spain, coccidiosis affects 96.1% goat kids fed milk (Ruiz et al. 2006). In northern Jordan, coccidiosis occurs in 85% local goats and 54% of them show concurrent infection with multiple *Eimeria* species. Incidence of concurrent infection was significantly higher in kids (66%) than in animals over 1 year of age (49%) (Mahmoud and Hossan 2003). Prevalence of coccidia in the U.S.A. was 100% ( $n = 318$ ); in Australia, coccidia were detected in 97% kids out of 497 (Reeg et al. 2005). In the Czech Republic, Strnadová et al. (2008) found coccidia in 54% kids less than 4 months old.

The course of clinical coccidiosis in goat kids can be acute, subacute, chronic or latent. The first symptoms of acute coccidiosis include raised temperature, watery diarrhea containing blood and particles of intestinal mucosa, mucosal anaemia, conjunctivitis and rhinitis. Mahmoud and Hossan (2003) reported up to 58% mortality of kids. Animals from 1 to 3 months of age are most susceptible and they shed oocysts most intensively between 5 and 8 weeks of age (Reeg et al. 2005). The course of infection is affected by the number of oocysts being shed. Coccidiosis is considered to be clinical when more than 100,000 *Eimeria* oocysts are detected in 1 g of faeces (Scott 2007).

Interaction between the parasite and the host leading to the development of clinical infection is affected by many important factors including nutrition. Overall zinc content in the organism significantly affects the body weight of the individual (Bell et al. 2007).

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In animal organism, zinc works as an activator of more than 200 enzymes and that is why its deficiency has a negative impact on the organisms as a whole (Evans and Halliwell 2001). Insufficient zinc concentrations cause decreased activity of T cells and thus zinc affects immunity both directly and indirectly (Shi et al. 1998). Low concentrations of zinc in blood were reported in parasitic infections (Tasci et al. 1995), as parasites utilize zinc for their metabolic and biochemical processes (Scott and Koski 2000).

Physiological range of zinc concentration in blood plasma of sheep and goats is 12.5–18 µmol/l. Zinc concentration in colostrum and milk is quite high and reflects the level of intake of dietary zinc. Zinc concentrations in milk of sheep and goats range between 3 and 5 mg/l, in colostrum between 15 and 25 mg/l. Zinc requirement in mammals is 40 to 100 mg/kg of dietary dry matter. Youngs that grow rapidly need more zinc. Requirement of zinc in adults is increased during pregnancy and high lactation (Jelínek et al. 2003).

Natural zinc sources include bran, grains and fodder yeast. The following forms of zinc supplementation are permitted by regulatory authorities: zinc chloride, monohydrate; zinc oxide; zinc lactate, trihydrate; zinc acetate, dihydrate; zinc sulphate, monohydrate; zinc sulphate, heptahydrate; zinc carbonate; chelated zinc and amino acids; and zinc propionate.

In case of zinc deficiency, it is advisable to administer higher doses of zinc than recommended (NRC 1985). This should improve weight gains and feed conversion ratio as well as ensure higher concentrations of zinc in the organism (Sharma et al. 2004).

In the present study, the effect of zinc supplementation in kids was evaluated in relation with shedding of *Eimeria* oocysts, weight gain, blood zinc concentrations and clinical signs of coccidiosis in the examined animals.

### Materials and Methods

In total, 22 goat kids (11 males and 11 females) were naturally infected with *Eimeria* oocysts and kept in stables of the Ruminant Clinic of the University of Veterinary and Pharmaceutical Sciences Brno. Kids and their mothers were randomly divided to 4 groups (A, B, C, D), each containing 5 or 6 kids. Mothers were divided in this way as early as 4 months before parturition.

Animals of the control group (A) received no additional zinc; group B was supplemented with inorganic zinc (zinc oxide) at the dose of 60 mg/kg dry matter of diet; group C received zinc lactate (zinc lactate trihydrate, Zinc Chelate, Agrobac, Czech Republic) at the dose of 60 mg/kg dry matter of diet; and group D were given zinc chelate (Bioplex Zn, Alltech, USA) at the dose of 60 mg/kg dry matter of diet. Goats received Zn-enriched diet 4 months before parturition. The diet of goats before parturition consisted of commercial pelleted feed for goats, hay *ad libitum*, mineral lick and zinc supplement. After parturition, mothers were given also grout and oat. In the first days, newborn kids sucked mother's milk only, but after about 7 days after the parturition, they started to eat also the diet intended for their mothers. Throughout the experiment, kids had free access to their mothers' diet.

As soon as kids reached the age of 3 weeks, samples of their faeces were taken weekly until they were 9 weeks old (altogether 7 times). Samples were obtained from kids using special pants for collection of faeces. The pants were put on in the morning and were left on kids for about 4 h.

Mixed samples of faeces from individual groups were examined quantitatively according to McMaster, and OPG

(Oocysts Per Gram). The samples were analyzed also by a basic flotation method and the intensity of infection was evaluated semiquantitatively as demonstrated in Table 1. In total, 28 group samples of faeces were examined from March to May 2007.

The kids were weighed every week throughout the experiment, starting on the parturition day. Blood samples were taken from kids in order to identify zinc concentrations. Blood was obtained from vena jugularis and collected into disposable heparinized plastic test tubes. Within 2 h of sampling the blood plasma was separated from the whole blood after centrifugation. Blood samples from kids were taken on the parturition day and on day 2 and 7 after birth. Afterwards, kids were sampled every second week (on weeks 3, 5, 7 and 9).

Zinc concentrations in blood plasma were determined in the laboratory of the Ruminant Clinic by the method of flame AAS at Solaar M6 (Unicam, Great Britain) following the previous deproteination

Table 1. Semiquantitative microscopic evaluation of *Eimeria* oocysts in feces of goat kids

Infection intensity	Number of oocysts
Negative	0 in the sample
+/-	1-2 in some visual field
+	1-3 in every visual field
++	4-6 in every visual field
+++	7 and more in every visual field

of sample by adding trichloroacetic acid at the 1:1 ratio. Zinc was determined in the supernatant after centrifugation.

The data samples were evaluated by parametric tests, namely one-way analysis of variance using Bartlett's test. Subsequent evaluation of data was done using Tukey-Kramer method. Non-parametric Kruskal-Wallis test and Steel-Dwass test were used in case of non-homogenous variances that occurred in some data samples.

## Results

In all groups supplemented with zinc (B, C, D), a slower onset of coccidiosis was observed. Kids from these groups started to shed oocysts several weeks later than kids from the control group. Kids from the control group (A) started to shed *Eimeria* oocysts first at 4 weeks of age. The highest number of oocysts 56650 were shed by kids from the group supplemented with inorganic zinc (group B). Kids from the group C supplemented with zinc lactate started to shed oocysts as late as in week 6. At this time, kids from this group shed the least number of oocysts throughout the experiment, i.e. 11.9% of the total number of *Eimeria* oocysts shed in all groups. Animals from group D supplemented with zinc in the form of chelate started to shed oocysts in week 5. Numbers of oocysts shed in 1 g of faeces of goats in individual groups during the whole experiment are summarized in Table 2.

Table 2. Number of oocysts in 1 g of faeces in goat kids sorted by age and groups

Age of kids (in weeks)	Group A (n = 6) Control	Group B (n = 6) Zn oxide	Group C (n = 5) Zn lactate	Group D (n = 5) Zn chelate
3	0	0	0	0
4	100 (+/-)*	0	0	0
5	18650 (+++)	0	0	4400 (++)
6	2500 (++)	6300 (++)	6000 (++)	1850 (+)
7	1550 (+)	25900 (+++)	1350 (+)	350 (+/-)
8	3000 (++)	17100 (+++)	1850 (+)	8250 (+++)
9	2700 (++)	7350 (+++)	4400 (++)	450 (+/-)
Total	28500	56650	13600	15300
Percentage (%)	25.0	49.7	11.9	13.4

\*Semi-quantitative evaluation according to Table 1

Groups of kids that received organic zinc (lactate, chelate) shed significantly less *Eimeria* oocysts ( $p \leq 0.05$ ) compared to the control group and the inorganic zinc group. Throughout the experiment, kids from the inorganic zinc group (group B) shed the most oocysts (49.7%). On the contrary, kids from the zinc lactate and chelate groups shed the least number of oocysts 11.9 (13.4%). Shedding of oocysts was not accompanied by clinical symptoms of coccidiosis (diarrhea) in any group.

Average weekly weight gains of kids, standard deviations, and numbers of animals in individual groups are summarized in Table 3. Statistical evaluation of the results indicated that in week 3, group D showed significantly higher weight gains compared to group B ( $p = 0.011$ ). The same situation was observed in week 4 ( $p = 0.004$ ). Moreover, group D showed significantly higher weight gains compared to group A ( $p = 0.018$ ) and group C ( $p = 0.005$ ). In week 5, animals from group D showed again significantly higher weight gains compared to group B ( $p = 0.021$ ) and group A ( $p = 0.026$ ). The situation was similar in week 6 and week 8. Kids from group D had significantly higher weight gain compared to group B ( $p = 0.045$ ,  $p = 0.011$ ). In weeks 1, 2, 7 and 9, the measured values were non-significant.

Average zinc concentrations in kids' blood plasma, standard deviations and numbers of animals in individual groups are demonstrated in Table 4. The results of the present study indicate that the highest bioavailability was observed in chelated zinc. Five out of seven measurements showed that zinc concentration was highest in blood of kids receiving

Table 3. Weekly weight gains in goat kids (kg)

Age of kids (in weeks)	Group A (n = 6) Control	Group B (n = 6) Zn oxide	Group C (n = 5) Zn lactate	Group D (n = 5) Zn chelate
	$\Phi \pm s$	$\Phi \pm s$	$\Phi \pm s$	$\Phi \pm s$
1	1.45 ± 0.12	1.62 ± 0.21	1.61 ± 0.14	1.54 ± 0.22
2	1.85 ± 0.12	1.74 ± 0.48	1.46 ± 0.34	1.65 ± 0.32
3	1.62 ± 0.30	1.15 ± 0.53 <sup>a</sup>	1.39 ± 0.34	2.08 ± 0.49 <sup>a</sup>
4	1.56 ± 0.24 <sup>b</sup>	1.41 ± 0.47 <sup>a</sup>	1.38 ± 0.33 <sup>c</sup>	2.28 ± 0.33 <sup>abc</sup>
5	1.45 ± 0.50 <sup>b</sup>	1.42 ± 0.33 <sup>a</sup>	1.73 ± 0.74	2.43 ± 0.40 <sup>ab</sup>
6	1.35 ± 0.53	0.70 ± 0.55 <sup>a</sup>	1.18 ± 0.64	1.66 ± 0.46 <sup>a</sup>
7	1.77 ± 0.37	1.50 ± 0.91	0.68 ± 0.83	1.64 ± 0.61
8	1.44 ± 0.48	0.33 ± 0.39 <sup>a</sup>	1.00 ± 0.93	1.8 ± 0.86 <sup>a</sup>
9	1.16 ± 0.64	1.43 ± 0.58	1.98 ± 1.05	2.04 ± 0.60

$\Phi$  – mean value calculated from the measured values; s - standard deviation;  
<sup>aa-c</sup> on the line of the table - significant differences between groups ( $p \leq 0.05$ )

Table 4. Zinc concentrations in goat kids' blood plasma ( $\mu\text{mol/l}$ )

Age of kids	Group A (n = 6) Control	Group B (n = 6) Zn oxide	Group C (n = 5) Zn lactate	Group D (n = 5) Zn chelate
	$\Phi \pm s$	$\Phi \pm s$	$\Phi \pm s$	$\Phi \pm s$
Day 0	5.22 ± 1.49	5.24 ± 2.01	6.23 ± 1.01	6.93 ± 2.02
Day 2	19.18 ± 2.58 <sup>a</sup>	11.31 ± 3.22 <sup>a</sup>	17.86 ± 2.86	16.64 ± 4.87
Day 7	17.51 ± 2.15 <sup>a</sup>	14.35 ± 1.60 <sup>a</sup>	17.30 ± 0.77	16.30 ± 2.56
Week 3	16.40 ± 1.36	14.03 ± 0.99	14.62 ± 3.26	16.80 ± 2.74
Week 5	13.41 ± 2.09	10.61 ± 0.74	13.13 ± 3.07	13.96 ± 1.95
Week 7	11.09 ± 0.40 <sup>a</sup>	10.86 ± 1.43 <sup>b</sup>	11.90 ± 0.86	14.18 ± 3.14 <sup>ab</sup>
Week 9	10.91 ± 1.14 <sup>a</sup>	10.42 ± 0.91 <sup>b</sup>	11.46 ± 0.67	12.88 ± 1.15 <sup>ab</sup>

$\Phi$  – mean value calculated from the measured values; s - standard deviation;  
<sup>aa, bb</sup> on the line of the table - significant differences between groups ( $p \leq 0.05$ )

chelate. Increased concentration of zinc in kids fed chelate was confirmed by statistical evaluation in week 7 and week 9 compared to the control group (week 7:  $p = 0.038$ ; week 9:  $p = 0.020$ ) and the inorganic zinc group (week 7:  $p = 0.024$ ; week 9:  $p = 0.004$ ). Increased blood concentrations of zinc were associated with markedly less intensive shedding of *Eimeria* oocysts in the zinc chelate group compared to the control group and the inorganic zinc group. On days 2 and 7, a significantly lower zinc concentration was found in kids of mothers fed inorganic zinc compared to the control group ( $p = 0.005$ ,  $p = 0.045$ ).

## Discussion

Impact of various forms of zinc on the number of *Eimeria* oocysts being shed, weight gain, and blood concentrations of zinc were compared in order to find out whether positive effects of zinc can be used for efficient prophylaxis against *Eimeria*, the most common protozoal parasite of gastrointestinal tract of kids.

Impact of zinc on a course of infection is connected with host immunity. Fekete and Kellems (2007) reported that zinc has both specific and nonspecific roles in the immune defence mechanism. Zinc regulates the maturation and function of immune cells. The effect of zinc on growth and immunocompetence was described by Davis et al. (2004) and the impact of zinc on reduction of growth depression in coccidiosis was studied by Khanagwal et al. (2000).

The lowest concentration of zinc in blood plasma of kids from goats supplemented with zinc oxide was found on days 2 and 7. Based on our results, we can presume that this form of zinc is inefficiently transferred from mother's body to colostrum and milk. The influence of food supplemented with different forms of zinc (lactate, chelate, amino acid-polypeptidic complex, oxide) on the zinc concentration in blood plasma and milk of goats was studied by Pechova et al. (2009). They found that the supplementation of different form of zinc did not influence the concentration of zinc in milk, but influenced the zinc concentration in blood plasma. The efficiency of different organic and inorganic forms of zinc was almost the same.

Animals from the inorganic zinc group shed the highest number of oocysts throughout the experiment and had lower weight gains in most weeks compared to the control group. In general, the inorganic form of zinc does not significantly affect the health of kids. Groups of kids that received organic zinc shed significantly less *Eimeria* oocysts compared to the control group and the inorganic zinc group.

No group of kids showed the typical symptom of coccidiosis. This corresponds also with the lower number of oocysts shed by kids. Maximum number of oocysts (OPG 25,900) was observed in week 7 in kids from the inorganic zinc group (B). Clinical coccidiosis is usually associated with OPG 100,000 (Scott 2007).

Weight gain is an important indicator of subclinical coccidiosis as well. Correlation between the number of shed *Eimeria* oocysts and kids' weight gain was demonstrated in this study. Weight gains decreased in weeks when kids were shedding the highest numbers of oocysts. This tendency was apparent in the inorganic zinc group where weekly weight gains markedly decreased during the onset of coccidiosis in week 6, and weight gains were also lower during the period of intensive shedding of oocysts (+++). This phenomenon was not observed in the group supplemented with zinc chelate. Numbers of oocysts did not reach the values observed in group B and the onset of coccidiosis in week 5 did not affect weight gains significantly. The effect of individual forms of zinc on weight gains was proved as well (Table 3). Significantly higher gains were observed in weeks 3, 4, 5, 6 and 8 in kids receiving zinc chelate compared to kids supplemented with inorganic zinc. Compared to the control group, kids from the zinc chelate group showed increased weight gains in weeks 4 and 5.

In addition, supplementation of chelated zinc clearly increased blood concentration of Zn, which may be important for its other functions in the organism. Buzadžić et al. (2002) reported that best bioavailability of zinc is ensured by administering zinc chelate together with peptides and amino acids. Bioavailability of zinc methionine chelate is higher by 206% compared to supplementation with zinc sulphate (Wedekind et al. 1992). Zinc administered in this form offers higher protection against free radicals compared to zinc salts (Bagchi et al. 1998).

Kids supplemented with lactate and chelate form of zinc shed the least number of *Eimeria* oocysts. Although kids receiving zinc lactate were the last to start to shed oocysts (and they shed the least number of them throughout the experiment), they did not show significantly higher weight gains compared to the control group and other groups, which is a primary indicator from the perspective of animal health and cost-effectiveness. It is clear from the results that although the intensity of coccidial infection was higher in the zinc chelate group compared to the lactate group, the chelate group showed the highest weekly weight gains.

Monitoring the effects of individual forms of zinc on the number of *Eimeria* oocysts being shed by goats, clinical signs of the disease and weight gains indicated that organic forms of zinc (namely chelated zinc) administered to mothers and their kids decrease the number of oocysts being shed and increase the kids' weight gain. The best results were achieved by using organic forms of zinc also by Wang et al. (2010).

Organic zinc is to be recommended to be used as prophylaxis against coccidiosis of goat kids.

### Vliv dotace anorganického a organicky vázaného zinku na kokcidiózu kůzlat

Cílem naší práce bylo posoudit vliv diet obohacených zinkem podávaných kozám a jejich kůzlatům na počet vylučovaných oocyst kokcidií, klinický průběh kokcidiózy, přírůsteky hmotnosti a koncentraci zinku v krevní plazmě kůzlat. Celkem 22 kůzlat bylo rozděleno do 4 skupin po 5-6 zvířatech. Kozy a kůzlata z kontrolní skupiny nedostávaly žádný přírůstek zinku, druhá skupina byla suplementována anorganickým zinkem (oxid zinečnatý), třetí skupina dostávala zinek ve formě laktátu a čtvrtá skupina dostávala zinek ve formě chelátu. Vzorky trusu kůzlat byly odebírány týdně od 3. do 9. týdne věku (tj. celkem 7 odběrů). Vzorky trusu kůzlat z každé skupiny byly vyšetřovány kvantitativní metodou na počet vylučovaných oocyst kokcidií. Každý týden byla kůzlata vážena a byla jim odebírána krev na stanovení hladiny zinku v krevní plazmě. Kůzlata ze skupiny dotované zinkem ve formě chelátu a laktátu vylučovala signifikantně ( $p \leq 0.05$ ) nižší počet oocyst kokcidií 13,4 % (resp. 11,9 % z celkového počtu vyloučených oocyst) v porovnání s kůzlaty kontrolními a dotovanými zinkem anorganickým (ta vyloučila 25 %, resp. 49,7 % z celkového počtu vyloučených oocyst). V žádné ze skupin nebyly zaznamenány klinické příznaky kokcidiózy. Kůzlata dotovaná chelátem zinku vykazovala ve srovnání s ostatními skupinami nejvyšší váhové přírůstky a vyšší koncentraci zinku v krevní plazmě. Organicky vázaný zinek tak může být doporučen k využití v rámci prevence kokcidiózy kůzlat.

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