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Original article

Effect of organic zinc supplementation on hematological, mineral, and metabolic profile in dairy cows in early lactation

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Abstract

The aim of the current trial was to evaluate the effect of organically chelated zinc – methionin (Zn-Met) supplementation (30 mg Zn /kg DM TMR) on hematological, biochemical, and mineral profile of dairy cows in early lactation (1 - 90 d p.p.). Twenty dairy cows were randomly allocated to one of two dietary treatments in a randomized design. Animals in group C were treated as control (no zinc supplementation); whereas animals in group S were supplemented with organic Zn.

Zn-Met supplementation had a significant effect on hematological parameters. White blood cell (WBC) counts 60 days p.p. and red blood cell (RBC) count, hemoglobin concentration (HGB), hematocrit level (HCT) and platelet count (PLT) on calving day, 30th- and 60th- day p.p were significantly higher in cows fed Zn than in the control group. In calves from supplemented mothers, there was a significant increase in RBC ($p \leq 0.001$), HCT ($p \leq 0.01$) and MCV ($p \leq 0.05$).

There was no difference in other parameters among the groups, except of the highly significant difference in Zn concentration in blood serum of the S-group during the entire experimental time. The results obtained confirm the beneficial effect on serum zinc level and hematological parameters with no negative effects of 30mg Zn/kg TMR addition on mineral and biochemical parameters.

Keywords*:* trace elements, zinc, hematocrit, biochemical parameters

Introduction

The prepartal dry off and early lactation are the most difficult periods for a dairy cow. The first 100 days of lactation is the phase of maximal milk production, high-yielding dairy cows face a negative energy balance and often suffer from ketosis, which is the most economically significant production disease (Payne et al. 1973). Many dairy cows, develop typically a primary ketosis, which is a manifestation of carbohydrate metabolism disorders during 2-6 postpartal weeks. Excess or lack of protein, deficiency of trace elements and vitamins, lack of coarse fiber, imbalance between intake and energy need after birth, high content of keto-

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genic substances in the ration, all contribute to the emergence of ketosis in this critical postpartal period. At the same time, the consumption of glucose for the formation of lactose is high, the reserves are small, and there is an intensive breakdown of proteins and fats in the liver, with the production of ketone bodies, which results in degeneration of the parenchymatous organs, especially the liver (Jagoš et al. 1985, Dirksen et al. 2006). The level of mineral substances and their proportions have an impact of the prevention of production diseases in cows, which tend to be more common in older dairy cows. Furthermore, feeding of corn silage as basis of total mixed ration (TMR) of dairy cattle often face an insufficient concentration of microelements in the feed ration, which causes withdrawal conditions in highly pregnant cows, ending in negative effect on fetal development (Ballantine 2002, Kellogg et al. 2004, Cope et al. 2009, Kinal et al. 2014, Caldera 2019).

Supplemental zinc (Zn) is usually added to mixed rations of farm animal diets in two different forms. Compared to inorganic forms such as Zn-oxide or Zn-sulphate, the organic form of Zn is absorbed efficiently and retained in the tissue to enhance growth, reproduction, performance, improve health and immunity (Spears 1996, Horst et al. 2019, Alhussien et al. 2021). National Research Council (NRC 2001) recommended the level 40-60 mg of Zn/kg dry matter (DM) of total mixed ration (TMR) for dairy cows as necessary. Some researchers have reported higher bioavailability of organic Zn sources, (Zn-Met, Zn-Lysin, Zn-proteinate, Zn-amino acid chelate) than those from inorganic ($ZnSO_4$, ZnO) zinc sources (Spears 1989, Wedekind et al. 1992, Spain 1993, Cao et al. 2000, Spears et al. 2002, Byrne and Murphy 2022). The inorganic sources of trace elements are not sufficiently absorbed and retained (Spears 2003). Trace element deficiencies of zinc, copper, manganese, or cobalt have been observed for many years (Illek 1987, Kleczkowski et al. 1995, Nagalkshmi et al. 2009, Osorio et al. 2016, Hussein 2018). These minerals play very important roles in in vitamin and hormone regulation, protein synthesis, carbohydrate metabolism, tissue connectivity, immune system, appetite regulation, fertility, reproduction, or growth (Miller et al. 1988, Chesters 1997, Droke et al. 1998, NRC 2001, Chen 2005, Dirksen et al. 2006, Suttle 2010, Osorio et al. 2016, Weiss 2017, Chen 2020). Many authors demonstrate beneficent effects of zinc on cattle lactation and reproductive performance, fertility, claw integrity of dairy cattle and calves (Neathery 1973, Malcolm-Callis et al. 2000, NRC 2001, Spears et al. 2002, Kellogg et al. 2004, Kincaid and Socha 2004, Nocek et al. 2006, Griffiths et al. 2007, Siciliano-Jones et al. 2008, Machado et al. 2013, Dresler et al. 2016).

It is known from the interaction principles of individual mineral substances that zinc and e.g., iron is in an antagonistic relationship, just like zinc and copper or zinc and calcium (Hamilton et al. 1979). Because copper has a significant effect on hematopoiesis, iron, as a structural component of hemoglobin in red blood cells, is associated with the development of anemia and zinc is significantly involved in the optimal state of immunological processes in the body (Nagalakshmi et al. 2009, Żarczyńska et al. 2021), it is possible to observe how the composition of the feed ration contributes to cows health of the body (Choi and Kim 2005). It is possible to trace how the hematocrit develops as a parameter monitoring anemia (Kardawa. 2020), inflammation, dehydration, diarrhea (Brown et al. 1978, Giugliano and Millward 1984). In high-producing dairy cows, the value of hemoglobin and hematocrit is lower, on the contrary, in dehydrated cows it is increased (Jones et al. 1982). For a more objective assessment of specific cases, it would be necessary to evaluate other blood parameters in the context of the clinical examination of each animal, which is not possible within the scope of this study.

Interactions of trace elements with other minerals, enzymes, hormones are the point of interest of many studies. Calcium (Ca) is known to interact with the red cell membrane, decreasing permeability and increasing hemoglobin retention. Zinc, as an opponent of calcium, seems to counteract the retention of hemoglobin in the erythrocyte membrane. Ca may play an important role in the pathogenesis of irreversibly sickled cells, zinc may improve the filterability of sickle cells (Dash et al. 1974). The metabolism of zinc and thyroid hormones thyroxine T3, T4 and thyroid stimulating hormone (TSH) are closely interlinked (Triggiani et al. 2009, Ambooken et al. 2013). Zinc is another element that is needed for the synthesis of thyroid hormones. A zinc deficiency can lead to hypothyroidism and because thyroid hormones are required for the absorption of zinc, hypothyroidism can lead to a zinc deficiency. During thyroid hormones supplementation is profitable to take zinc supplement too (Chen et al. 2010). The formation of insoluble complexes with calcium, fiber, and phytates markedly decrease the intestinal absorption of zinc. Compared to plasma or serum zinc level, there are other tissues, where the concentration of zinc is a more reliable indicator of chronic zinc deficiency, like hair, liver or bone tissue (Gude 2011). It seems likely that zinc is essential for the biological functioning of thyroid hormone and related receptors. The active form of thyroxine – T3 can be initiated precisely by binding to the T3-receptor containing two zinc atoms in the core of its target tissue. T3 receptors lose their ability to bind to DNA, when

Table 1. Ingredient composition of total mixed ration (TMR).

Ingredients	Dry cows TMR 1 [kg]	Pre-fresh cows TMR 2 [kg]	Fresh cows TMR 3 [kg]	Lactating cows TMR 4 [kg]
alfalfa haylage	10	8	9	11
pea haylage	4	5	7	7
corn silage	6	10	14	14
corn LKS*	0	2	4	6
corn CCM*	4	3	3	3
leaven	0	3	3	3
meadow hay	3.5	1.5	2	2
feed straw	3	1	1	1
concentrate	1	3.5	4.5	7.5
pea corn	0	0.3	0.5	0.5
barley groats	0	2	2	3
Mineral mix *Table 1a	0	0.16	0.16	0.16
Monofos Ca-P	0.25	0	0	0
feed sugar	0	0	0	0.3

* CCM = corn cob mix, LKS = Liesch Kolben Silage

zinc is removed by chelation (Miyamoto et al. 1991, Freake et al. 2001). Cebulska et al. (2021) investigated the extend of heavy metal contamination of the environment by determination of concentrations of heavy metals (selenium, zinc, copper, and cadmium) in the liver of selected wild animal species (red fox, wild boar, red deer) in Poland and reported about not exceeded standard safe values for consumers.

Yokus and Cakir (2006) reported seasonal and physiological variations of copper, zinc, magnesium (Mg), iron (Fe), sodium (NA), chlorine (Cl), potassium (K), calcium (Ca), phosphorus (P), urea, alkaline phosphatase (ALP), creatinine (CR), aspartate aminotransferase (AST), alanine aminotransferase (ALT), cholesterol, albumin, globulin, lactate dehydrogenase (LDH), and total protein (TP) concentrations in cattle. They have evaluated, that some parameters differ according to physiological and seasonal conditions like ALP, copper, magnesium, and potassium concentrations, copper concentration is increased through the pregnancy, other parameters are not influenced neither by the seasonal nor the physiologic variations (zinc, iron, sodium, chlorine, calcium, urea, creatinine, albumin, and globulin values).

The aim of the current study was to evaluate the effect of supplementing Zn-Met source on serum Zn, Cu, Mn, Se, Fe status, mineral profile (Na, K, Ca, P, Mg), hematological and biochemical parameters in dairy cows in early lactation and their newborn calves, to ascertain the utility of these parameters, as a biochemical marker to determine the bioavailability of Zn from organic source Zn-Met.

Materials and Methods

Institutional Review Board Statement

All study procedures were approved in accordance with the "Act on the protection of animals used for scientific purposes" of the Czech Republic. This act is in accordance with the EU Directive (No. 2010/63/EU) on the protection of animals used for scientific purposes and with the decision of the Ministry of Agriculture of the Czech Republic, No. 22036/2019-MZE-18134.

Experimental design

The experiment was carried out in the VOS agricultural farm in Velké Opatovice, Czech Republic in accordance with the Animal Protection Act No. 246/1992 Coll. Twenty Holstein-Friesian dairy cows (10 animals in a group) were randomly divided into one of two groups according to productivity, age and number of lactation – supplemented (S) and control (C) group for the experimental period from day 21 ante partum (a.p.) until the end of the third month of lactation, 90th day post-partum (p.p.). Holstein cows were placed in free group housing of 40 animals after calving due to the same metabolic needs under the same microclimatic conditions. the animals of the two balanced groups were kept in groups. During the lactation period, there were 40 dairy cows in one group. All animals had ad libitum access to the mixed feed ration (TMR). The base TMR contained 37 mg Zn/kg DM. The exact

Table 1a. Composition of Mineral mixture for cattle in 1 kg; DM = 95%.

Calcium [%]	19.5	Copper [mg/kg]	460	Iodine [mg/kg]	15
Phosphorus [%]	10	Zinc [mg/kg]	470	Vitamin A [IU/kg]	800000
Sodium [%]	4.8	Manganese [mg/kg]	480	Vitamin D3 [IU/kg]	80000
Magnesium [%]	5	Cobalt [mg/kg]	57	Vitamin E [IU/kg]	8000

Mikrop M®; Mikrop Čebín, Czech Republic

Table 2. Nutrient composition of ad libitum - TMR in individual periods of the experiment.

		Dry off	Pre-fresh cows	Fresh cows	Lactating cows I.
Dry matter	g	11320.0	15120.0	20112.0	24132.0
Crude protein	g	1472.0	2088.0	4132.0	4382.0
PDI - N	g	826.0	1356.0	2488.0	2574.0
PDI - E	g	721.1	1204.0	2412.0	2612.0
Crude fat	g	245.2	312.2	885.3	988.3
Crude fiber	g	3135.0	2865.8	3365.0	3268.0
Starch	g	1523.0	2516.0	4472.2	4922.3
Non-fiber Carbohydrate	g	122.0	622.3	1021.0	1132.0
NEL	MJ	58.1	90.3	169.3	178.6
Crude Ash	g	926.0	954.6	1785.0	1836.0
Calcium Ca	g	28.08	31.15	57.08	62.15
Phosphorus P	g	14.55	20.53	28.13	30.40
Sodium Na	g	4.58	7.40	20.10	23.20
Potasium K	g	46.55	56.15	82.20	90.50
Magnesium Mg	g	8.28	12.15	18.65	18.83
Copper Cu	mg	128.00	147.15	216.30	221.55
Manganese Mn	mg	405.75	533.00	608.00	658.00
Zinc Zn	mg	420.50	534.00	741.25	809.00
Selenium Se	mg	3.03	3.58	4.60	4.88
Iodine I	mg	4.08	9.05	13.53	13.83
Vitamín A	IU	45.75	58783.00	80363.00	91.33
Vitamín D	IU	804.00	10281.25	16130.25	16714.00
Vitamín E	IU	165.00	309.00	316.25	330.25
Niacin	mg	783.50	1581.25	2466.25	2582.00

ingredient composition is showed in Table 1 and Table 1a, respectively.

The nutrient composition was count according to the nutritional requirements of each stage of gravidity or lactation, respectively, due to dry matter intake (Table 2).

Supplementation with an organic form of zinc

The supplementation plan is showed in Table 3, the trial lasted from the 21st day before the planned parturition up to 90th day post partum (p.p.). The experimental group was fed organic zinc bound to the amino acid methionine (Zn-Met) (BIOPLEX Zn® Alltech, USA) at a dose of 30 mg per kg of dry matter (DM) of TMR. The dosage of 1.5% - premix of Zn-Met was controlled by the amount of dry matter received in individual periods (Table 3.).

Blood sample collection and analysis

For the hematological analysis venous blood samples were collected at the beginning of the trial, at the day of calving, at 30th, 60th day post partum (p.p.) and at the end of the Zn-Met addition, on 90th day p.p. The newborn calves were involved in the trial on the

Table 3. Plan of Zn-Met supplementation in individual periods of the experiment.

	Dry matter intake	Zinc	Premix	Premix per Group
	kg/head/day	mg/head/day	g/head/day	g/10 heads/day
Dry off	11.32	340	22.7	227
Pre-Fresh Cows	15.12	454	30.3	303
Fresh Cows	20.11	604	40.3	403
Lactating Cows I	24.13	724	48.3	483

Premix Zn-Met = 1.5% = 15000 mg Zn/kg; Supplementation = 30 mg Zn / kg DM TMR

day of birth, during the first 2 hours after birth, before the first drinking of colostrum. The median caudalis vein was chosen for blood sampling in mothers, the jugular vein in newborn calves. The blood collection for the biochemical and mineral profile was performed on 21st day ante partum (a.p.), 30th, 60th, 90th and 120th day p.p. (one month after Zn-Met addition). For hematological examinations plastic sample tubes with an integrated needle - 27 mm length, Ø 1.2 mm (Hemos®-type II; Gama Group a.s., Brno, Czech Republic) were used, including 0.08 ml anticoagulant (Heparin-Natrium-5000-ratiopharm®). For biochemical and mineral examinations, the second blood sample was taken using tubes without additives for serum.

Hematology

In the laboratory of the Department of Animal Morphology, Physiology and Genetics at Mendel University in Brno, Czech Republic, following hematological parameters were measured in blood samples of the whole blood on the Element HT5 device (Scil animal care company GmbH, Viernheim, Germany): the number of erythrocytes (RBC), leukocytes (WBC) and platelets (PLT), hemoglobin concentration (HGB), hematocrit value (HKT) and erythrocyte indices (MCV, MCH, MCHC). Three analytic methods are used for 15 µL of aspired heparinized full blood: laser-assisted flow cytometry for the detection of WBC, the impedance method for the detection of RBC and PLT, and the colorimetric measurement for HGB.

Blood chemistry and mineral parameters

Blood samples were centrifuged at a speed of 3000/min for 10 minutes. Serum was separated and frozen at a temperature of -20°C to collect the minimum number of samples required for the biochemical sets of the Roche Hitachi 917 Disk Chemistry Analyzer (F. Hoffmann-La Roche; Basel, Switzerland) in the laboratory of VETLABFARM s.r.o., Brno, Czech Republic. The device works on the principle of two-stage spectrophotometric analysis. The samples are placed in the five-position racks of the analyzer reservoirs. Measuring cuvettes are placed in a circle in a water bath in 37°C. Automated stations suck the reaction mixture from the individual cuvettes, the sample and reagent are mixed for photometry. The following selected parameters were tested: total protein (CB), albumin (ALB), urea (UREA), glucose (GLU), bilirubin (BIL), alkaline phosphatase (ALP), aspartate-aminotransferase (AST), gamma-glutamyl--transferase (GMT or GGT) and thyroxine (T_4). The macroelements sodium (Na), potassium (K), calcium (Ca), phosphorus (P), magnesium (Mg) and the microelements zinc (Zn), copper (Cu), manganese (Mn), iron (Fe) and selenium (Se) were determined by the method of atomic absorption spectrophotometry (AAS).

Data analysis

Raw data were statistically analyzed using Microsoft EXCEL® (Microsoft Office, Microsoft Corporation, Washington, USA) and Statistica™ (Dell Software,Inc., Round Rock, USA); 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, $p \leq 0.01$ and $p \leq 0.001$ as highly significant.

Results

Hematological profile

Total mathematical-statistical characteristics of the hematological values studied are given in Table 4. Hematological parameters RBC ($p \leq 0.001$), HCT ($p \leq 0.01$) and MCV ($p \leq 0.05$) of newborn calves showed significantly higher values in the experimental (S) group. The number of RBC of the control (C) group was outside the physiological range. Its sublimit level is related to the too low value of HGB, HCT, PLT and all erythrocyte indices. The supplemented group of calves does not show these features of microcytic hypochromic anemia just after birth. Zinc deficiency in pregnant cows can lead to suboptimal provisioning of newborn calves, resulting in a higher propensity

Table 4. Hematological parameter in calves and their mothers.

Parameter/Unit/ Physiological range			Cows 21 d a.p.	Calves Day of Birth	Cows Partrus	Cows 30 d p.p.	Cows 60 d p.p.	Cows 90 d p.p.
WBC [10^3/mm^3] <5.0;10.0> cows <4.0;12.0> calves	S-group	x	5.86	9.34	6.54	8.18	7.95	5.49
		SD	0.91	2.26	3.00	2.52	0.75	0.78
	C-group	x	5.81	7.34	8.35	9.58	5.95	5.82
		SD	0.74	2.85	2.83	2.31	0.86	1.29
		p	*	*	*	≤0.05	≤0.01	*
RBC [10^6/mm^3] <5.0;10.0>	S-group	x	6.48	7.05	6.20	6.22	6.34	4.41
		SD	0.56	1.26	0.44	0.97	0.52	0.52
	C-group	x	6.50	4.78	5.47	5.40	4.81	4.37
		SD	0.48	1.17	0.49	0.67	0.31	0.35
		p	*	≤0.001	≤0.01	≤0.05	≤0.001	*
HGB [g/dl] <9.0;14.0>	S-group	x	11.08	9.38	10.60	10.16	10.12	9.09
		SD	0.54	1.30	0.68	0.68	0.68	0.66
	C-group	x	11.11	8.25	9.53	9.81	9.29	8.86
		SD	0.49	1.35	1.00	0.88	0.78	1.37
		p	*	*	≤0.01	≤0.01	≤0.05	*
HCT [%] <28.0;38.0>	S-group	x	32.61	29.28	30.56	29.47	27.98	22.32
		SD	2.11	5.29	1.77	2.50	2.24	1.97
	C-group	x	33.05	22.12	25.52	26.17	21.96	21.22
		SD	1.98	5.85	1.33	1.81	1.81	2.41
		p	*	≤0.01	≤0.001	≤0.01	≤0.01	≤0.01
PLT [10^3/mm^3] <300;800>	S-group	x	361.25	321.90	353.10	320.20	324.30	416.00
		SD	61.23	75.95	111.77	65.94	58.82	68.85
	C-group	x	370.29	309.08	233.33	296.37	296.27	340.00
		SD	73.07	71.14	99.74	61.78	48.22	105.91
		p	*	*	≤0.01	≤0.05	≤0.05	*
MCV [fl] <46.0;65.0>	S-group	x	51.37	41.50	49.42	53.40	44.26	48.10
		SD	2.43	1.64	4.25	3.03	3.70	4.62
	C-group	x	50.95	39.40	47.10	48.25	45.64	44.02
		SD	2.09	2.30	2.94	2.69	2.62	2.80
		p	*	≤0.05	*	≤0.01	*	≤0.05
MCH [pg] <11.0;17.0>	S-group	x	14.69	13.43	17.12	23.80	16.04	14.85
		SD	1.27	0.81	1.25	1.78	1.30	1.57
	C-group	x	15.06	15.41	17.91	22.11	19.35	15.85
		SD	2.01	3.37	2.54	1.56	1.05	1.35
		p	*	*	*	*	≤0.001	*
MCHC [g/dl] <31.0;34.0>	S-group	x	32.56	32.34	34.68	30.87	36.30	42.80
		SD	0.98	2.29	1.72	0.58	0.72	2.74
	C-group	x	32.09	29.32	37.20	34.56	42.36	45.88
		SD	1.65	1.32	4.12	2.67	0.66	2.35
		p	*	*	*	≤0.001	≤0.001	≤0.05

* Not significant difference ($p \geq 0.05$); *p* = significant difference ($p<0.05$); S = supplemented; C = control; x = mean value; SD = standard deviation WBC = white blood cells; RBC = red blood cells; HGB = hemoglobin; HCT = hematocrit; PLT = platelets; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration.

for respiratory disease during the period of postnatal adaptation to a new environment.

In comparison with the hematological parameters of cows of the control group on the day of delivery, which are in the physiological range (WBC, RBC, HGB, PLT, MCV), the hematocrit value of the control group is too low and even significantly lower ($p \leq 0.01$) than the value of the experimental group, which is normal on this day. RBC, HGB, HCT and PLT values show statistically significantly higher levels in the zinc-supplemented group in the next 90 days, i.e., for the entire rest of the trial period.

Thirty days p.p., the C-group showed lower values of RBC ($p \leq 0.05$), HGB ($p \leq 0.01$), HCT ($p \leq 0.01$), PLT ($p \leq 0.05$), MCV ($p \leq 0.01$) and MCHC ($p \leq 0.01$). HCT was below the reference minimum and HGB was within the norm at the same time in the C-group. While the WBC level of the experimental group is within the norm, the control group is close to the upper limit of the physiological range.

On 60^{th} day p.p., the hematological values of the C-group dairy cows return to the reference optimum. The HCT value of the S-group is significantly higher ($p \leq 0.01$) compared to the control animals, which are below the reference minimum. In our experiment, two months after delivery, we also found significantly higher values in HGB ($p \leq 0.05$), PLT ($p \leq 0.05$), MCH ($p \leq 0.001$) and MCHC ($p \leq 0.001$). WBC were measured higher in S-group than those of the control ($p \leq 0.01$). Both groups were within the reference range.

In the last month of the trial, 90 days p.p., many parameters were decreased under the reference limit – RBC, HCT, or at the lowest limit – WBC, HGB in both groups. HCT was significantly higher in S-group ($p \leq 0.01$), MCV as well ($p \leq 0.01$), on contrary, MCHC was currently lower ($p \leq 0.05$).

Biochemical profile and Mineral profile (Table 5 and 6)

The concentrations of total protein in blood serum were at the same level in both groups throughout the entire experimental period, no significant differences between groups were found. The lowest value was measured 21 d a.p., the highest on the day of calving, and then the values of both groups slowly decreased.

Twenty-one days a.p. and 30 days p.p. there is a significant increase in blood urea in Zn-Met group ($p \leq 0.05$). Four months p.p., both groups showed values above the physiological maximum, which can be related to the high content of crude protein in the ration. On the day of birth there was a significant increase in Zn ($p \leq 0.01$) and Mg concentration ($p \leq 0.05$) in blood serum in S-group. 30 days p.p. there was a significant reduction of K ($p \leq 0.01$) and Mn ($p \leq 0.05$) levels and an increase in the Zn level in S-group. 60 days p.p. Zn was significantly higher ($p \leq 0.001$) in S-group. 90 days p.p. there were shown three statistically relevant differences in Zn ($p \leq 0.001$), P ($p \leq 0.05$) and Mn ($p \leq 0.001$) concentration. At the end of the experiment there was an increase in Zn levels ($p \leq 0.001$) and decrease of Ca concentration ($p \leq 0.05$) in the S-group.

Discussion

All measured biochemical and mineral parameters (Table 5, Table 6) correspond to the physiological levels of these parameters in the period around calving and early lactation during the first three postpartal months. Kovačević et al. (2021) reported in their study dispersion of reference intervals for hematological, biochemical, and mineral parameters in cows in early lactation and a great range and magnitude of intraindividual variation in healthy individuals. Some parameters showed large intraindividual variation probably due to metabolic rearrangement and stress response in early lactating cows. Metabolic rearrangement is characterized by lipolysis and increased hepatocyte load (AST, ALT, GGT), and stress leads to a change in the leukogram of cows (WBC). High variability for these parameters may be a consequence of magnitude change in the value of these parameters in the weeks after calving. Macro-elements (Ca, P and Mg) showed low intraindividual variation in the study of Kovačević et al. (2021), because of homeostatic mechanisms after calving maintain their concentration in a narrow range of values.

The direct relationship between zinc concentration in blood serum and hematological parameters was subjected to correlation analysis. A direct linear relationship (r_{xy}=Zn;HCT)) was found between Zn levels and HCT $r=0.281$. The correlation coefficient between serum Zn concentration and RBC was recorded as $r=0.210$, which is just above the established minimum limit ($r = 0.2$). A direct linear dependence could be observed in the case of the effect of Zn concentration on the amount of hemoglobin $r=0.465$. For the other hematological parameters, the correlation coefficient was lower than $r=0.2$, so neither a direct nor an indirect relationship was confirmed. Šrejberová et al. (2006) and Hussein (2018) observed the correlation dependence of zinc and copper concentration in blood plasma on hematological parameters of sheep and cattle. She concluded that direct and indirect linear dependencies were found in dairy cattle and less so in sheep, mainly in cases where one of the mineral substances is in deficit or excess in the body. Hussein (2018) tested in 75 sheep with clinical signs of zinc and copper deficiency in Iraq and compared them to the control without

Table 5. Biochemical parameters in prepartal dairy cows and during early lactation.

Parameter/Unit/ Physiological range			21d a.p.	partus	30d p.p.	60d p.p.	90d p.p.	120d p.p.
TP [g/l] <60;80>	S-group	x	69.09	76.53	73.04	72.59	74.17	74.34
		SD	2.49	1.99	1.28	1.12	1.19	3.10
	C-group	x	67.25	76.30	73.16	73.45	74.51	74.83
		SD	3.04	2.62	1.48	1.21	0.92	1.54
		p	*	*	*	*	*	*
ALB [g/l] <30;40>	S-group	x	34.36	35.04	33.63	33.22	33.53	33.35
		SD	1.07	0.74	0.47	0.77	0.59	1.46
	C-group	x	34.20	34.51	33.77	33.83	33.82	33.44
		SD	1.00	0.99	0.51	0.70	0.50	0.78
		p	*	*	*	*	*	*
UREA [mmol/l] <3.3;5.0>	S-group	x	3.83	3.46	4.45	5.11	5.03	5.28
		SD	0.19	0.22	0.32	0.20	0.31	0.37
	C-group	x	3.77	3.32	4.24	5.35	5.14	5.45
		SD	0.19	0.23	0.21	0.28	0.35	0.34
		p	*	*	≤ 0.05	*	*	*
GLU [mmol/l] <2.2;3.3>	S-group	x	3.72	3.96	3.09	2.99	3.11	3.13
		SD	0.15	0.15	0.09	0.10	0.08	0.04
	C-group	x	3.58	4.17	3.12	2.98	3.15	3.19
		SD	0.24	0.41	0.13	0.09	0.09	0.03
		p	*	*	*	*	*	≤ 0.01
BIL [μmol/l] ≤ 8.5	S-group	x	3.56	5.96	4.13	3.98	3.71	3.29
		SD	0.39	0.57	0.36	0.21	0.47	0.42
	C-group	x	3.37	5.61	3.73	3.66	3.85	3.65
		SD	0.45	0.98	0.38	0.45	0.26	0.31
		p	*	*	≤ 0.05	≤ 0.05	*	*
AST [μkat/l] ≤1.334	S-group	x	1.38	1.46	1.49	1.52	1.38	1.43
		SD	0.04	0.04	0.07	0.08	0.26	0.05
	C-group	x	1.39	1.47	1.52	1.57	1.39	1.42
		SD	0.04	0.03	0.07	0.07	0.03	0.04
		p	*	*	*	*	*	*
GGT [μkat/l] ≤0.834	S-group	x	0.24	0.26	0.38	0.40	0.34	0.34
		SD	0.03	0.03	0.09	0.07	0.05	0.04
	C-group	x	0.25	0.31	0.42	0.42	0.40	0.39
		SD	0.05	0.04	0.07	0.06	0.05	0.03
		p	*	≤ 0.01	*	*	≤ 0.01	≤ 0.001
ALP [μkat/l] ≤5.0	S-group	x	0.36	0.40	0.60	0.63	0.52	0.53
		SD	0.07	0.07	0.10	0.10	0.07	0.05
	C-group	x	0.34	0.38	0.56	0.67	0.58	0.59
		SD	0.05	0.05	0.10	0.07	0.10	0.08
		p	*	*	*	*	*	*
T_4 [nmol/l] <49;106>	S-group	x	78.09	78.85	72.78	63.44	47.94	39.69
		SD	7.16	4.79	6.22	3.94	8.43	4.23
	C-group	x	83.78	83.60	72.34	61.89	45.27	37.88
		SD	8.70	4.11	3.74	5.98	6.61	3.66
		p	*	≤ 0.05	*	≤ 0.05	*	*

* Not significant difference; *p* = significant difference ($p<0.05$); S = supplemented; C = control; x = mean value; SD = standard deviation; a.p. = ante partum; p.p. = post-partum; TP = total protein; ALB = albumin; BIL = bilirubin; AST = aspartate aminotransferase; GGT = gamma glutamyl transferase; ALP = alkaline phosphatase; T4 = thyroxin.

Table 6. Mineral profile in prepartal dairy cows and during early lactation.

Parameter/Unit/ Physiological range			21d a.p.	partus	30d p.p.	60d p.p.	90d p.p.	120d p.p.
Na [mmol/l] <135;150>	S-group	x	143.50	148.10	144.20	144.40	145.50	144.60
		SD	2.74	1.62	1.53	1.82	1.67	2.14
	C-group	x	143.20	146.90	144.40	144.60	145.10	143.60
		SD	2.44	2.54	2.01	1.87	1.78	1.82
		p	*	*	*	*	*	*
K [mmol/l] <4.0;5.0>	S-group	x	4.43	4.30	4.36	4.50	4.54	4.53
		SD	0.16	0.12	0.09	0.07	0.12	0.20
	C-group	x	4.36	4.29	4.52	4.52	4.50	4.56
		SD	0.13	0.12	0.10	0.13	0.14	0.10
		p	*	*	≤ 0,01	*	*	*
Ca [mmol/l] <2.0;3.0>	S-group	x	2.40	2.12	2.30	2.36	2.37	2.37
		SD	0.07	0.12	0.05	0.02	0.05	0.03
	C-group	x	2.49	2.13	2.32	2.35	2.38	2.40
		SD	0.06	0.11	0.05	0.04	0.03	0.03
		p	*	*	*	*	*	≤ 0,05
P [mmol/l] <1.6;2.3>	S-group	x	1.95	1.82	2.04	2.02	2.05	2.08
		SD	0.07	0.08	0.05	0.06	0.04	0.06
	C-group	x	1.96	1.86	2.06	2.04	2.07	2.07
		SD	0.09	0.06	0.11	0.07	0.05	0.06
		p	*	*	*	*	≤ 0,05	*
Mg [mmol/l] <0.70;1.77>	S-group	x	0.97	0.93	0.97	0.95	0.98	1.02
		SD	0.08	0.05	0.03	0.03	0.04	0.05
	C-group	x	0.96	0.91	0.98	0.94	0.96	1.00
		SD	0.05	0.04	0.05	0.02	0.04	0.05
		p	*	≤ 0,05	*	*	*	*
Zn [µmol/l] <12.2;26.0>	S-group	x	10.28	11.09	13.34	14.38	14.76	15.15
		SD	1.23	0.80	0.78	0.78	0.91	0.89
	C-group	x	10.39	9.56	11.21	11.30	11.02	10.70
		SD	1.17	0.92	1.21	1.06	1.13	1.04
		p	*	≤ 0,01	≤ 0,001	≤ 0,001	≤ 0,001	≤ 0,001
Cu [µmol/l] <8.0;39.0>	S-group	x	12.04	11.21	13.15	13.01	13.29	12.62
		SD	0.98	0.86	0.91	0.35	0.57	0.88
	C-group	x	11.72	11.05	12.99	13.00	13.09	13.10
		SD	0.82	0.87	0.83	1.01	0.81	1.05
		p	*	*	*	*	*	*
Mn [µg/l] <7.0;19.0>	S-group	x	8.34	8.25	8.04	8.61	8.55	9.91
		SD	0.91	0.53	0.30	0.68	0.59	1.28
	C-group	x	8.33	8.07	8.32	9.01	10.12	10.60
		SD	1.28	0.92	0.39	0.63	1.08	1.21
		p	*	*	≤ 0,05	*	≤ 0,001	*
Se [µg/l] <92;247>	S-group	x	105.59	99.02	103.17	104.03	102.89	105.99
		SD	10.63	5.44	6.87	8.70	9.07	7.75
	C-group	x	102.47	92.67	102.13	103.53	102.69	105.21
		SD	13.69	8.15	8.98	10.11	7.33	7.85
		p	*	*	*	*	*	*
Fe [µg/l] <92;247>	S-group	x	25.16	19.38	20.94	21.69	23.14	24.38
		SD	1.17	0.87	0.61	1.11	1.26	0.79
	C-group	x	24.92	18.76	21.05	22.46	23.04	23.95
		SD	1.09	0.63	0.82	1.08	1.17	0.68
		p	*	*	*	*	*	*

* Not significant difference; *p* = significant difference ($p<0.05$); S = supplemented; C = control; x = mean value; SD = standard deviation; a.p. = ante partum; p.p. = post partum; Na = sodium; K = potassium; Ca = calcium; P = phosphorus; Mg = magnesium; Zn = zinc; Cu = copper; Mn = manganese; Se = selenium; Fe = iron.

symptoms. The hematological parameters showed significant decrease ($p<0.05$) in RBC, HGB, HCT, and no difference in MCV and MCHC, which means normocytic normochromic anemia. The same effect was evaluated in our study too. In his study, Kincaid (1999) evaluated the differences between methods for diagnosing microelement deficiencies in ruminants and concluded that metallothionein or RBC concentration in the blood is a suitable indicator for zinc deficiency, as their levels hardly change during infection. As other indicators of zinc saturation, he mentions the concentration of zinc in lymphocytes, neutrophils, granulocytes, or blood platelets.

Considering that zinc is contained in the blood as part of enzymes in erythrocytes, platelets and is bound to albumin and globulin (Milne et al. 1985, Underwood et Suttle 1999), the values of HGB, HCT, RBC and PLT should be higher in the supplemented group. Our results confirm this thesis. Compared to the S-group, hypochromic hypovolemic anemia was observed in newborn calves of the C-group. Zinc deficiency in pregnant cows can lead to suboptimal provisioning of newborn calves, resulting in a higher propensity for respiratory disease during the period of postnatal adaptation to a new environment (Kalaeva et al. 2020). Significantly higher values of RBC, HGB, HCT and PLT of cows of S-group on the day of parturition, 30th, 60th day p.p. and demonstrably higher values of RBC and HCT in newborn calves of Zn-supplemented mothers confirm the results of several earlier studies (Sobhanirad, Naserian et al. 2012, Mattioli et al. 2019). The HCT value is highly positively correlated with the HGB concentration (Šoch et al. 2011), which also corresponds to our results. Cope et al. (2009) observed the effect of zinc chelate supplementation on the same hematological parameters of cows as our experiment but did not find any demonstrable difference in the RBC and WBC among four groups supplemented for 14 weeks with a low or NRC (2001) recommended dose of Zn in organic and inorganic form. Kleczkowski et al. (1995) in his work captures the negative effect of insufficient supply of zinc and copper to the body on the number of red blood cells, hemoglobin concentration and hematocrit value of cows and calves. Šoch et al. (2010) deals with the relationship between microelements and hematological parameters in cows. According to the experimental results of their observation, not only the concentration of zinc and copper in the blood plasma depends, but also the corresponding hematological parameters fluctuate depending on the altitude, the health status of the organism, the content of microelements in the ration, race, age, sex of animals and climatic conditions of breeding. They do not confirm the assumption that the concentration of hemoglobin will rise in proportion to increasing altitude. The highest concentration of zinc and hemoglobin in the blood of Holstein cows was measured at an altitude of 550 m above sea level.

Application of Zn-Met significantly increased the level of bilirubin in S-group on 30th and 60th d p.p. There was no directly positive effect of Zn addition manifested on total protein, albumin, GLU concentration and AST, GGT, ALP activity in serum, throughout the entire experimental period. 120 d p.p. there was a decrease of liver enzymes activity of AST, ALP and GGT. ALP was higher, as expected, in S-group in the first 3 months of the trial, then in the C-group on 60-120th d p.p., but not significantly, which did not confirm the expected impact of Zn-Met supplementation. Nagalakshmi et al. (2099) reported increased ($p<0.01$) alkaline phosphatase (ALP) activity on 75 d of trial in Zn proteinate-supplemented lambs with 15 ppm Zn added to basal diet containing 29.28 ppm Zn.

The positive correlation of Zn and Mg confirmed significant increase of Mg ($p\leq0.05$) at 0 days p.p. in S-group. The Zn-Met supplementation had significant influence on Zn level in blood serum ($p\leq0.01$, $p\leq0.001$), observed in S-group from parturition up to the end of the experiment. The increasing trend of Zn concentration in S-group corresponds to increasing Zn-Met supplementation according to DM intake in early lactation. The other studies reported positive effect of organic Zn addition on Zn serum concentration (Kinal et al. 2011, Sobhanirad et Naserian 2012, Dresler et al. 2016). There were also significant changes in K, Mn, and P levels. There was a significant decrease in Ca concentration ($p\leq0.05$) and Zn increase ($p\leq0.001$) in S-group on 120th d p.p. The Ca decrease may be related to the known antagonism of Ca:Zn. The other elements (Na, Cu and Se) demonstrated no changes.

Conclusions

It was confirmed in the present study, that organic zinc supplementation (Zn-Met) at the recommended level (NRC 2001) has a significantly positive effects on Zn level in blood serum, hematological parameters RBC, HCT, HGB and PLT in calves and cows during the entire experimental period. The biochemical and other mineral levels were not significantly affected.

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