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The Impact of Copper and Zinc Deficiency on Milk Production Performances of Intensively Grazed Dairy Cows on the North-East of Romania

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***Abstract*—**The influence of copper and zinc supplements on milk production performances and health indicators was tested in a 20-week feeding trial, with 40 Holstein-Friesian lactating cows, divided in four groups (copper, zinc, copper-zinc and control). Correlations of the Cu and Zn plasma values with some animal performance criteria of health (body condition score and somatic cell counts) and production (milk yield, peak milk yield, fat and crude protein content) were done. During the 140 days of the experiment, the two added minerals caused a statistically significant increase ($p < 0.05$) of their plasma values after the peak of the cows' lactations. It was also observed that subjects that have received copper and zinc supplements had the lowest number of somatic cell counts in milk. The Pearson correlation test showed a positive correlation ($p = 0.007$, $r = +0.851$) between the plasma Zn and the milk production. The improvement of the nutritional status improved the milk production performances of the cows as well as their health performances.

***Keywords*—**Copper, dairy cows, health, milk production, zinc.

I. Introduction

THE status of copper and zinc and their interrelationships in the livestock of Romania is little known. Some researches on the trace elements in animals were done before [15], [23] in the Southern and Western areas but not in the North-East of the country. In the N-E of Romania, cow's husbandry is one of the main occupations among the dominant agricultural activities. Enhancing of the cows performances here is an important way of increasing the profit of the local farmers, so that studying of the Cu and Zn status in dairy cows might add value to it. Previous researches showed that adequate copper and/or zinc input may be used as a strategy to optimize immune system function by the reduction of the metabolic and oxidative stress [8], and milk production performances [13].

The objective of this study was to evaluate the impact of copper and zinc deficiency to intensively grazed lacting dairy cows in Romania, on health and production performance.

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II. Materials and Methods

A. Study Area

The study was done in a large dairy farm located at 47°9′24″ N and 27°38′50″ E in an area where the climate is characterized by hot and dry summers and cold winters. Average annual rainfall totals about 550mm characterized by a patchy distribution. The soil of this region has a low concentration of Cu and Zn, 10-20ppm and < 50ppm, respectively [1]. Drinking water in the region has an average Cu concentration of 0.022mg/L and of 0.156mg/L Zn [11] well below the maximum concentrations approved by the Council Directive No. 83 [9].

B. Experimental Design and Diets

The present 20-week study was conducted on a large dairy farm according to the veterinary legal regulations for the farm animal protection [10] and involved 40 postpartum lactating (10 to 20 days of lactation) nonpregnant Holstein- Friesian cows, in good health condition. They were divided in two groups: copper-zinc and control. The Copper-Zinc group was given 1g CuSO4 5H2O and 4g ZnSO4 7H2O/cow/week for 20 weeks. The Cu and Zn substrates were dissolved in water before being orally administered to each subject. The Control group received no mineral supplement. The ration was analyzed every 4 weeks.

Standardized clinical examinations [19] were performed on each cow every week by a unique operator. Once a month, the body condition of each subject was scored as described by Edmondson et al. [12] whose method is based on a five-point scale (1 = emaciated; 5 = over-fat) and it is used as a preventive approach procedure of the production diseases.

Individual milk yields were recorded daily and milk samples collected every 28 days. Milk samples were analysed for fat and crude protein content using an IR spectrometric method (FTS). Somatic cell counts (SCC) were determined using the Flow Cytometry Method (Bentley FTS/FCM).

Blood samples were collected from the coccidian vein in lithium-heparin coated tubes (Sarsted) for further plasma copper and zinc, for quantitative evaluations. Samples were drawn before minerals were administered on the first day (t0), on days 28 (t1), 56 (t2), 84 (t3), 112 (t4) and 140 (t5). The tubes were centrifuged at 2500×*g* for 20min. Plasma was removed and frozen at −20˚C. Plasma, after thawing, was deproteinized with 0.6 M solution of trichloroacetic acid and hydrochloric acid (1:1, v:v). The mixture was placed on a

water bath at 90˚C for 15min and then separated by centrifugation at 3000×*g* for 10 minutes [25]. Cu and Zn concentrations were determined by a flame atomic absorption spectrophotometry method [28].

Skin biopsies by excision of the periorbital area were made to some subjects of all groups at the end and the beginning of the study. Fragments of tissue (about $1cm^2$) were displayed on filter paper and fixed in 10% buffered formalin solution for 12 hours. The staining method of the skin sections was hematoxylin eosin methylene blue (HEA).

C. Statistical Analysis

Mean values of all data, standard deviations of the mean, and analysis of the differences between groups were determined using the SPSS 17.0 statistical package program. Differences with $p < 0.05$ were considered significant. Normal distribution of data was checked with the Kolmogorov-Smirnov test. The normal distribution parameters were analyzed with the One Way ANOVA method followed by the Tukey test for multiple comparisons of the average values. Parameters with an abnormal distribution were analyzed with the Kruskall-Wallis test. If significant differences were observed, the mean values were compared, two by two, with the non-parametric Mann-Whitney-Wilcoxon test. Correlation of plasma parameters with health and production indicators were made using the Pearson's test for a normal distribution of data or the Spearman test for their abnormal distribution. Data on SCC were log10 transformed before statistical analysis to enhance linearity.

III. Results

A. Diet Intake

During the 20 weeks study, the daily diet including 29-31 kg maize silage, 7-8kg alfalfa hay, 300g concentrate mixed feed/L milk, provided 17.2 ± 1.1kg dry matter, 1563 ± 50.5g protein, 91.1 ± 3.2g Ca, 59 ± 1.8g P, 24.1 ± 0.9g Mg, 28 ± 8.4 g Na, 711.3 ± 39.2ppm Mn, 3 ± 0.4ppm Cu, 9 ± 1.1ppm Zn and 78000 ± 1800 UI vitamin A, for each subject. The daily quantity of Cu and Zn intake was higher for supplemented cows e.g. Copper-Zinc group (303.1 ± 7mg Cu/cow and 1065 ± 34mg Zn/cow), in comparison to the Control group (48.5 ± 5.6mg Cu/cow and 154.8 ± 24.6mg Zn/cow).

B. Health Indicators

During the present study, five cows out of 40 were slaughtered, as follows: one in the Copper group, two in the Zinc group, one in the Control group and one in the Copper-Zinc group. The five cows, representing 12.5% of the entire lot, showed an increased weight loss. The body condition score (BCS) of the cows revealed a statistically significant increase ($p < 0.01$) from the first day till the 140th day of study in all groups (Table I). During the entire experiment, no statistically significant differences were observed between cows supplemented with minerals and those of the Control group. The variation of both plasma copper and zinc in the cows during the study is illustrated in Figs. 1 and 2. In this experiment, as expected, 60% of the cows were copper and zinc deficient at t0. Since the 112th day of the experiment, there was a significant increase ($p < 0.01$) of the plasma Cu in the Copper group (1.31 ± 0.16mg / L) compared to that in the Control group (0.92 ± 0.31mg / L). There was a statistically significant increase of plasma copper concentration in cows of Copper group (1.41 ± 0.23mg / L) reported to that in the Control group (1.09 ± 0.09mg / L, $p <0.01$) and Zinc group (1.17 ± 0.20mg / L, $p <0.05$) on the 140th day of the experiment. Also, a statistically significant higher ($p < 0.01$) plasma zinc concentration in cows of two groups that received 10 g ZnSO4/week (Zinc and Copper-Zinc) compared to that in the cows of the groups that have not received this mineral at t2, t3 and t5. Cows in the Control group also had a substantial change in the plasma Cu and Zn, but their values returned to the previous levels one month later, compared to the cows whose diet was supplemented with the two minerals. In our experiment, the Cu: Zn ratio in the plasma ranged between 0.67 and 1.25.

TABLE I
VARIATION OF THE BODY CONDITION SCORE COWS DURING THE 140-DAY EXPERIMENT

Health indicator	Time (days)	Groups			
		Copper	Zinc	Copper-Zinc	Control
The body condition score	t_0	2.32±0.10 n=10	2.5±0.20 n=10	2.35±0.12 n=10	2.32±0.12 n=10
	t_1	2.32±0.12 n=10	2.3±0.19 n=10	2.30±0.16 n=9	2.22±0.15 n=9
	t_2	2.46±0.21 n=10	2.43±0.11 n=8	2.47±0.15 n=9	2.44±0.16 n=9
	t_3	2.66±0.12 n=9	2.56±0.11 n=8	2.69±0.16 n=9	2.61±0.13 n=9
	t_4	2.83±0.12 n=9	2.75±0.13 n=8	2.88±0.18 n=9	2.69±0.24 n=9
	t_5	**3.00±0.17**** n=9	**2.91±0.13**** n=8	**3.00±0.17**** n=9	**2.87±0.21**** n=9

n=number of cows, ** Significant differences ($p <0.01$) within each group comparing t_0 to t_5

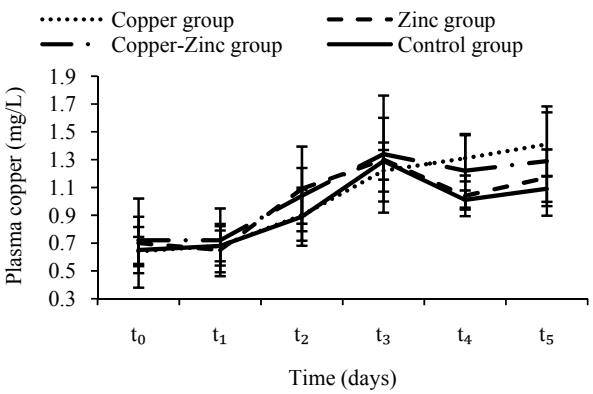

Fig. 1 Variation of the plasma copper of the dairy cows during the 140-day experiment
t_0= first day of study; t_1= 28th day of study; t_2= 56th day of study; t_3= 84th day of study; t_4= 112th day of study; t_5= 140th day of study

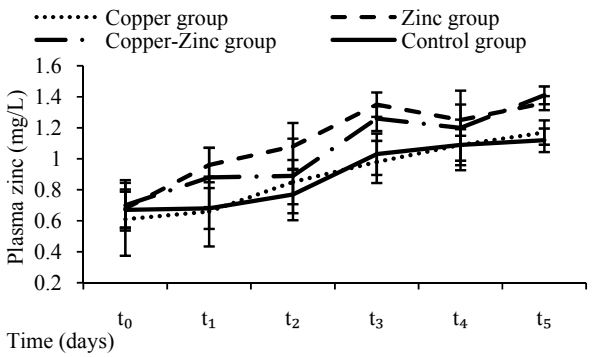

Fig. 2 Variation of the plasma zinc of the dairy cows during the 140-day experiment
t_0= first day of study; t_1= 28th day of study; t_2= 56th day of study; t_3= 84th day of study; t_4= 112th day of study; t_5= 140th day of study

Histological features of the skin in cows are shown in the Figs. 3 (a), (b), and 4 (a), (b). The cows of Control group opposed to supplemented cows, presented at t5 different degrees of periorbital acromotrichia with few melanocytes in the epidermis, agglutination of melanocytes in the dermis, hair follicle degeneration and poorly pigmented melanocytes in the hair follicle sheaths.

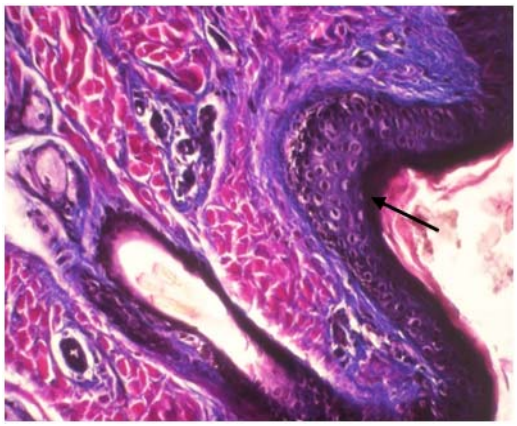

Fig. 3 (a) Normal cow skin microscopic; HEA stain x 400

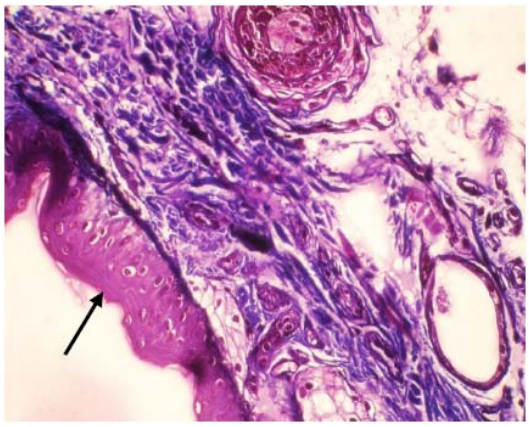

Fig. 3 (b) Cow skin microscopic with acromotrichia; HEA stain x 400

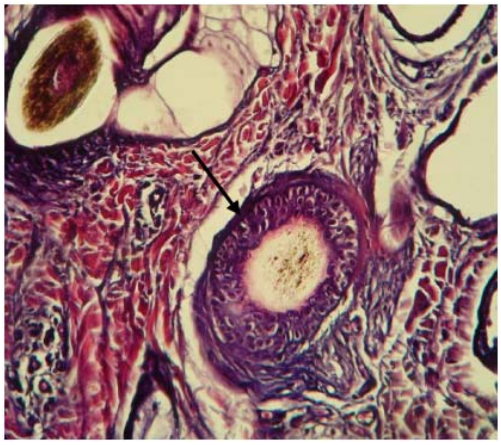

Fig. 4 (a) Skin microscopic structure of a five-year cow with normal hair follicle; HEA stain x 400

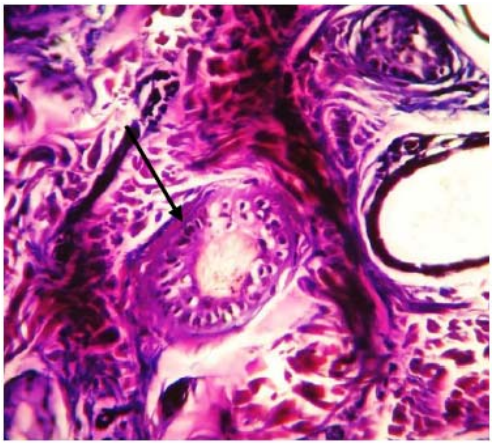

Fig. 4 (b) Skin microscopic structure of a five-year cow with acromotrichia, degenerated hair follicle; HEA stain x 400

C. Production Indicators

Variation of the somatic cell counts [SCC (log10)] in the milk samples from the experimental dairy cows is shown in Fig. 5.

The 35 remaining cows had an increased SCC in their milk, more than 200,000 cells / mL.

In the present experiment, the milk SCC in the Copper-Zinc group showed a decreasing trend until the 140th day of study without any statistically significant differences.

The highest average values in the present experiment were observed in the Control group on the 84th day of study (5.79) and the lowest in the Copper-Zinc group on the 112th day of study (5.51), but Kruskal-Wallis test showed no significant differences between the mineral supplemented groups and the Control group. Spearman test showed no correlation between copper and zinc plasma and the milk SCC.

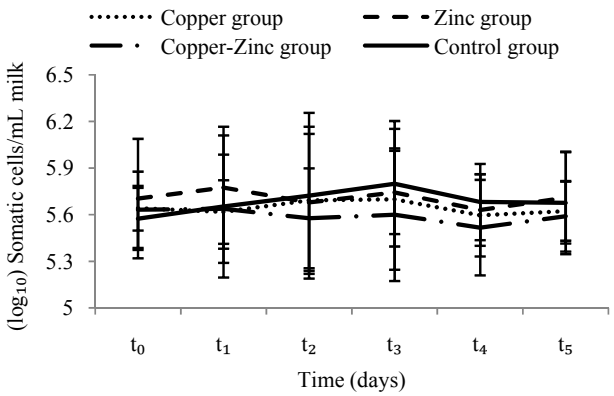

Fig. 5 Variation of SCC (log_{10}) in milk samples of the experimental groups and Control
t_0= first day of study; t_1= 28th day of study; t_2= 56th day of study; t_3= 84th day of study; t_4= 112th day of study; t_5= 140th day of study

The variation of the milk production, milk protein and milk fat of the cows during the experiment is presented in Table II. There was a statistically significant increase ($p < 0.05$) of the milk yield in the Zinc group (23.78 ± 2.48 L and 22.25 ± 2.75 L) compared to that in the Copper group (20.05 ± 3.52 L and 18.21 ± 3.34 L) on the 56th and 84th day of the experiment. Also, a statistically significant higher ($p < 0.05$) milk production in cows of the Zinc group (22.2 ± 2.38 L) compared to that in the cows of the Control group (19.81 ± 2.24 L) at t5 was observed. The Pearson correlation test showed a positive correlation ($p = 0.007$, $r = + 0.451$) between plasma Zn and the milk production in cows on t3, but there was no correlation between the plasma Cu and the milk production. The Pearson correlation test did not show a correlation between the body condition score and the milk production.

TABLE II
VARIATION OF THE MILK PRODUCTION, MILK PROTEIN AND MILK FAT IN THE EXPERIMENTAL AND CONTROL GROUPS DURING THE140-DAY EXPERIMENT

Production indicator	Time (days)	Groups			
		Copper	Zinc	Copper-Zinc	Control
Milk production (L/day)	t_0	21.22±6.35	23.1±4.08	23.94±4.49	19.63±5.32
	t_1	21.26±6.90	22.52±1.31	20.12±4.64	21.74±4.18
	t_2	20.05±3.52	23.78±2.48*	20.95±2.20	20.04±3.07
	t_3	18.21±3.34	22.25±2.75*	21.63±2.18	19.94±3.44
	t_4	20.31±4.25	21.78±2.87	21.01±4.51	20.22±4.33
	t_5	17.57±4.88	22.2±2.38#	21.12±4.93	19.81±2.24
Milk protein (%)	t_0	3.3±0.17	3.24±0.29	3.29±0.23	3.24±0.20
	t_1	3.19±0.17	3.16±0.22	3.25±0.11	3.17±0.15
	t_2	3.17±0.17	3.16±0.11	3.20±0.18	3.03±0.25
	t_3	3.17±0.14	3.2±0.10	3.18±0.14	3.14±0.21
	t_4	3.22±0.11	3.26±0.06	3.25±0.11	3.21±0.13
	t_5	3.26±0.14	3.28±0.06	3.27±0.12	3.23±0.14
Milk fat (%)	t_0	4.02±0.15	4.09±0.28	4.18±0.21	4.01±0.23
	t_1	3.76±0.16	3.98±0.28	3.98±0.23	3.78±0.17
	t_2	3.79±0.15	3.9±0.08	4.18±0.17	3.76±0.14
	t_3	4.01±0.09	3.9±0.13	3.96±0.15	3.98±0.13
	t_4	4.1±0.10	4.07±0.06	4.11±0.07	4.09±0.13
	t_5	4.14±0.07**	4.13±0.04	4.15±0.06	4.1±0.15

t_0= first day of study; t_1= 28th day of study; t_2= 56th day of study; t_3= 84th day of study; t_4= 112th day of study; t_5= 140th day of study; * Significant differences ($p < 0.05$) between Zinc group and Copper group at t_2 and t_3; # Significant differences ($p < 0.05$) between Zinc group and Control group at t_5; ** Significant differences ($p < 0.01$) within the group comparing t_0 to t_5

During the experiment, two cows of the Copper group, one of the Zinc group and one of the Control group presented a sudden drop in their milk yield, followed soon by its recovery, apparently without any reasonable explanation, e.g. disease. Cows of the Control group had a slightly lower milk protein content on t5 (3.23 ± 0.14%) than other groups, as follows: the Copper group: 3.26 ± 0.14%, the Zinc group: 3.28 ± 0.06%, the Copper-Zinc group: 3.27 ± 0.12%) but the differences were not statistically significant.

The milk fat of the cows in the Copper-Zinc group had higher values than the other groups but the differences were not statistically significant. Significant increases ($p < 0.01$) of the milk fat were observed in the cows of the Copper group on t5 (4.14 ± 0.07%) compared to t1 (3.76 ± 0.16%). Pearson test showed no correlation between copper and zinc plasma and the milk fat. Also, the Pearson test showed no statistically significant correlations between the milk protein and fat, on one hand and the body condition score on the other.

IV. DISCUSSION

Good quality milk production requires a balanced diet. Adequate mineral nutrition may be used as a strategy to optimize immune system function by the reduction of metabolic and oxidative stress; and therefore it may have a positive effect on the defense mechanisms of mammary gland against mastitis [34]. In the case of the present experiment carried on in a region known for imbalances of the soil trace elements, diet supplementation with Cu and Zn was necessary.

Both cupric sulphate ($CuSO_4$ $5H_2O$) and zinc sulphate ($ZnSO_4$ $7H_2O$) are accepted as feed additives by the European Union [6]. Maximum content of the element in the total diet of cows is 35mg/kg for copper and 150mg/kg for zinc [6]. In this experiment the basal diet provided less than 0.3g of Cu / day and 1g of Zn / day, and plasma copper and / zinc before supplementation was around 0.6mg / L, suggesting that cows had inadequate copper and/zinc status. The Cu and Zn content were in accordance with the recommendations of NRC [26] for the group supplemented with both minerals. The supply of these trace minerals Cu and Zn, impacts several aspects of cows performance and health, such as lactation and immune function [26]. Zinc deficiency causes loss of appetite and disturbances in metabolism as zinc is involved in protein synthesis, carbohydrate metabolism and nucleic acid metabolism. The soundest diagnosis of mineral deficiency is provided by a clinical or production response to supplements of the mineral or minerals thought to be lacking [31].

In our study, the body condition score of cows was very little influenced by treatment and averaged between 3 in the cows of Copper-Zinc group and 2.87 in Control group, on t5, but the statistical test showed no correlation between zinc plasma and the body condition score. The range of the ideal body condition score is 2.5-3.0 (one month postpartum), 3.0 (mid lactation) and 3.25-3.75 (end of lactation) according to Braun et al. [4].

During the 140 days of study, the copper and/or zinc sulphate addition to the diet caused a statistically significant increase of the plasma minerals. This blood mineral increase occurred only beyond the peak of lactation (at t1) and the blood levels of copper were found within the normal limits of 0.97 - 1.57mg / L according to Radostits et al. [29] and those of zinc within the normal limits of 0.8 - 1.2mg / L according to Suttle [33], starting at t2 and remained so until the end of the experiment. Many factors can influence concentrations of trace elements in blood. For example, inflammation stress and infection may increase plasma Cu and decrease plasma Zn [17]. These difficulties in interpreting the results can be ruled out by discarding results for samples with high Cu: Zn ratios (>3-4) [2]. In our experiment, the Cu: Zn ratio in the plasma was well below 3-4 in every subject.

In this experiment the cows with acromotrichia were copper and zinc deficient. Acromotrichia is the result of the reduced tyrosinase activity that inhibits the conversion of tyrosine into melanin. The effect of copper on the skin pigmentation is exercised by stimulating the conversion of tyrosine into melanin by tyrosinase or other enzymes containing Cu [24].

Zn and Cu supplementation has been associated with higher antioxidant capacity resulting in reduced somatic cell count (SCC) [35]. Average SCC during the first 140 days of lactation had a tendency to be lower for the group fed with inorganic Zn and Cu. Kinal et al. [22] associated the reduction of SCC with quick formation of keratin in teat canal provided by the supplementation of Zn. Scaletty et al., [30] found Cu sulfate supplemented cows, had decreased severity of clinical mastitis cases. SCC is a primary indicator of mastitis and milk quality in dairy herd [35], [20].

The 40 cows had an increased total number of somatic cells in their milk, more than the highest value for the milk from a healthy mammary gland, that is, 200,000 cells / mL milk [5].

The high SCC values in this study suggested the high incidence of the incipient subclinical masititis. Deficiencies of Cu and Zn have been associated with increased incidence and severity of intra-mammary infections, increased clinical mastitis cases and higher somatic cell counts in individual cows and bulk tank milk [21], [30].

The control of mastitis is based on two principles: reduction of the nipple extremity exposure to pathogens and maximizing the defense system of the milking cow. In this farm, the routine milking is done in the confining places where the bedding is thin (1kg sawdust/ cow/ day), and thus the exposure of nipples to pathogens is increased [3]. In our study, the cows that received Zn sulfate had higher milk production compared to that in the cows of the groups that have not received this mineral; this gain in milk yield was positively correlated with increase in the plasma zinc level. That milk protein was not influenced by the copper and/or zinc supplementation is in accordance with Formigoni et al. [14]. However, Griffiths et al. [16] reported an increase in milk protein in cows fed a combination of organic sources of Zn, Mn, Cu and Co.

That feeding inorganic Zn and Cu increased milk fat content during the first 140 days of lactation is in accordance with Nocek et al. [27], but not consistent with others [32], [7] who found that milk fat level was not influenced by treatment. Also, Hullar and Brand [18] reported that increasing the forage content of the diet leads to increased milk fat and depressed milk protein as well as milk yield, and vice versa.

V. Conclusion

Based on the results of this study it may be concluded that feeding inorganic source of Cu and Zn had a tendency to reduce SCC during the first 140 days of lactation, and a improvement of milk production performances in dairy cows. Nevertheless, the serious errors of management concerning housing of the animals probably masked a better benefit of the copper and/or zinc supplementation in this farm.

Further studies and monitoring are necessary to evaluate potential deficiencies of copper and zinc in cows as the reports of the trace elements status are controversial in Romania.

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References

[1] P. Andar, I.N. Robu, R. Nicolescu, U. Siewers, and H. Larenz. *Geochemical atlas of Romania*, Geological Institute of Romania-IGR. Bucharest, Hannover, 2006.

[2] A.H. Andrews, R.W. Blowey, H. Boyd, and R.G. Eddy. *Bovine medicine diseases and husbandry of cattle*, 2nd edition. Blackwell Science. Oxford, 2004, pp. 249-265.

[3] A. Anton. *Researches regarding copper and zinc deficiency in cattle* (in Romanian), PhD thesis. Faculty of Veterinary Medicine. USAMV Iasi, 2010.

[4] R.K. Braun, G.A. Donovan, T.Q. Tran, J.K. Shearer, E.L. Bliss, D.W. Webb, D.K. Beede, and B. Harris. "Body condition scoring dairy cows as a herd management tool", *Comp. Cont. Educ. Pract*., vol. 9, 1987, pp. 192-200.

[5] C. Burvenich, E. Monfardini, J. Mehrzad, A.V. Capuco, and M.J. Paape. „Role of neutrophil polymorphonuclear leukocytes during bovine coliform mastitis: physiology or pathology?" *Koninklijke Academie voor Geneeskunde van Belgie*, vol. 66: 2004, pp. 97-150.

[6] Commission Regulation. „Commission Regulation No 1334/2003 of 25 July 2003 amending the conditions for authorisation of a number of additives in feedingstuffs belonging to the group of trace elements". *Annex. Official Journal of the European Union*. L 187/11, 26.7.2003, Brussels.

[7] C.M. Cope, A.M. Mackenzie, D. Wilde, and L.A. Sinclair. „Effects of level and form of dietary zinc on dairy cow performance and health", *J. Dairy Sci.* vol. 92, 2009, pp. 2128–2135.

[8] C.S. Cortinhas, B.G. Botaro, M.C.A. Sucupira, F.P. Renno, and M.V. Santos. „Antioxidant enzymes and somatic cell count in dairy cows fed with organic source of zinc, copper, and selenium", *Livest. Sci*. vol. 127, 2010, pp. 84-87.

[9] Council Directive. 1998. „Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption". *Annex I. Official Journal of the European Communities*. L 330/32, 5.12.98, 32-54.

[10] Council Directive. „Council Directive 98/58/CE of 20 July 1998 concerning the protection of animals kept for farming purpose". *Official Journal* L221, 08.08.1998, 23-27.

[11] D.E. Diaconu. *Some physical-chemical study of drinking water sources with sanitary and pharmaceutical implications in Moldavia* (in Romanian), PhD thesis. Faculty of Farmacy. UMF Iasi, 2009.

[12] A.J. Edmondson, I.J. Lean, L.D. Weaver, T. Farver, and G. Webster. „A body condition scoring chart for Holstein dairy cows", *J. Dairy Sci.*,vol. 72, 1989, pp. 68–78.

[13] E. M. Evans and B. Halliwell. „Micronutrients oxidant/antioxidant status", *Brit. J. Nutr*., vol. 85, 2001. pp. s57-s74.

[14] A. Formigoni, M. Fustini, L. Archetti, S. Emanuele, C. Sniffen, and G. Biagi. „Effects of an organic source of copper, manganese and zinc on dairy cattle productive performance, health status and fertility", Anim. *Feed Sci. Tech.*, vol. 164(3-4), 2011, pp. 191-198.

[15] S. Ghergariu. *Oligominerale și oligomineraloze*, Academiei, 1980, pp. 59-189

[16] L.M. Griffiths, S.H. Loeffler, M.T. Socha, D.J. Tomlinson, and A.B. Johnson. „Effects of supplementing complexed zinc, manganese, copper and cobalt on lactation and reproductive performance of intensively grazed lacting dairy cattle on the South Island of New Zealand", *Anim. Feed Sci. Tech.*, vol. 137, 2007, pp. 69-83.

[17] H.T. Herdt, W. Rumbeiha, and W.E. Braselton. „The use of blood analyses to evaluate mineral status in livestock", *Vet. Clin. N. Am.-Food A.* vol. 16, 2000, pp. 423-444.

[18] I. Hullar, and A. Brand. „Nutritional factors affecting milk quality, with special regard to milk protein: a rewiew", *Acta Vet. Hung*., vol. 41 (1-2): 1993, pp.11-32.

[19] P. Jackson, and P. Cockcroft. *Clinical examination of farms animals*, Blackwell Science. Malden, 2002, p. 313.

[20] S.Z. Janosi, and Z.S. Baltay. „Correlations among the somatic cell count of individual bulk milk, result of the California Mastitis test and bacteriological status of the udder in dairy cows", *Acta Vet. Hung*., vol. 52 (2), 2004, pp. 173-183.

[21] D.W. Kellogg. „Zinc methionine affects performance of lactating cows", *Feedstuffs*, vol. 62 (35), 1990, p.15.

[22] S. Kinal, D. Korniewicz, D. Jamroz, A. Korniewicz, M. Slupczynska, M. Bodarski, R. Zieminski, S. Osieglowski, and I. Dymarski. „The effectiveness of zinc, copper and manganese applied in organic forms in diets of high milk yielding cows", *J. Food Agric. Environ*., vol. 5, 2007, pp. 189–193.

[23] A. Lionide, D. Curca, M. Samarineanu, A. Cismileanu, D. Nica, C. Toca, and D. Botus. „Efectul suplimentarii rației cu zinc organic la vi□ei asupra raspunsului imun umoral", *Scientific Works Veterinary Medicine*, C Series LII, 2007, pp. 291-298.

[24] L.R. McDowell. *Minerals in anumal and human nutrition*, Elsevier Science. Amsterdam, 2003, pp. 260-270

[25] D. Mihele. *Medical Biochemistry* (in Romanian). 2nd edition. Medicala. Bucharest, 2006.

[26] National Research Council. *Nutrient requirements of dairy cattle*. 7th edition. National Academy Sciences. Washington. D.C., 2001, pp. 236-267.

[27] J.E. Nocek, M.T. Socha, and D.J. Tomlinson. „The effect of trace mineral fortification level and source on performance of dairy cattle", *J. Dairy Sci.*,vol. 89, 2006, pp. 2679–2693.

[28] P.J. Parsons, and F.Jr. Barbosa. „Atomic spectrometry and trends in clinical laboratory medicine", *Spectrochimica Acta Part B*, vol. 62, 2007, pp. 992-1003.

[29] O.M. Radostits, C.C. Gay, D.C. Blood, and K.W. Hinchcliff. *Veterinary Medicine. A textbook of the diseases of cattle, sheep, pigs, goats and horses*, 10th edition. Saunders W.B. Philadelphia. 2007, pp. 1707-1732.

[30] R.W. Scaletti, D.S. Trammell, B.A. Smith, and R.J. Harmon. „Role ofdietary copper in enhancing resistance to Escherichia coli mastitis", *J. Dairy Sci.*, vol. 86, 2003, pp. 1240–1249.

[31] M.C. Sharma, and C. Joshi. „Therapeutic eficacy of zinc sulphate used in clustered model treatment in alleviating zinc deficiency in cattle and its effect on hormones, vitamins and production parameters", *Vet. Res. Comm.* Vol. 29, 2005, pp. 609-628.

[32] J.L. Siciliano-Jones, M.T. Socha, D.J. Tomlinson, and J.M. DeFrain. „Effect of trace mineral source on lactation performance, claw integrity, and fertility of dairy cattle", *J. Dairy Sci.* vol. 91, 2008, pp. 1985–1995.

[33] N.F. Suttle. „Trace element disorder", in: *Diseases and husbandry of cattle*, Blackwell Publishing. Oxford, 2004, pp. 294-308.

[34] W.P. Weiss. „Relationship of mineral and vitamin supplement with mastitis and milk quality". in: *National Mastitis Council Annual Meeting Proceeding*, Orlando, USA, 2003, pp. 37–44

[35] W.P. Weiss, and D.J. Wyatt. „Effects of feeding diets based on silage from corn hybrids that differed in concentration and in vitro digestibility of neutral detergent fiber to dairy cows", *J. Dairy Sci.*, vol. 85, 2002, pp. 3462–3469.