IMPACT OF THE COMBINATION OF SYNERGISTICALLY ACTING PROBIOTIC CULTURES, ORGANIC ACIDS AND ADSORBENTS MIXTURE TO DAIRY COW'S PERFORMANCE AND MILK COMPOSITION

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Abstract. This paper reviewed issues regarding the use of probiotics (feed additive) in animal feeding. Probiotics are increasingly used in commercial animal production operations improve animal health and productivity. The major outcomes from using probiotics include improvement in productivity and milk quality. A total of 20 Lithuanian Black and White cows (10 animals in the control group and the test group) were selected for feeding test and fed with the experimental diets for 90 days. The experimental group of cows fed with a diet added by the combination of synergistically active probiotic cultures, organic acids and adsorbents mixture (20 g/day). The results shown that mixture increased these parameters: cows productivity 18.77% ($P<0.05$), fat content in the milk 0.37% ($P>0.05$), protein content – decreased by 0.13% ($P>0.05$) and lactose content decreased by 24% ($P<0.05$) compared to the control group. The results of this study clearly demonstrate that combination of synergistically acting probiotic cultures, organic acids and adsorbents mixture had positively effect on productivity and milk quality of dairy cows.
Keywords: milk yield, milk quality, feed additives, probiotics.

Introduction. Milk economy is a priority and competitive sector in Lithuania, which provides producers with income and consumers with dairy products that are of good quality (Lee et al., 2003). The demand for milk and dairy products is increasing. As a general rule milk consumption is high in developed countries and low in the developing ones, and appears to be particularly low in tropical and subtropical climates. Based on country-specific estimates of per capita milk consumption, the following three categories have been defined: High, more than 150 kg per capita/year: Argentina, most CIS countries, Costa Rica, Ecuador, Europe, Honduras, Israel, Lebanon, North America, Oceania, Turkey, Uruguay and others such as Pakistan and Sudan. Medium, 30-150 kg per capita/year: India, Japan, Republic of Korea, North and Southern Africa, most countries of the Middle East and Latin America (except Argentina, Ecuador and Uruguay). Low, less than 30 kg per capita/year: China, Ethiopia, Yemen and most countries of Central Africa and East and Southeast Asia. So to satisfy increasing demand of dairy products milk producing enterprises should increase their production and cows' productivity as well (Jeroch et al., 2010).

Milk production is one of the most complex branches of agricultural production. The profitability of dairy farming is determined by many factors. Profitability of milk production is reduced if we ignoring any element of complex milk production technology. The key factors that determine the cost-effectiveness of milk production are genetic potential of animals, feed and feeding condition, housing and care technologies. The meaning of separate elements is uneven (Želvytė et al., 2006). In order to increase the productivity of cows, it is important to ensure their welfare, in particular - optimal functioning of the digestive system due to the peculiarities of the large rumen ecosystem and the specific composition of microflora (Bahari, 2017). For this purpose, various feed additives are often used: probiotic preparations, organic acids, adsorbents (Kudlinskienė et al., 2016; Zebeli et al., 2010).

Probiotics are microbial food supplements that apply beneficial effects on the host through improving the intestinal microbial balance. During the last decades, various feed additives have been used in ruminant nutrition. Production benefits, together with lower incidence of digestive disorders, better body condition of the animals, and reduced feed cost. For the last 10 years, increasing consumer concern about the long-term effects of antibiotics has led to a more focused interest in probiotics (Muehlhoff et al., 2013).

Dairy cows should get with feed enough dry matter, which are full of energy, protein, vitamins and minerals. Minerals are very important for all vital processes for animal‘s organism, but they do not have an energy value.

Analyzing experiments conclusions from scientistic literature, different nutritional elements, we can see that there is a positive effect for dairy cow's healthy and productivity. In scientist literature about *Saccharomyces cerevisiae, Aspergillus oryzae, Kluyveromyce smarxianus* and malic acid, there is absence about supplements effect for dairy cow's production quality, sensors analysis. **So the aim** of our research was to determine the influence of the combination of synergistically acting probiotic cultures, organic acids and adsorbents mixture on on dairy cow's performance and milk composition.

Material and methods

The research was carried out complying with the Law of the Republic of Lithuania on Animal Care, Housing and Use (No. XI–2271) as well as complying with the amended Order of State Food and Veterinary Service "On Approval For Requirements For Housing, Care and Use of Animals for Experimental and Other Scientific Research" (No. B1-872 of 24-09-2015).

For the trial, 20 Lithuanian Black-and-White cow's with analogous characteristics were selected. Control and experimental group dairy cow's housed and fed in equal conditions. Feeding trial was divided in two periods – preparatory (14 days) and experimental (90 days).

The cow's of experimental group were fed a conventional diet supplemented by the combination of synergistically active probiotic cultures, organic acids and adsorbents mixture.

Table 1. **Feeding scheme of preparatory period (14 days)**

Group	Number of cows in group	Feeding characteristics
Control	10	Basic diet
Experimental	10	Basic diet +10 g combination of synergistically active probiotic cultures, organic acids and adsorbents mixture

Table 2. **Feeding scheme of experimental period (90 days)**

Group	Number of cows in group	Feeding characteristics
Control	10	Basic diet
Experimental	10	Basic diet +20 g combination of synergistically active probiotic cultures, organic acids and adsorbents mixture

Table 3. **Specification of the combination of synergistically active probiotic cultures, organic acids and adsorbents mixture**

Hydrated sodium calcium aluminosilicates (HSCAS)	72,46 %
Tannines	2,20 %
Fermentation extracts of *Saccharomyces cerevisiae, Aspergillus oryzae, Kluyveromyces marxianus*	7,89 %
Carrier & anticaking	7,65 %
BHT (butylated hydroxytoluene)	0,10 %
Calcium propionate	0,50 %
Malic acid (E296)	8,35 %
Essential oils, plant extracts and derivatives	0,85 %

Table 4. **Energy and nutritional value of the control and experimental group diet (1 kg DM)**

Indicator	Units	Control and experimental group's diet's
Net energy per lactation (NEL)	MJ NEL/kg DM	6.79
Crude protein	g/kg DM	162
Crude fibre	g/kg DM	156
Crude fat	g/kg DM	36
Starch	g/kg DM	277
Sugar	g/kg DM	33

As we can see from the data in Table 4, the diets of cows in control and experimental group have the same energy values: 6.97 MJ NEL per kilogram of dry matter. The diets are based on the needs for energy, food, minerals and vitamins. The energetic and nutritional value of the diets is based on the computerized feeding program HYBRIMIN® Futter 2008.

Milk yield was determined by control milking. Milk samples were taken according to sampling requirements. Analysis was carried out by SE „Pieno tyrimai", using „Lactoscope 550" ir „LactoScope FTIR" (FTI.O.2001; Delta Instruments). Milk samples were analysed for milk fat, milk protein, lactose, urea concentration and somatic cells content.

Dairy producers are paid for milk based on the milk weight in kilograms, on the basic norms of milk protein and fat's, as well as on the milk qualitative indicators. The whole milk obtained, according to the formula, is converted to the basic milk quantity.

The acquired data of productivity, quality and composition of the milk processed using Microsoft Office Excel 2010. Arithmetic averages of characteristics and the deviations of the averages will be calculated. The reliability of arithmetic averages difference (P) is determined according to the Student. The data are considered to be statistically significant when P<0.05.

Results and discussion

The cows of one breed and one herd, which are treated on equal conditions, usually produce not equal amount of milk of different composition (Vibhute et al., 2011). Studies by researchers from different countries have found that feeding and keeping conditions make cow's productivity levels about 50-60%. Cows can fully realize their genetic abilities only when they have enough energy and all the necessary nutrients in the diet. If there is a shortage in the daily feed dose for a long time, the bodys reserves are exhausted, metabolism is disturbed, productivity decreases, and the composition of the milk deteriorates (Yasuda and Fukata, 2004).

Factors affecting the composition of milk are genetics, lactation stage, level of productivity, age of cows, environmental conditions, health and nutrition. Inheritance affects milk composition by 55%, the rest - 45% remain to environmental factors such as feeding (Schoeder, 2012).

Table 5. **The change in productivity of cows during the experimental period, kg**

Group	Beginning of the preparatory period	Beginning of the experimental period	After 30 days	After 60 days	After 90 days
Control	34.8±4.76	32.2±6.09	25.71±4.47	28.03±4.00	26.24±3.90
Experimental	34.6±4.83	33.86±10.04	29.27±5.17	33.29±4.93	29.12±4.95

As we can see from Table 5, during the experimental period, the increase of milk yield was observed in the experimental group comparing to the control group. At the preparatory period, on average, there was less milk by 0.2 kg or by 0.57% from the experimental group than from the control group of cow's (P>0.05). At the beginning of the experimental period, on average, there was more milk by 1.66 kg or by 5.16 % than in the control group of cow's (P>0.05). However, after the first experimental month, on average, there was more milk by 3.56 kg or by 13.85% in the experimental group than in the control group of cow's (P>0.05). In the second month of the experiment, cow's of the experimental group gave more milk by 5.26 kg or by 18.77% than control cow's (P<0.05). In the third month of experiment, there was more milk from the experimental group by 2.48 kg or by 9.31% than from the control group of cow's (P<0.05). The same increase of milk yield as mentioned by Vibhute et al. (2011) in their research.

During the whole experimental period, the average milk yield from cow's of experimental group was more by 11.7 kg or by 14.63% than in the control group of cow's (P>0.05).

As we can see from Table 6, during the whole experimental period, the milk of the experimental group was more by 3766.2 kg or 11.51% than in the control group of cow's (P>0.05).

Table 6. **Quantity of basic milk during the experimental period, kg**

Group	Quantity of basic milk during the experimental period, kg
Control	3377.4
Experimental	3766.2

Table 7. **Average quantity of basic milk during the preparation and experimental period, kg/d**

Group	Beginning of the preparatory period	Beginning of the experimental period	After 30 days	After 60 days	After 90 days
Control	43.4	39.04	31.74	39.64	37.45
Experimental	43.96	38.4	33.63	46.24	41.85

As we can see from Table 7, at the beginning of the preparatory period, it was received more milk by 0.56 kg or by 1.29% in the experimental group than in control group of cow's (P>0.05). However, at the beginning of the experimental period, it was received by 0.64 kg or 1.63%. less of milk from the experimental group as compared to the control group. During the first month of trial, the cow's of the experimental group gave more of basic milk by 1.89 kg or by 5.95% than the cow's of the control group; during the second month, it was more respectively by 6.6 kg or by 16.64% (P>0.05) than in the control group of cow's (P>0.05). In the third month of the experiment, the cow's of experimental group gave more of basic milk by 4.4 kg or by 11.74% than the cow's of the control group (P>0.05).

Fat content in milk during trial

Fat is one of the most important ingredients in milk. It is easily assimilated and is necessary for the human body as an energy source. Fat in milk consists of small l-5 μm diameter spheres visible only through a microscope. The size of the bulbs depends on the cow's breed, the lactation phase and individual characteristics. Milk fat contains about 60 per cent of saturated fatty acids and about 40 per cent of unsaturated fatty acids (Schroeder, 2012).

Table 8. **Analysis of milk fat during the preparation and experimental period, per cent**

Group	Beginning of the preparatory period	Beginning of the experimental period	After 30 days	After 60 days	After 90 days
Control	4.52±0.9	4.1±0.85	4.16±0.76	4.95±1.08	4.7±0.97
Experimental	4.74±0.97	3.9±0.76	3.89±0.62	4.84±1	5.13±1.1

As we can see from Table 8, the fat content in milk throughout the experimental period had been changing in milk of all cow's. At the beginning of the preparatory period, the fat content in milk of the experimental group was 0.22% (P>0.05) higher as compared to the control group. At the beginning of the experimental period, the fat content in milk of the experimental group was less by 0.2% as compared to the control group (P>0.05). After the first trial month, the milk of the experimental group had a fat content lower by 0.27% than in the control group (P>0.05). After the second experimental month, the fat content of cow's milk of the control group was higher by 0.1% than in the milk of the experimental group (P>0.05). However, at the end of the experiment, the fat content of cow milk was higher by 0.36% in the experimental group than the control group (P>0.05). During the whole experimental period, the fat content in milk of the experimental group increased by 1.24% (P>0.05). Yasuda and Fukata (2004) reported that the amount of total fat in milk of treated group was significantly higher than for control group.

Analysis of protein quantity in milk

Nutritional factors have a significant influence on the milk composition of dairy cow's. Nutrition provides the most effective means of changing the composition of milk. Among the milk ingredients (fat, protein, lactose, minerals and vitamins), fats and proteins are most susceptible to cow nutrition changes (Santos, 2002).

Protein concentrations in milk vary from about 3.0 to 4.0 per cent. It depends on the cow breed. The protein content in milk is proportional to the fat content in milk. There is a relationship between the two components of the milk: the higher the fat in the milk is, the higher the protein content (Theurer et al., 1995).

Table 9. **Analysis of milk protein during the preparation and experimental period, per cent**

Group	Beginning of the preparatory period	Beginning of the experimental period	After 30 days	After 60 days	After 90 days
Control	3.19±0.22	3.33±0.32	3.41±0.31	3.52±0.2	3.62±0.3
Experimental	3.16±0.32	3.17±0.26	3.23±0.25	3.5±0.29	3.49±0.29

As we can see from the data of Table 9, the protein content in the milk of the control and experimental groups of cows did not significantly differ during the first month of the experiment. The increase in milk protein in the middle of the experiment was not found in experimental group; and at the end of the experiment, the increase in milk proteins was determined in the control and experimental group as compared with the beginning of the experiment. Proteins in the experimental group increased by 0.33% (P>0.05) during the whole trial period. It can be assumed that supplementation of diets with the combination of synergistically acting probiotic cultures, organic acids and adsorbents mixture had a positive effect on the protein content in milk. While in research conducted by Singh and Kumar showed that addition of probiotic increases milk protein by 11.90, 21.43, and 21.43% (Singh and Kumar, 2007).

Analysis of somatic cells content in the milk

Somatic cells are white blood cells (leukocytes), epithelial cells of the body and secretion (Staniškienė et al., 2007). The uterine epithelial cells, in the course of normal processes of the body, constantly evolve and renew. When there is no inflammation in the mammary gland, the number of somatic cells ranges from 10 to 200,000 predominantly epithelial cells. Naturally, they are always multiplied by the time they stop feeding, once they start lice once a day, as well as in the first days after calving (Barlowska et al., 2009).

Table 10. **Analysis of somatic cells content in the milk during the preparation and experimental period, thou/ml**

Group	Beginning of the preparatory period	Beginning of the experimental period	After 30 days	After 60 days	After 90 days
Control	33.8±6.23	59.6±15.9	149±21.48	104±15.28	141±7.71
Experimental	60.2±10.11	46.22±14.52	64.1±14.87	76.5±21.34	107.2±19.33

As we can see from Table 10, the number of somatic cells in cows' milk changed significantly during the experimental period. It was found that the number of somatic cells in the milk of cows of the control group was higher as compared with the experimental group during the first month of the experiment.

At the beginning of the preparatory period, the lowest number of somatic cells was recorded in the milk of the control group at 33.8 thou/ml in the experimental group it was recorded 60.2 thou/ml of somatic cells. However, at the beginning of the trial period, in the experimental group, the number of somatic cell was 13.38 thou/ml or less by 22.45% than in the control group. After the first month of the experiment, the number of somatic cells in milk of dairy cow's in the experimental group was 64,1 thou/ml or 56.98% as compared to the control group (P>0.05). After the second month of the experiment, the number of somatic cells in the experimental group was 27.5 thou/ml or less by 26.44% as compared to the control group (P>0.05). In the last month of the experiment, the number of somatic cells in the experimental group was 33.80 thou/ml or less by 23.97% as compared to the control group (P>0.05). To sum up the obtained results, we can conclude that the combination of synergistically acting probiotic cultures, organic acids and adsorbents mixture reduced the number of somatic cells in the experimental group.

Analysis of milk lactosis

Milk lactose is the most important milk carbohydrate, otherwise called as milk sugar. Lactose in the cow's milk makes in average of 4.7%, it makes about 30% of the total milk energy value. It is known that milk lactose reflects the amount of glucose (sugar) in the blood. Therefore, a lower level of lactose in milk indicates that cows lack energy (Masek et al., 2008).

Table 11. **Analysis of lactose quantity in the milk during the preparation and experimental period, per cent**

Group	Beginning of the preparatory period	Beginning of the experimental period	After 30 days	After 60 days	After 90 days
Control	4.56±0.14	4.76±0.14	4.58±0.14	4.6±0.15	4.54±0.11
Experimental	4.65±0.37	4.78±0.13	4.62±0.08	4.7±0.07	4.51±0.13

As we can see from the data of Table 11, the lactose content in the milk of the control and experimental groups of cows did not significantly differ during the period of the experiment.

At the beginning of the preparatory period, in the cow's milk of the experimental group it was found more lactose by 0.09% as compared to the control group (P>0.05). At the beginning of the trial period, in the cow's milk of the experimental group it was found more lactose by 0.02% as compared to the control group (P>0.05). After the first experimental month, in the experimental group the lactose concentration was higher by 0.04% (P>0.05) than in the cows' milk of the control group. After the second trial month, in the experimental group the lactose concentration was higher by 0.1% (P>0.05) than in the cow's milk of the control group. At the end of the experiment, in the experimental group lactose concentration was lower by 0.03% (P>0.05) than in the milk of the control group.

Analysis of urea content in milk

Milk urea is the final product of nitrogen metabolism, which shows in milk the balance of nitrogen in the body and the cow's "sourcing" with energy feed and protein. Urea is important not only for the diagnosis of diseases, but also for the assessment of cow's feeding. The urea rate in milk is between 15 and 25 mg%, and for productive cows it is 30 mg%. If less than 15 mg% of urea is found in the milk, it means that there is a shortage of protein in the cow‘s diet. By increasing the protein content in the diet will increase not only the cow‘s productivity, but also the protein content of milk, the cow's will be less likely to suffer from metabolic diseases. If more than 30 mg% of urea is found in milk, it can be suspected that cow's are fed with high protein feed and their feeding is ineffective, causing loss to the dairy farm, as the feed is not fully consumed (Lock and Michael, 2012).

Table 12. **Analysis of urea concentration during the preparation and experimental period, mg%**

Group	Beginning of the preparatory period	Beginning of the experimental period	After 30 days	After 60 days	After 90 days
Control	21.1±6.49	18±5.6	21.2±4.05	23.7±4.57	16.2±4.66
Experimental	26.67±7.87	20.5±4.62	23.1±7.05	24.1±1.23	17.4±8.39*
*- data statistically significant at P<0.05					

As we can see from Table 12, the concentration of urea in dairy cow's milk changed significantly during the experimental period. It is found that from the beginning of the experiment, the urea content tended to decrease in the experimental group, from the beginning of the preparatory period up to the end of the experiment, the concentration of urea in dairy cow's milk decreased by 36.26% (P<0.05) in the experimental group. Therefore, we can say that the combination of synergistically acting probiotic cultures, organic acids and adsorbents mixture influenced the concentration of urea in dairy cow's milk. While other researchers observed no differences in the concentration of urea between diets containing probiotics and the control treatment (Bruno et al., 2009: Masek et al., 2008; Abo El-Nor and Kholif, 1998) reported that the elevated levels of urea nitrogen in ruminants are caused due to feeding by probiotics.

Conclusion

The result of this study clearly demonstrate that feed additive synergistically acting probiotic cultures, organic acids and adsorbents mixture can positively affect milk productivity, results shows increase of milk productivity and fat content. The feed additive had no significant effect on the lactose content but feed supplement mixture reduced the concentration of urea and somatic cells in milk.

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