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**Effects of glycine chelated Zn, Cu, Mn and Fe supplementation on some milk parameters and serum trace elements levels in dairy cows**

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***Abstract.*** The paper presents data regarding the impact of some trace mineral supplementation on milk production and quality parameters and also the status of some minerals in the serum of glycine chelated mineral supplemented dairy cows. The study was made on thirty Holstein multiparous dairy cows, having arround 62 ± 4.5 months old divided randomly in two groups: C – Control receiving normal diet without glycine mineral supplementation and one experimental group (E) that received supplement of glycine chelated Cu, Zn, Mn and Fe as follows: 15 mg/kg Cu, 60 mg/kg Zn, 20 mg/kg Mn and, 100 mg/kg Fe, added in concentrate feed. The experiment starts from day 30 of lactation until day 100 of lactation and were assesed the milk production and milk quality parameters (milk fat, protein, lactose, freezing point and not-fat solids percentages, somatic cell count, total bacterial count), and serum levels of iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), and selenium (Se). In experimental group we observed significant ($P \leq 0.05$) increase of milk yield and fat, and a not significant ($P \geq 0.05$) increase of protein and not-fat solids (NFS) percentage, as well as a significant decrease of somatic cell count (SCC) and total bacterial count (TBC). The serum content of selected minerals in day 30, 65 and 100 of lactation we observed to be significantly ($P \leq 0.05$) increased for Se, Zn and Fe, especially in the day 100 of lactation and decreased for Cu and Mn. We can conclude that introduction of a chelated mineral supplement, could increase the milk production and the milk quality parameters.

***Key words:*** milk parameters, dairy cows, supplements, minerals

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**Introduction.**

To ensure a optimal transition from pregnancy to lactation is very important to supply a adequate amount of macro and microminerals (Andrieu S, 2008). Trace minerals proven to be essential for important physiological processes like health maintenance, antioxidant protection, and maximization of productivity of dairy cows (Faulkner MJ and Weiss WP, 2017; Wu G, 2018) while the deficiency of trace minerals can depress imunity of dairy cows (Spears JW and Weiss WP, 2008).

Ruminants are most frequently subject to a severe dietary deficiencies of trace elements such as cobalt, copper, iodine, selenium, zinc, and manganese (Hidiroglu M, 1979), which can have profound effects on the reproductive performance of cattle (Mackenzie AM et al., 2001).

Copper, zinc and selenium seems to be deficient in ruminants and this trace minerals could impair the production and reproduction parameters of cows (McDowell LR and Arthington JD, 2005; Leon-Cruz M et al., 2020).

There were previous reported some positive responses to supplementation of trace minerals or different organic forms of trace minerals to dairy cows which include a greater milk production (Rabiee AR et al., 2010), decreased Somatic Cells Count (Kellogg DW et al., 2004), and also the decreased lameness, and improved foot health of dairy cows (Nocek JE et al., 2000; Siciliano-Jones JL et al., 2008; Overton TR and Yasui T, 2014).

The aim of the presented study was to emphasize the influence of glycine chelated minerals on some milk quality parameters and the blood level of selected minerals.

**Materials and methods.**

**Object of research.** Holstein multiparous dairy cows with the age arround 62 ± 4.5 months, weighing 462 ± 23 kgю Голштинские многоплодные молочные коровы в возрасте около 62 месяцев и весом 462±23 кг.

The inclusion of the animals in the study as well as the experimental procedures were approved in the Scientific Committee, Decision no. 62 /15.11.2020. In addition, all procedures in this study fully comply with the EU Directive on Animal Experiments (Directive 2010/63/ EU). During the research, measures were taken to ensure a minimum of animal suffering and to reduce the number of experimental samples studied.

**Research scheme.** The study was carried out on a number of thirty *Holstein* multiparous dairy cows, with the age arround 62 ± 4.5 months, weighing 462 ± 23 kg, being housed in individual tie stalls. The study was carried out within a private zootechnical unit from Bihor County, Romania. The cows were randomly distributed in two experimental groups (n=15) as follows: C – Control receiving normal diet without glycine mineral supplementation and one experimental group (E) that received supplement of glycine chelated Cu, Zn, Mn and Fe (E.C.O. Trace®, Biochem, Germany) as follows: 15 mg/kg Cu, 60 mg/kg Zn, 20 mg/kg Mn and, 100 mg/kg Fe, added in concentrate feed.

The experiment starts from day 30 of lactation until day 100 of lactation, the cows being milked twice a day. The milk analyses were performed weekly and collection of blood for serum trace elements analysis was sampled in day 0 (day 30 of lactation) in the middle and end of experiment (day 65 and day 100 of lactation) from the tail vein.

The feeding was twice a day, in an individual front of 75 cm/head consist in 5 kg of concentrates, 8 kg of alfalfa hay and 21 kg of corn silage. The total chemical content of the ratio is presented in table 1.

Table 1. **Content of ratio for dairy cows used in experiment**

| Parameter | Unit | Values |
|---|---|---|
| DM | % | 48.2 |
| Crude Protein (CP) | % | 16.96 |
| Soluble protein | % from CP | 49.5 |
| NDF | % | 31.19 |
| ADF | % | 20.06 |
| NE | Mcal/kg | 1.65 |
| Ca | % | 1.02 |
| P | % | 0.31 |
| Mg | % | 0.38 |
| K | % | 1.07 |
| Na | % | 0.33 |
| Cl | % | 0.32 |
| S | % | 0.28 |
| Vitamin A | IU/kg | 11 400 |
| Vitamin | IU/kg | 3100 |
| Vitamin E | IU/kg | 35200 |
| Fe | ppm | 208.33 |
| Zn | ppm | 26.91 |
| Cu | ppm | 9.14 |
| Mn | ppm | 24.27 |
| Se | ppm | 0.19 |

**Technical equipment.** The levels of the main bioelements manganese (Mn), selenium (Se), zinc (Zn), copper (Cu), and iron (Fe) in serum were analyzed using a furnace with pyrolitic tube for Mn, Se and Cu, and flame analisys for Zn and Fe by atomic absorption spectroscopy (AAS) using a ContrAA800 spectrophotometer (Analytic Jena, Germany). The analyses for milk fat, protein, lactose, freezing point and not-fat solids percentages were assessed by FTIR spectroscopy on Milkoscan MARS (Foss, Hilleroed, Denmark), somatic cells count (SCC) assessed by flow cytometry on Fossomatic FC (Foss, Hilleroed, Denmark), and total bacterial count (TBC) was assessed by flow cytometry using a Bactoscan FC (Foss, Hilleroed, Denmark).

**Statistical processing.** The results were statisticaly analyzed by one-way ANOVA with the Bonferroni correction considering the differences are statistically provided when $P \leq 0.05$ or lower being expressed as mean ± SD. The software used was GraphPad Prism 6.0 for Windows (GraphPad Software, San Diego, USA).

**Results and discussions**

Trace minerals such as Cu, Zn, Mn, Se and Fe act as co-factors for many anti-oxidant system enzymes (Méplan C, 2011) which are directly involved in all metabolic processes in living organisms, thus are essential for cell metabolism and many other body functions, including energy production, growth, and reproduction.

As is presented in table 2, in our study we observed that the milk yield was significantly ($P \leq 0.01$) increased in cows that received supplementation with chelated minerals (E/C: +10.52%). Another significant ($P \leq 0.05$) increase was recorded regarding the protein content (E/C: +5.12%). In our study we also recorded a significant ($P \leq 0.05$) decrease of SCC (E/C: -12.47%), and TBC (E/C: -8.33%), while the rest of studied parameters were not significantly ($P \geq 0.05$) modified in experimental group comparative with control, respectively we noted an increase of protein (E/C: +3.03%), NFS (E/C: +0.23%), and a decrease of freezing point (E/C: -0.18%).

Table 2. **Milk parameters in dairy cows, control and mineral glycine chelated supplemented group**

| Group | Parameter | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Milk production (l/day)** | **Fat (%)** | **Protein (%)** | **Lactose (%)** | **NFS (%)** | **SCC ($x10^3$/ml)** | **TBC (log)** | **Freezing point ($^0$C)** |
| C | 38±1.8 | 3.9±0.1 | 3.3±0.1 | 4.58±0.08 | 8.68±0.3 | 58.5±3.2 | 2.4±0.1 | -0.531±0.02 |
| E | 42±2.5** | 4.1±0.1* | 3.4±0.1 | 4.58±0.1 | 8.70±0.4 | 51.2±1.8* | 2.2±0.1* | -0.530±0.01 |

Note: * – $P \leq 0.05$, ** – $P \leq 0.01$

There are authors that pointed out the positive effect of trace minerals supplementation on the milk performance in dairy cows. In this aim, Ballantine HT et al. (2002) reported a similar increase in lactation performance in cows when portions of the Zn, Mn, Cu, and Co from sulfate sources were replaced with chelated minerals. Also, Kincaid RL and Socha MT (2004), and Siciliano-Jones JL et al. (2008) observed a increase in lactation performance during peak lactation, and also the increase of protein content in response to replacing the inorganic Zn, Mn, Cu, and Co with chelated minerals.

The incrase of milk production correlated with the decrease of SCC could be attributed to the improved udder health (Kellog DW et al., 2004; Osorio JS et al., 2016), this studies being in accordance with our findings. Similar findings were obtained by Roshanzamir H et al. (2020) in Holstein cows that received organic Mn, Zn and Cu. Hackbart KS et al. (2010) did not observed a benficial efect on milk production in dairy cows supplementd with organic trace metals to provide 40, 26, 70, and 100% of supplemented Zn, Mn, Cu, and Co, respectively, but observed a increase protein and fat percentage.

At the beginning of the experiment, in the day 30 of lactation, we did not observed significant changes in the serum content of the studied trace minerals. We recorded only a slight, but not significant

(P≥0.05) increase of Zn (E/C: +3.71%), Se (E/C: +1.96%) and Mn (E/C: +9.52%), and a decrease of Fe (E/C: -0.58%) and Cu (E/C: -2.80%).

Table 3. **Serum levels of studied trace minerals in dairy cows, control and mineral glycine chelated supplemented group**

| Day of lactation | Group | Parameter (μg/dl) | | | | |
|---|---|---|---|---|---|---|
| | | Zn | Se | Fe | Mn | Cu |
| 30 | C | 83.4±2.5 | 2.54±0.8 | 274.1±15.2 | 0.63±0.02 | 78.4±3.8 |
| | E | 86.5±3.8 | 2.59±0.9 | 272.5±10.5 | 0.69±0.11 | 76.2±4.8 |
| 65 | C | 81.5±3.7 | 2.65±0.7 | 267.4±14.4 | 0.59±0.12 | 72.11±4.5 |
| | E | 92.1±4.5*, # | 3.11±0.8*, # | 288.4±12.3*, # | 0.54±0.21 | 69.14±1.4* |
| 100 | C | 84.2±5.2 | 2.88±0.8 | 271.1±10.3 | 0.61±0.13 | 74.5±3.2 |
| | E | 114.2±5.8**, $ | 5.54±0.5**, $$ | 314.1±12.7*, $ | 0.57±0.24 | 69.2±1.9* |

Comparision E/C: * – P≤0.05, ** – P≤0.01
Comparision with day 30: # – P≤0.05
Comparision with day 65: $ – P≤0.05, $$ – P≤0.01

In the middle of exeriment, in day 65 of lactation comparing the experimental group with the control we noted a significant increase of Zn (E/C: +13.01%), Se (E/C: +17.35%) and Fe (E/C: +7.85%), while the rest of studied elements were decreased, not significant (P>0.05) for Mn (E/C: -8.47%), and statisticaly significant (P≤0.05) for Cu (E/C: -4.11%).

In the day 100 of lactation we noted a significant increase Zn (E/C: +35.62%, P<0.01), Se (E/C: +92.36%, P≤0.01), and Fe (E/C: +15.86%, P≤0.05). For the other two studied trace elements we recorded a decrease, not significant (P≥0.05) for Mn (E/C: -6.55%) and significant (P≤0.05) for Cu (E/C: -7.11%)

Comparing the serum leves of selected trace minerals in day 65 of lactation with those of day 30 of lactation we observed that were significant changes. We noted a significant (P≤0.05) increase of Zn (65/30: +6.47%), Se (65/30: +20.07%) and Fe (65/30: +5.83%). For Mn and Cu we observed a not significant (P>0.05) decrease (Mn 65/30: -21.73%, Cu 65/30: -9.26%).

Making a comparision between day 65 and day 100 of lactation we observed approximatively the same dynamics as previous, the significant increase of Zn (100/65: +23.99%, P<0.05), Se (100/65: +78.13%, P≤0.01), and Fe (100/65: +8.91%, P≤0.05), with the exception of Mn and Cu which present a slight, but not significant (P≥0.05) increase in the daay 100 of lactation (Mn 100/65: +5.55%, Cu 100/65: +0.08%).

The minerals play indisputable significant roles in herd fertility, and the minerals that are of particular interest are categorized into major and trace elements such as: iodine, iron, copper, manganese, cobalt, zinc, molybdenum and selenium (Boland MP, 2003). Complexes of trace minerals with amino acid are more bioavailable and, by this, are better retained by the body than inorganic sources of them (Siciliano-Jones JL et al., 2008).

Selenium and copper concentrations are generally low in local forage, and are supplemented by farmers, therefore, animals that lives outside on local forage and are not supplemented with these trace elements is possible to be relatively low in both minerals (Top AMvd, 2005).

The interactions between Cu and Zn within the intestinal tract are well known and important for the absorbance of these elements, because increased of Zn concentrations can be followed by the synthesis of thionein, a protein that binds Zn or Cu, by this forming metallothionein (Cousins RJ, 1985), but it is not clear as to what extent Zn-induced thionein formation is able to influence Cu absorption from the intestine of ruminants (Top AMvd, 2005).

Similar findings were obtained by Roshanzamir H et al. (2020), which observed a increase of blood Mn and Zn in cows dietary supplemented with Cu, Mn and Zn as methionine, sulphate or glycine.

**Conclusion.**

Administration of supplements with chelated minerals was followed by a significant increase of milk production and milk quality parameters, and also changes in the serum levels of studied trace minerals, especially the increase of Zn, Se, and Fe, while was observed a slight decrease of Cu and Mn.

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