

Influence of cyanobacteria *Arthrospira (Spirulina) platensis* biomass additives towards the body condition of lactation cows and biochemical milk indexes

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Abstract. The objective of the research was to estimate the influence of cyanobacteria *Arthrospira (Spirulina) platensis* biomass additive on cows' body condition, milk productivity and biochemical indexes at the beginning of lactation. Two parallel groups of Lithuanian black and white cows in their early lactation period were used for the experiment.. During the 90-day experimental period, they were fed on almost the same ration: the experimental group received 200 g of cyanobacteria *Arthrospira (Spirulina) platensis* additives daily, mixed with the combined feed. The body condition of cows was scored according to a 5-point scale system in their dry period, after calving, and after the first, second or third month of lactation. Their productivity, milk composition and quality indexes were estimated during control milking: milk yields – on the farm and rates of composition and quality – in the laboratory of SE 'Pieno tyrimai'. The experiment showed that the cows of the experimental group which received 200 g of cyanobacteria *Arthrospira (Spirulina) platensis* daily during the experimental period became 8.5-11 percent fatter ($P < 0.01$). Each gave on average 34 kg milk per day in the beginning of their lactation, or 6 kg more than those from the controlled group ($P < 0.05$). Throughout the 90-day experiment, the average income from the milk of one cow from the experimental group was 378 Lt, or 21% more than from the controlled group. The use of cyanobacteria additives was economically effective, because 1 Lt costs for 'Spirulina platensis' increased income from the milk by 8.4 Lt.

Key words: cyanobacteria *Arthrospira (Spirulina) platensis*, 'Spirulina platensis' additive, cow, body condition, milk productivity and quality

INTRODUCTION

The profitability of dairy farming primarily depends on the productivity of the cow herd, which, in turn, depends on conditions of nutrition. Productivity of cattle in Lithuanian farms has been growing through recent years and in 2007 the average milk productivity from one cow was 4484 kg, and in specialized farms and corporations – 5632 kg of milk (Lithuanian annual statistics, 2007). The positive impact was the result of genetic characteristics, improved nutrition and keeping conditions of the cattle, and the experience of dairy farmers. While productivity is growing, the nutritional needs also change depending on the animal's age, physiological state and lactation period

(Ulbricht et al., 2004). In addition, most nutritional substances in the ration have to be in the proper proportion; as a result, balancing rations for cows of high productivity is not an easy task. The rations made of local production forage and grains often lack energy and nutritional components, causing the cow to compensate from its body reserves, resulting in decreased milk productivity and physical condition. Proper body condition of cows in all lactation stages increases milk yield. Physical condition affects the amount of stored energy reserves and differing reserves in different lactation periods. To evaluate a dairy cow's body condition, it is important to know its stage of productivity and to estimate the condition standard limits for the particular period (Broster & Broster, 1998). It has been established that the stage of body condition at calving period is a crucial index, predicting milk productivity during the next lactation (Wallace, 2000). Cows scoring less than 3.0 at calving produce less milk (Kim & Suh, 2003); others concur that milk productivity is smaller from attenuated cows (Contreras et al., 2004). To achieve higher milk productivity, changes in body condition should be controlled. If the body condition at calving matches the standards, and in the first weeks after calving the rations are properly balanced, it would be possible to avoid decrease of milk production (Dechow et al., 2002). Periodic evaluation and analysis of a cow's body condition increases understanding the changes of energy balance in the animal organism, allows pre-diagnosis of health disorders, forecasting of milk productivity and helps ascertain errors in feeding (Buckley et al., 2003).

Arthrospira (Spirulina) platensis is also used as an additive for other livestock. In rabbit growth, trials of diet efficiency with the inclusion of *Arthrospira (Spirulina) platensis* show that the final weight, weight gain and feed efficiency did not differ significantly ($P > 0.05$) among the dietary treatments, but an *Arthrospira (Spirulina) platensis* inclusion level of 10% gave the highest feed intake. The DM, OM, CP, GE, NDF and ADF digestibility of the control diet were higher than those of the *Arthrospira (Spirulina) platensis*-containing diets (Peiretti & Meineri, 2008). Various supplements, additives, pre-mixes, etc. are used for balance/compensation of rations. Currently, significant attention is being paid to natural and safe additives, which increase cow productivity, and do not harm animal and human health (Jeroch et al., 2008). The cyanobacterium *Arthrospira (Spirulina) platensis* has even been used by humans because of its nutritional and possible medicinal effects (Colla et al., 2007).

Scientists from around the world have been looking for ways to use the biomass of oceans and fresh water for many years; one possibility is creating new, safe food and forage supplements and additives. For this reason, examining low organisms such as blue-green algae as a nutritional source for animals is receiving considerable attention. Algae are single-celled and multi-cellular organisms, mainly lower order water plants, growing in the seas, lakes, rivers, ponds and swamps, and are also found on humid soil, banks, and wet stones. To date, more than 25 000 species of algae have been counted. Algae are significant in protecting oceans and other water sources from damaging substances and compounds. (Praskevicius et al., 2006) have stated that algae and other water plants produce about 80% of all organic substances as a result of photosynthesis; earth plants produce only the remaining 20%. Therefore, from 1 hectare of sea it is possible to get about 10 tons of algae dry mass. Through the evolutionary process algae have adapted to concentrate many chemical elements that serve as a raw material, with

easily absorbed elements that make them useful for food, forage and the pharmacy industry. Some sea algae (i.e. sea cabbage), with iodine removed, are used for animal feed (Simkus et al., 2007).

In fresh waters green algae and blue algae - cyanobacteria - predominate, but can also be grown in artificial, enclosed ecological systems. Green algae *Chlorella vulgaris*, widely used in the pharmacy industry (Praskevicius, 2006), is common in alkaline reaction water ponds in South America, Asia, and Africa which offer enough warmth and light. Although *Arthrospira (Spirulina) platensis* is one of the oldest algae, its appearance dating back approximately 3.5 billion years, it has recently become of interest to nutritionists. Its cells' differentiation is low but, with its unique chemical composition, can be a preferred raw material for the pharmacy, food and feed industries. *Arthrospira (Spirulina) platensis* organic materials consist of proteins – 60-70%, carbohydrates – 10-20%, fat – 5% and fibre – 2% (Table 1). According to the literature (Kupras et al., 2003, Paulauskas et al., 2007), dry biomass of *Arthrospira (Spirulina) platensis* has more protein than barley, wheat and *Chlorella vulgaris* (Table 1).

Table 1. The chemical composition of *Arthrospira (Spirulina) platensis*, *Chlorella vulgaris*, *Hordeum vulgare* and *Triticum aestivum* (Hendrickson, 1989 by Kupras et al., 2003)

Components	<i>Arthrospira (Spirulina) platensis</i> Cyanobacteria	<i>Chlorella vulgaris</i> Green algae	<i>Hordeum vulgare</i> Barley	<i>Triticum aestivum</i> Wheat
Proteins, %	65	58	12-14	13-15
Carbohydrates, %	18	23	23 - 40	54
Fat, %	5	9	2 – 5	4
Humidity, %	5	5	5	5
Vitamins, 10 g of substance				
Beta carotene, TV	23000	5550	-	-
C, mg	0.5	1	33	31
B ₁ , mg	0.31	0.17	0.13	0.10
B ₂ , mg	0.35	0.43	0.28	0.20
B ₃ , mg	1.46	2.38	1.06	0.75
B ₅ , µg	10	130	250	240
B ₆ , µg	80	140	30	128
B ₉ , µg	1	2.7	64	108
B ₁₂ , µg	32	13	-	4.3
Minerals, 10 g of substance				
Calcium, mg	100	22	111	52
Phosphorus, mg	90	90	60	52
Potassium, mg	120	90	89	143
Iron, mg	15	13	1.6	5.7
Zinc, mg	0.3	7	0.7	0.5
Copper, mg	120	10	140	140
Sodium, mg	60	0	78	143
Pigments, 10 g of substance				
Phycotian, mg	1500	-	-	-
Chlorophyll, mg	115	280	149	55

Compared with proteins of other raw materials, the proteins of *Arthrospira (Spirulina) platensis* have more valuable amino acids as well as biologically active substances such as vitamins, minerals, poly-saccharids, which are able to form compounds with metals, etc. Among biologically active compounds, isoprenoids are especially important, positively influencing activity of various enzymes, as well as the synthesis of nucleic acids and photosynthesis. Antioxidant characteristics of cyanobacteria are based on contained phytohormones and enzymes. The blue pigment phycocyanin of cyanobacteria stimulates neural and immune systems (Simkus et al., 2006). Because of its biologically active substances, unique chemical composition, valuable proteins, correct proportion of amino acids, and amount of vitamins and minerals, biomass of *Arthrospira (Spirulina) platensis* can be successfully used in animal nutrition (Wallace, 2000). According to data of the experiment carried out at the Lithuanian Veterinary Academy, cow's milk productivity increased an average of 7.6 % after receiving feed additive of *Arthrospira (Spirulina) platensis* (Simkus et al., 2005). It can be grown in special photosynthetic blocks, in certain nutritional media. The cyanobacteria *Arthrospira (Spirulina) platensis* is approximately 100 times larger than green algae *Chlorella vulgaris*. Accordingly, *Arthrospira (Spirulina) platensis* is more productive, is more easily grown and does not need to be centrifuged like *Chlorella vulgaris* (Praskevicius et al., 2006).

Lithuanians have recently started to grow cyanobacteria *Arthrospira (Spirulina) platensis* artificially for preparation in additives for animal fodder. According to the producer – UAB 'Vingrunė', this additive matches the requirements of directive ES 82/471/EEB, and is registered in the State license office of the Lithuanian Republic. The main raw material for producing the additive is biomass of cyanobacteria *Arthrospira (Spirulina) platensis*. Cyanobacteria is grown in bio-photoreactors and their biomass is recorded in a technological process using fructose and molasses as media. The final product, named 'Spirulina platensis', designed for combined fodder enrichment with valuable nutritive elements, is produced after inserting 5% of biomass of cyanobacteria *Arthrospira (Spirulina) platensis* raw material into the residue of linseed or sunflower. Information about its use as an additive is still insufficient in Lithuania and neighbouring countries.

The aim of the research is to estimate and evaluate the influence of fodder additive 'Spirulina platensis' (cyanobacteria *Arthrospira (Spirulina) platensis*) on the body condition of cows, fodder intake, milk productivity and biochemical indices.

MATERIALS AND METHODS

The research on the influence of fodder additive 'Spirulina platensis' (cyanobacteria *Arthrospira (Spirulina) platensis*) on the body condition of cows, fodder intake, milk productivity and quality was carried out at the Lithuanian Academy of Veterinary for 90 days in the period of April – June 2007 (end of stable period, beginning of pasture period). Two parallel groups of Lithuanian black and white cattle, of similar productivity and body condition scoring, estimated in their dry period (30 days before calving), were formed (I–control and II–experimental).

Cattle of both groups in stables were bound, given 2 hours of movement every day, were fed individually, given water from automatic waterholes and after calving were milked twice per day (at 5 am and 4 pm). In both groups cows received 15 kg silage and haylage, 2 kg of hay and an additional 350 g of combined fodder per 1 litre of milked milk after calving. In pastures the average ration per cow was 60 kg of grass, 100 g vitamin-mineral supplements and 300 g of combined fodder per 1 litre of milked milk. Each cow of the experimental group additionally received 200g of 'Spirulina platensis' additive dry biomass (UAB 'Vingrunė'), which was mixed manually into combined fodder. The ration of fodder matched standards set for cattle feeding (Jatkauskas et al., 2002). The fodder intake was estimated by weighing before ingestion and removing any remains.

The cows' body condition was scored according to a 5-point scale (Jones & Heinrichs, 2004) and was estimated 5 times: in the dry period (30 ± 10 days), after calving (1-2 days), in early lactation after 1 month (30 ± 10 days), after 2 months (60 ± 10 days) and at the peak of lactation period (90 ± 10 days). The evaluation was made after morning milking and feeding, examining and frisking the back, and girth, loin and tail areas of standing animals, in order to estimate the body condition score.

The milk productivity (yield of milk, kg) and biochemical indexes (concentration of fat, proteins and lactose in milk %; concentration of urea mg/% and number of somatic cells thousand/ml) were measured for cows from both groups ($n = 20$). The milk productivity was estimated on the farm, the quality rates – in the laboratory of state enterprise 'Pieno tyrimai' using devices 'Lacto Scope FTIR (FT 1.0.2001)' and 'Soma Scope (CA-3A4, 2004)', according to standard methodology. The experimental data were evaluated statistically using ANOVA by 'Win Excel' (Juozaitienė & Kerziene, 2001). The standard error and significant differences between averages of variables by P test were established.

RESULTS AND DISCUSSION

The milk yields of experimental cows increased progressively: during the first month their milk yield average was by 4 kg or 15% more, and during the second and the third months, respectively, by 6 kg (21%) and 7 kg (24%) more than from the control group ($P < 0.05$). During the entire treatment period, the average milk yield of experimental cows was by 6 kg or 21% larger than that of the controlled herd. The milk yields of control group cows increased only at the beginning of grazing and remained the same during the next two months of the experiment (Fig. 1).

The positive influence on productivity of the experimental group of cows could be a subsequence of 'Spirulina platensis' additives chemical composition and biological activity on the micro-organic activity of the cattle's fore-stomachs. According to literature (Kupras et al., 2003; Simkus et al., 2005) 'Spirulina platensis' consists of alkaline elements and other substances which can change the cattle fore-stomachs' reaction to the alkaline side. That is highly significant, because the activity

of fore-stomachs' micro-organisms and the fermentation process is the most effective when the fore-stomachs' reaction to the inner environment is neutral. It is also crucial that enough volatile fatty acids are formed during the fermentation process because they are necessary for cow organism functions and milk synthesis (Jeroch et al., 2004).

In order to achieve maximum milk production it is also important to secure proper cow body condition. Data presented in Table 2 show that body condition of cows from both groups fulfilled the standard at the dry period and calving, however, after calving the body condition of experimental group cows remained 15% higher than that of the control group. An analogous tendency remains during lactation, when cows from the experimental group (fodder with 'Spirulina platensis' additives) exhibit better body condition by 8.5-11% than cows from the control group ($P < 0.01$). At the peak of lactation, the body condition of both groups decreased. The cows of the control group did not even match recommended standards and were inferior by almost 10% compared with the experimental group (Table 2).

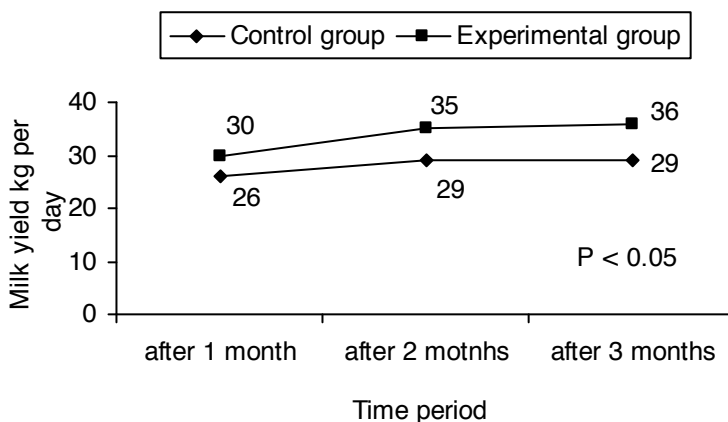


Fig. 1. Dynamics of milk yields (average milk yield per day, kg)

Table 2. The body condition of cows in various productivity periods and recommended standards

Cycle of productivity	Phase of lactation, days	Body condition, points		Recommended condition (according to Jones and Heinrichs, 2004)
		Control group (n = 20)	Experimental group (n = 20)	
Dry period	30 ± 10	3.58	3.61	3.50 ± 0.25
Calving	1 st -2 nd	3.00	3.45	3.50 ± 0.25
Beginning of lactation (after 1 month)	30 ± 10	2.71	2.98	3.00 ± 0.25
Beginning of lactation (after 2 months)	60 ± 10	2.57	2.79	2.75 ± 0.25
Peak of lactation (after 3 months)	90 ± 10	2.36	2.62	2.75 ± 0.25
P (for control and experimental groups)		-	<0.01	-

Milk composition and quality indexes were similar in both groups of cows; hence concentration of fat was rather lower in the experimental cow group during the first and the third month of the experiment (Fig. 2). Nutritional factors, especially low concentration of crude fibre in fresh grass and relatively high air temperature, could have had the greatest impact in the transitional period from stables to pastures. However, milk yield and total milk fat quantity of the experimental group of cows were significantly higher compared with control.

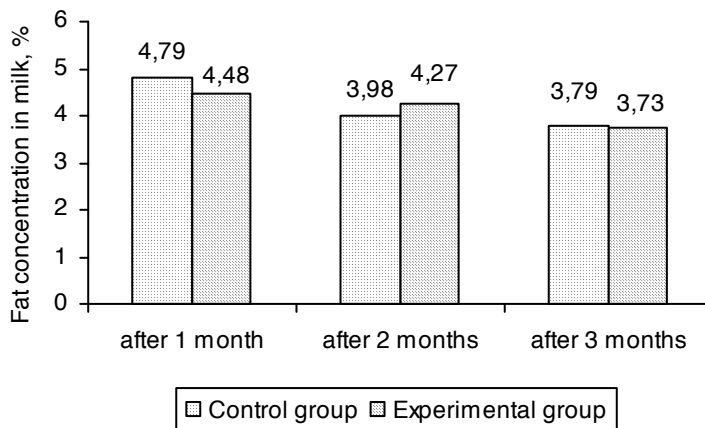


Fig. 2. Milk fat concentration, %

The concentration of protein in milk of both groups of cows did not differ significantly during the experimental period. However, the protein concentration in milk of the experimental group had a tendency to increase during the second and third months of the experiment (Fig. 3). Additionally, the milk protein quantity of the experimental group was bigger because of higher milk yield and protein concentration during the second and third months of the experiment.

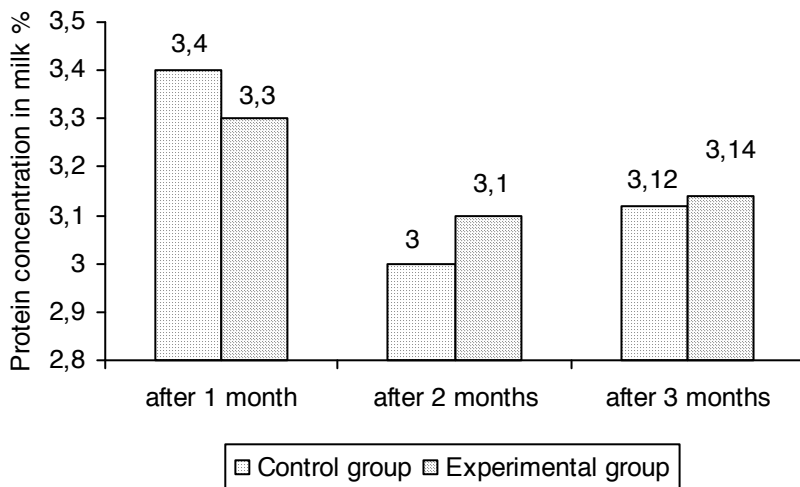


Fig. 3. Protein concentration in milk, %

Analysis of the concentration of lactose established that the concentration of lactose increased constantly during the experiment in milk of both groups of cows, and was within the mark. However, the lactose concentration in the milk of the experimental group was slightly higher (Fig. 4).

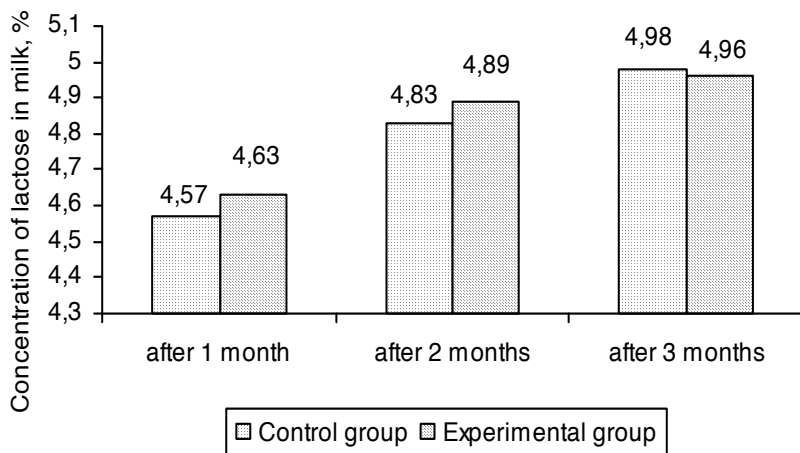


Fig. 4. The concentration of lactose in milk, %

Lactose is the main carbohydrate in milk which influences milk secretion (Jukna et al., 1994). It has an essential impact on the synthesis of milk dry material (about 52%) and the synthesis of the liquid fraction in the mammary gland. Lactose characteristically binds a lot of water, and determines the amount of synthesized milk. Each microgram of lactose binds approximately 10 times more water, therefore the concentration of lactose in the milk and mammary gland is one of the most important factors of milk productivity (Vattio, 2004). Lactose is synthesized in the mammary gland and depends on nutrition, genetics and other factors. For its synthesis the main raw material is glucose, which is synthesized in the liver. However, the main raw material for the synthesis of glucose is propionic acid in the fore-stomachs during the fermentation process: synthesis of milk depends not only on nutritional ration, but also on the activity of micro-organisms in the fore-stomachs and metabolic activity in the organism (Jeroch et al., 2004, Jukna et al., 1994), which was positively influenced by the ‘*Spirulina platensis*’ additive used in our experiment.

The next important index in cows’ milk is the amount of urea. Urea concentration in milk indicates the fermentation process in the fore-stomach and the level of nutrition. If the concentration of urea in milk is low, the ration is lacking proteins; if high, protein is in excess. The amount of urea in milk can determine if the ration has sufficient energy and proteins, a proper proportion of these indicators in the fodder, etc. Long-lasting high concentration of urea in milk is a signal that continuing inadequate nutrition could lead to cows’ health disorders, decrease of productivity and decline of reproductive features. It was noticed (Ulbricht et al., 2004) that cows with long-lasting increased urea concentration in milk had been more difficult to inseminate. In our experiment (Table 3), more substantial influence of *Arthrospira (Spirulina) platensis* on urea concentration in milk has not been estimated.

In the early pasture time, the urea concentration in milk was slightly higher than the set standard in the milk of both groups, but the urea concentration in both groups’ milk later decreased and satisfied the standard (Table 3).

Table 3. Concentration of urea in milk, mg/%

Group	Length of experiment			Standard (Kabasinskiene et al., 2007)
	1 month	2 months	3 months	
Control	43 ± 2.99	30 ± 5.60	23 ± 2.41	20 – 30
Experimental	44 ± 4.14	37 ± 5.13	22 ± 1.86	
P	> 0.05	> 0.05	> 0.05	-

Note. ± Standard error

The number of somatic cells (SLS) in the milk of both groups of cows has a tendency to vary. In some cows in both groups, milk SLS increased (i.e. control group – inv. No. 01945578, 02832541; in the experimental – inv. No. 02832535, 02190827). However, SLS in milk of both groups of cows was within the mark (Kabasinskiene et al., 2007) during the experimental period. According to the data of our experiment, the direct influence of ‘Spirulina platensis’ additive to SLS in milk was not established. Hence the tendency of SLS decrease in milk of experimental group of cows was monitored, but statistically reliable differences were not established (Fig. 5).

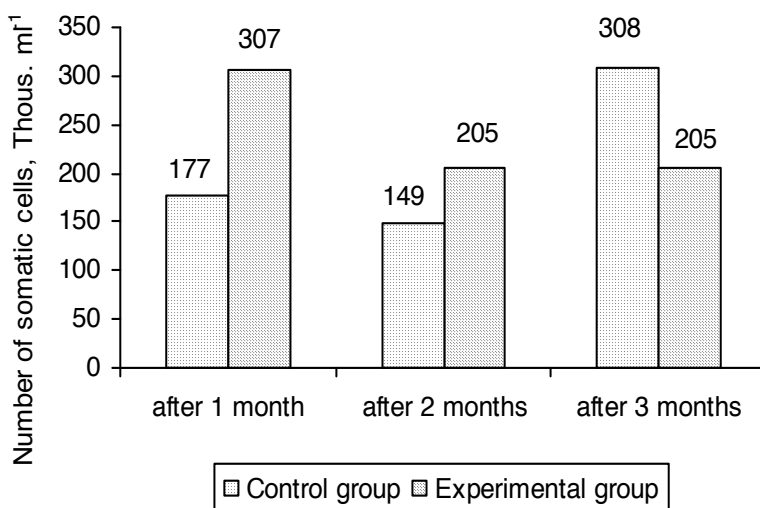


Fig. 5. Number of somatic cells, thous. ml⁻¹, P > 0.05

The analysis showed that during the 90-day period, the cows of the experimental group were distinguished by better milk productivity by 540 kg more (average milk yield was 3060 kg) compared with the control herd. It was noticed that cows of the experimental group were more bouncy, and their estrus was sharper than that of controlled cattle. That positive influence was stimulated by ‘Spirulina platensis’ additive, and has been confirmed by other authors (Kupras et al., 2003, Simkus et al., 2005; 2006) as well. It was established that production and economical indexes of the experimental group of cows were higher than that of the control herd (Table 4). Significantly increased milk productivity of the experimental group of cows during the experiment with no changes of fat and protein concentrations in its milk allowed us to maintain that the positive influence on these indexes was developed by ‘Spirulina platensis’ additive. The milk of the experimental group of cows had more lactose,

which positively influenced milk quality. Therefore, the received data confirmed that combined fodder additive ‘Spirulina platensis’ positively influenced milk productivity, lactose concentration in milk, body condition of cows and estrus processes. The use of ‘Spirulina platensis’ additive was economically efficient. The income from the milk increased on average by 378 Lt from each cow of the experimental group compared with the control herd. Accordingly the costs for ‘Spirulina platensis’ additive for one cow compounded at just 45 Lt. Therefore, the cost for the ‘Spirulina platensis’ additive for 1 Lt yielded 8.4 Lt of additional income (Table 4).

Table 4. Efficiency of cow feed with ‘Spirulina platensis’ additive technology

Index	Control group (n = 20)	Experimental group (n = 20)	%, compared with Control group
Average yield per day, kg	28 ± 4.07	34 ± 4.80	121*
Average fat concentration in milk, %	4.19 ± 0.48	4.16 ± 0.49	99.3
Average protein concentration in milk, %	3.17 ± 0.35	3.18 ± 0.37	100.3
Average lactose concentration in milk, %	4.79 ± 0.66	4.83 ± 0.55	101
Average urea amount in milk, mg/%	32 ± 4.02	34 ± 3.83	106
Average number of somatic cells in milk, thous./ml.	211 ± 49	239 ± 98	113
Cow’s milk yield during a 90-day period, kg.	2520	3060	121
Income for milk, Lt	1764	2142	121
The use of supplement ‘Spirulina platensis’ for 1 cow, kg	-	18	-
Price of additive, Lt	-	45	-
Increased extra income after spending 1 Lt for ‘Spirulina platensis’ additive	-	8.4	-

Note. * - P < 0.05

Research data show that during the experiment, welfare of both groups of cows was similar, though the experimental group of cows had better appetite, and adapted better to changed nutrition conditions during the transitional period from stable to pasture. The milk productivity of this group was higher; their milk had a higher concentration of lactose; the body condition and estrus of these cows were clearer. In our opinion special composition of combined fodder additive ‘Spirulina platensis’ positively influenced activity of micro-organisms in the big fore-stomach and fermentation and metabolism processes in cattle organism. The above-mentioned changes positively influenced synthesis of lactose, milk productivity as well as particular body condition and reproductive processes.

CONCLUSIONS

The combined fodder additive 'Spirulina platensis' in cows' early lactation period had a positive influence on increase in milk productivity by 21 % ($P < 0.05$). That increased joint yield of milk fat, protein and lactose.

Milk composition and quality indexes were similar in both groups of cows: concentration of fat was rather lower in the experimental group during the first (0.06%) and third (0.31%) month and somewhat higher (0.29%) during the second month of the experiment; accordingly the protein concentration in milk of the experimental group of cows has a tendency to decrease (by 0.1%) during the first month and to increase during the second (0.1%) and third (0.02%) months of the experiment; the concentration of lactose increased constantly in milk of both groups of cows, however, in the milk of the experimental group, concentration of lactose was higher (0.06%) and during the third month of the experiment it was slightly lower (0.02%) compared with the control group of cows.

'Spirulina platensis' additive positively influenced processes of cow estrus and body condition (8.5-11 %).

Fodder additive 'Spirulina platensis' cyanobacteria *Arthrospira (Spirulina) platensis*, used in the early lactation period, was economically effective, compared to the cows feeding without that additive.

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