

Dietary effects of zinc, copper and manganese chelates and sulphates on dairy cows

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Abstract

The experiment was carried out on 90 dairy cows in the period from 6 weeks before calving up to 305th day of lactation. The animals randomly divided into 3 groups were fed with diets containing different chemical forms of zinc, manganese and copper applied in mineral mixtures. The control group received these trace elements as sulphates, but in experimental groups 20 or 30% of daily needs were covered by chelated forms. Cows receiving 30% of needed Zn and Mn (each 315 mg/day) and Cu (63 mg/day) in amino acids-bound forms were characterized by higher (6.5%) milk production than the control animals. In these cows also a decrease of somatic cell contents in milk (34%) was observed. The application of chelated forms of trace elements to the diets given to animals affected positively the total immunoglobulins amount in colostrum and in blood serum of new-born calves. The significant ($P \leq 0.05$) increase of both zinc and copper levels in blood of cows receiving organically-bound Zn, Mn and Cu was stated.

Key words: Dairy cows, zinc, copper, manganese, chelates, milk, blood, serum, colostrum, calves.

Introduction

The nutritional support of high yielding cows depends not only on precisely balanced basic nutrients but also on minerals, which contents in feeds vary in particular years and depend on soil type and harvest time^{5,6}. The important factor, that affects both the absorption and utilization of trace elements in the metabolic pathways, is their chemical form. In respect to many biological and biochemical functions of zinc, the interest on this trace element is growing dynamically. In many experiments the effectiveness of zinc utilization from oxides and chlorides as well as from complexes with amino acids and proteinate was estimated. However, the data obtained in these studies are unclear. When rations given to chickens were deficient in zinc, the absorption of this element from Zn-methionine or soya proteinate was higher by about 17 or 77 %, respectively, than the absorption from zinc sulphate³⁵. From a research on sheep author concluded that absorption of zinc was similar from Zn-AA and zinc oxide²⁵. In an experiment on dairy cows²⁴ where diet used was rich in corn, the bioavailability of zinc calculated in relation to ZnSO₄ (100%) reached 61% from ZnO and even 206% from Zn-AA. Also Close⁸ stated in an experiment on pigs that copper was absorbed better from Cu-lysine than from CuSO₄. Baker and Ammerman² found that copper from chelates or other organic compounds was better absorbed by animals than from inorganic salts. Spears²⁵, Campbell et al.⁷, Olson et al.¹⁹ and Uchida et al.³³ in the experiments on dairy cows obtained similar effects, but concerning other trace elements.

Increased share of HF in dairy cows genotype and increased milk yield require consideration of these facts in precise composing of feeding rations in context of their mineral composition. The market of mineral additives (minerals and mineral-vitamin mixtures) is going to be enlarged and various forms of offered trace element complexes are used. Taking into account that the data obtained in experiments performed on cows^{4,15-18,26} not always is unequivocal and shows differences in absorption

of trace elements from organo-metal compounds, we decided to make another experiment. The purpose was to compare the effect of Zn, Cu and Mn from sulphates and organo-metal compounds applied in high milking cow rations on productivity, composition and quality of milk and colostrum, as well as on biochemical indices of blood serum of cows and their calves.

Materials and Methods

The experiment was performed on 90 dairy cows of Black-and-White breed upgraded with 50-70% of HF. The average body weight of cows was 620±40 kg and average milk yield in the last lactation about 6,500 kg. The animals were kept on straw in the stanchion barn belonging to the Agricultural Experimental Station of Smardzów Wrocławski. The experiment was commenced in dry period of cows (6 weeks before calving) and was continued up to 305th day of lactation. The cows were successively divided into three experimental groups (30 animals in each) according to the calving date (from January to June), percentage share of HF genes (50-70%), sequence of lactations and earlier yield of milk. Each group of animals chosen by analogue method consisted of ones in 1st lactation (8 heifer-cows), 2nd - 3rd lactation (11 cows) and 4th - 7th lactation (11 cows). The cows were milked twice a day. All cows were fed with the same diets in summer and winter time (Table 1). The cows producing more than 25 kg of milk/day were fed additionally with 1 kg of concentrate per each 2 kg of surplus milk.

At the beginning of the experiment and during it the basic chemical analysis of feedstuffs was made once a month according to the conventional methods¹ and obtained analytical data allowed to calculate the nutritive value of diets according to INRA'88 standards (Table 1). In feed samples, after previous wet mineralisation (in HNO₃, H₂SO₄ and HClO₄ mixture) using TH-3 mineralisator the contents of Ca, Mg, Zn, Cu and Mn were determined using atomic absorption spectrophotometer (AAS-3

EA-30 Carl Zeiss). The phosphorus was estimated using vanadomolybdate method and Specol-11 Carl Zeiss apparatus¹.

The rations for dry cows provided (depending on season) 10.2–10.6 kg of dry matter. The diet given to cows producing 25 kg of milk contained about 18 kg of dry matter. The nutrients were balanced according to INRA'88 standards (Table 1). According to the feeding recommendations used in Poland^{10, 13}, the requirement for trace minerals calculated to 1 kg of dry matter is similar: for zinc and manganese 50 and for copper 10 mg/kg of dry matter. The daily requirement of dry cows and cows producing 25 kg of milk according to INRA'88 standards amounted 900 mg for zinc or manganese and 180 mg for copper. The feeding groups were diversified with respect to the chemical forms of Zn, Cu and Mn which were applied in mineral mixtures, that were made exclusively for experimental purposes and given individually to the animals at dose of 175 g/day/head, always during morning feeding (Table 2).

The control group (I) received mineral mixture containing zinc, copper and manganese as sulphate salts while in experimental groups 20% (II) or 30% (III) of daily needs for microelements (Zn, Mn each 315 mg, Cu 63 mg) was covered by organic forms of these trace elements – chelates with amino acids* (Table 3). Total amounts of trace elements were the same in all groups. The level of those elements in the organic compounds calculated to 1 kg of diets' dry matter, did not exceed the levels generally accepted by EU countries (Directive No.70/524), thus there was no group fed with trace minerals only as chelates. The provided rations for cows were totally eaten.

The milk production was controlled once per month by weighing of total obtained milk. In the milk and in colostrums (taken from 8 cows per group), on the 1st day after calving the content of total protein and fat were estimated using instrumental methods and MilcoScan 138B apparatus. The somatic cells content was determined using Somacont Bantley 150 apparatus. The samples of colostrums and milk were taken proportionally to the weight of daily produced milk and colostrums.

The density of colostrum was determined using the Densimeter DIVH 35 apparatus; total immunoglobulins level was estimated according to Schulz and Waldmin³¹. After previous wet mineralisation of milk the mineral ingredients Ca, P, Mg, Zn and Cu were determined using atomic absorption spectrophotometer¹.

During experiment the blood samples from the jugular vein were taken four times (1st, 2nd, 3rd and 6th month) from 8 cows of each group in the same phase of lactation, and selected biochemical parameters were estimated. In blood serum the calcium, magnesium and phosphorus were estimated using chemotests POCh Gliwice. After previous wet mineralization, the samples of blood serum were analyzed for zinc, copper and manganese using atomic absorption spectrophotometer. Moreover, from the calves got from the experimental cows (a dozen per group) and fed colostrum and milk on the second and sixth day of their life there the blood samples from jugular vein were taken out, and in separated serum the protein level using Abbe's refractometer and also the immunoglobulins using sodium sulphate – "turbidity-test" according to Schulz and Waldmin³¹ were determined.

Calves within a period from birth up to 3rd month of life were kept under the same environmental conditions and were fed as follows: to the 6th day with colostrums and then up to the 60th day of life with the milk replacers. Additionally from 30th to 60th day of life the calves were fed with concentrate mixture (0.6-2.2 kg) and with hay (0.3-1.0 kg). During this period the body weight as well as the daily gains of calves were controlled. The purpose of the body mass registration was to estimate the growth rate of animals gets from experimental cows, which during dry period (6 weeks before calving) were fed with different forms of Zn, Cu and Mn.

All results were statistically analysed with one-way variance analysis and Duncan's multiple range test using of software StatSoft - Statistica²⁷.

Results and Discussion

The dairy cows received Zn and Mn (at the amounts of 315 mg) and Cu (at amount of 63 mg) in chelates reached slightly higher milk production. The milk yield for 305 days of lactation and average daily milk secretion were highest ($P \leq 0.05$) in group III, in which 30% of daily needs for zinc, copper or manganese were covered from organic forms. In groups I and II close but lower results than in group III were obtained (Table 4). No significant differences between groups I and II in milk yield expressed as FCM (0.66 kg) were found, however the differences between control and group III (1.34 kg) were statistically significant ($P < 0.05$). This result could be obtained as an effect of better absorption of trace elements from organo-metal forms, as also stated in other studies^{8, 24, 30, 35}. In contrast, in experiments conducted by Ward and Spears³⁴ and Spears²⁵ the influence of these forms of trace elements on cows productive parameters was not observed.

Application of the organic forms of trace elements in the diets given to cows of group II and III did not affect the total amounts of produced fat and the fat and protein content in milk (Table 4). The effective reproduction of cows is one of the main factors of profitability and production in dairy herds and depends on many factors, in them feeding as well. In some experiments the attention was paid to the decreasing of the fertilizing index with simultaneous increasing of milk yield, especially in heifers²⁸. The application of the organic complexes containing Zn, Cu and Mn, when compared to control group, have had a positive effect on the reproduction indices, what was proved by the slightly better insemination index of cows from both experimental groups (II and III) (Table 4). Obtained data are similar to the results published by Campbell et al.⁷. On the other hand O'Donoghue et al.¹⁸ giving to Friesian cattle the rations containing organic forms of zinc and copper stated the tendencies of the higher conception rate. In similar experiments⁴ such results were not confirmed.

The organo-metal compounds of the examined trace elements significantly ($P \leq 0.01$) reduced the amount of somatic cell content (SCC) in milk of cows from group II and III by about 25 and 34%, respectively in comparison to the control group (Table 4). It was also stated that the content of somatic cells in milk from cows of control group was over 30% higher in winter time than in summer months. Application of Zn, Cu and Mn chelates in mixtures for cows during winter season had a greater influence on reduction of SCC than in the summer time (Table 4). Organic forms of zinc and copper that were applied in the experiment by O'Donoghue et al.¹⁸ significantly decreased the SCC in milk. Also Boland et al.⁴ who gave Cu, Zn and Mn Bioplexes coupling with selenium

* Amino acid complexes (Zinpro Company): Availa Cu-100, Availa Zn-100, Availa Mn-80

Table 1. The composition and nutritive value of the basic rations for dry cows and for cows producing 25 kg of milk per day estimated according to INRA standard.

Item	Feeding rations			
	Dry cows		Cows producing 25 kg of milk	
	Summer feeding	Winter feeding	Summer feeding	Winter feeding
Maize silage, kg	20	20	30	30
Lucerne fresh, kg	20	-	20	-
Brewers' grains, kg	-	10	2	2
Wilted grass silage, kg			-	10
Concentrate mixture *, kg			5	5
Mineral-vitamin mixtures, g			175	175
Content in ration				
Dry matter, kg	10.6	10.2	18.4	18.4
UFL	7.5	7.5	13.9	13.8
PDIN, g	725	545	1455	1313
PDIE, g	672	568	1419	1321
LFU	12	12.2	14.3	14.5
Zinc, mg			900	900
Manganese, mg			900	900
Copper, mg			180	180

* cows producing more than 25 kg of milk were fed additionally with 1 kg of concentrate mixture per each 2 kg of milk.
 UFL – unit for lactation, PDIN – protein digestible in the intestine from nitrogen (protein true digestible in the intestine, calculated when N is not limiting in the rumen), PDIE – protein digestible in the intestine from energy (protein true digestible in the intestine, calculated when energy is not limiting in the rumen), LFU – lactating cows fill unit (energy unit for milk production)

Table 3. Quality of trace elements delivered from special mineral mixtures and diets.

Form of trace elements		The content in daily ration mg/day/animal				
		I – control		II	III	
Zinc, Zn	inorganic	525		315	210	
	organic	-		210	315	
Copper, Cu	inorganic	105		63	42	
	organic	-		42	63	
Manganese, Mn	inorganic	525		315	210	
	organic	-		210	315	
Minerals		Roughage	Full mixture	Mineral mixture	Total	In 1 kg of dry matter
		g/day/animal				
Macroelements						
Ca		80	45	20	145	8.0
P		19	42	26	86	4.8
Mg		25	20	13	58	3.2
		mg/day/animal				
Microelements						
Cu		78	30	105	213	11.8
Zn		484	60	525	1069	59.4
Mn		516	77	350	943	52.4

Table 4. The performance of cows accounted for the period 305 days of lactation.*

Item	Feeding groups			SEM
	I	II	III	
Milk yield in 305 days of lactation, kg	6466 ^a	6520 ^a	6816 ^b	250.6
Daily milk yield, kg	21.20 ^a	21.38 ^{ab}	22.35 ^b	0.58
Milk yield, FCM/kg/day	20.51 ^a	21.17 ^{ab}	21.85 ^b	0.58
Total milk fat in 305 days of lactation, kg	250.2	258.2	266.5	9.41
Content of fat in milk, %	3.87	3.96	3.91	0.072
Daily fat yield, g	820 ^a	847 ^{ab}	874 ^b	15.7
Total protein milk in 305 day of lactation, kg	211.4	210.6	219.5	7.56
Content of protein in milk, %	3.27	3.23	3.22	0.027
Daily protein yield, g	693	690	720	9.43
Insemination index	2.4	2.2	2.2	-
Somatic cell content in milk, 10 ³ /ml	409 ^A	306 ^B	270 ^B	18.3
Summer season (April-September)	337 ^a	308 ^b	284 ^b	21.5
Winter season (October-March)	435 ^A	304 ^{Ba}	260 ^{Bb}	19.3

Differences between mean values in the rows designed with A, B were significantly different at $P \leq 0.01$

Differences between mean values in the rows designed with a, b were significantly different at $P \leq 0.05$

* in each group n = 30

(Selplex) in the cows rations stated significant (40%) decrease of the SCC.

On the basis of the analysis of a huge population of cows, Rajcewic and Potocnik ²¹ concluded that the SCC in milk is affected by a great amount of various factors. After analysis of 163,000 milk samples from 21,746 cows in 2,148 herds, Bogucki

Table 2. The content of mineral ingredients and vitamins in 1 kg of used mineral-vitamin mixtures for cows.*

Item	Feeding group			
	I – control	II	III	
Macroelements, g/kg				
Calcium	Ca	110	110	110
Phosphorus	P	150	150	150
Magnesium	Mg	75	75	75
Sodium	Na	55	55	55
Trace elements, mg/kg				
Copper sulphate	Cu	600	360	240
Copper chelate	Cu	-	240	360
Zinc sulphate	Zn	3,000	1,800	1,200
Zinc chelate	Zn	-	1,200	1,800
Manganese sulphate	Mn	3,000	1,800	1,200
Manganese chelate	Mn	-	1,200	1,800
Iron	Fe	600	600	600
Cobalt	Co	10	10	10
Selenium	Se	5	5	5
Iodine	I	30	30	30
Vitamin A, IU		600,000	600,000	600,000
Vitamin D ₃ , IU		120,000	120,000	120,000
Vitamin E, mg		1,800	1,800	1,800
Vitamin B ₁ , mg		9	9	9
Vitamin B ₂ , mg		2	2	2
Vitamin B ₆ , mg		6	6	6
Panthenic acid, mg		200	200	200
Nicotinic acid, mg		500	500	500
Yeast, g		200	200	200

* Premix was prepared by Dolnośląskie Wytwórnice Pasz "Dolpasz", Poland

and Sawa ³ stated that the increase of the SCC in milk is usually accompanied by decreasing of daily milk yield. This fact could be confirmed by the results of Dorynek et al. ¹¹ who stated that the cows with the lowest SCC in milk are characterized by higher milk yield and lower protein content in milk.

According to Campbell et al. ⁷, application of Cu, Mn, Co and Zn bound with amino acid had no effect on SCC in milk. However, in other experiments ²³ it was stated that the milk glandule epithelium was regenerated to a greater extent after application of Zn bound with amino acid or proteinates. One of the many defensive mechanisms that prevent mastitis is teats' self-cleaning mechanism depending on the presence of the keratinous plug. Formation of keratin is associated with the level and form of zinc. Thus, it could be stated that organic forms of zinc could affect the quickness of keratin plug reconstruction in udder teats between milking, and it can be connected with decreasing of somatic cell content.

In colostrum from cows fed organic forms of microelements (group II and III) slightly higher dry matter, total protein and fat ($P \leq 0.05$) levels were stated, but the content of lactose was significantly ($P \leq 0.05$) higher in cows' colostrum from group I (Table 5). Slight differences were also found in density of colostrum. Moreover, in the colostrums of cows from group III, the higher content of immunoglobulins as well as lowered SCC were detected (Table 5). The changes in colostrum composition could be important for calves rearing in relation to the better absorption of immunoglobulins^{9, 31, 32}.

The content of macroelements calcium, phosphorus, magnesium and sodium in milk was in all groups similar (Table 6). The higher zinc and copper ($P \leq 0.05$) levels

Table 5. Colostrum composition.*

Item	Feeding group			SEM
	I	II	III	
Dry matter, %	17.0	18.9	20.1	1.152
Crude protein, %	9.42	10.91	11.37	1.038
Crude fat, %	5.54 ^a	5.57 ^a	6.43 ^b	0.493
Lactose, %	2.67 ^a	2.23 ^{ab}	1.63 ^b	0.150
Density, g/cm ³	1.043	1.044	1.049	0.002
Immunoglobulins, mg/ml	42.18	44.72	57.46	4.572
Somatic cell content, 10 ³ /ml	1584	1346	1086	205.51

Differences between mean values in the rows designed with a, b were significantly different at $P \leq 0.05$ * in each group n = 8

Table 6. The composition of milk and blood serum of cows and some biochemical indices.

Item	Feeding group			SEM
	I	II	III	
Content in milk *				
Lactose, %	4.63	4.69	4.81	0.495
Urea, mmol/l	15.84	15.55	15.24	1.14
Calcium, g/kg Ca	0.81	0.84	0.85	0.204
Phosphorus, g/kg P	0.93	0.88	0.88	0.015
Magnesium, g/kg Mg	0.097	0.095	0.097	0.002
Sodium, g/kg Na	0.48	0.47	0.49	0.009
Zinc, mg/kg Zn	3.18	3.21	3.35	0.088
Copper, mg/kg Cu	1.62 ^a	2.04 ^b	2.13 ^b	0.092
Content in blood**				
Glucose, mmol/l	2.79	2.74	2.84	0.054
Urea, mmol/l	3.97	3.96	3.61	0.116
Total protein, g/l	77.4	79.2	79.3	0.20
AlAT, IU	57.8	56.1	53.2	1.15
AspAT, IU	28.9	30.8	29.5	0.204
Calcium, g/kg Ca	2.54	2.48	2.53	0.028
Phosphorus, g/kg P	2.64	2.42	2.54	0.110
Magnesium, g/kg Mg	1.97	1.94	1.94	0.089
Zinc, μ mol/l Zn	17.7 ^a	18.7 ^b	20.5 ^b	0.84
Copper, μ mol/l Cu	12.9 ^a	13.0 ^a	15.5 ^b	1.08
Manganese, μ mol/l Mn	0.0023	0.0024	0.0036	0.00

Differences between mean values in the rows designed with a, b were significantly different at $P \leq 0.05$ * in each group n = 30, ** in each group n = 8

Table 7. The body weight protein and immunoglobulin level in blood serum of calves derived from cows from group I, II and III. *

Item	Feeding group of cows			SEM
	I	II	III	
The weight after birth, kg	33.7	34.0	34.1	0.40
The weight in 3rd month, kg	85.2	89.5	88.9	6.2
The weight gain for 3 month, kg	51.5	55.5	54.8	2.54
Daily gain, g	570	611	609	22.6
Total protein, g/l				
Days of life				
- 2	4.53	4.63	4.79	0.172
- 6	4.96	5.05	5.15	0.109
Immunoglobulins, g/l				
Days of life				
- 2	5-15	5-15	10-15	-
- 6	10-15	10-15	>15	-

* in each group n = 8, ** Differences between groups were insignificant

related to control were detected in milk of cows of both experimental groups (II and III) (Table 6). This result might indicate the better availability of zinc and copper from chelates, what could prove the better availability of those elements from organic forms. It was also confirmed by the results obtained in other experiments^{14, 29}.

Biochemical parameters, such as level of glucose, urea, total protein, macroelements as well as AlAT and AspAT activity determined in blood and blood serum were similar in all groups (Table 6). Obtained values were close to the data published by Winnicka³⁶. However, in blood serum from cows fed with Zn, Cu and Mn in organic forms, especially from group III, the higher

($P \leq 0.05$) zinc and copper levels were estimated. Statistically insignificant differences in manganese concentration in blood serum of cows from group III in comparison to animals from group I and II were determined.

Obtained data confirms the results of authors^{14, 20, 29} who stated that the application of trace elements as chelates or yeast enriched in these microelements improve their bioavailability for rumen microorganisms and on the same way the possibility of the better transferring of trace elements to the bloodstream. Other authors also presented similar results after application of various forms of zinc and copper^{12, 19, 25}.

The content of total protein as well as the level of immunoglobulins in the calves' blood serum estimated on second and sixth day of their life were in group II and III slightly higher than in control animals (Table 7), however, the differences were not confirmed statistically. It could be a result of better utilization of immunoglobulins from the colostrum from cows fed during dry period with trace elements as chelates. The main source of immunoglobulins for calves is colostrum, especially during first hours after birth^{22, 37}. There is a possibility of the increase of antibodies levels in colostrum by proper nutrition of cows, what is the most important, especially in periparturient period^{32, 37}.

The body weight of calves derived from experimental cows estimated after birth and in 3rd month of life was similar in group II and III, however, the calves of cows fed with chelates were characterized by 7% higher body weight gain than control animals (Table 7). These differences were not statistically confirmed. In the experiment by Stanton et al.²⁶ on the effectiveness of application of different levels (low and high) of inorganic and organic forms of trace elements in feeding of the Angus breed cows it was stated that calves of cows with the same body weight and in the same lactation, which received organic forms of trace element, were characterized by higher body gains in comparison to calves of cows fed inorganic forms of those elements.

Conclusions

Obtained data concerning high yielding cows within 305-days lactation indicates that application of trace elements in chelate form at the dose of Zn and Mn 315 mg and Cu 63 mg in daily ration for cows increases the milk production by 6.5% during 305-day lactation, the zinc and copper concentration in blood serum of cows, affects positively the immunoglobulin content in colostrums and in blood serum of calves and improves the daily body mass gain of calves during first 3 months of life. Also as an effect of application of chelates a decrease of somatic cell content by 34% was observed. The lower level of minerals from chelates applied in group II (Zn and Mn 210 mg; Cu 42 mg) had no such markedly better productive effects.

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