

Influence of organic zinc on lactation performance and somatic cell count in dairy cowsJosef Illek¹, Sylva Dresler², Miloslav Šoch², Naděžda Kernerová²,
Křtinyňa Šimák Líbalová², Eliška Zevlová², Nikola Havrdová²¹University of Veterinary Sciences Brno, Faculty of Veterinary Medicine, Large Animal Clinical Laboratory, Brno, Czech Republic²University of South Bohemia in České Budějovice, Faculty of Agriculture and Technology, Department of Animal Husbandry Sciences, České Budějovice, Czech Republic

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Abstract

The objective of the study was to evaluate the effect of organically chelated zinc methionine (Zn-Met) supplementation on lactation performance of dairy cattle, i.e. milk yield (MY), milk components, and the somatic cell count (SCC) in early lactation. Eighty multiparous cows received two treatments (n = 40 per group) from 21 days prepartum throughout 14 weeks post partum (p.p.). Treatments consisted of 1) supplemental Zn-Met at the dose of 30 mg Zn/kg dry matter (DM) of the total mixed ration (TMR) for the supplemented (S) group; and 2) basal TMR without supplementation for the control (C) group. Daily MY of Zn supplemented cows 30 days p.p. was not significantly affected ($P > 0.05$) by the treatment. After 60 days, the MY of the S-group was significantly higher (S: 44.70 ± 4.24 kg vs. C: 42.08 ± 4.60 kg, respectively); ($P < 0.05$). Ninety days p.p. there was a highly significant difference ($P < 0.005$) as well (S: 43.22 ± 4.47 kg vs. C: 39.98 ± 4.80 kg). Supplemented cows also produced significantly more milk fat and milk protein than the control on days 60 and 90 of the trial. There was a significant drop and a decreasing trend during the entire trial in SCC in S-group. Milk quality and udder health were positively affected by the Zn-Met treatment. This finding has an important implication for veterinary practice in an era of antibiotic reduction.

Trace elements, cattle, milk quality

Trace element deficiencies of minerals such as zinc (Zn), copper (Cu), manganese (Mn) or cobalt (Co) have been observed and studied for many years. They play very important roles in protein synthesis, vitamin metabolism, tissue connectivity, immune system, hormone regulation, appetite regulation, growth, fertility, quality of skin and claw integrity (Hidiroglou 1979; Illek 1987; Miller et al. 1989; Shankar and Prasad 1998; NRC 2001; Illek 2003; Tomlinson et al. 2004; Slavík et al. 2006; Dresler et al. 2016; Weiss 2017; Chen et al. 2020; Guimaraes et al. 2022). Zinc is a microelement, which has an essential function for health and performance by livestock. Zinc supplementation has an effect on cattle performance and health, such as fertility, lactation, claw integrity and immunity (Neathery et al. 1973; Malcolm-Callis et al. 2000; Spears and Kegley 2002; Kellogg et al. 2004; Kincaid and Socha 2004; Nocek et al. 2006; Griffiths et al. 2007; Siciliano-Jones et al. 2008; Pavlík et al. 2010; Machado et al. 2013; Horst et al. 2019).

Zinc is an integral part of many enzyme systems, or it works as an enzyme activator. It affects milk production, reproduction, the immune system, and animal growth through the enzyme activity and gene expression of proteins influenced by zinc (Chesters 1997). Zinc plays catalytic and structural roles in over 300 enzymes and transcription factors (Lipscomb and Sträter 1996). The function of metalloenzymes such as carbonic anhydrase, lactate dehydrogenase, alcohol dehydrogenase, alkaline phosphatase, carbonic anhydrase,

Address for correspondence:MVDr. Sylva Dresler
Department of Animal Husbandry Sciences
Faculty of Agriculture and Technology
University of South Bohemia in České Budějovice
Studentská 1668, 370 05 České Budějovice, Czech RepublicPhone: +49-9137-3690412
E-mail: sylva.dresler@gmail.com
<http://actavet.vfu.cz/>

Cu/Zn-superoxide dismutase (SOD), carboxypeptidase-A, -B or DNA-, RNA-polymerase is negatively influenced by zinc deficiency (Miller and Miller 1962; Kirchgessner et al. 1975; Chesters 1992; Chahabra and Arora 1993; Underwood and Suttle 1999; Illek 2003; Chen et al. 2020).

As a part of insulin, Zn affects the activity of some hormones such as glucagon or corticotropin; that is the way in which zinc intervenes in the protein and energetic metabolism (Illek 2003).

Zinc has two most direct connections to the immune function, the role of Zn as an antioxidant, i.e., SOD and the importance of Zn to cell replication and proliferation (Droke et al. 1998; Spears and Weiss 2008; Nagalakshmi et al. 2009; Dresler et al. 2016; Chen et al. 2020; Alhussien et al. 2021). Zinc is essential for leukocyte creation and function; it has an influence on antibody creation and phagocytosis and is important in the cell apoptosis process (Droke and Spears 1993; Illek 2003). Corinthas et al. (2010) reported a positive effect of the organic source of Zn on the somatic cell count (SCC), but no effect on antioxidant enzymes – Zn/Cu-SOD, glutathione peroxidase (GSH-Px). However, Chen et al. (2020) compared four different Zn-Met treatments (0, 20, 40, 60 mg/kg of dry matter [DM]) and reported a significant increase in Zn/Cu-SOD and total antioxidant capacity in cows supplemented with > 40 mg of Zn/kg of DM compared to the control group. Similarly, Dresler et al. (2016) demonstrated the positive effect of Zn-Met on SOD activity in weaned calves.

Zinc status in dairy cows is essential in keratinization within the teat canal, so teat health is related to zinc (Andrieu 2008). Compared to traditional feeding of inorganic sources of Zn (oxide, sulphate), organically bound Zn has higher bioavailability (Wedekind et al. 1992; Kincaid and Socha 2004; Caldera et al. 2019) and can increase Zn concentration in tissues (Ivanišínová et al. 2016) and plasma or reduce SCC in milk (Cousins 2006; Illek et al. 2007; Wang et al. 2013; Neja et al. 2016). Whitaker et al. (1997) reported on the influence of Zn on immune system, finding higher SCC and mastitis rates in Zn-deficient dairy cows. Hansen (1992) reported on a zinc supplementation of 2.5 g Zn proteinate/day, which caused a great decrease of SCC of $100 \times 10^3/\text{ml}$ by herds with high SCC in average ($400 \times 10^3/\text{ml}$). Kelllogg et al. (2004) and Sobhanirad et al. (2010) experienced a reduced SCC in cows treated with Zn-Met. Nocek et al. (2006) reported similar reproductive and health performance in cows supplemented by 100% of National Research Council (NRC) 2001 requirements (Subcommittee on Dairy Cattle Nutrition, NRC, Washington, D.C.) for complexed trace minerals as the cows fed on the same elements in the inorganic form. However, milk yield and milk solid amount were higher. Krebs (1998) confirmed the increased need for Zn during lactation compared to pregnancy, especially in the first weeks p.p. The study of Kováč et al. (2007) reports that high SCC correlates to two acute phase proteins, haptoglobin and serum amyloid A in serum and milk, as a mastitis marker. Egyedy et al. (2022) confirm that cows with high SCC prior to drying off have a higher incidence of periparturient disease, associated with immunosuppression (mastitis, metritis, ketosis, retained placenta, and lameness), lower content of milk solids and milk yield than healthy cows.

Therefore, the objectives of this study were to assess the effect of organic Zn supplementation on the milk yield, milk quality (milk solids), and SCC as a mammary health indicator.

Materials and Methods

The study was conducted in a free-stall barn on a commercial farm with 400 Holstein dairy cows in VOS Zemědělců a.s., Velké Opatovice, Czech Republic. The previous lactation 305-d mature equivalent milk yield amounted to 9,247 kg up to 10,150 kg. In the last 12 months before the experiment the average SCC was $186 \pm 92 \times 10^3/\text{ml}$, milk fat $3.77 \pm 0.17\%$ and milk protein $3.30 \pm 0.23\%$.

A total of 80 late-pregnancy multiparous Holstein-Friesian dairy cows (40 cows per treatment) entering their second and third lactation (20 primiparous and 20 multiparous) were randomly allocated to one of two treatments: S-group and C-group. The experiment lasted four months (21 days prepartum up to the end of the 3rd month of lactation). A basal mixed ration for all cows included a mixture of roughage and production concentrate (Tables 1 and 2). The diet was formulated to meet NRC 2021 requirements for dairy cattle. All cows were given the same basal total mixed ration (TMR). Zinc concentration in the basal diet was 37 mg Zn/kg DM. On average 21 days prior calving, all cows of the Zn-supplemented group (S-group) were given 30 mg Zn-Met/kg DM (Bioplex® Zn, Alltech, USA) for 90 days p.p. Cows of the control group (C-group) were fed the same diet without any additional Zn source. The experimental cows were given 33–724 mg/head/d, according to the DM intake (dry off - 11.32 kg; before calving - 15.12 kg; lactation start - 20.11 kg; milk production - 24.13 kg DM TMR/day), to provide 100% of the NRC (2021 recommended supply).

Table 1. Ingredients of the basal diet.

Ingredients	kg DM/d per head	Ingredients	kg DM/d per head
Alfalfa-grass heylage	4.80	Straw	0.85
Corn silage	4.20	Wheat	1.70
CCM corn silage	0.78	Production concentrate	4.00
Lupine heylage	1.50	Melavit® (molasses + propylenglycol)	0.88
Meadow hey	1.7		

DM = dry matter, d = day, CCM = Corn-Cob-Mix

Table 2. Ingredients of the production concentrate.

Macroelements		g/kg	Trace elements		mg/kg
Calcium	Ca	15.00	Iron	Fe	194.50
Phosphor	P	9.00	Copper	Cu	62.52
Sodium	Na	16.00	Cobalt	Co	1.13
Kalium	K	14.00	Manganese	Mn	376.80
Chlorine	Cl	8.50	Zinc	Zn	395.30
Magnesium	Mg	4.80	Iodine	J	5.88
Sodium Chloride	NaCl	2.00	Selenium	Se	1.17

All health events, such as retained placenta, metritis, mastitis, ketosis or displaced abomasum were recorded in each group over the whole experimental time.

All cows were milked twice a day and the milk weight was recorded automatically every day. The evening and morning samples were preserved and analysed in the VOS laboratory (Zemědělců a.s., Velké Opatovice, Czech Republic) with the Fossomatic™ (Hilleroed, Denmark) device. The individual milk samples were taken twice a month to test milk components and the somatic cell count. Milk was sampled and analysed according to the standard procedure for milk solids (milk protein, lactose, and milk fat) determination ČSN EN ISO 8968-4 (570528), ČSN ISO 22662 (570524), ČSN ISO 488 (570517) and for SCC determination ČSN EN ISO 13366-2 (570531), the fluor-opto-electronic method for cell-counting on milk.

All obtained results were processed by a statistical analysis using the Microsoft-Office-365® Excel® software (Microsoft Corp., Redmond, USA). The different data sets were compared by two-choice paired Student's *t*-test for the median value and two-choice *t*-test with equal of unequal variance, respectively. Values of $P \leq 0.05$ and $P \leq 0.01$ were considered as significant and highly significant, respectively.

Statement by the Institutional Review Board

All procedures in study (REFNO: ES_1-2023_Illek) were following national legislation of the Czech Republic (Act No. 246/1992 Coll., on the Protection of Animals Against Cruelty, as amended) in agreement with EU legislation (Directive 2010/63/EU revising Directive 86/609/EEC on the protection of animals used for scientific purposes, as amended). All procedures were of routine agricultural practice and the Animal Experimentation Ethics Committee of the University of Veterinary Sciences Brno was informed of all procedures and agreed to the study.

Results

From the first day post calving up to the last day of 3 months trial the daily milk yield was recorded. Cows of the S-group tended to produce more milk than the control in all 3 months (Fig. 1).

The daily milk yield of cows fed the recommended Zn supply (100% NRC 2021) in the organic form was not affected ($P > 0.10$) by the treatment in 30 days post calving. In 60 days, the difference of the average milk yield of cows of the S-group was > 2 litres higher compared to control cows ($P < 0.05$). At the end of the trial, 90 days p.p., there was a highly significant difference ($P < 0.005$) in the lactation performance (8%) in the S-group as well.

The SCCs in the C-group were higher in all three months of the trial than in the cows of the S-group (Fig. 2). The difference was significant in all three months of lactation. In the first and the second month, SCC was significantly lower ($P < 0.05$) in cows receiving the organic Zn supplementation (S-group) compared to those of the C-group. In 90 days of lactation, the SCC in the organically supplemented S-group was highly significantly lower ($P < 0.001$).

Milk composition seems to be affected by the supplementation of organically bound Zn as well (Fig. 3). In the first 30 days p.p. there was no significant difference in the amount of milk solids ($P > 0.1$). However, in the second and the third month of lactation (after 60 and 90 days of trial) there was a significant difference in the protein and fat content in milk. Sixty days p.p., the amounts of milk fat ($P < 0.01$) and milk protein ($P < 0.05$) were higher in the S-group of cows, and 90 days p.p. there was a difference in average protein and fat amount (both $P < 0.01$).

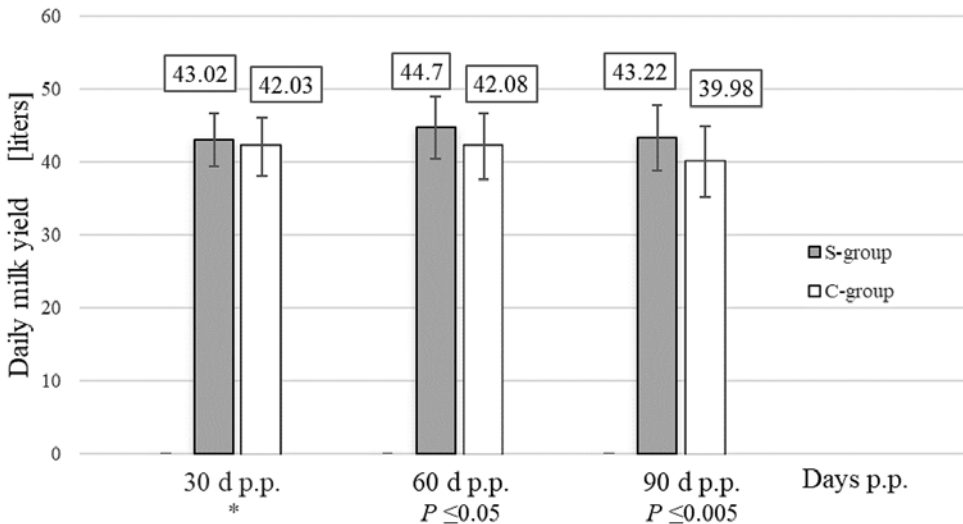


Fig. 1. Effect of zinc supplementation on milk yield over the 90-day trial period

S-group - supplemented group, C-group - control group, p.p. - post partum, P - significant difference ($P \leq 0.05$),

* - non-significant difference ($P > 0.05$)

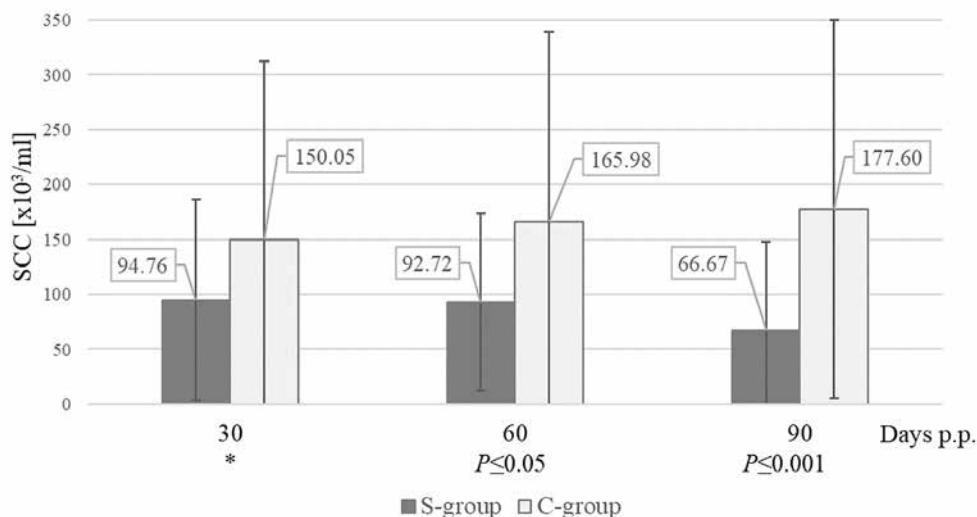


Fig. 2. Somatic cell count

S-group - supplemented group, C-group - control group, p.p. - post partum, SCC - somatic cell count, P - significant difference ($P \leq 0.05$), * - non-significant difference ($P > 0.05$)

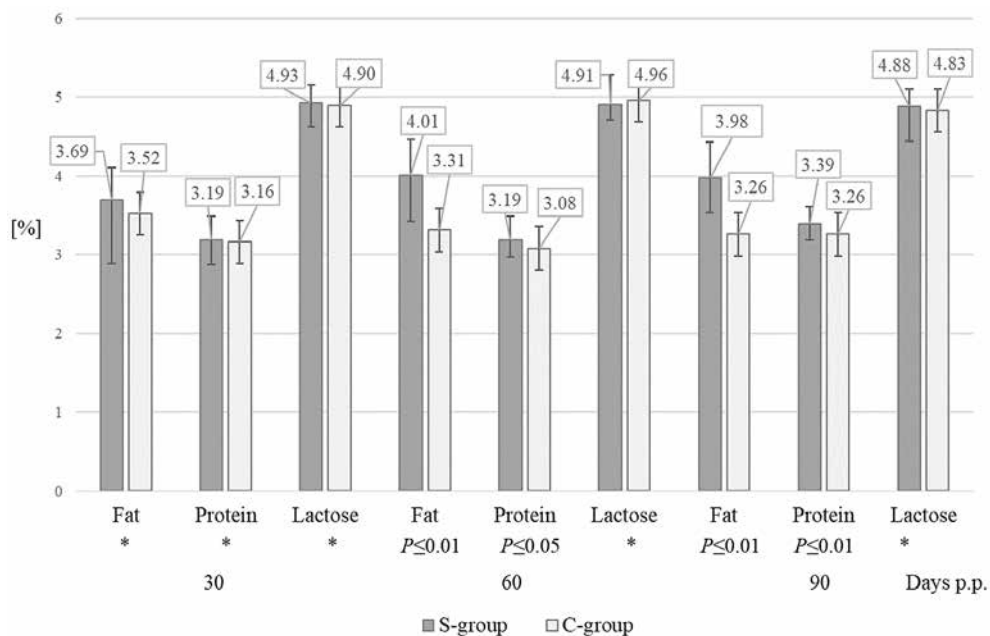


Fig. 3. Milk components in 30-, 60- and 90-days p.p.

S-group - supplemented group, C-group - control group, p.p. - post partum, P - significant difference ($P \leq 0.05$), * - non-significant difference ($P > 0.05$)

Discussion

Lactation performance seems to be positively affected by the organic Zn supplementation in most of the indicators tested in our trial. The daily milk yield in the supplemented group tended to increase during the entire experimental period. At the same time, SCC tended to decrease compared to the control group, which showed the opposite tendency in both parameters. A similar result was reported by Nocek et al. (2006) who found the SCC in cows supplemented by (100% NRC) complexed zinc source to be 46.5% lower than the SCC of cows supplemented by (100% NRC) inorganic zinc, and 47.7% lower than the SCC of cows supplemented by (75% NRC) organic zinc. In contrast, no positive effect on the milk composition and milk yield by the level or source of mineral supplementation was reported by Kincaid and Socha (2004), however, a significant increase of IgG in colostrum was reported in cows treated with organic Zn. No increase in lactation performance was observed in the study of Uchida et al. (2001). Kincaid and Socha (2004) observed higher lactation performance in peak lactation, but not in early lactation. Janů et al. (2007) reported an increase of SCC with increasing milk yield in a similar, high-yielding herd (10,000 litre/lactation) like the S-group in our trial. The decrease of SCC in the S-group may indicate a positive impact of Zn-Met on the mammary gland health. Kinal et al. (2005a) described a positive effect of organic zinc supplementation in the form of a decrease in SCC only in cows with high SCC. Singh et al. (2019) noted a beneficial effect of concurrent supplementation with Zn sulphate ($ZnSO_4$) and biotin on hoof health, milk yield, and milk solids when comparing clinically lame cows and non-lame healthy cows. The increase of milk protein and the decrease of SCC in their study could have been related to an improvement of hoof health.

According to Moore et al. (1988) and Cope et al. (2009), (30 and 50% NRC 2001, respectively), the addition of the organic form of Zn did not affect the milk composition and SCC but it did have an increasing effect on the milk yield (Cope et al. 2009). An increase in the milk yield was described by Kinal et al. (2005b) in cows treated with Zn chelates; additionally, a 20–30% reduction in SCC was observed. A positive effect of 100% NRC complexed Zn supplementation on protein and fat or milk yield was reported by Kellogg et al. (2003, 2004), Kinal et al. (2005a), Nocek et al. (2006) and Chen et al. (2020). Uchida et al. (2001) reported a higher content of milk protein after complexed Zn supplementation as well; the milk fat amount stayed unaffected. Similarly to our study, Ballantine et al. (2002) compared organic and inorganic sources of Zn, Mn, Cu and Co 21 days before calving to 250 days p.p., in order to determine the effect of these trace elements (TE) on reproduction and lactation performance in dairy cattle as well as on the incidence and severity of claw disorders. Osorio et al. (2016) confirmed the positive response in milk yield and milk protein in cows fed organic TE (Zn, Cu, Mn, Co), which could be explained by the beneficial effects of TE combination on postpartal DM intake driven by better liver and immune function because of a better antioxidant status. The organic source treatment increased the milk yield, improved all indicators of reproduction performance, and decreased claw lesions. Also, Kellogg et al. (2003) reported a positive effect of organic form of TE on reproduction performance, milk yield increase, and SCC decrease.

No significant differences were found in health indicators of both experimental groups ($P > 0.05$). Treatment by organic Zn had only a numerical effect on the incidence of health disorders. The same results were reported by Ferguson et al. (2004) and Nocek et al. (2006).

In conclusion, organic Zn supplementation at the recommended level had a significantly positive effect on the daily milk yield, milk components, such as milk fat and milk protein, and SCC of dairy cows in early lactation. The positive impact of Zn-Met on mammary gland health and milk quality is very valuable, especially at a time when reduction of antibiotics is of utmost importance in veterinary practice.

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