THE EFFECTS OF EXTRUDED LUPINS *(LUPINUS SPP.)*, FABA BEANS *(VICIA FABA)* AND PEAS *(PISUM SATIVUM)* ON THE RUMINAL FLUID PARAMETERS IN DAIRY COWS

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Abstract. The aim of our research was to determine the influence of extruded lupins', extruded fodder beans and extruded peas on fermentation processes in the rumen of dairy cows. Lithuanian Black-and-White cows with analogous characteristics were selected and randomly allocated into 4 groups (control and experimental's), 10 animals each. The control group was fed a conventional diet consisting mainly of grass silage, ground barley grain, enriched extruded full fat soybean, and mineral premix. The experimental groups were fed a similar diet, but instead of 1.5 kg of soybean meal, the cows were given the same amount of the extruded lupins, extruded fodder beans and extruded peas. Raw material's for the trial was extruded by SC „Kauno Grūdai". The samples were collected from three randomly selected animals of each group with a stomach tube (Sederevičius, 2000) 3 hours after the supplementary feeding with extuded lupins, extruded fodder beans and extruded peas. The rumen fluid was analyzed for the pH, total volatile fatty acid (VFA) content, total and ammonia nitrogen, reduction activity of bacteria and protozoa count. The results of this study shown that soybeans replacement with lupins *(Lupinus spp.)*, fodder beans *(Vicia faba)* and peas *(Pisum sativum)* in dairy cows rations, had no negative influence on rumen's fermentatative indexes and warranted it's optimal activity.
Keywords: extruded lupins, extruded fodder beans, extruded peas, dairy cows, rumen fluid

Introduction

In many animal production systems feed is the biggest single cost and profitability can depend on the relative cost and nutritive value of the feeds available (McDonald et al., 2002). In pursuit of sustainable and economically-viable farming systems, there is a need for livestock farmers to reduce reliance on imported feedstuffs, such as soya bean, which are subject to world market price fluctuations and have a high environmental footprint.

Soybean is widely used in conventional intensive animal feeding systems because of its known high protein content (38-42%) and good amino acid balance and digestibility (Baker, and Stein, 2009; Cervantes-Pahm et al., 2008; Hartwig, et al., 1997; Johnson, 2008; Opapeju et al., 2006). However soybean meal costs and availability are strongly related with the price development of agricultural commodities on the world market (Jezierny et al., 2010). Factors which may influence world market prices include variations in population and economic growth, changes in consumer's product preferences, but world market prices are also dependent on weather conditions (Gill, 1997; Trostle, 2008). The worry of the public opinion about the widespread use of genetically modified (GMOs) feedstuffs in animal feeding, such as soybean, has lead to focus on the possibility to replace soybean with alternative legume grains able to satisfy animal dietary protein requirements.

Consequently, livestock farmers worldwide are under increasing pressure to maximise their use of home-grown feeds. Grain legumes crops represent a great resource in organic agriculture both to satisfy the nutritional content of organic livestock feeding and to maintain soil fertility. The commercial availability of organic grain legume is decreasing, the costs are high and the GMO contamination risk is particularly high for soya bean, used to achieve the high protein values required by the animals. So, the cultivation of grain legumes such as sweet lupin (*Lupinus albus*), field bean (*Vicia faba var. minor*), high protein pea (*Pisum sativum*) on farm could solve the problem and improve the sustainability of the farm (Singh et al., 2007).

The most commonly in dairy cows nutrition used legume grains are peas (*Pisum sativum*), fodder beans (*Vicia faba*), and lupins (*Lupinus spp.*), it is characterised by high energy density allowed to the high protein, starch and/or fat concentrations, as more than sufficient is their calcium concentration. Within the grain legumes, lupins have higher amounts of crude protein (324–381 g/kg dry matter), compared to faba beans (301g/kg dry matter) and peas (246 g/kg dry matter). Jezierny et al. (2007) reported similar contents of crude protein in different batches of lupins, faba beans and

peas averaging 387, 308 and 249 g/kg dry matter, respectively. Many researches had been done in order to investigate legume grains effects on dairy cows performance and milk composition, nevertheless, there haven't been done many researches in pursuance to investigate its effects on fermentation processes in the rumen of dairy cows.

So the aim of our research was to determine the influence of extruded lupins', extruded fodder beans and extruded peas on fermentation processes in the rumen of dairy cows.

Material and methods

Dairy cows feeding trial. For the trial, 40 Lithuanian Black-and-White cows with analogous characteristics were selected. The animals selected were divided in 4 groups (control and experimental's), 10 animals each. Feeding trial were divided in two periods - preparatory (14 days) and experimental (90 days). Feeding scheme in the experimental period is provided in Table 1. Raw material's for the trial was extruded by SC „Kauno Grūdai".

Table 1. **Feeding scheme in the experimental period (90 days)**

Group	**Number of cows, n**	**Feeding characteristics**		
Control	10	Diet	+1,5 kg extruded soybean, cow per day	-
1 experimental	10	Diet	+1,5 kg extruded lupins, cow per day	+300 g extr. soybean, cow per day
2 experimental	10	Diet	+1,5 kg extruded beans, cow per day	+400 gr. extr. soybean, cow per day
3 experimental	10	Diet	+1,5 kg extruded peas, cow per day	+700 gr. extr. soybean, cow per day

The control group was fed a conventional diet consisting mainly of grass silage, barley grain, enriched extruded full fat soybean flour, and mineral premix. The experimental groups was fed a similar diet, but instead of 1.5 kg of soybean meal, the cows were given the same amount of the extruded lupins, extruded fodder beans and extruded peas.

Table 2. **Diets for control and experimental groups, their energy and nutritional values**

Feedstuff	**Units**	**Groups**			
		Control (extruded soybeans)	**1 experimental (extr.lupins+ soybeans)**	**2 experimental (extr.beans+ soybeans)**	**3 experimental (extr.peas+ soybeans)**
Maize silage	kg	10,0	10,0	10,0	10,0
Perennial grass silage	kg	12,0	12,0	12,0	12,0
Grass silage	kg	12,0	12,0	12,0	12,0
Barley flour	kg	5,0	5,0	5,0	5,0
Straw	kg	2,0	2,0	2,0	2,0
Molasses	kg	1,0	1,0	1,0	1,0
Extruded soybeans	kg	1,5	0,3	0,4	0,7
Extruded lupins	kg	-	1,5	-	-
Extruded beans	kg	-	-	1,5	-
Extruded peas	kg	-	-	-	1,5
Minerals and vitamins supplements	kg	0,83	0,83	0,83	0,83
1 kg diet DM contains:					
Net energy per lactation (NEL)	MJ	5,70	5,73	5,75	5,78
Crude protein	g	130	129	128	128
Crude fibre	g	196	195	192	191
Crude fat	g	39	30	30	33
Starch	g	190	203	208	211
Sugar	g	38	36	36	36

Feed samples were analyzed for dry matter, crude protein, crude fibre, crude fat, nitrogen-free extracts, calcium and phosphorus. The energy value of feeds was expressed as net energy for milk production (NEL). The analyses were carried

out at the Analytical laboratory of the LHSU Institute of Animal Science using ordinary methods (AOAC, 2000). Energy and nutritional values of the diets were calculated with the feeding software HYBRIMIN® Futter 2008.

Sampling and analyses of rumen fluid. The rumen fluid samples were collected three times (once a month) in the experimental period. The samples were collected from three randomly selected animals of each group with a stomach tube (Sederevičius, 2000) 3 hours after the supplementary feeding with lupins, faba beans and peas. The rumen fluid was analyzed for the 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), ammonia nitrogen – by titrimetric method with the preliminary distillation (Behr steam distillation unit S1, Germany). 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, LHSU Veterinary Academy.

Statistical analysis. SPSS software, version 15.0 (Chicago, IL, USA, 2006) was used for the statistical analysis of the data.

Results and discussion

Rumen fluid's fermantation processes' activity is not permanent, it can depend on feeding time, ration, feed's quality, time that passed after feeding, rumen fluid's pass to other sections of digestive tract.

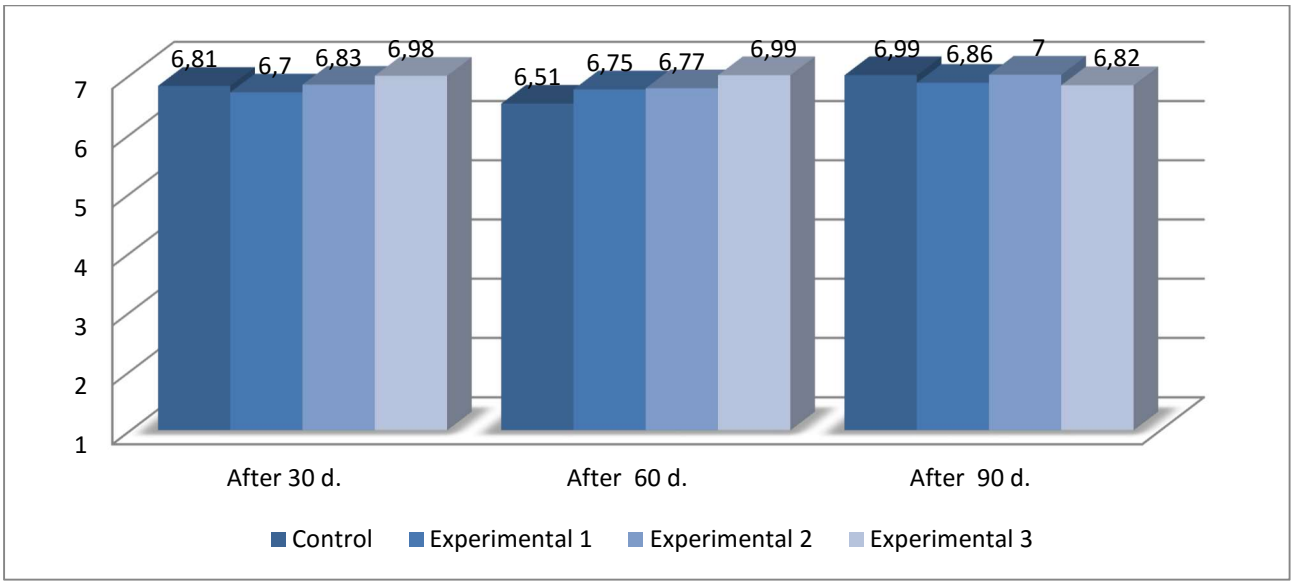

Figure 1. **Rumen fluid pH**

One of the most important organism homeostasis' indexes is pH. According to Cerrato-Sánchez and Calsamiglia (2007), rumen pH normally ranges between 6.5-6.8, and according to Carter and Grovum (1990), physiologically normal rumen pH is from 5.5 to 7.0. From the data given in Figure 1, it can be observed that control and experimental group's rumen fluid's pH did not range significantly during all experimental period and matched physiological norms (Sederevičius, 2004) ($P>0.05$). This made beneficial conditions for rumen protozoa growth and development. In other research, Soto-Navarro et al. (2004) reported minimal changes in pH when feeding increasing levels of field peas in medium-concentrate diets.

After the research it was determined that using extruded lupins, extruded fodder beans and extruded peas in cow's ration had no statistically significant influence on rumen fluid's bacteria reduction activity ($P>0.05$).

Protozoa are very active in the protein degradation, protozoa population are fewer than rumen bacteria population (Ørskov, 1988). Observing range of protozoa quantity in rumen's fluid during experimental period, it was perceived that after first month of the experiment the quantity of protozoa between control and II – III experimental groups was very similar (258.59 – 263.29 $x10^3$/ml), though it was determined that Ist experimental group's cows, that were fed with extruded lupins and soybean, quantity of protozoa in rumen fluid was significantly lesser comparing to control group, which is 145,04$x10^3$/ml or 56,74 % lesser ($P>0.05$).

After analyzing 2nd experiment's month results, we observed that the quantity of protozoa in I experimental group's cow's rumen fluid increases during the experiment, and in the end of the experiment it is 242.7 $x10^3$/ml or 68.13 % bigger comparing to it's quantity in the beginning of the experiment. The same tendency is observed in II experimental group. Opposite reaction proceeds in III experimental group cow's, which ration was formed from extruded peas and soybean.

It was observed that during the experimental period the quantity of protozoa in rumen fluid of this experimental group tendentiously decreases and is 66.15 x10^3/ml or 25.2 % lesser than after first month of the research (P>0.05). The obtained results confirm the data of other authors that the total protozoa count in the rumen fluid of cows directly depends on forage preparation technologies ration composition and structure (Moskalenko, Kuznecov, 2003).

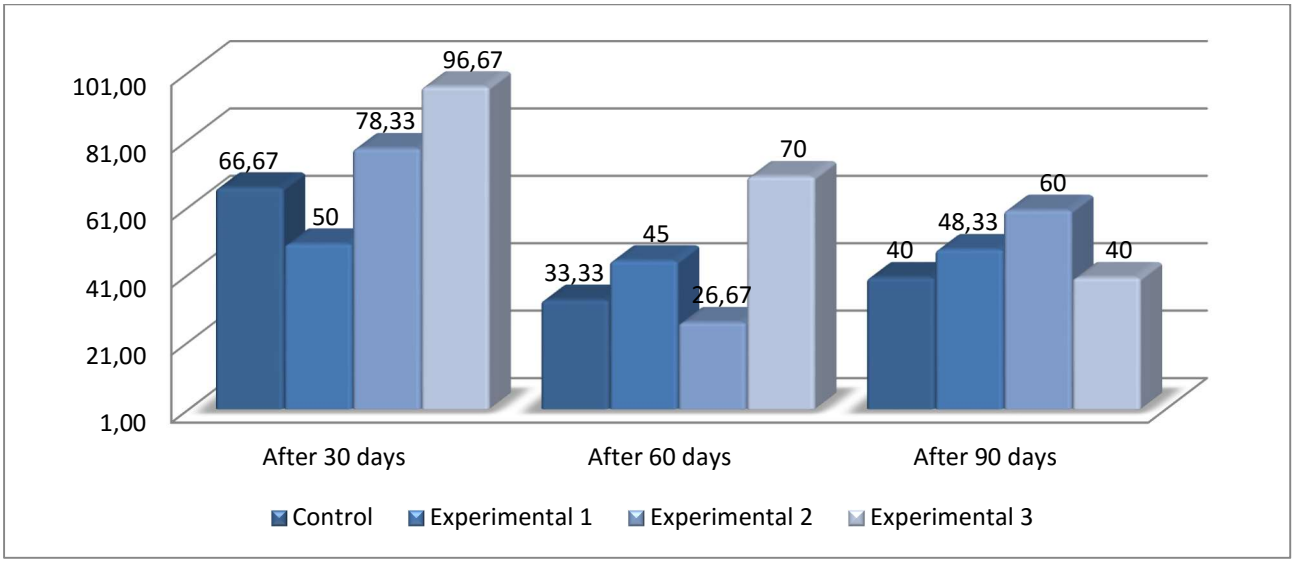

Figure 2. **Reduction activity of bacteria, s**

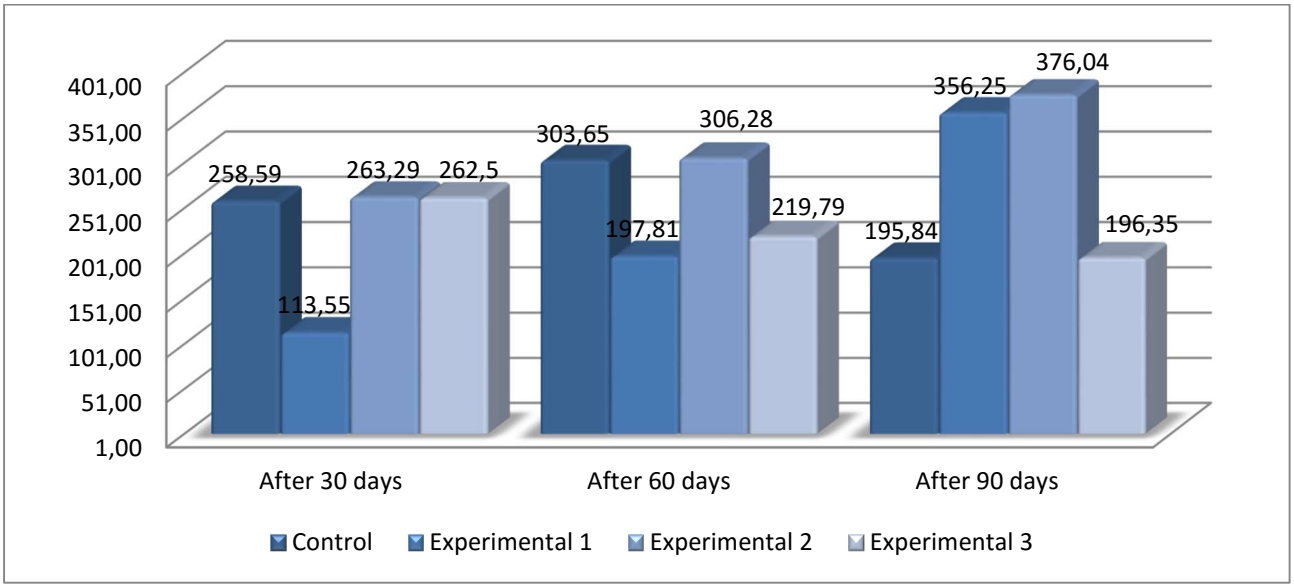

Figure 3. **Protozoa count, x10^3/ml**

It is known that changing the proportion of forage and concentration in diets affects ruminal fermentation characteristics such as volatile fatty acids (VFA) and ruminal pH (Schwartzkopf-Genswein et al, 2003). After analysing alterations of volatile fatty acids during experimental period, we observed that the concentration of volatile fatty acids during whole experimental period was physiologically within the mark in both control and experimental groups. After a month of experiment the concentration of volatile fatty acids in I experimental group‘s rumen fluid was 3.66 % higher, II experimental group‘s – 10.95 % and III experimental group‘s – 14.56 % lower comparing to control group (P>0.05). After two months of the experiment the concentration of volatile fatty acids in I experimental group‘s cow‘s rumen fluid was 4 % higher, II experimental group – 3.2 % higher, and III experimental group – 4 % lower, comparing to control group (P>0.05). In the end of the experiment concentration of volatile fatty acids in I experimental group‘s rumen fluid was 6.9 % lower, II and III experimental group‘s – 3.45 % higher comparing to control group‘s cow‘s rumen fluid (P>0,05).

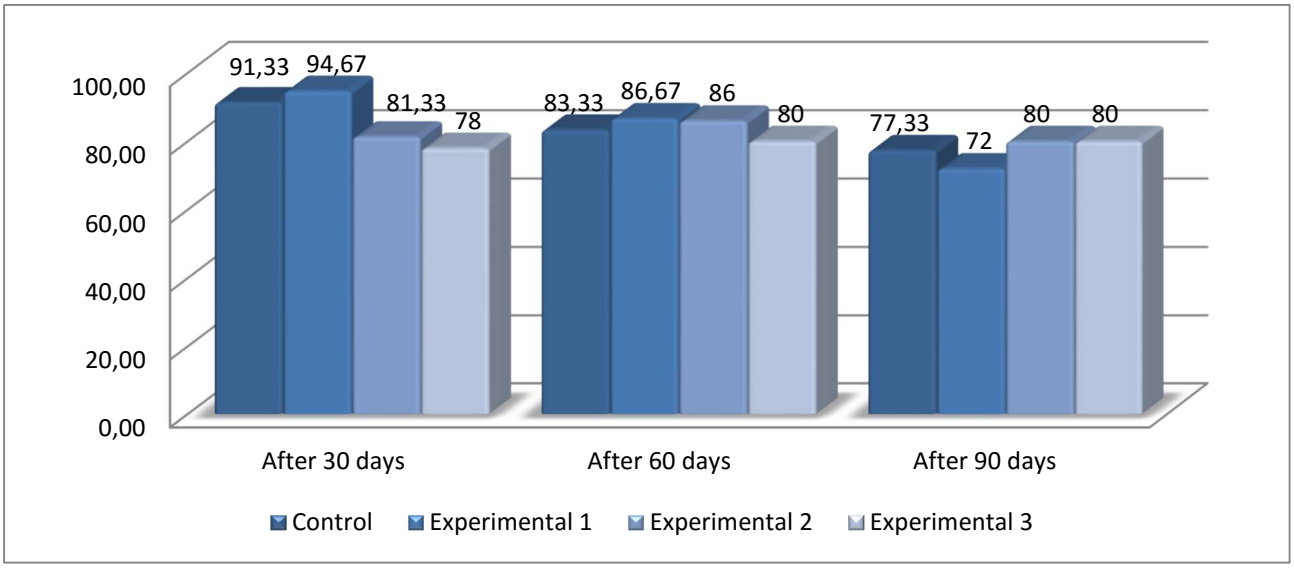

Figure 4. **VFA, mmol/l**

Reed et al. (2004) reported a cubic increase in total VFA with increasing field pea level in growing diets for beef cattle. In contrast, Soto-Navarro et al. (2004) observed no differences in ruminal fermentation variables when feeding increasing levels of field pea to cattle. Ruminal pH was similar among treatments and therefore a change in total VFA concentration was unexpected.

The feed nitrogen compounds (NPN and true protein) are exposed to rumen microbial enzymatic activity and are subjected to partial or total degradation. In general, the simple nitrogen compounds and highly soluble as the non protein nitrogen (NPN), are degraded by rumen microorganisms resulting in an increase in the ammonia rumen concentration. Furthermore, the proteins are partly degraded by the action of microorganisms proteolytic enzymes, resulting in peptides and amino acids, which by the action of peptidases and desaminase of these microorganisms, produce ammonia, volatile fatty acids (VFA) and carbon dioxide (Annison, Lewis, 1981; Bach, Calsamiglia, 2002). Nitric substances‘ fermentation‘s in rumen analysis showed, that total amount of nitrogen and ammonia during the experimental period varied differently between groups.

While analysing alterations of total amount of nitrogen during experimental period, it was observed that both control and all experimental group‘s had it‘s concentration in rumen fluids physiologically within the mark, the whole experimental period. After first month of the experiment the total amount of nitrogen in I experimental group‘s (fed with extruded lupins and soybean) rumen fluid was 7.73 % higher, II experimental group‘s (fed with extruded beans and soybeans) – 11.74 % lesser, and III experimental group‘s (fed with extruded peas and soybeans) – 15.75 % lesses, comparing to control group‘s cow‘s rumen fluid (P>0.05).

While analyzing results of 2nd month of the experiment, it was determined that control group’s cows, which ration was formed from extruded soybean, had total amount of nitrogen in rumen’s fluid increase by 58.45 % comparing to results of the first month. Besides that, we determined that I experimental group’s cow’s rumen fluids total amount of nitrogen was 43.94 %, II experimental group’s – 37.97 %, and III experimental group’s – 44.84 % lesser comparing to control group (P>0.05).

The ruminal ammonia nitrogen (N-NH_3) concentration is a consequence of the balance between production, absorption and utilization by microorganisms (Silveira et al., 2009). While analysing ammonia nitrogen alteration tendencies during experimental period, it was determined that I experimental group‘s cows (fed with extruded lupins and soybeans) had it‘s concentration in rumen‘s fluid 4.2 % higher after the first month of the experiment, II experimental group‘s (fed with extruded beans and soybeans) – 10.2 % lesser comparing to control group‘s cow‘s rumen fluid (P>0.05). III experimental group‘s (fed with extruded peas and soybeans) cows had the same ammonia nitrogen amount as control group‘s cows.

While analysing results of 2nd month of experiment it was determined that control group‘s cows, which were additionally given extruded soybean, total amount of nitrogen in rumen fluid increased by 21.11 % comparing to the results of first experimental month, the same tendency was seen with total ammonia nitrogen in rumen fluid. Besides that, it was determined that I experimental group had 49.25 % of ammonia nitrogen in rumen fluid, II experimental group‘s – 21.9 %, and III experimental group‘s – 25.85 % lesser comparing to control group‘s cow‘s rumen fluids (P>0.05).

In the end of the experiment control group‘s cows distinguished for gaving significantly decreased amount of total nitrogen in rumen‘s fluids, comparing to second month of the experiment (39.92 and 42.26 %) It was determined that I experimental group‘s rumen fluid total amount of ammonia nitrogen was 11.11 %, II experimental group‘s – 63.88 % bigger comparing to control group (P>0.05).

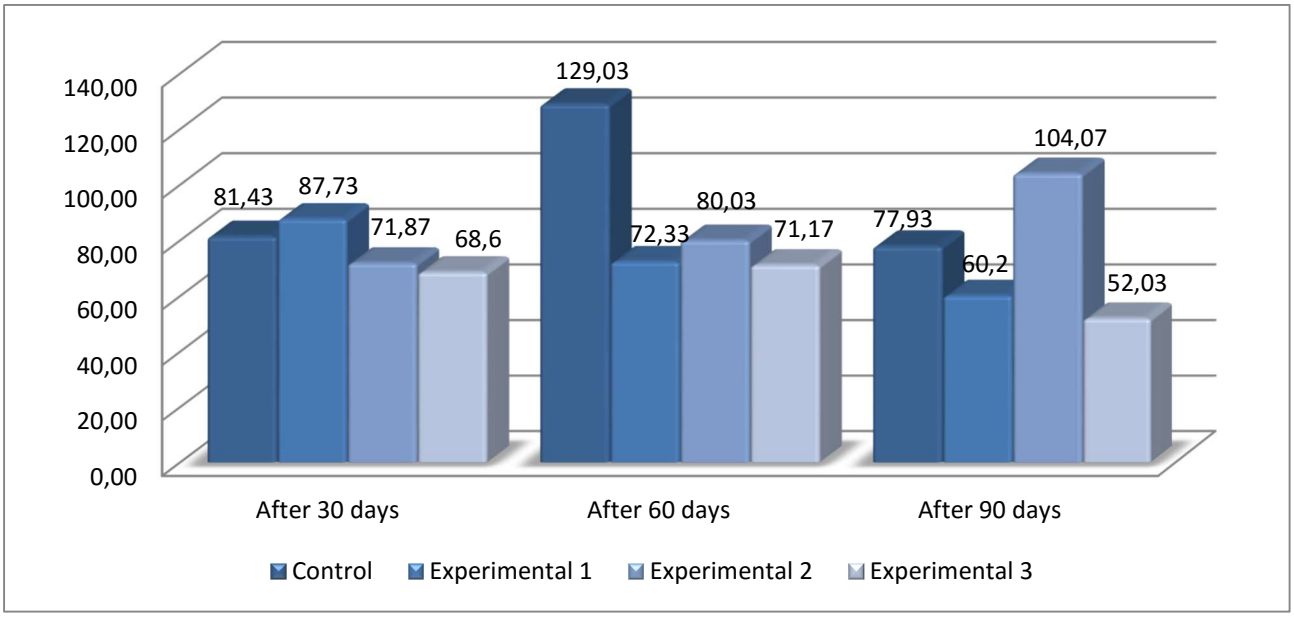

Figure 5. **Total nitrogen, mg/100 ml**

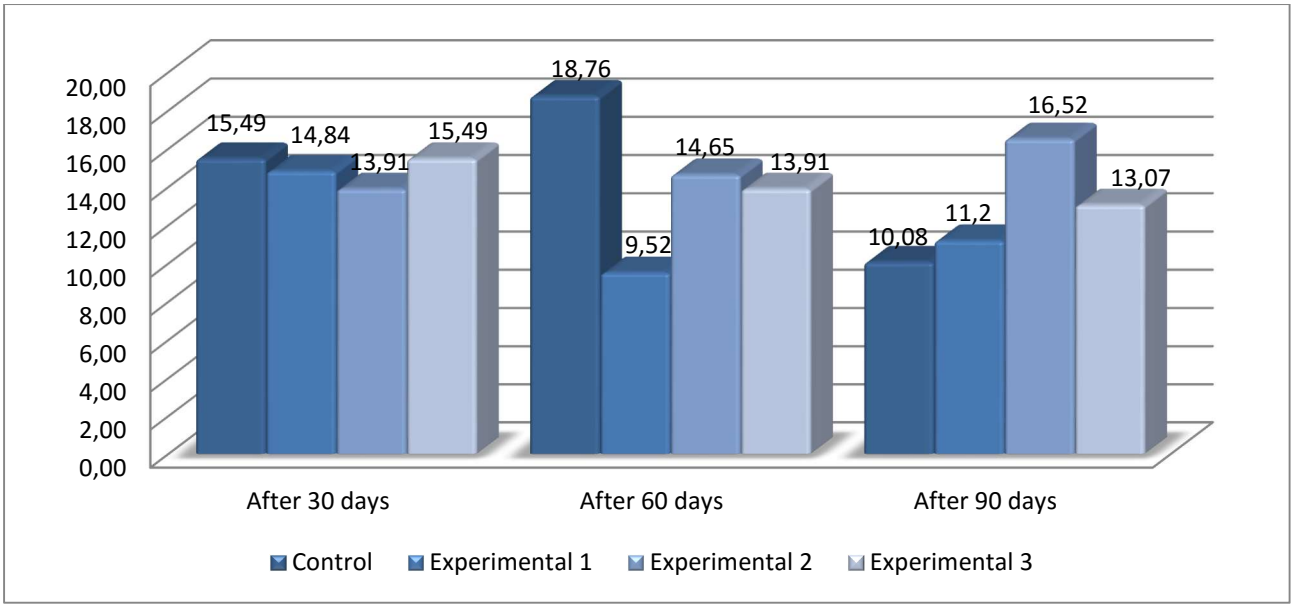

Figure 6. **Ammonia nitrogen, mg/100 ml**

The level considered optimal for ruminal ammonia concentration (10 mg/100 mL) cannot be considered static, since the ability of bacteria to synthesize protein and capture ammonia depends on the fermentation rate of carbohydrates (Van Soest, 1994). In this sense, Ítavo et al. (2002) estimated maximum concentrations of ammonia nitrogen (N-NH_3) at 22.93 mg/100 mL of ruminal fluid, with 14.14% of concentrate and 1.89 hours after the feed intake. The values found for the maximum concentrations of N-NH3 of diets, in function of the time of collection, are in agreement with Mehrez et al. (1977), who suggested that the maximal fermentative activity would occur when the concentrations of ammonia nitrogen (N-NH_3) are between 19 and 23 mg/100 mL of ruminal fluid.

Conclusions

The results of this study shown that extruded soybeans replacement with extruded lupins *(Lupinus spp.)*, extruded fodder beans *(Vicia faba)* and extruded peas *(Pisum sativum)* in dairy cows rations, had no negative influence on rumen‘s fermentative indexes and warranted it‘s optimal activity.

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