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# Effects of liquid organic mineral complex (MultiMix®) on milk yield, composition and cheesemaking capacity of milk in dairy cows

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***Abstract.*** *The objective of this experiment was to investigate the effects of liquid organic mineral complex (MultiMix®) on the milk yield, composition and cheesemaking capacity of milk in dairy cows. Thirty Holstein cows (8 primiparous and 22 multiparous) were used in a randomized complete block design experiment with 15 cows per treatment. Feeding was ad libitum targeting 5% refusals. Milk yield data and samples for fat, true protein, solids not fat (SNF), lactose content and parameters characterizing the milk's coagulation properties were collected throughout the experiment. Data suggested that MultiMix® administered through the cows' drinking water had a positive effect on the milk productivity with a high degree of significance (p<0.001). A positive effect on the indicators characterizing the quality composition of milk has also been observed (p<0.001). Additionally, the cheesemaking capacity of milk was enhanced by supplementing animal water with MultiMix® (p<0.001). Overall, the new liquid organic mineral complex used in the present experiment showed promising results for improvement of milk production and composition in dairy cows but further studies are needed to unveil the physiologic mechanisms underlying these beneficial effects*.

**Keywords:** cheesemaking capacity of milk, Holstein cows, liquid organic mineral complex, milk composition

## Introduction

It has been shown that at least 25 inorganic elements are required by animal species for bone growth and maintenance, osmotic balance, nerve and muscle function, hormones, enzymes and many other functions. The efficiency of mineral absorption varies according to the mineral and the mineral source. On the other hand the animal productivity has increased significantly in recent years, which in turn has led to an increase in the demand for more effective animal feeding strategies. Specific areas of research are focused on the effect of the structure of minerals, on their retention and absorption by the body, as well as their relationship with the health and productivity of dairy cows (Nocek et al., 2006; Siciliano-Jones et al., 2008).

The impact of the supply of organic trace elements on the milk yield and composition is different. Nocek et al. (2006) observed a positive effect of organic mineral sources both on milk yield and on its composition, and Ashmead et al. (2004) reported a cumulative effect on milk yield during three lactations with the addition of organic minerals. In contrast, Weiss et al. (2010), indicated that the addition of organic iron had no significant effect on the milk yield when supplemented to dairy cows.

In their meta-analysis study Rabiee et al. (2010) reported an increase in fat and protein content and daily milk yield, as well as improved reproductive capacity by supplementation with organic source of minerals. Contrary results were reported by Cortinhas et al. (2012) who found that the addition of either organic or inorganic sources of Zn, Cu and Se did not affect the intake of dry matter and nutrients, metabolic profile in the blood, milk yield and quality of milk in cows. However, a slight effect of these supplements was observed after prolonged period of use and the positive effect was found on all of the studied parameters.

A study conducted by Del Valle et al. (2015) evaluated the effects of organic and inorganic sources of minerals in rations for dairy cows on milk yield and composition, dry matter intake, blood parameters, microbial protein synthesis and energy and protein balance. The results indicated that the organic mineral source increased milk fat without altering the milk yield, dry matter intake or overall digestibility of feed. Blood parameters, microbial protein synthesis, and energy and protein balance were not affected by mineral sources.

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Trace elements are in two principle forms and most of them are naturally present in the diet of cattle, but basically in their inorganic form they are not well absorbed by animals. Referring to this fact, Bassil and Rizk (2018) evaluated the influence of organic trace elements supplementation on their absorption, milk yield and composition, by adding Zinc (0.026%), Manganese (0.016%) and Iron (0.04%) to the basic feed ration of dairy cows. The results of their study showed that the addition of zinc has the best effect and absorption on dairy cows' milk yield, and in terms of milk fat, iron-treated groups had a higher fat content in milk. Hackbart et al. (2010) also concluded that replacing a portion of inorganic supplemental trace minerals with an equivalent amount of these organic trace minerals (Zn, Mn, Cu, and Co) for dairy cows increased milk production during the mid-lactation.

We hypothesized that the addition of organic minerals in acetate form would have superior effect on milk yield and composition when supplemented to dairy cows. Thus, the aim of the present study was to investigate the effects of liquid organic mineral complex (MultiMix®) on the milk yield, composition and cheesemaking capacity of milk in dairy cows.

## Material and methods

*Animals and experimental design*

The study was a 8-wk design experiment with 2-wk adaptation period and was conducted at the Dairy Research farm of Agricultural Institute in Stara Zagora, Bulgaria. Thirty Holstein cows averaging 65±14 days in milk at the start of the experiment (8 primiparous, 532±46.0 kg and 22 multiparous, 618±52.5 kg) were used in the experiment with 15 cows per treatment. Basal diet provided to both groups of animals was fed as Total mixed ration (TMR) and consisted of (%, DM basis): 52% corn silage, 30% concentrated feed (incl. minerals: Mg, Mn, Zn, I, Fe, Co, Ca, Se and vitamins: A, $D_3$, E), 10% grape pomace, 8% grass hay. Feeding was ad libitum targeting 5% refusals. Cows within block were randomly assigned to one of 2 experimental treatments: (1) basal diet and tap water, not supplemented (control), and (2) basal diet and administration of liquid organic mineral complex applied through the cows' drinking water at a dosage of 1 L per 5 tons of water (MultiMix®). The organic mineral complex MultiMix® contained Fe (20,000 mg/l), Mn (20,000 mg/l), Zn (20,000 mg/l), Ca (16,000 mg/l), Mg (8,000 mg/l), P (8,000 mg/l), Na (5,000 mg/l), Cu (5,000 mg/l), Cl (1,250 mg/l), Calodat (500 mg/l), Se (15 mg/l), K (10 mg/l) and in a dendritic monohydrate acetate form.

*Milk production and composition*

Milk production of the cows was recorded daily. Milk samples were collected weekly, during the morning milking (without the addition of a preservative) throughout the 60 days' experimental period (205 milk samples). Milk samples were analyzed for fat, true protein, solids not fat (SNF), lactose content and parameters characterizing the milk's coagulation properties - rennet coagulation time (RCT), curd firming time ($k_{20}$) and curd firmness ($a_{30}$). The analysis of the qualitative composition of the milk was performed in the laboratory of the Agricultural Institute - Stara Zagora, using infrared spectroscopy (Lactoscan MCCW - V1, Milktronic Ltd., Bulgaria). Milk coagulation properties were determined using a Computerized Renneting Meter (Polo Trade Ltd., Italy). Milk samples (10 mL) were heated to 35°C, and 200 μL of a rennet solution (NATUREN Plus 215/0.8l with milk coagulation activity - 215 IMCU/ml).

*Statistical analysis*

All data were analyzed using the statistical software products Systat 13 and Pest (Groeneveld, Netherlands). The resulting phenotypes were adjusted for the main factors influencing milk yield on an individual control day. A model was used in which each daily milk control was considered as a separate observation and to achieve an unbiased assessment of the features set in the hypothesis, a mixed linear model was used:

- vector of the observation for the quantity of milk in kg for the respective control day of each individual included in the analysis; fatty and protein substances, solids not fat (SNF), rennet coagulation time (RCT), curd firming time ($k_{20}$) and curd firmness ($a_{30}$);

- vector of the fixed effects: treatment, consecutive lactation, age in days as of the date of the control day, lactation days as of the respective control day of the respective lactation of the animal.

$Y_{ijklm} = Group_i + Par_j + Testdim_k + Age_l + e_{ijklm}$,

Where:

$Y_{ijklm}$ - m-number of the corresponding observation of the indicator;

$Group_i$ - fixed effect of the $i^{-th}$ group;

$Par_j$ - fixed effect of the j-th consecutive lactation;

$Testdim_k$ - random regression effect of the $k^{-th}$ lactation days to the respective control day of the respective lactation of the animal;

$Age_l$ - effect of the $1^{st}$ age of successive calving;

$e_{ijklm}$ - random effect of unobserved factors.

Statistical significance was declared at p≤0.05 and tendency was at 0.05<p≤0.10.

## Results and discussion

*Milk production and composition*

During the experimental period, the general statistical characteristics of the studied parameters characterizing the milk production showed that MultiMix® applied through the cows' drinking water had marked positive effect on milk production (29.96 kg) compared with the control (25.84 kg), and the highest maximum milk yield was achieved in the experimental group (Table 1). The lowest standard deviation and the lowest standard error – 3.254 and 0.336, respectively, were recorded in the control group.

**Table 1.** General statistical characteristics of the studied parameters characterizing the milk's quality composition

| Parameters | N | Mean | Min | Max | Var. | Std. Dev. | Std. Err. |
|---|---|---|---|---|---|---|---|
| Milk yield, kg, avg. | 205 | 28.07 | 14.00 | 48.00 | 30.156 | 5.491 | 0.384 |
| Milk yield, kg - MultiMix® | 111 | 29.96 | 14.00 | 48.00 | 39.108 | 6.254 | 0.594 |
| Milk yield, kg - Control | 94 | 25.84 | 20.00 | 34.00 | 10.587 | 3.254 | 0.336 |
| Milk fat, %, avg. | 205 | 4.01 | 2.14 | 7.39 | 0.852 | 0.923 | 0.064 |
| Milk fat, % - Multimix® | 111 | 4.08 | 2.14 | 7.39 | 1.018 | 1.009 | 0.096 |
| Milk fat, % - Control | 94 | 3.92 | 2.35 | 7.14 | 0.650 | 0.806 | 0.083 |
| Milk true protein, %, avg. | 205 | 3.26 | 2.76 | 3.79 | 0.025 | 0.159 | 0.011 |
| Milk true protein, % - MultiMix® | 111 | 3.19 | 2.76 | 3.79 | 0.024 | 0.154 | 0.015 |
| Milk true protein,% - Control | 94 | 3.34 | 3.04 | 3.59 | 0.016 | 0.128 | 0.013 |
| Solids not fat (SNF), %, avg. | 205 | 8.88 | 7.54 | 9.83 | 0.185 | 0.430 | 0.030 |
| SNF, % - MultiMix® | 111 | 8.69 | 7.54 | 9.83 | 0.161 | 0.401 | 0.038 |
| SNF, % - Control | 94 | 9.11 | 8.31 | 9.79 | 0.119 | 0.345 | 0.036 |
| Lactose, %, avg. | 205 | 4.88 | 4.14 | 5.38 | 0.056 | 0.236 | 0.016 |
| Lactose, % - MultiMix® | 111 | 4.77 | 4.14 | 5.38 | 0.048 | 0.22 | 0.021 |
| Lactose, % - Control | 94 | 5.00 | 4.56 | 5.38 | 0.036 | 0.188 | 0.019 |

In line with the results obtained in the present study Rabiee et al. (2010) also reported an increase in yield of fat, true protein and daily milk production, after supplementing dairy cows with organic mineral source.

The milk fat concentration of both groups of animals was relatively high, and the highest average values were obtained at MultiMix® treated group (4.08%) compared with the control (3.92%). Del Valle et al. (2015) observed similar results after addition of organic mineral sources in the diet of dairy cows. The authors stated that the organic minerals increased milk fat content without altering the milk yield, dry matter intake or overall digestibility of feed.

The highest average values of concentration of true protein, solids not fat (SNF) and lactose were reported for the milk produced by the animals in the control group - 3.34%, 9.11% and 5.00%, respectively.

The results of the average differences and significance between the experimental and control group of the studied parameters characterizing the milk's quality composition are presented in Table 2. The analysis of the significance between the groups based on milk yield, milk protein content, SNF and lactose, showed that there was a highly significant difference between the values in the group supplemented through drinking water with the liquid organic mineral complex (MultiMix®) and the control group (p<0.001). Only for the percentage of milk fat content no significant difference was observed between the variables in the group treated with MultiMix® compared with the control.

**Table 2.** Mean differences and significance between the experimental and control group of the studied parameters characterizing the milk's quality composition

| Variable | Mean Difference | 95.00% Confidence interval | | Standard Deviation of Difference | t | df | *p*-value |
|---|---|---|---|---|---|---|---|
| | | Lower limit | Upper limit | | | | |
| Milk yield, kg - MultiMix®<br>Milk yield, kg - Control | 4.702 | 3.310 | 6.094 | 6.798 | 6.706 | 93.000 | 0.000*** |
| Milk true protein, % - MultiMix®<br>Milk true protein, % - Control | -0.147 | -0.189 | -0.106 | 0.203 | -7.048 | 93.000 | 0.000*** |
| Milk fat, % - MultiMix®<br>Milk fat, % - Control | 0.109 | -0.147 | 0.365 | 1.252 | 0.845 | 93.000 | 0.401 |
| SNF, % - MultiMix®<br>SNF, % - Control | -0.434 | -0.543 | -0.325 | 0.533 | -7.902 | 93.000 | 0.000*** |
| Lactose, % - MultiMix®<br>Lactose, % - Control | -0.241 | -0.300 | -0.181 | 0.291 | -8.011 | 93.000 | 0.000*** |

Means with different number of star symbols differ at: *p<0.05, **p<0.01q, ***p<0.001

*Milk coagulation properties*

There was no difference in the first parameter characterizing the milk coagulation properties - rennet coagulation time (RCT) betweeen treatments, 14.71 and 14.25 min for MultiMix® and the control, respectively (Table 3). The highest value in the second parameter of the milk coagulation properties - curd firmness ($a_{30}$, mm) was reported in the milk produced by the animals from the control group - 36.94 mm. Lower values of this parameter were reported in the group supplemented with MultiMix® - 32.59 mm.

The analysis of significance between the groups based on the characteristics of the milk coagulation properties showed that there was a significant difference between the experimental and the control group (p<0.01), in regard to the curd firmness ($a_{30}$, mm) (Table 4). There was no difference in rennet coagulation time (RCT, min) and CF of 20 mm ($k_{20}$, min) between the groups (p<0.05).

**Table 3.** Mean differences and significance between the experimental and control groups of the studied parameters characterizing the individual milk coagulation properties

| Parameters | N | Mean | Min | Max | Var. | Std. Dev. | Std. Err. |
|---|---|---|---|---|---|---|---|
| Rennet coagulation time (RCT), min, avg. | 205 | 14.50 | 3.09 | 30.00 | 33.444 | 5.783 | 0.404 |
| RCT, min - Multimix® | 111 | 14.71 | 4.56 | 30.00 | 39.437 | 6.28 | 0.596 |
| RCT, min - Control | 94 | 14.25 | 3.09 | 30.00 | 26.600 | 5.158 | 0.532 |
| Curd firmness ($a_{30}$), mm, avg. | 205 | 34.58 | 10.00 | 69.00 | 149.137 | 12.212 | 0.853 |
| Curd firmness ($a_{30}$) - Multimix® | 111 | 32.59 | 10.00 | 69.00 | 158.827 | 12.603 | 1.196 |
| Curd firmness ($a_{30}$)/ - Control | 94 | 36.94 | 10.00 | 67.00 | 128.921 | 11.354 | 1.171 |
| Curd firming time ($k_{20}$), mm, avg. | 205 | 1.07 | 0.20 | 4.34 | 0.978 | 0.989 | 0.069 |
| Curd firming time ($k_{20}$) - Multimix® | 111 | 1.11 | 0.20 | 4.34 | 1.155 | 1.075 | 0.102 |
| Curd firming time ($k_{20}$) - Control | 94 | 1.02 | 0.20 | 3.49 | 0.773 | 0.879 | 0.091 |

*Curd firmness ($a_{30}$); Curd firming time ($k_{20}$)

**Table 4.** Mean differences and significance between the experimental and control groups of the studied parameters characterizing the individual milk coagulation properties

| Variable | Mean Difference | 95.00% Confidence interval | | Standard Deviation of Difference | t | df | *p*-value |
|---|---|---|---|---|---|---|---|
| | | Lower limit | Upper limit | | | | |
| Rennet coagulation time (RCT)- Multimix®<br>Rennet coagulation time (RCT)- Control | 0.341 | -1.469 | 2.151 | 8.837 | 0.374 | 93.00 | 0.709 |
| Curd firmness ($a_{30}$) - Multimix®<br>Curd firmness ($a_{30}$) - Control | -4.426 | -7.869 | -0.982 | 16.812 | -2.552 | 93.00 | 0.012** |
| Curd firming time ($k_{20}$) - Multimix®<br>Curd firming time ($k_{20}$) - Control | 0.133 | -0.152 | 0.417 | 1.389 | 0.926 | 93.00 | 0.357 |

*Curd firmness ($a_{30}$); Curd firming time ($k_{20}$)

The results obtained in the present study demonstrated that supplementation of liquid organic mineral complex MultiMix® applied through the cows' drinking water had some positive effects on lactation performance in dairy cows. These results are in agreement with previously reported in some studies which indicated that dairy cows require Zn, Cu and Se to maintain the antioxidant activity of their immune system (Weiss et al., 2005). Thus, the use of trace minerals from organic sources in animal nutrition (i.e. complex, chelated amino acids, proteins), which have higher bioavailability (Spears, 2003) compared to those from inorganic sources, can be an important tool for increasing milk production and maintaining the health status of animals (Cortinhas et al., 2012). Additionally, source and amounts of trace minerals can affect ruminal fermentation (Faulkner and Weiss, 2017). Organic trace minerals (Cu, Zn, Mn, Se, and Co) had no effect on nutrient digestibility by dairy heifers but increased total VFA production compared with sulfate minerals (Pino and Heinrichs, 2016). Therefore, the energy supplied to the cow's body might be increased and this is likely a factor how trace minerals affect the daily milk production. Faulkner and Weiss (2017) also suggested an improved rumen fermentation and microbial populations, which may in turn positively affect the nutrient digestibility.

Over the last decade, significant advances have been made in understanding the effects of vitamins and trace mineral elements in the nutrition of dairy cows (Feng-Li Yang et al., 2010). Their studies showed that vitamin A and β-carotene supplementation have had positive effects on udder health and milk production. The addition of Zinc (Zn) increased lactation efficiency and reduced the number of somatic cell count in milk. This in turn was associated with better indicators of the milk coagulation properties. As early as 1996, Auldist et al. (1996) found that milk with a high content of somatic cells (over 500,000 units/ml) reduced the yield of cheese, reduced the concentration of fat, casein and resulted in high content of whey proteins. Bobbo et al. (2016) investigated the relationship between the number of somatic cells and some technological properties of milk. The results of their study showed that the higher number of somatic cells had a proven negative effect on almost all of the studies characterizing the milk coagulation properties. There was an improvement of some of the milk coagulation properties after supplementing the cows' drinking water with MultiMix®. However, these promising results for improvement of milk production and composition in dairy cows must be confirmed in further studies in order to unveil the physiologic mechanisms underlying all these beneficial effects.

## Conclusion

Data suggested that MultiMix® administered through the cows' drinking water had a positive effect on the milk productivity with a high degree of significance ($p<0.001$). A positive effect on the indicators characterizing the quality composition of milk have also been observed ($p<0.001$). Additionally, the cheesemaking capacity characterized by the milk coagulation properties was enhanced by supplementing animal water with MultiMix® ($p<0.001$). Overall, the new liquid organic mineral complex used in the present experiment showed promising results for improvement of milk production and composition in dairy cows but further studies are needed to unveil the physiologic mechanisms underlying these beneficial effects.

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