INFLUENCE OF EXTRUDED RAPESEEDS AND FABA BEANS MIXTURE ON PRODUCTIVITY, PRODUCTION QUALITY AND RUMEN FLUID PARAMETERS OF DAIRY COWS

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Abstract. The purpose of this study was to assess the influence of a mixture of extruded rapeseeds (70%) and fodder beans (30%) for dairy cows on fermentation processes of rumen fluid, milk productivity and quality. For the purposes of this study, 30 holsteinizated Lithuanian cows of Black-and-White breed of II-IV lactation were selected and divided into 2 groups (15 cows each) applying the principle of analogous groups. The number of infusoria, the rumen reduction activity of bacteria, pH, total volatile fatty acid (VFA), content total and ammonia nitrogen were investigated during the whole experimental period. Milk quantity, composition, and quality indicators were estimated during control milking; fat, protein, lactose, and urea were assessed with the LactoScope FTIR instrument (FT1.0. 2001; Delta Instruments, the Netherlands). The investigation showed no crucial influence of the extruded rapeseeds and faba beans mixture on microbiological and biochemical indicators as well as milk composition and quality indicators of dairy cows' rumen content. However, the milk yield of the experimental cow group increased by 2.35 kg/d, i.e. 10.96 % ($P<0.05$), while the control group showed an increase of only 0.59 kg/d, or 2.94 % ($P>0.05$). During the whole investigation period, the experimental group of cows produced 8.02% more milk compared with the controlled group of cows.

Keywords: dairy cow, mixture of rapeseeds and faba beans, rumen, milk production

Introduction. The most important task for the dairy farming sector today is to get cost-effective production of high quality. In order to enrich the diets, there is a demand for cheaper local raw materials amply containing all the necessary nutrients: protein, fat, carbohydrates, etc. The rumen of ruminants is especially important since in it cellulose is broken down to glucose and sugar, and these carbohydrates are necessary for bacteria growth and protein synthesis (Nocek and Tamminga, 1991). The number of microorganisms in a rumen reaches to 10^{11} of viable cells/mL (Yeoman et al., 2011). Without these microorganisms, ruminants would get 15% less nutrients from their forage. The microorganisms of a rumen are also digested in the small intestines of ruminants and remain as a nutrient source, i.e. transform into valuable microbial proteins (Leschine, 1995). There are hundreds of different bacteria in the rumen that take part in digestive processes (Yun et al., 2006). Feeding ruminants with forage that contain little fibrous materials results in easy digestion of carbohydrates; however, this is also the reason for gram-negative bacteria to appear in the rumen (Plaizier et al., 2008).

Ruminal pH influences activity of microorganisms. Optimal pH of dairy cows' rumen content is 6.3–6.8. When pH of rumen content is lower than 6.0, the growth of anaerobic fungi and infusoria slows down. Increased acidity in a rumen decreases effectiveness of forage digestibility; when pH is lower than 5.5, infusoria die and bacteria activities are disrupted (Sederevičius et al., 2001). Due to their great number and mobility, infusoria mix and loosen the content of a rumen, which results in a greater surface area of forage and better conditions for activities of bacteria and enzymes. Infusoria accumulate reserved polysaccharides, which are not only vital for their own life but are also important to the cattle nutrition. Infusoria actively break down cellulose, hemicellulose, and starch, and participate in digestive processes in the rumen (Karim and Santra, 2002).

Volatile fatty acids are the main energy source for cows that provide approximately 70% of energy and consist in the rumen during fermentation. During presence of microorganisms in the rumen, carbohydrates ferment, amino acids break down, and other processes take place.

Concentration of volatile fatty acids in the rumen varies from 60 to 150 mmol/L, and it is strongly influenced by ration composition (Aschenbach et al., 2011).

The aim of this study was to assess the influence of a mixture of extruded rapeseeds (70%) and fodder beans (30%) for dairy cows on fermentation processes of rumen fluid, milk productivity and quality.

Material and methods

The investigation was carried out in a dairy cow farm in Marijampole district. The experiment was divided into 2 periods: preparatory 14 days and experimental 90 days. Selected dairy cows were divided into 2 groups (control and trial), 15 animals each, according to the principle of analogous groups (considering lactation, parity,

productivity during former lactation, animal's weight, milk production). The cows were tethered, watered from automatic water troughs, milked using milking pipelines and fed twice a day. The experiment was carried out with 30 holsteinizated Lithuanian Black-and-White dairy cows of II-IV lactation. The groups, number and feeding strategy of cows are presented in Table 1.

Table 1. **Feeding scheme**

Group	Number of cows per group, units	Feeding characteristics
Control	15	Basic ration
Trial	15	Basic ration + 0.800 kg of extruded rapeseeds (70%) and faba beans (30%) mixture for a cow per day

Temperature of the extrusion was kept constant, i.e. 140ºC±5ºC. Prior to the extrusion, beans and rapeseeds were ground using a hammer mill (2/3 mm sieves). Water consumption during the production was approx. 70 L/t. Humidity of the products after the extrusion was approximately 16%, and after cooling, it was 9–10%.

The experiment was carried out complying with the Law of the Republic of Lithuania on animal care, housing and use No. XI-2271 of 03-10-2012 and 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)

In the agricultural company, dairy cows are fed with compound feed mixtures produced in the farm. Grass and corn silage is produced and crops are grown for nutrition purposes. Rations for both groups were balanced based on the need of feed, mineral substances and vitamins (see Table 2).

Nutritional and energy value of the rations was calculated using a computer feeding programme HYBRIMIN® Futter 2008.

Table 2. **Averaged day ration of control and trial groups**

Forage	Control group	Trial group
Grass haylage, kg	14.00	14.00
Maize silage, kg	23.00	23.00
Straws, kg	0.50	0.50
Molasses, kg	1.00	1.00
Silage from sugar beet pulp, kg	10.00	10.00
Compound feed (+extruded rapeseeds (30%) and faba beans (70%) mixture), kg	-	8.00
Ration contains:		
Dry matter, kg	24.94	24.96
NEL (net energy for lactation), MJ/kg SM	6.72	6.78
Crude proteins, g/kg SM	167	170
Crude fat, g/kg SM	26	29
Crude fibre, g/kg SM	165	166
Crude ashes, g/kg SM	85	84

Methods/technique

Nutritional value assessment of cereal grain was carried out at the Lithuanian University of Health Sciences, Institute of Animal Raising Technology, Laboratory of Animal Productivity, Department of Animal Breeding and Nutrition, Laboratory of Animal Nutriciology Research and Department of Systemic Evaluation of Nutrigenomics and Animal Husbandry Processes as well as the Institute of Husbandry.

During the experiment, control milking was performed once a month. Control milking showed the milk yield. The milk samples were assessed as follows: the number of somatic cells in the milk samples was determined using SomaScop MK2 (Delta Instruments, the Netherlands), which functions based on the fluorine-opto-electronic method; milk fat, protein, lactose and urea were determined using LactoScope FTIR (FT1.0. 2001, Delta Instruments, the Netherlands) based on the method of absorption of infrared radiation medial region rays; milk testing was performed at State Enterprise 'Pieno tyrimai'.

The samples were collected from 3 randomly selected animals of each group with a stomach tube (Sederevičius, 2000) 3 hours after morning feeding. The rumen fluid was analysed for pH, total volatile fatty acid (VFA) content, total and ammonia nitrogen, reduction activity of bacteria and protozoa count. Ruminal pH was measured immediately after sampling, using a handheld pH-meter (Horiba - Twin pH, Spectrum Technologies). Total VFA was defined by rumen fluid distillation in a Marcgamus apparatus according to the method of Pustovoj (1978). Total nitrogen was analysed by Kjeldahl procedure (Behr system, Germany), and ammonia nitrogen by titrimetric method with the preliminary distillation (Behr steam distillation unit S1, Germany). Glucose fermentation reaction and reduction activity of bacteria were evaluated according to the method described by Bakūnas (2004).

Fuchs-Rosenthal counting chamber (Blaubrand, Wertheim, Germany) was used for enumeration of protozoa by Olympus microscope (BX43, Hamburg, Germany). The rumen fluid analyses were carried out at the Research Centre of Digestive Physiology and Pathology of the Department of Anatomy and Physiology, Lithuanian University of Health Sciences Veterinary Academy.

Statistical analysis

Statistical analysis was carried out by the means of SPSS for Windows software, version 15.0 (SPSS Inc., II, USA, 2006). It includes calculation of arithmetic mean values of the variables errors of the mean values, and the obtained results are statistically significant when $P<0.05$.

Results

Table 3 shows that at the beginning of the experiment the difference between the rumen content pH of the control group cows (pH 6.83) and the trial group cows (pH 6.81) was 0.02. During the experiment and at the end of it, the rumen content pH of both groups showed no significant differences and was within the physiological norm.

Table 3. **Variation of rumen parameters over the experimental period**

pH variation over the experimental period			
Group	**Beginning**	**Middle**	**End**
Control group	6.83	6.54	6.78
Trial group	6.81	6.68	6.80
Variation of reduction activity of rumen bacteria over the experimental period, s			
Control group	153±57.74	120±55.68	113±20.82
Trial group	163±23.09	77±8.54	123±46.19
Variation of protozoa number in the rumen content, x10^3/mL			
Control group	135.94±34.48	362.50±15.86*	285.45±61.17
Trial group	314.59±13.29*	122.92±14.52	325.00±72.08
Glucose fermentation reaction in the rumen content, cm^3/h			
Control group	1.67±0.76	1.98±0.96	1.87±1.00
Trial group	1.30±0.82	1.83±0.58	1.79±0.76
Variation of the number of volatile fatty acids in the rumen content, mmol/L			
Control group	66.67±5.77	86.67±23.09	83.33±5.77*
Trial group	66.67±11.54	103.33±15.27*	76.67±15.27
Variation of total nitrogen in the rumen content, mg/100 mL			
Control group	60.90±8.77	109.67±24.47	134.40±37.11
Trial group	60.20±19.33	86.33±11.21	125.30±29.40
Variation of ammonia nitrogen in the rumen content, mg/100 mL			
Control group	15.21±4.04	29.49±9.26	23.89±7.00
Trial group	16.24±1.56	21.93±5.52	27.81±11.96
*P<0.05			

Throughout the entire experimental period, the reduction activity of rumen content bacteria of both groups of cows was practically the same and within the limits.

Analysis of protozoa number variation in the rumen content showed that the number of the protozoa was different at the beginning of the experiment, halfway through the experiment and at the end of it. At the beginning of the experiment, the number of protozoa in the trial group of cows was 178.7 x 10^3/mL, and at the end it was 39.6 x 10^3/mL higher compared with the protozoa number of the control group of cows ($P<0.05$). In the middle of the experiment, the number of protozoa was higher 239.6 x 10^3/mL in the control group of cows (362.50 x 10^3/ml) compared with the trial group (122.92 x 10^3/mL) ($P>0.05$).

Throughout the entire experimental period, glucose fermentation reaction was similar in both groups of cows and within the physiological norm from 1.30 to 1.98 cm^3/h ($P>0.05$).

Analysis of number variation of VFA at the beginning of the experiment showed that the number of volatile fatty acids in both groups of cows was similar at the beginning as well as at the end of the experiment, i.e. 66.67 mmol/L at the beginning for the control group of cows and 83.33 mmol/L at the end of the experiment. For the trial group, the number of VFAs was the same as for the control group of cows (66.67 mmol/L), and at the end of the experiment it was 76.67 mmol/L**.** In the middle of the experiment, the content of VFAs was lower in the control group (86.67 mmol/L) compared with the trial group of cows (103.33 mmol/L) ($P<0.01$).

Analysis of fermentation of nitrogen substances in the rumen showed that the variation of the content of total nitrogen and ammonia nitrogen comparing the two groups was different throughout the experiment.

Analysis of variation of total nitrogen content over the experimental period showed that the concentration of the total nitrogen in the rumen content in the control group as well as in the trial group was within the physiological norm throughout the whole experimental period. For the

control group of cows, the total nitrogen content in the rumen content was 60.90 mg/100 mL at the beginning, and 134.40 mg/100 mL at the end of the experiment. For the trial group, the content was similar to the control group at the beginning, i.e. 60.20 mg/100 mL, while at the end of the experiment it was 125.30 mg/100 mL (P>0.05).

Analysis of the total ammonia nitrogen content during the first experimental month revealed that the content in the rumen content of both groups was 15.21 mg/100 mL in the control group and 16.24 mg/100 mL trial group. Halfway through the experiment, the ammonia nitrogen content of the control group of cows was 7.56 mg/100 mL higher than in the trial group of cows. At the end of the experiment, the content of total ammonia nitrogen was similar in both groups: 23.89 mg/100 mL in the control group of cows and 27.81 mg/100 mL in the trial group of cows. Comparison of the content of total ammonia nitrogen during the 3 stages of the experiment shows that the mean content of ammonia nitrogen was within the physiological norms.

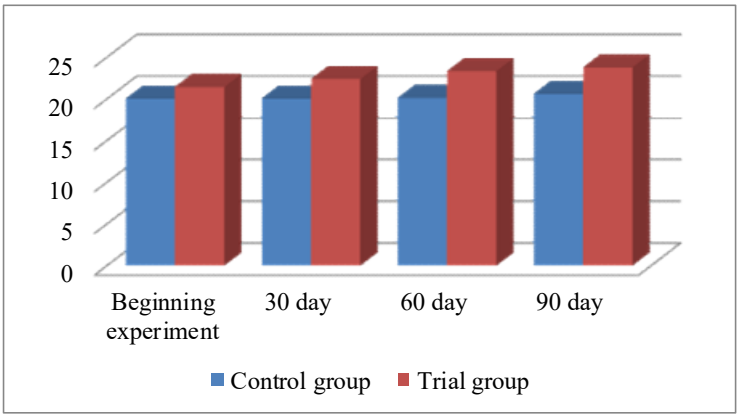

Fig. 1. **Milk yield variation during experimental period, kg per day**

Comparison of the beginning and the end of the experiment shows that the milk yield of the control group of cows increased by 0.59 g/d or 2.94 % (P>0.05), while for the trial group of cows the increase was 2.35 kg/d or 10.96 % (P<0.05). Throughout the entire period of the experiment, the trial group of cows produced 8.02 % more milk compared with the control group of cows.

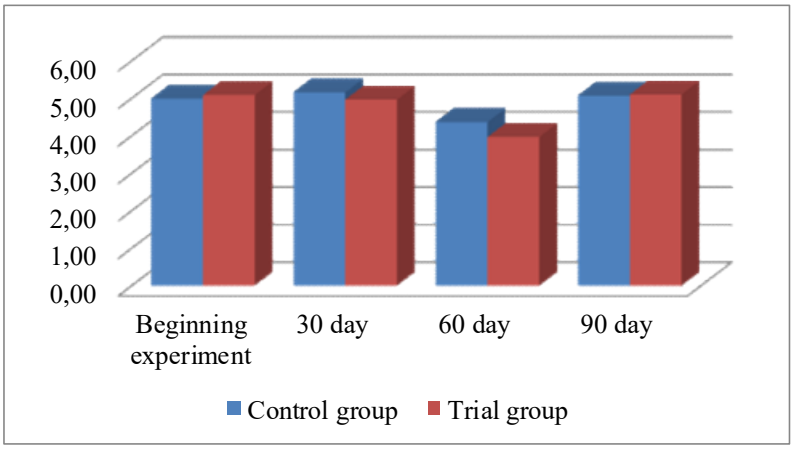

Fig. 2. **The dynamics of milk fat, %**

As Figure 2 shows, fat content was similar in both groups of cows at the beginning and at the end of the experiment. At the beginning of the experiment, the content of milk fat in the control group of cows was 5.00%, and in the trial group of cows it was 5.10%; at the end of the experiment, it was 5.08% and 5.12% (P>0.05), respectively.

Comparison of the control group and the trial group with respect to milk protein content shows that the variation of the content of milk protein throughout the entire experimental period was not identical. For the control group, the highest content of protein in milk was at the end of the experiment (4.17%), and the lowest content was half-way through the experiment (3.72%); comparing the beginning and the end of the experiment, it was by 0.38% higher. For the trial group, the highest content of protein was at the end of the experiment as well (3.91%), and the comparison between the beginning and the end of the experiment showed that the content of milk protein was higher by 0.33% at the end of experiment.

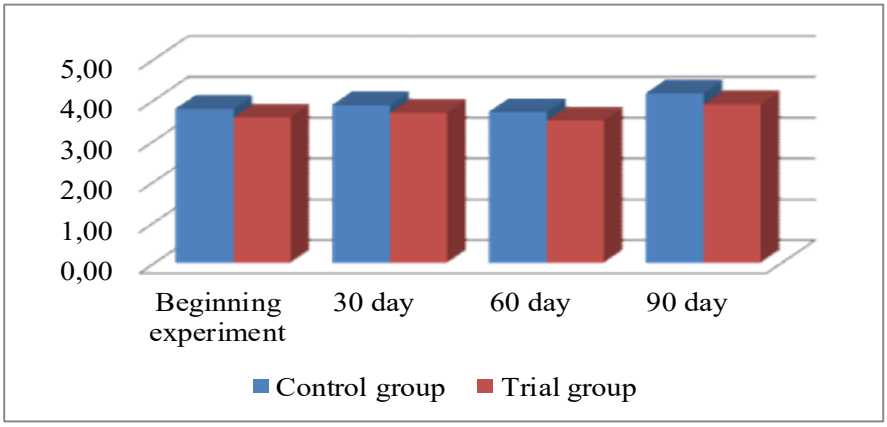

Fig. 3. **The dynamics of milk proteins, %**

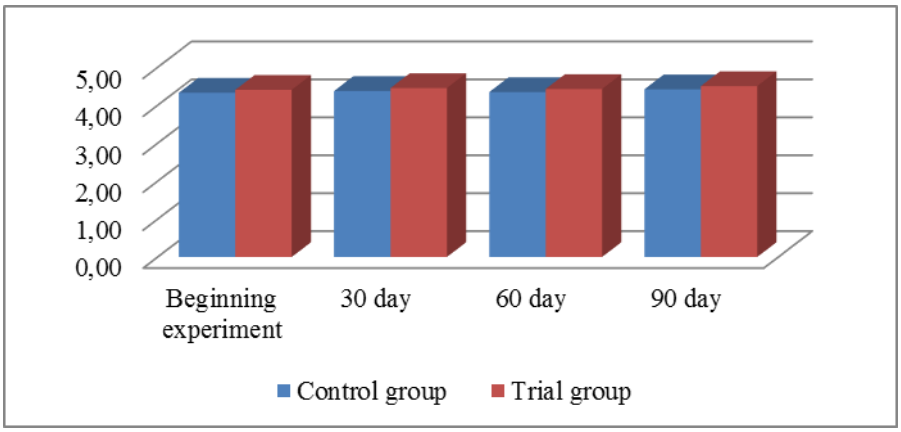

Fig. 4. **The dynamics of milk lactose, %**

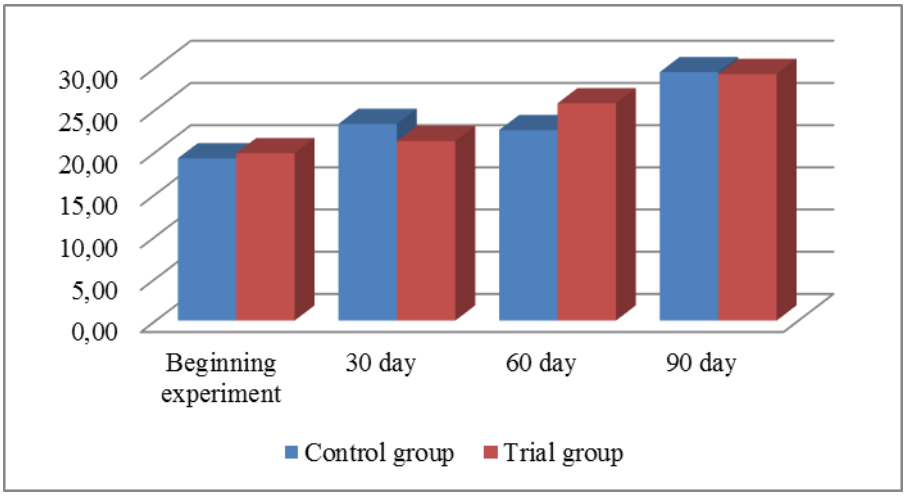

Fig. 5. **The dynamics of urea, mg%**

Concentration of lactose in milk of cows of both groups was almost the same and varied evenly during the whole experiment. Comparison between the beginning of the experiment and the end shows that lactose content from milk of the control group increased 0.11% ($P<0.05$), and the trial group had an increase of 0.09% ($P<0.05$).

The data in Figure 5 show that the concentration of urea in milk of both group of cows varied differently throughout the entire experiment, yet it was within the physiological norms. At the beginning of the experiment, the content of urea was 19.22–19.83 mg%, and at the end it was 29.43 and 29.22 mg%.

Discussion

Activity of fermentation processes of rumen content is unstable. It could depend on the time of feeding,

composition of the ration, quality of forage, the amount of time after feeding, the rate the rumen content is passed to other parts of the digestive tract, etc. (Laugalis et al., 2007).

A. Sederevičius et al. (2001) discussed that the optimal pH of the rumen is 6.5–6.8. The medium of the organism becomes more acid when pH drops lower than 5.5; infusoria die, activities of bacteria are disturbed. There are times when pH is even lower. Throughout the entire experiment, the rumen content pH of the trial group of cows varied from 6.68 to 6.81 and was within the norms (Dijkstra et al., 2012). pH of the control group of cows varied from 6.54 to 6.83.

A reagent (methylene sand) is reduced by rumen content microorganisms of healthy cows during no longer than 3 minutes (180 s) when the ambient temperature is 20–22°C (Sederevičius et al., 2004). During this experiment, the reductive activity of rumen content bacteria was not higher than 163 s.

The comparing between the beginning and the end of the experiment showed that the amount of protozoa in the rumen content of the trial group of cows increased by 3.3% and was 325.00 x 10^3/mL. Population of the rumen content microorganisms is constituted of bacteria, protozoa and fungi. They break down starch, cellulose and hemicellulose. Bacteria synthesise the main part of microbial biomass. It constitutes from 4 to 9 kg of biomass depending on the composition of forage ration, feeding technology, etc. (Jeroch et al., 1999).

Glucose fermentation reaction is an important indicator in the rumen content. During this reaction, gas is produced in the rumen, approx. 0.5–1 L per minute. Greater part of this gas is composed of carbon dioxide (60–70%), methane (30–40%), also nitrogen, hydrogen, oxygen, etc. The lack of carbohydrates and protein in the ration results in low production of gas. If the fermentation process in the rumen is within the normal range, 1–2 cm^3 /h of gas are produced during 1 hour (Czerkawski, 1986).

The rate of glucose fermentation reaction in the rumen content of the control group of cows and in the trial group of cows was within the normal range at the beginning of the experiment as well as at the end of the experiment (1.30–1.98 cm^3/h) and agreed well with data of other authors (Yang et al., 2001).

VFAs are the main source of cow energy. Cows obtain approximately 70% of energy from this source. VFAs are produced in the rumen during the fermentation process. During this experiment, the variation of the content of VFAs was similar in the rumen content of the control group of cows as well as of the trial group of cows (from 66.67 mmol/L to 103.33 mmol/L) and was within the physiological norms. The content of ration determines the VFA concentration in the rumen and it varies from 60 to 150 mmol/L (Aschenbach et al., 2011).

There is a possibility that productive cows will get too much nitrogen compounds with the forage that break down quickly to ammonia and, while accumulating in the organism, distort the liver. In such a way, metabolism is impaired, especially of minerals. Synthesis of vitamins becomes worse, the level of magnesium in the blood drops and the cow is diagnosed with tetany. Throughout the experiment, total nitrogen concentration in the rumen content of the control group of cows as well as in the trial group was within the physiological norms (60.20–134.40 mg/100 mL) and agreed well with data of other authors (Reynolds, 2005).

Analysis of the obtained data showed that the content of ammonia nitrogen in the rumen of the trial group of cows was unstable and increased from 16.24 to 27.81 mg/mL. Calsamiglia et al. (2010) also declares that concentration of ammonia nitrogen in the rumen content is usually unstable. The optimal content of ammonia nitrogen in the rumen content is from 3 to 25 mg/100 mL (Boucher et al., 2007). At the end of the experiment the content of ammonia nitrogen in the trial group was by 2.81 mg/100 mL higher; however, it did not influence the fermentation processes of the rumen since the excess ammonia nitrogen removed from the organism with urine (Agle et al., 2010).

The obtained results also show that the trial group of cows produced 8.02% more milk compared with the control group. It was determined that at the end of the experiment, as opposed to the beginning, the yield of the trial group of cows produced by 2.35 kg, i.e. 10.96% more milk. Similar results were obtained by other authors. For example, Mordenti et al. (2007) claims that insertion of field beans into the ration positively influenced the milk production as well as composition and quality indicators.

It was determined that the compound feed used in the research and containing 10% of extruded rapeseed and soybean mixture did not essentially influenced indicators of milk composition and quality throughout the entire period of the experiment. Over the entire experimental period, the content of milk fat increased by 0.02%, protein by 0.33%, and lactose concentration by 0.09%. There was no significant difference between the data of milk composition.

Throughout the entire experimental period, concentration of urea in the milk of the trial group of cows was within the norms (15–30 mg%) (Bannink et al., 2013); at the beginning of the experiment, the concentration was 19.83 mg%, and at the end of the experiment it was 29.22 mg%.

After analysis of the obtained results, it can be concluded that the rapeseed and soybean mixture used in the experiment had no significant influence on microbiological and biochemical indicators of the rumen of dairy cows.

Conclusion

The supplement of extruded rapeseeds (70%) and faba beans (30%) to the mixture used in the experiment had no considerable influence on milk composition and quality indicators. Throughout the entire experimental period, the milk yield increased by 2.35 kg/d, i.e. 10.96 % ($P<0.05$), while the milk yield of the control group of cows increased only by 0.59 kg/d or 2.94 % ($P>0.05$). Over the entire period of the experiment, the trial group of cows produced 8.02% more milk than the control group of cows.

After the investigation, it can be concluded that when the cows were fed the ration with the compound feed containing 10% of rapeseeds and faba beans mixture (70% rapeseeds and 30% faba beans) the rumen fermentation indicators were within the norms throughout the whole experiment.

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