Influence of vegetable biostimulators on physical and chemical parameters of cows’ milk

Katerina Silchenko¹, Taisiya Ryzhkova²

¹Lugansk National Agrarian University, Department of Animal Husbandry and Food Technology, Slobozhanska 68, Starobilsk, Luhansk Region, Ukraine, 9270392
²Kharkiv State Zooveterinary Academy, Department of Processing Technology, Standardization and Technical Service, Akademychna 1, Mala Danylivka, Derhachiv district, Kharkiv Oblast, Ukraine, 62341

SUMMARY
The paper presents the results of a comparative case study on the effect of the plant biostimulators ‘Biosvit’ and ‘Megasvit’ on the physical and chemical composition of cow’s milk. The milk was sampled from dairy cows of the Ukrainian Black-and-White breed from the Vidrodzhennya +1881 dairy farm, Zolochivskyi district, Kharkiv Oblast.

Two batches of biological products were used in the case study. The first product, ‘Biosvit’ was made from the dry mixture ‘Eleven Tigers Herbal Bar Energizer’ by Eleven Tigers. The mixture consisted of 7 species of foreign plants. ‘Megasvit’ was the other product, made from a dry mixture of 7 plants of domestic origin.

The experimental groups of animals, E1 and E2, were injected subcutaneously with ‘Biosvit’ and ‘Megasvit’ at the rate of 3-4 mg of active substance per kg of body weight (on average 15 ml per animal). A control group of cows was injected subcutaneously with 15 ml of isotonic NaCl solution per animal. Milk samples were taken three times: at the start of the experiment, before the first injection; 72 hours after the first injection; and 30 days after the fifth (final) injection. The physical and chemical parameters of samples of cow’s milk were assessed.

The physical and chemical parameters of E1 and E2 milk samples were shown to have changed after 30 days of the experiment in the groups injected with ‘Biosvit’ and ‘Megasvit’. Milk fat content by weight increased by 0,21 p.p. and 0,32 p.p., respectively, as compared to 4,03% in the control group (C); true protein increased by 0,12 p.p. and 0,37 p.p., compared to 2,71% in group C; lactose increased by 0,17 p.p. and 0,20 p.p. as compared to group C; dry matter increased by 0,10 p.p. and 0,13 p.p. compared to group C; dry nonfat milk residue increased by 0,08 p.p. and 0,14 p.p. as compared to 8,27% in group C; and crude protein

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increased by 0.24 p.p. and 0.28 p.p. as compared to 2.86% in C. There was no significant difference in the freezing point across the groups.

KEY WORDS: plant biological stimulants, dairy cows, milk, physical and chemical parameters

INTRODUCTION

The development of dairy farming in the agricultural sector of the Ukrainian economy is currently an extremely important task (Lorenzo et al., 2019). Statistical data from the most recent years confirms a downward trend in the number of cattle (Kuz, 2016). Unfortunately, increasing milk yield in cows does not ensure an increase in overall milk production by the sector. Therefore, farmers face the task of intensifying the development of dairy farming through new technologies. One way to increase cows’ productivity is the use of biological stimulants aimed at improving their metabolic processes (Arefiev et al., 2008).

High-intensity metabolic processes in animals affect their physiological parameters, which naturally results in lower resistance, as well as inhibition of other vital physiological functions. This in its turn leads to a significant deterioration of animal health and reduced milk yield (Lorenzo et al., 2019; Veselov et al., 2019). It can also lead to a reduction in reproductive capacity and shorten the duration of economic use. The cows’ productivity and the physical and chemical parameters of milk are affected as well (Petrichenko, 2016).

The development of innovative technologies aimed at increasing production of high-quality milk should be considered a potential solution to these problems (Cherven et al., 2017). An example is dairy farming technology involving the use of biological stimulants of plant origin, as it increases the productivity of dairy cows and the average daily gains of calves (Kochetkova et al., 2005).

The aim of the study was to determine the effect of the biological stimulants ‘Biosvit’ and ‘Megasvit’ on the physical and chemical parameters of cow milk. The biostimulator ‘Biosvit’ was made following V. P. Filatov’s method (Novitskaya and Levit, 2015; Kalikinskaya - AIF E., 2013), using vegetable raw materials imported from Thailand in the form of the dry mixture ‘Eleven Tigers Herbal Bar Energizer’, produced by Eleven Tigers. The mixture includes 7 plants.

The main components of ‘Eleven Tigers Herbal Bar Energizer’ are herbs with a range of pharmacological properties. They influence metabolic processes, affect the immune system, stimulate the digestive system, improve blood circulation, induce strength and energy, strengthen and stimulate the body, and normalize the hormonal balance (Balym, et al., 2012). The plants have different chemical properties, but all have positive effects on the body.

Research has shown that the healing effect of some medicinal plants can be enhanced by placing them for a long period in adverse environmental conditions (such as cold, darkness, malnutrition or ionizing radiation). Biological and chemical processes can take place in isolated parts of plants or in the whole organism. Moreover, substances with non-specific properties, such as biogenic stimulants, are produced (Khomlyak, 2006).
**MATERIALS AND METHODS**

The paper is part of the research project of the Department of Processing Technology Standardization and Technical Service of Kharkiv State Zooveterinary Academy named ‘Improvement of existing and creation of new cattle breeds and technologies for livestock production and product processing’ (state registration no. 0116U002794). The study was conducted on the Vidrodzhenya+1881 dairy farm, Zolochivskyi district, Kharkiv Oblast.

The experiment was conducted on cows of the Ukrainian Black-and-White breed in their third or fourth lactation, two months after calving in autumn. The animals were housed and fed under the same conditions during the experiment. Groups of cows were formed on the principle of analogous pairs. One control group (C) and two experimental groups (E1 and E2) were formed. There were 5 cows in each group (15 cows). Their live weight was about 450-500 kg, and their initial annual productivity was 5000-5500 kg of milk.

The next step of our experiment was the practical application of the biostimulators ‘Biosvit’ and ‘Megasvit’ on the dairy farm Vidrodzhenya+1881.

To create biological products aimed at increasing the productivity of dairy cows and improving the physical and chemical parameters of milk (e.g. the protein and fat fractions), the BioLab laboratory of experimental veterinary medicine in Kharkiv produced two experimental biological products: ‘Biosvit’ and ‘Megasvit’. ‘Biosvit’ was made according to the method described by V. P. Filatov (Novitskaya and Levit, 2015; Kalikinskaya - AlF E., 2013), from raw vegetable materials of foreign origin (BFO), while ‘Megasvit’ was made from raw vegetable materials of domestic origin (BDO).

Simultaneously with the cows’ diet, both the control and experimental groups were given 5 injections of the biological preparations at intervals of 72 hours.

Milk samples were taken three times: during morning milking prior to the injections, 72 hours after the first injection, and 30 days after the fifth and final injections of ‘Biosvit’ and ‘Megasvit’. Milk samples were sent to the Testing Centre of the Institute of Animal Husbandry of NAASU for determination of their physical and chemical parameters.

Table 1 shows the names, origin and dosage in grams of the plants in the dry mixes used to produce of the two biological products. The mixture used to prepare ‘Megasvit’ included plants that grow in Ukraine and have similar chemical and pharmacological properties (Silchenko, et al., 2020) as the plants included in the herb mixture Eleven Tigers Herbal Bar Energizer, produced by Eleven Tigers, which was used for the production of ‘Biosvit’. The weight of the dry mixture was 20.5 gm for both preparations. It was selected based on the weight of the contents of one package of ‘Eleven Tigers Herbal Bar Energizer’.
Table 1
Names of plant names used to make an aqueous extract of biological products from a dry mixture

| Plants included in biological products | 'Biosvit' Amount, g | 'Megasvit' Amount, g |
|---------------------------------------|---------------------|---------------------|
| Ginseng root (*Pánax ginseng*)        | 3,0                 | Branches and leaves of cherry (*Prunus cerasus*) | 2,5 |
| Extract of winter jasmine (*Jasminum nudiflorum*) | 2,5 | Seeds and leaves of dill (*Anethum graveolens*) | 2,0 |
| Ginger root (*Zingiber officināle*)   | 1,5                 | Leaves of fireweed (*Chamaénérion angustifolium*) | 4,5 |
| Indian snakeroot (*Rauvólfia serpentina*) | 2,5 | Angelica roots (*Angelica archangelica*) | 2,0 |
| Eucommi root (*Eucommia ulmoides*)    | 4,5                 | Flowers of medical chamomile (*Matricaria recutita*) | 4,5 |
| Anaxagoras (*Anaxagorea luzonensis A. Gray*) | 4,5 | Stevia leaves (*Stévia*) | 2,5 |
| Angelica (*Angelica archangelica*)    | 2,0                 | Liquorice root (*Glycyrrhiza glabra*) | 2,5 |
| Total                                 | 20,5                | 20,5                |

The control group of cows (C) was injected subcutaneously in the neck with isotonic sodium chloride solution at the rate of 3-4 mg per kg body weight in the amount of 15 ml per cow. These injections were made to control for the effects of stress on the cows’ productivity and milk quality.

The experimental groups of animals, E1 and E2, were injected with the biological products ‘Biosvit’ and ‘Megasvit’ at the rate of 3-4 mg of active substance of the biological product per kg of body weight of animals in the amount of 15 ml per cow, five times at 72 h intervals.

Table 2 presents the number of cows of the Ukrainian Black-and-White Spotted dairy breed included in the experiment as well as the dosage and frequency of injections.

Table 2
Injection dosage for two types of biological products in the experiment carried out on cows of the Ukrainian black-and-white spotted dairy breed

| Group | Number of cows | Biological products                     | Dosage | Time between injections (5 injections) |
|-------|----------------|----------------------------------------|--------|---------------------------------------|
| C (control) | 5             | Isotonic sodium chloride solution (NaCl) | 15 ml  | 72                                    |
| E1    | 5             | 'Biosvit'                               | 15 ml  | 72                                    |
| E2    | 5             | 'Megasvit'                              | 15 ml  | 72                                    |
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Table 2 shows that the same doses of the biological products were used in the experiment, in the same number and at the same intervals. Plants were used in the amount of 0.5±0.1 g to prepare a single dose of ‘Biosvit’ and ‘Megasvit’. In total, 375 doses were prepared for the experiment.

Milk samples were taken from the control and experimental cow groups and sent to the Testing Centre of the Institute of Animal Husbandry of the National Academy of Agricultural Sciences of Ukraine (NAASU) for determination of physical and chemical parameters.

Comparative analysis of the physical and chemical composition of the plants included in the biological products included the following parameters: moisture content, dry matter, ash, crude fat, nitrogen, total crude fibre, NFE (nitrogen-free extracts), calcium, and phosphorus. The parameters of the both foreign and domestic plant materials were determined in the laboratory of the Institute of Animal Husbandry of the National Academy of Agricultural Sciences of Ukraine.

Statistical data processing of the results was performed with Microsoft Excel.

Difference likelihood was evaluated using Student’s t-criterion for three confidence levels with different degrees of freedom. The results were considered significant at P < 0.01. The data is shown in tables 2, 4, 5.

RESULTS AND DISCUSSION

Two batches of biological products, made from two types of aqueous solutions, were tested in the study. One batch was made from a dry extract of the herb mixture ‘Eleven Tigers Herbal Bar Energizer’ by Eleven Tigers, which consisted of seven plants of foreign origin. The other product was made from seven plants of domestic origin.

Table 3 shows the physical and chemical compositions, on a dry-matter basis, of the plants used to produce aqueous extracts of the biological preparations ‘Biosvit’ and ‘Megasvit’. Table 3 shows the physical and chemical composition of the dry matter of plants used to obtain aqueous extracts of ‘Biosvit’ and ‘Megasvit’. The data represents the results of laboratory analysis conducted by the laboratory of the Research Institute of Animal Science of NAASU.

In selecting the plants of domestic origin for the mixture and subsequent preparation of an aqueous extract, in addition to the physical and chemical composition, we also took into account the effects of their individual characteristics on the vital functions of the experimental animals and their overall resistance. This approach to the formulation of the plant mixture allows us to expect the biostimulator to have a beneficial effect by improving the physical and chemical parameters of the cows’ milk and their productivity.
### Table 3

Comparative composition of the two plant dry extracts: ‘Biosvit’ of foreign origin and ‘Megasvit’ of domestic origin

| Parameters (percentage weight) | Composition of dry mixture of plants used in 2 biological products | Differences |
|-------------------------------|---------------------------------------------------------------|--------------|
|                               | Composition of the dry plant mixture used in Biosvit (foreign origin) | Composition of the dry plant mixture used in Megasvit (domestic origin) | |
| Moisture, %                   | 9.52±0.30                                                     | 13.40±0.68   | +3.88* |
| Dry matter, %                 | 90.48±3.61                                                   | 86.22±2.13   | -4.26* |
| Ash, %                        | 6.30±0.25                                                    | 7.85±1.41    | +1.55* |
| Crude fat, %                  | 2.55±0.10                                                    | 2.84±0.36    | +0.29* |
| Total nitrogen, %             | 1.21±0.04                                                    | 1.96±0.29    | +0.75* |
| Total protein, %              | 7.58±0.30                                                    | 12.27±1.84   | +4.69** |
| Crude fibre, %                | 27.00±1.08                                                   | 15.66±3.31   | -11.34* |
| NFE, %                        | 47.05±1.88                                                   | 47.60±3.93   | +0.55* |
| Calcium, %                    | 0.61±0.02                                                    | 1.14±0.14    | +0.53* |
| Phosphorus, %                 | 0.24±0.03                                                    | 0.29±0.04    | +005* |

* P < 0.05, **P < 0.01

Table 4 presents the physical and chemical parameters of milk samples collected from the three groups (control group injected with an isotonic (0.9% NaCl) solution, and experimental groups E1 and E2, injected with biological products prepared from materials of foreign origin (BFO) and domestic origin (BDO), 72 hours after the first injection.

### Table 4

Physical and chemical parameters of cows’ milk samples 72 hours after the first injection with biological products (n = 5)

| Mass fraction, % | Control group of cows injected with 0.9% NaCl solution | (E1) Cows injected with ‘Biosvit’ (BFO) | (E2) Cows injected with ‘Megasvit’ (BDO) |
|------------------|----------------------------------------------------------|----------------------------------------|----------------------------------------|
| Fat              | 4.02±0.10                                                | 4.19±0.02*                             | 4.23±0.06*                             |
| True protein     | 2.72±0.03                                                | 2.86±0.02*                             | 2.87±0.029*                            |
| Lactose          | 4.72±0.16                                                | 4.83±0.02*                             | 4.84±0.03*                             |
| Dry matter       | 12.36±0.28                                               | 12.74±0.07*                            | 12.84±0.019*                           |
| Dry skim milk residue | 8.25±0.27                                              | 8.42±0.02*                             | 8.46±0.01*                             |
| Crude protein    | 2.87±0.11                                                | 3.02±0.02*                             | 3.14±0.01**                            |
| Freezing point, ºC | -0.53±0.05                                              | -0.539±0.07*                            | -0.542±0.09*                           |

NB. * P < 0.01; **P < 0.05
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Analysis of the data in Table 4 indicates minor differences in the physical and chemical parameters of cows’ milk between the control group C and the experimental groups. The fat fraction in the milk samples from cows receiving the biological products ‘Biosvit’ (BFO) and ‘Megasvit’ (BDO) increased by 0.17 p.p. and 0.21 p.p., respectively, compared to the control sample of milk (P < 0.01). True protein in the milk samples increased by 0.14 p.p. and 0.15 p.p. with the use of the BFO and BDO biological products, respectively, compared to the control group C, (P < 0.01).

Protein has by far the highest content of biologically important organic substances in milk, as amino acids, which are formed as a result of protein breakdown, build the body’s cells, enzymes, antibodies, and hormones.

Lactose content in milk samples affected by the BFO and BDO biological products was 0.11 p.p. and 0.12 p.p. higher, respectively, than in samples from the control group (P < 0.01). Dry matter content in the milk samples from cows receiving the BFO and BDO products increased by 0.38% and 0.48%, respectively (P < 0.01). Dry skim milk residue in the milk samples obtained following the use of the BFO and BDO products increased by 0.17 p.p. and 0.21 p.p., respectively (P < 0.01). Protein consists of two very important and useful types of protein: casein and whey proteins. The share of crude protein in the milk samples from cows receiving biological products made from raw materials of foreign and domestic origin increased by 0.15 p.p. (P < 0.01) and 0.27 p.p. (P < 0.05), respectively. The freezing point decreased slightly due to the increase in dry matter in the experimental samples (P < 0.01).

Table 5 presents the changes in the physical and chemical parameters of cows’ milk samples 30 days after the fifth (final) injection of the biological stimulants ‘Biosvit’ and ‘Megasvit’.

Table 5

| Mass fraction, %         | C Control group of cows injected with 0.9% NaCl solution | (E1) Cows injected with ‘Biosvit’ (BFO) | (E2) Cows injected with ‘Megasvit’ (BDO) |
|--------------------------|----------------------------------------------------------|----------------------------------------|----------------------------------------|
| Fat                      | 4.03±0.11                                                | 4.24±0.08**                           | 4.35±0.21*                             |
| True protein             | 2.71±0.04                                                | 2.83±0.02**                           | 3.08±0.12*                             |
| Lactose                  | 4.70±0.17                                                | 4.87±0.04*                            | 4.90±0.02*                             |
| Dry matter               | 12.23±0.29                                              | 12.33±0.41*                           | 12.36±0.44*                            |
| Dry skim milk residue    | 8.27±0.27                                               | 8.35±0.13*                            | 8.41±0.15*                             |
| Crude protein            | 2.86±0.10                                               | 3.10±0.04**                           | 3.14±0.04*                             |
| Freezing point, °C       | -0.536±0.051                                            | -0.539±0.02                           | -0.539±0.02                            |

*p < 0.01; **p < 0.05

The data indicates significant differences in the physical and chemical parameters of the cows’ milk from the experimental groups, induced by the biological products, in comparison to the control group.
Table 5 shows that the mass fraction of fat in the milk samples obtained following the use of the BFO and BDO products increased by 0.21 p.p. and 0.32 p.p. respectively compared to the control milk sample (P < 0.05). The fat in the milk samples from experimental group E2, injected with the BDO product, increased by 0.11 p.p. compared to experimental group E1, which was injected with the BFO product (P < 0.05). Protein in the milk samples affected by the BFO and BDO products increased by 0.12 p.p. (P < 0.05) and 0.37 p.p. (P < 0.01), respectively, compared to the control group. True protein content in the milk samples from group E2, treated with the biological product ‘Megasvit’, was higher by 0.25 p.p. compared to the milk samples affected by ‘Biosvit’ in group E1 (P < 0.05). The mass fraction of lactose in the milk samples from cows receiving the biological products of foreign and domestic origin increased in comparison to the control by 0.17 p.p. and 0.20 p.p., respectively (P < 0.01). The difference between these parameters in the experimental groups injected with the biologicals is minor (0.03 p.p.), with a higher value of 4.90 ± 0.02 recorded in the group of animals injected with ‘Megasvit’. Dry matter in the milk samples from cows receiving the BFO and BDO products increased in comparison to the control group by 0.10 p.p. and 0.13 p.p. (P ≥ 0.05). The difference between these parameters in experimental groups E2 and E1 was 0.03 p.p. Dry skimmed milk residue in the milk samples from cows receiving the biological products ‘Biosvit’ and ‘Megasvit’ increased by 0.08 p.p. and 0.14 p.p., respectively, in comparison to the control group C (P < 0.01). The difference between parameters in experimental groups E2 and E1 was 0.06 p.p. Crude protein in the milk samples from cows receiving the BFO and BDO products increased by 0.24 p.p. (P < 0.05) and 0.28 p.p., respectively (P ≥ 0.01), in comparison to the control group, and the difference between experimental groups E2 and E1 was 0.04 p.p.. There was no significant difference between the freezing point in the control and experimental milk samples.

It should be noted that simultaneously with the sampling of milk from the control and two experimental groups of cows for determination of its physical and chemical parameters, blood samples were taken as well (Silchenko et al., 2020). Their morphological and biochemical parameters were determined in the above-mentioned laboratory. Data on the analysis of cows’ blood in the control and experimental groups and the effect of the biological stimulants ‘Biosvit’ and ‘Megasvit’ on its morphological and biochemical parameters is provided in a previously published article (Silchenko et al., 2020). The analysis indicated a positive effect of both biological products. According to that paper, all physiological processes occurring in animals are to some extent associated with protein metabolism and the proportions of protein fractions. Analysis of the protein composition of the blood can be used to monitor animal health and also to identify potential links to productivity.

Mamenko and Portiannyk (2020) proposed combining the use of the antitoxic mineral and vitamin premix MP-A with subcutaneous injection of the biologically active drug BP-9 of plant origin to protect cows from the toxic effects of heavy metals and promote excretion of pollutants in the urine, thus reducing their content in raw milk. This can help to produce milk that meets domestic and international quality standards. Bukalova and Prilipko (2013) noted that the stability of milk depends on inhibition of the activity of its microflora by substances with bactericidal action. These can be products of organic synthesis, which can be added to newly drawn milk. It also can be achieved through controlled feeding of animals with plants containing active phytostabilizers. Salamon and Hrytsyna (2019), suggest feeding animals specially harvested plants that increase appetite, promote biochemical feed processing in the pancreas, abomasum
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and intestine, normalize bacterial and enzymatic processes in the gastrointestinal tract, and enhance the hormonal functions of the lactating animal.

Based on the research results presented in the discussion, it can be concluded that the general stimulating effect of herbal preparations is based on the principles of metabolic processes that play a role in dairy farming. Therefore, future research will focus on the long-term impact of the use of the biological products ‘Biosvit’ and ‘Megasvit’ on cows’ productivity and the technological properties of milk.

CONCLUSIONS

1. Two experimental biological products were produced: ‘Biosvit’, made from plant materials of foreign origin (BFO), and ‘Megasvit’, made from plant materials of domestic origin (BDO). Their injection into the body of dairy cows (groups E1 and E2) had a positive effect on milk quality in comparison to the control group (C).
2. Injection of the biological product ‘Megasvit’ had a more significant impact on the mass fraction of fat and protein, which were increased by 0.11 p.p. and by 0.25 p.p., respectively.
3. The biological products ‘Biosvit’ and ‘Megasvit’ can be recommended for practical use on dairy farms to increase cows’ productivity and improve milk quality.
4. Further research will focus on the impact of the biological products ‘Biosvit’ and ‘Megasvit’ on the productivity and technological properties of dairy cows’ milk.

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