Effects of organic zinc supplementation in weaned calves

Sylva Dresler[1], Josef Illek[2], Ladislav Zeman[1]

[1]Mendel University in Brno, Faculty of Agronomy, Department of Animal Nutrition and Forage Production, Brno, Czech Republic
[2]University of Veterinary and Pharmaceutical Sciences Brno, Large Animal Clinical Laboratory, Brno, Czech Republic

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Abstract

The aim of the study was to investigate the effect of organic zinc supplementation in calves on serum zinc (Zn) concentrations, selected metabolic profile indicators and serum immunoglobulin (Ig) concentrations. The trial included 2 groups (n = 10) of weaned female calves. The Zn-Methionin calves (group Zn-Met) were supplemented with 30 mg Zn-Met/kg dry matter (DM)/day (BIOPLEX® Zn, Alltech, USA) for 90 days; the control calves (group C) received the same diet without organic zinc supplementation. Compared to the control group, organic Zn treatment significantly increased serum Zn concentration ($P < 0.05$), superoxide dismutase (SOD) activity ($P < 0.01$) and total Ig ($P < 0.01$) in the group Zn-Met at the beginning (7 days from the start of Zn-Met supplementation) of the trial. At the end of the trial (day 90) serum total protein (TP) ($P < 0.05$), albumin ($P < 0.01$), urea ($P < 0.01$), SOD ($P < 0.01$), copper (Cu) ($P < 0.05$), Zn ($P < 0.01$) and Ig ($P < 0.05$) concentrations were significantly higher in the Zn-Met calves. In the control group alkaline phosphatase (ALP) activity was significantly ($P < 0.01$) higher on day 90. A positive correlation between zinc concentrations, ALP, and SOD activities in serum, and a negative correlation between zinc and copper concentrations were demonstrated. Dietary Zn-Met supplementation in calves markedly influenced the metabolic profile and serum immunoglobulin concentrations. Compared to the control group, the Zn supplemented group showed a significantly ($P < 0.05$) lower ALP and significantly greater SOD serum activity ($P < 0.01$) at the end of the trial. Total Ig concentrations were significantly higher in the Zn treated group (day 7: $P < 0.01$ vs. day 90: $P < 0.05$).

Trace element, biochemical indicator, immune response, SOD, cattle

The enzyme activity and gene expression of proteins influenced by zinc as an essential trace element that plays structural and catalytic roles in over 300 enzymes and transcription factors (Lipscomb and Sträter 1996; Illek 2003; Suttle 2010) affect reproduction, growth, milk production, and the immune system of animals (Illek 1987; Chesters 1997; Andrieu 2008). Zinc sources of greater bioavailability such as zinc methionine (Zn-Met) (Wedekind et al. 1992; Kincaid et al. 1997) can increase concentrations of Zn in tissues such as thymus, bone marrow (Heilig et al. 2014), and serum (Cousins and Leinart 1988) more markedly than traditional Zn sources. The role of Zn as an antioxidant and its importance for cell replication and proliferation are the two most direct connections between Zn and the immune function (Spears and Weiss 2008; Nojiri et al. 2011). Therefore, the objective of this study was to assess the effect of zinc supplementation on serum Zn concentrations, metabolic profile indicators and serum immunoglobulin concentrations.

Materials and Methods

The study was performed in a herd of 300 Holstein cows with a mean milk yield of 9,318 kg per 305 day lactation (mean fat content 3.79%; mean protein content 3.31%). In total there were 236 calves: 110 pre-weaning (milk replacer fed) and 126 post-weaning. Calf loss due to mortality reached 3.4%. The calves did not show

Address for correspondence:
Doc. MVDr. Josef Illek, DrSc., Dipl. ECBHM
Large Animal Clinical Laboratory, Faculty of Veterinary Medicine
University of Veterinary and Pharmaceutical Sciences Brno
Palackého třída 1946/1, 612 42 Brno, Czech Republic
Phone: +420 731 512 909
E-mail: illekj@vfu.cz
http://actavet.vfu.cz/

any clinical signs of Zn deficiency. During the whole pre-weaning period the calves were housed in individual pens. Twice a day they received 4 litres of Lactosan® (Schaumann, Germany) milk replacer (40 mg Zn/kg DM as $ZnSO_4$), and Calf Starter Feed MLSTEL® (Farmtec, a.s., Czech Republic), (30 mg Zn/kg DM, 22% CP). They had free access to meadow hay and water *ad libitum* between meals. The calves were weaned at 8 weeks of age. The trial included 20 weaned female calves, randomly allocated into 2 separately housed groups of 10 animals. The diet consisted of Calf Starter Feed 2 (19.5 % CP, 30 mg Zn/ kg DM), meadow hay and water *ad libitum*. After a 21 day adaptation period the experimental Zn supplementation was launched. The zinc treated calves (group Zn-Met) (n = 10) received an organic zinc source (Zn-Met) for 3 months, at 30 mg Zn-Met/kg DM/day (BIOPLEX® Zn, Alltech, USA). The control calves (group C) (n = 10) received the same diet without organic zinc supplementation. During the experimental period, the general health status of calves was monitored.

At the beginning and end of the experiment, i.e. on days 7 and 90 of Zn-Met supplementation, blood samples were taken from the jugular vein of calves in both groups for biochemical and immunological analyses (serum concentrations of total protein [TP], albumin [ALB], urea, alkaline phosphatase [ALP], superoxide dismutase [SOD], vitamin A, vitamin E, copper [Cu], zinc [Zn], and total immunoglobulin [Ig]). The metabolic profile indicators, microelements Cu and Zn (by atomic absorption spectrophotometry AAS), vitamin A and E (by spectrofluorometry), and total serum immunoglobulin (by turbidimetry) concentrations were analyzed with commercial biochemical kits with the Hitachi 917 analyzer following the manufacturer's instructions (F. Hoffmann-La Roche, Germany) in Vetlabfarm s.r.o., Brno, Czech Republic. The decisive criterion for the diagnosis of Zn deficiency in calves is a decreased serum Zn concentration. Zinc concentrations lower than 12 μmol/l were regarded as deficient, Zn concentrations within the range of 12–18 μmol/l were considered to be the optimum Zn status. Zinc concentrations higher than 18 μmol/l were regarded as indicating a slightly excessive Zn supply. Raw data were statistically analyzed using Microsoft EXCEL® and Statistica®; the data sets were compared by two-choice Student's *t*-test with equal or unequal variance. The correlation was expressed by correlation coefficient and its significance was evaluated by Student's *t*-test. The value of $P \leq 0.05$ was considered as significant, $P \leq 0.01$ as highly significant.

Results

At the beginning of the experimental period two indicators were outside the physiological range. Both the supplemented (Zn-Met) and the control group showed low serum copper and total immunoglobulin concentrations. Significant differences in Ig ($P \leq 0.01$), Zn ($P \leq 0.05$) and SOD ($P \leq 0.01$) concentrations were demonstrated on day 7. The zinc supplemented group showed higher concentrations of the three indicators than the control group.

At the end of the trial (day 90) the Zn-Met (P) and control (C) calves showed significant differences in following indicators: TP ($P \leq 0.05$), albumin ($P \leq 0.01$), urea ($P \leq 0.01$), ALP ($P \leq 0.01$), SOD ($P \leq 0.01$), copper ($P \leq 0.05$), zinc ($P \leq 0.01$), and total Ig ($P \leq 0.05$). Serum urea and Zn levels in the control group, and Cu concentration in both the groups were outside the physiological ranges. Total protein, albumin (P: ($P \leq 0.05$), immunoglobulins, and vitamin A showed a significant increase ($P \leq 0.01$). Urea ($P \leq 0.01$), ALP ($P \leq 0.01$), SOD ($P \leq 0.05$), vitamin E ($P \leq 0.01$) and Zn ($P \leq 0.05$) concentrations were decreased in both groups. Initial and final values are summarized in Table 1.

The experimental calves did not show any clinical signs of Zn deficiency or other diseases at the beginning or at the end of the trial.

Serum total protein (TP) and albumin concentrations increased with age of the calves. Both experimental groups showed significant differences in the initial and final concentrations of TP. The Zn-Met group showed slightly higher final TP and albumin concentrations than group C (TP: $P \leq 0.05$, albumin: $P \leq 0.01$).

At the end of the trial period there was a significant difference between the initial and final urea concentrations both in the control ($P \leq 0.01$) and the Zn-Met ($P \leq 0.01$) group. A significant final difference ($P \leq 0.01$) in final urea concentrations between the groups was observed. Group C showed a greater drop below the lower limit of the physiological range than the group Zn-Met.

Alkaline phosphatase and SOD activities decreased with age of the calves. There was a significant difference between the groups. The final ALP activity was significantly higher in group C compared to Zn-Met ($P \leq 0.01$). The measurements performed at the beginning and end of trial revealed a significant drop ($P \leq 0.01$) in serum ALP activity in each group.

Table 1. Biochemical indicators of calves on days 7 and 90 of the trial.

Indicator	Reference values	Unit		Day 7		Day 90	
				Zn-Met	C	Zn-Met	C
Total protein (TP)	50–70	g/l	x	57.52	58.48	68.47*	65.94*
			SD	2.61	1.5	1.87	2.42
Albumin	30.3–42	g/l	x	30.55	30.53	35.95**	32.73**
			SD	1.68	1.45	1.4	1.04
Urea	2.5–6.6	mmol/l	x	4.37	4.23	2.92**	2.48**
			SD	0.38	0.5	0.3	0.21
Alkaline phosphatase (ALP)	max 20	µkat/l	x	10.56	11.82	7.1**	9.06**
			SD	1.99	1.48	1.3	1.65
Superoxide dismutase (SOD)	-	U/ml	x	487.92**	456.39**	471.26**	426.1**
			SD	22.87	28.67	29.69	33.55
Vitamin A	> 0.7	µmol/l	x	1.26	1.3	2.37	2.55
			SD	0.15	0.23	0.6	0.48
Vitamin E	> 0.64	µmol/l	x	7.3	7.07	2.97	2.65
			SD	1.65	1.35	0.55	0.37
Copper (Cu)	12.2–18.9	µmol/l	x	10.98	11.35	10.8*	9.88*
			SD	1.29	1.38	1.24	1.2
Zinc (Zn)	12.6–45.9	µmol/l	x	15.36*	13.74*	14.27**	11.25**
			SD	0.83	2.26	1.16	2.52
Total immunoglobulin (Ig)	> 12	g/l	x	14.51**	7.46**	27.77*	24.31*
			SD	1.08	0.69	3.97	1.7

*significant difference a t$P <$ 0.05, **highly significant difference at $P < 0.01$, SD = standard deviation, x = mean value

Both ALP and SOD are Zn-depended enzymes, therefore we investigated the relationship between these two indicators. Significantly positive correlation of ALP activity and serum Zn concentration was demonstrated by regression analysis as r = 0.389, ($P \leq 0.01$), which may imply a favourable influence of Zn-Met on serum ALP activity. We found significant differences between the initial and final SOD values in each group (P: $P \leq 0.01$ vs. C: $P \leq 0.003$) and a significantly positive correlation coefficient r = 0.279 ($P \leq 0.05$). A more pronounced drop of SOD in group C is shown in Table 1.

The differences between the experimental groups in the initial and final concentrations of vitamin A and E (days 7 and 90, respectively) were not significant. However, there was a significant increase ($P \leq 0.01$) from the initial to final plasma vitamin A concentration in each group (Table 1). Vitamin E concentrations were significantly decreased ($P \leq 0.01$) in both groups. The Zn-Met group tended to show higher initial and final vitamin E concentrations compared to group C.

All calves showed marginal copper deficiency throughout the trial. Initial and final serum Cu concentrations in both experimental groups were outside the physiological range (Table 1). A significantly negative correlation between Zn and Cu concentrations reflects the suppressive effect of increasing Zn concentrations on Cu concentrations that were decreasing in both the groups at the same rate; r = -0.493; ($P \leq 0.05$).

By evaluating serum Zn concentration we found significant differences in serum Zn concentrations in Zn-Met and control calves on day 7 ($P \leq 0.05$) and day 90 ($P \leq 0.01$). During the trial a significant drop ($P \leq 0.05$) in serum Zn concentrations occurred in group C. The final serum Zn concentration was below the physiological range in group C, whereas in the Zn-Met group it was high above the lower physiological limit. The Zn-Met group showed a decrease as well, but non-significant.

During the trial there was a significant increase in total serum Ig in each group ($P \leq 0.01$). On day 7, in neither group total Ig values reached the lower limit of physiological range (>18 g/l). The Zn-Met group showed significantly higher serum Ig concentrations than group C both at the beginning and the end (day 7: $P \leq 0.01$, day 90: $P \leq 0.05$), with final serum Ig concentrations being more increased than the initial ones.

Discussion

During the 90-day trial we observed a physiological TP increase, qualified as normoproteinaemia that is to be achieved at the age of 3–4 months (Dirksen et al. 2006). Another effect we observed was the reduction of Zn-dependent ALP activity with increasing age. Generally, growing animals show higher ALP activity because of the bone isoenzyme ALP activity. Zinc is involved in bone mineralization and calcium mobilization. Positively correlated ALP activity is decreased in Zn deficient animals (Heilig et al. 2014). At the end of the trial the control calves were marginally Zn deficient, although ALP activity was higher compared to the Zn supplemented group. Spears (1989) demonstrated an increased ALP activity in heifers supplemented with organic and inorganic Zn sources. A decrease in ALP activity at the end of this trial was smaller in group C. Andrieu (2008) reported that Zn deficiency leads to a decreased ALP activity in cattle. Heilig et al. (2014) found reduced serum Zn and ALP concentrations in milk fever cows compared to a healthy control group. However, the ALP activity is influenced by many other factors and in cattle it shows great individual variability (Dirksen et al. 2006; Chesters 1997). Therefore, ALP is not recommended as a suitable indicator of Zn concentration in the body status of the animal (NRC 2001).

The increase in vitamin A concentrations and decrease in vitamin E concentrations could be associated with dietary changes. Vitamin A concentration in group C tended to be higher on days 7 and 90. Dang et al. (2013) demonstrated a positive effect of Zn/Cu/vitamin E micronutrient supplementation on the phagocytic activity of neutrophils, modulated immune cell function, and enhancement of their production in peripartum cows. A significant ($P < 0.01$) increase in the B lymphocyte proliferation was observed in the Zn supplemented group and Zn/Cu/vitE supplemented cows on day 15 pre-calving, as well as in the copper-supplemented group of cows during the *post partum* period ($P < 0.01$). This confirms that it is vital to provide sufficient amounts of copper and safeguard the optimal function of the antioxidant system in cattle.

A drop of in urea concentrations below the physiological limit in the control calves at the end of the trial was surprising. Otherwise, the calves were prosperous. The reason for the serum urea drop could be an insufficient dietary crude protein (CP) supply. Spears (1989) explained a serum urea drop in heifers supplemented with Zn-Met by higher amino acid utilization for protein synthesis. Also Kessler et al. (2003) reported a serum urea drop in fattening bulls.

Unlike in this study, Kessler et al. (2003) did not observe any significant differences in metabolic serum indicators (TP, ALB, ALP) between the C and Zn-Met supplemented groups at the end of their trial. The regression analysis demonstrated a positive correlation between Zn and SOD concentrations in serum. As compared to the control calves, the Zn-supplemented group showed significantly higher Zn concentrations and SOD activity in serum; a similar drop in each indicator was demonstrated. Singh and Singha (2003) and Nojiri et al. (2011) reported a positive effect of Zn supplementation on SOD activity as well. A previously known negative correlation between serum Zn and Cu concentrations was demonstrated. Similar results were reported by Sikka et al. (2002).

The supplementation of Zn-Met showed a significant effect on serum Ig concentrations in Zn-supplemented calves. Similarly, Droke et al. (1998) and Dang et al. (2013)

demonstrated the positive effect of Zn, Cu, and vitamin E supplementation on the immune response in cattle, and so did Chirase and Greene (2001) who compared organic Zn-Met with zinc oxide (ZnO) supplementation in calves infected with infectious bovine rhinotracheitis. Kincaid et al. (1997) did not demonstrate any effects of Zn-Met, Zn-Lys and ZnO supplementation on the cytotoxic activity and phagocytic activity of neutrophils. Spears and Kegley (2002) were not successful either. Sikka et al. (2002) reported a significant positive interaction between serum zinc concentration and vitamin A/D/E supplementation, and higher total Ig concentrations in buffalo calves supplemented with Zn, Cu, Ca, Mg, Cu, Mn, Fe, Na, Cl, Co, I, and F without vitamin A/D/E treatment compared to calves treated with all the micronutrients. Their dams were supplemented *pre partum* with vitamins A, D, and E. In our study a significant positive correlation between serum Zn and serum total Ig concentrations was demonstrated. Engle et al. (1997) reported a more pronounced cell response in marginally Zn deficient calves supplemented with Zn-Met and Zn-Lys than in calves supplemented with $ZnSO_4$. Nemec et al. (2014) reported a greater improvement of the immune status in lactating cows receiving organic Zn compared to those supplemented with inorganic Zn, Cu, and Mn sources. The neutrophil phagocytosis was positively affected in the group supplemented with chelated minerals.

Our results suggest that Zn-Met supplementation favourably influenced serum Zn concentrations. Souza et al. (2007) demonstrated a significantly higher retention of zinc in rats treated with Zn-Met compared to those given zinc sulphate ($ZnSO_4$) and zinc oxide (ZnO), which indicates an increase in Zn bioavailability reflected in its higher retention in the body.

The results of the experiment showed a favourable effect of the organic Zn source (Zn-Met, Bioplex®) supplementation of calves on serum Zn concentrations, total serum Ig concentrations, SOD activity, and serum TP and albumin concentrations. A positive correlation between Zn concentrations and ALP and SOD activities and a negative correlation between Zn and Cu concentrations were demonstrated. Serum Zn concentrations were slowly decreasing during the experimental period in both groups. The control group showed a highly significant drop in Zn concentrations. Significant differences in serum TP and albumin related to the calves' age were observed. In the Zn-Met supplemented group a significantly smaller SOD activity drop was observed at the end of the trial period. Initial and final total immunoglobulin concentrations were higher in the Zn treated group.

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