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ORIGINAL PAPER

MIXTURES OF EXTRUDED RAPESEED *(BRASSICA NAPUS)* AND FABA BEANS (*VICIA FABA*) IN DIET OF DAIRY COWS – RUMINAL AND MILK PARAMETERS*

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Abstract

The purpose of this experiment was to study the effect of adding mixtures of extruded rapeseeds and faba beans to dairy cows' rations on milk production and ruminal parameters An experiment was carried out on 75 Holsteinizated Lithuanian Black-and-White dairy cows divided into 3 groups. All cows were fed the same basal balanced diet, but the difference between the treatment was in the adding of extruded rapeseed 30% and extruded faba beans 70% mixture (trial group – T-1) and extruded rapeseed 70% and extruded faba beans 30% mixture (trial group – T-2) while the control group (C-0) was fed the basal diet only. Comparing the beginning and the end of the experiment, total milk yield (P<0.05), content of fat (P<0.05) and protein (P<0.05) increased in both groups fed extruded products. The difference in the lactose content in milk was statistically significant (P<0.05) at the end of the experiment between groups T-1 and T-2 relative to C-0. The total content of saturated, monounsaturated and polyunsaturated fatty acids was affected by the treatment (P<0.05). The content of Palmitic acid and Oleic acid was increasing during the whole experiment in all the groups (P<0.05). The mixtures of extruded rapeseeds and faba beans did not have significant influence on the rumen fluid parameters or on *in vitro* digestion (P>0.05).

Keywords: extruded feed, faba beans (*Vicia faba*), fatty acid, dairy cows, milk, rumen.

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INTRODUCTION

Feeding factors (type, quantity and quality of forage) are the main contributors to the composition of milk (Hall et al. 2010). The quality and amount of milk produced by an animal is largely affected by what it is fed on and how the feeding is done. The composition of milk is also affected by genetic traits and the stage of lactation (Pustovoj 1978, Zwierzchowski et al. 2016). In addition to selecting good breeds, the proper feeding, housing and handling of dairy cows are prerequisites of good yields. It is important to ensure correct rations, as dairy cows need balanced feeds that provide energy (carbohydrates and fats), protein, minerals, vitamins, fibre, and water in sufficient amounts. Studies have reported a positive influence of including rapeseeds or faba beans in dairy cows' diets (Folch et al. 1957). There is increasing evidence that rapeseeds and faba beans are excellent protein supplements for dairy cows (Focant et al. 1998). Protein is extensively and rapidly degraded in the rumen, and the protein not broken down in the rumen should be accessible later in the intestinal tract (Poncet, Rrémond 2002). The highly soluble protein content in faba beans (that makes them easily degraded in the rumen) will provide a pulse of nitrogenous substrate for rumen microbes, and feeds need to be formulated to best harness this supply in order to provide essential amino acids for the ruminant itself (Třináctý et al. 2016). Extrusion cooking was developed to gelatinize cereal starch by using a process where the principle is to grind, add moisture, heat and pressure by forcing the grain through (Kalač, Samkova 2010). It has been confirmed that extrusion processing increases microbial fermentation by reducing the particle size and/or promoting starch gelatinization (Negi, Rani 2015), but decreases degradation of dry matter (DM) and crude protein (CP) in the rumen without affecting the intestinal digestibility (Neves et al. 2009).

The objective of our study was to evaluate the effects of adding mixtures of extruded rapeseed and faba beans to dairy cows' rations (in different proportions) on milk production and ruminal parameters

MATERIALS AND METHODS

All experimental procedures and animal management were in accordance with the guidelines of the Law of the Republic of Lithuania on animal care, housing and use No. XI-2271 of 03-10-2012, also 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).

Experimental design and animal feeding management

The investigation was conducted at a dairy cow farm. The experiment was divided in two periods – adaptation (14 days) and experimental stage (90 days). After 14 days of adaptation (the first day of which was denoted as day 0), the proper experiment began. Selected, clinically healthy dairy cows were divided into 3 groups (control group 0 and two trial groups T-1 and T-2). Holsteinizated Lithuanian Black-and-White dairy cows (n=75) were divided in three groups of 25 animals each, according to the days of lactation (2-3), parity and the milk yield. All cows were fed the same basal diet (Table 1) twice a day at 05.00 a.m. and 04.00 p.m. and had an unlimited access to fresh water.

Table 1

Ingredients and nutrient composition of the diets supplied to dairy cows

Diet composition	Weight (g kg^{-1} as feed)		
	C-0	T-1	T-2
Grass haylage	14.00	14.00	14.00
Maize silage	23.00	23.00	23.00
Straws	0.50	0.50	0.50
Molasses	1.00	1.00	1.00
Silage from sugar beet pulp	10.00	10.00	10.00
Extruded rapeseed 30% and faba beans 70% mixture	–	0.800	–
Extruded rapeseed 70% and faba beans 30% mixture	–	–	0.800
	nutrient content (g kg^{-1} as feed)		
Dry matter	531.8	527.2	531.9
Netto energy for lactation, MJ	147.2	147.19	146.91
Crude fat	42.2	41.5	41.6
Crude protein	263.4	288.5	251.8
Crude fibre	51.2	65.5	63.6
Crude ash	76.4	45.2	29.9
Starch	271.5	271.1	304.7
NDF	115.7	125.7	104.5
ADF	79.8	89.6	84.3
ADL	28	29.6	19
Glikozinolates, (μmol g^{-1})	-	6.87	6.37
Ca:P	1.62:1	1.61:1	1.61:1

C-0 – control (basal diet), T-1 – basal diet plus extruded rapeseed 30% and faba beans 70% mixture, T-2 – basal diet plus extruded rapeseed 70% and extruded faba beans 30% mixture, NDF – neutral detergent fibre, ADF – acid detergent fibre, ADL – acid detergent lignin, Ca – calcium, P – phosphorus

The treatment differences were achieved by adding extruded rapeseed 30% and faba beans 70% mixture (T-1) and extruded rapeseed 70% and extruded faba beans 30% mixture (T-2). Control group was fed the basal diet only. All three diets were balanced for minerals and vitamins to meet the nutritional requirements of the cows according to NRC (McNamee et al. 2002).

Preparation of extrusion

Extrusion of rapeseeds and faba beans was performed in an Insta-Pro Model MS3000 Medium Shear Extruder (Insta-Pro International, USA) at temperature regimes 90-105°C. Before extrusion, dry matter of samples was measured. After extrusion, samples were dried in a rotary type oven.

Milk yield and quality

The cows were milked twice a day (5:00 a.m. and 4:00 p.m.) and individual daily weights of milk yield were automatically recorded. The milk yield and milk fat, protein, lactose, and urea were determined with the instrument "LactoScope FTIR" (FT1.0. 2001; Delta Instruments, the Netherlands) using the method of absorption of infrared radiation medial region rays. 4% FCM (fat corrected milk) values were calculated from the formula: FCM = (0.4 x kg milk) + (15 x kg fat). The calcium content in the milk samples was determined by the hydrogenation method and by the atomic absorption spectrometry (ASA) technique after mineralization. The samples were mineralized twice in a mixture of nitric and perchloric acid using a microwave mineralizer (AOAC, 2006).

Analyses of fatty acids in milk

Control milking was performed twice during the experiment. Samples were collected in 80 ml vials to evaluate fatty and amino acids composition. Fat extraction was based on the Folch method (Diaz et al. 2006). Milk fatty acid methyl esters were prepared according to ISO 15884: 2002 and determined according to ISO 15885:2002 with GC series GC-210 plus (Shimadzu), equipped with a Shimadzu GCMS QP2010 ultra mass-selective detector. The compounds were separated in a RXi-5ms Restek column (30 m × 0.25 mm × 0.25 μm) with a helium flow of 0.92 ml min^{-1}. The temperature program started at 40°C, increased to 260°C, and the total run time was 60 min. The GC-MS interface was maintained at 270°C. Mass spectra were obtained by electron impact at 70 eV. Mass spectra data of fatty acids were acquired in the range of 33-400 m/z. The identification and calculation of fatty acids were based on the comparison of retention times and peak area of standard methyl esters in the Supelco 37 fame mix (Sigma-Aldrich Co. LLC).

Sampling and analyses of rumen fluid

Rumen fluid samples were collected twice: on the last day of adaptation period (0 day) and at the end of the experiment (day 90). The samples were collected randomly from three dairy cows in each group, using a stomach tube 3 h, after the morning feeding. The ruminal fermentation parameters such as pH, total volatile fatty acids (VFA), total nitrogen (N), ammonia nitrogen (NH_3-N), L(+)- and D(-)-lactic acid were investigated. Ruminal pH was measured immediately after sampling, using a pH-meter (Horiba – Twin pH, Spectrum Technologies). Total N was analysed by the Kjeldahl procedure (Behr System, Germany), NH_3-N – by a titrimetric method with preliminary distillation (Behr steam distillation unit S1, Germany). The concentrations of L(+)- and D(−)-lactic acid were evaluated with an enzyme test kit R-biopharm (AG - Roche, Germany), as reported elsewhere. The following microbiological parameters of the rumen fluid were studied: protozoa number, total count of aerobic and facultative anaerobic microorganisms, total count of enterobacteria, total count of enterococci. Protozoa number was counted in a Fuks-Rozental chamber (Blaubrand, Wertheim, Germany). Total count of aerobic and facultative anaerobic microorganisms was determined on plate count agar (CM0325, Oxoid, UK) after incubation at 30°C for 72 hours. The number of microorganisms was calculated and expressed as log10 of colony-forming units per millilitre (log10 CFU mL^{-1}).

In vitro digestion with ANKOM technology method

In vitro digestion was performed twice: on day 0 and on day 90, using rumen fluid from the three groups (C-0, T-1, T-2). The rumen fluid from each group was used separately for digesting: crude rapeseeds, crude faba beans, extruded rapeseed 30% and extruded faba beans 70% mixture and extruded rapeseed 70% and extruded faba beans 30% mixture.

Statistical analysis

The statistical analysis of data was performed using the IBM SPSS 25.0 statistical package (SPSS Inc., Chicago, IL, USA). We used the Linear Mixed Model to analyze the data. The model investigated the influence of two factors – the dairy cows' ration and the time factor that evaluated the replicability of the study. Differences between rations were expressed by calculating means and standard errors of the studied traits, and the statistical significance of the differences was evaluated by the Fisher's LSD criterion (Least Significant Difference). Differences were considered statistically significant when $p<0.05$

RESULTS

The effect of mixtures of extruded rapeseeds and faba beans on milk production and composition

At the beginning of the experiment (day 0), no statistically significant differences were noticed in parameters of milk production and composition between the control and trial groups (Table 2). The milk yield (day 90) increased in both trial groups (T-1 and T-2) – $P<0.05$. The milk fat content tended ($P=0.06$) to be higher for cows fed basal diets as compared with those fed supplemented diets (with either mixture of extruded rapeseeds and

Table 2

Influence of mixtures of extruded rapeseeds and faba beans on feed intake, milk production and milk composition of dairy cows

Specification	C-0		T-1			T-2		*p*-value	
	mean	SEM	mean	SEM	mean	SEM	C-0 *vs* T-1	C-0 *vs* T-2	T-1 *vs* T-2
Milk parameters									
Day 0									
($kg\ d^{-1}$)	19.5	0.50	20.27	0.69	20.54	0.73	0.45	0.29	0.76
	52.0	0.14	4.81	0.15	5.13	0.15	0.07	0.73	0.14
4% FCM ($kg\ d^{-1}$)	19.31	0.43	18.62	0.39	19.48	0.39	0.23	0.77	0.13
Protein (%)	3.79	0.09	3.60	0.08	3.63	0.06	0.09	0.15	0.14
(%)Lactose	4.39	0.03	4.41	0.03	4.45	0.03	0.62	0.13	0.78
Urea ($mg\ dL^{-1}$)	22.92	0.98	23.68	1.43	22.04	1.08	0.65	0.60	0.33
Day 90									
Yield ($kg\ d^{-1}$)	21.27	0.48	24.24	0.62	23.75	0.61	0.002	0.002	0.55
Fat %	4.30	0.11	4.15	0.19	4.37	0.14	0.77	0.26	0.16
4% FCM ($kg\ d^{-1}$)	18.59	0.28	18.35	0.89	19.52	0.35	0.35	0.91	0.30
Protein (%)	3.66	0.07	3.56	0.10	3.46	0.05	0.37	0.71	0.37
Lactose (%)	4.35	0.03	4.38	0.03	4.46	0.03	0.50	0.01	0.67
Urea ($mg\ dL^{-1}$)	22.44	1.06	22.33	0.90	23.52	0.90	0.94	0.42	0.38

C-0 – control (basal diet), T-1 – basal diet plus extruded rapeseeds 30% and faba beans 70%, T-2 – basal diet plus extruded rapeseeds 70% and faba beans 30%, C4:0 butanoic acid methyl ester, C6:0 hexanoic acid methyl ester, C8:0 octanoic acid methyl ester, C10:0 decanoic acid methyl ester, C12:0 dodecanoic acid methyl ester, C14:1 n5 methyl myristoleate, C14:0 methyl tetradecanoate, C16:1 cis-9 hexadecanoic acid methyl ester, C16:0 hexadecanoic acid methyl ester, C18:1 n9 cis-9-oleic acid methyl ester, C18:1 n9 trans-9-elaidic acid methyl ester, C18:0 methyl stearate, C20:0 eicosenoate methyl ester, C22:0 docosanoate methyl ester, SFA – saturated fatty acids, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids, C-0 *vs*. T-1 – control group 0 *vs*. trial group T-1, C-0 *vs* T-2 – control group 0 *vs*. trial group T-2, T-1 *vs* T-2 – trial group T-1 *vs* trial group T-2, SEM – standard error of the mean.

extruded faba beans). The results of the ANOVA test revealed that a significant effect (comparing the beginning and the end of the experiment) of supplementing dairy cows' rations with mixtures of extruded rapeseeds and extruded faba beans (mixed in different proportions) on milk yield (P<0.05), and on the content of fat (P<0.05) and protein (P<0.05) in milk. The milk protein concentration was similar for all groups per entire experiment. Differences in the content of fat, protein and Ca in milk were not statistically significant in all groups during the whole experiment, same as urea (P>0.05), meanwhile the lactose content in milk differed statistically significantly between C-0 and T-2 groups at the end of experiment (P<0.05).

The effect of mixtures of extruded rapeseeds and faba beans on fatty acid composition

Fatty acid composition of milk is shown in Table 3. The average of Palmitic acid (C16:0) was the highest among fatty acids in milk in all experimental groups during the whole experiment. The oleic acid (C18:1) average was the highest among unsaturated fatty acids in C-0 (29.9%), T-1 (28.1%) and T-2 (27.8%). The average of MUFAs were: C-0 (34.1%), T-1 (32.3%) and T-2 (31.1%); average of PUFAs – C-0 (5.3%), T-1 (5.5%) and T-2 (4.5%) of milk fatty acids. From the beginning to the end of the experiment, the total amount of SFA in all of our groups decreased as follows: C-0 – 5.7%, T-1 – 6% and T-2 – 6.9%. Comparing C-0 to group T-2 and T-1 to T-2, most of the values of fatty acids were statistically significant (P<0.05).

The effect of mixtures of extruded rapeseeds and faba beans on the rumen fluid parameters

It was noticed that feeding dairy cows (T-2) with the mixture composed in 70% of extruded rapeseeds and in 30% of extruded faba beans increased the total count of aerobic and facultative bacteria (P<0.05) in rumen fluid in comparison with C-0 at the end of the experiment (day 90) – Table 4. The C-0 feeding had a significant influence on the L(+)-lactate concentration in rumen fluid as it decreased by 0.36 mmol L^{-1} (P<0.05) after 90 days of feeding. Adding mixtures with different proportions of extruded rapeseeds and extruded faba beans to the diet had no influence on ruminal pH, the average of which was 6.9 at the beginning and 6.5 at the end of the experiment. No other significant changes in ruminal fermentation and microbiological parameters were detected; fluctuations were in normal ranges during the experimental (day 0-90) period (P>0.05). A significant effect was found at the beginning of the experiment on these parameters: protozoa number $\times 10^5$ ml^{-1} (P<0.002), Enterococci log10 ml^{-1} CFU (P<0.002), Enterobacteria log10 ml^{-1} CFU (P<0.002).

Table 3

The effect of mixtures of extruded rapeseeds and faba beans on the fatty acid composition in milk of dairy cows

Fatty acid	C-0		T-1		T-2		*p*-alue		
	mean	SEM	mean	SEM	mean	SEM	C-0 vs T-1	C-0 vs T-2	T-1 vs T-2
Day 0									
C4:0	1.45	0.11	1.33	0.21	1.42	0.14	0.68	0.29	0.58
C6:0	0.98	0.14	0.16	0.12	1.24	0.05	0.22	0.002	0.002
C8:0	0.99	0.09	0.18	0.11	1.09	0.07	0.83	0.13	0.18
C10:0	0.99	0.09	0.18	0.11	2.51	0.33	0.57	0.003	0.003
C12:0	2.62	0.29	0.80	0.20	3.56	0.53	0.69	0.002	0.004
C14:1	0.54	0.06	0.11	0.02	0.93	0.21	0.99	0.02	0.02
C14:0	8.63	0.80	13.05	0.42	11.56	0.38	0.97	0.02	0.02
C16:1	1.57	0.81	3.60	0.04	3.12	0.39	0.8	0.003	0.004
C16:0	29.55	2.00	34.88	0.27	31.76	1.96	0.38	0.95	0.41
C18:1	27.27	0.59	23.14	0.09	24.04	0.38	0.66	0.19	0.09
C18:1	2.34	0.93	2.49	0.32	4.19	0.44	0.69	0.65	0.40
C18:0	12.64	0.77	11.59	0.71	11.16	0.44	0.19	0.03	0.33
C20:0	0.24	0.04	0.22	0.01	0.12	0.01	0.45	0.10	0.34
C22:0	0.17	0.06	0.08	0.03	0.07	0.02	1.00	1.00	1.00
SFA	61.15	0.82	63.09	0.63	66.17	1.40	0.45	0.01	0.002
MUFA	32.74	1.48	31.89	0.74	31.66	1.05	0.10	0.003	0.17
PUFA	5.17	0.82	5.05	0.19	3.58	0.08	0.63	0.16	0.72
Day 90									
C4:0	1.32	0.25	1.57	0.03	2.07	0.31	0.72	0.29	0.48
C6:0	0.59	0.07	0.60	0.21	1.09	0.07	0.97	0.09	0.10
C8:0	0.62	0.03	0.69	0.19	1.03	0.05	0.79	0.16	0.24
C10:0	1.58	0.18	1.51	0.21	2.05	0.11	0.87	0.25	0.19
C12:0	1.47	0.14	2.09	0.71	3.14	0.18	0.36	0.03	0.13
C14:1	0.35	0.13	0.49	0.15	0.69	0.17	0.48	0.11	0.33
C14:0	8.37	1.31	8.37	0.16	8.61	0.28	0.99	0.84	0.84
C16:1	1.53	0.13	0.81	0.62	1.51	0.69	0.26	0.97	0.27
C16:0	27.67	0.98	31.46	0.86	31.31	0.92	0.003	0.002	0.80
C18:1	27.31	1.78	29.31	1.78	27.34	0.65	0.89	0.33	0.40
C18:1	1.47	0.22	2.01	0.49	4.52	0.17	0.19	0.89	0.23
C18:0	12.52	1.75	11.65	1.15	10.46	0.75	0.13	0.04	0.48
C20:0	0.13	0.07	0.19	0.12	0.04	0.04	0.57	0.41	0.14
C22:0	0.24	0.02	0.14	0.08	0.17	0.04	0.14	1.00	0.14
SFA	57.69	0.72	59.32	1.54	61.59	0.75	0.41	0.07	0.26
MUFA	31.57	1.84	33.08	2.08	32.77	0.26	0.83	0.43	0.57
PUFA	5.74	0.49	6.93	1.55	6.64	0.81	0.88	0.94	0.82

C-0 – control (basal diet), T-1 – basal diet plus extruded rapeseeds 30% and faba beans 70%, T-2 – basal diet plus extruded rapeseeds 70% and faba beans 30%, C4:0 butanoic acid methyl ester, C6:0 hexanoic acid methyl ester, C8:0 octanoic acid methyl ester, C10:0 decanoic acid methyl ester, C12:0 dodecanoic acid methyl ester, C14:1 n5 methyl myristoleate, C14:0 methyl tetradecanoate, C16:1 cis-9 hexadecanoic acid methyl ester, C16:0 hexadecanoic acid methyl ester, C18:1 n9 cis-9-oleic acid methyl ester, C18:1 n9 trans-9-elaidic acid methyl ester, C18:0 methyl stearate, C20:0 eicosenoate methyl ester, C22:0 docosanoate methyl ester, SFA – saturated fatty acids, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids; C-0 *vs*. T-1 – control group 0 *vs*. trial group T-1, C-0 *vs* T-2 – control group 0 *vs*. trial group T-2, T-1 *vs* T-2 – trial group T-1 *vs* trial group T-2, SEM – standard error of the mean.

Table 4

Effect of mixtures of extruded rapeseeds and faba beans on the biochemical and microbiological parameters on rumen fluid of dairy cows

Parameters	C-0		T-1		T-2		*p*-value		
	mean	SEM	mean	SEM	mean	SEM	C-0 *vs*. T-1	C-0 *vs* T-2	T-1 *vs* T-2
Day 0									
pH	6.83	0.08	6.96	0.13	6.91	0.192	0.46	0.51	0.64
Total VFA (mmol L^{-1})	66.7	3.33	66.0	0.14	66.7	6.67	1.73	7.95	51.28
Total N (mg dL^{-1})	60.9	5.06	133.7	29.5	60.2	11.16	0.64	0.77	0.85
NH_3-N (mg dL^{-1})	15.2	2.33	19.8	2.30	16.2	0.90	0.009	0.81	0.01
L(+)-lactic acid (mmol L^{-1})	0.52	0.13	0.27	0.06	0.25	0.03	0.63	0.18	0.09
D(−)-lactic acid (mmol L^{-1})	<0.001		<0.001		<0.001				
Protozoa number ($\times 10^5$ mL^{-1})	135.9	19.91	176.7	13.92	314.6	7.67	0.001	0.002	0.04
Enterococci (log10 mL^{-1} CFU)	4.28	0.19	3.05	0.13	3.14	0.03	0.002	0.002	0.62
Enterobacteria (log10 mL^{-1} CFU)	4.28	0.19	3.05	0.13	3.14	0.03	0.001	0.002	0.62
Aerobic and facultative (log10 mL^{-1} CFU)	4.75	0.28	3.95	0.03	3.86	0.07	0.17	0.11	0.72
Day 90									
pH	6.68	0.21	6.24	0.23	6.25	0.07	0.32	0.49	0.73
Total VFA (mmol L^{-1})	73.3	3.33	86.7	6.67	76.7	8.82	0.80	0.62	0.48
Total N (mg dL^{-1})	134.4	21.43	109.2	21.06	125.3	16.97	0.34	0.72	0.54
NH3-N (mg dL^{-1})	16.9	4.04	25.7	3.22	27.8	6.91	0.76	0.53	0.73
L(+)-lactic acid (mmol L^{-1})	0.16	0.08	0.14	0.09	0.21	0.14	0.47	0.87	0.67
D(−)-lactic acid mmol L^{-1}	<0.001		<0.001		<0.001				
Protozoa number (×105 mL^{-1})	285.4	35.32	295.8	20.02	325.0	41.61	0.02	0.37	0.003
Enterococci (log10 mL^{-1} CFU)	3.26	0.12	3.64	0.41	3.62	0.23	0.37	0.40	0.95
Enterobacteria (log10 mL^{-1} CFU)	3.28	0.03	.10	0.37	3.76	0.20	0.05	0.22	0.36
Aerobic and facultative (log10 mL^{-1} CFU)	5.78	0.19	5.53	0.28	5.93	0.16	0.05	0.01	0.10

C-0 – control (basal diet), T-1 – basal diet plus extruded rapeseeds 30% and faba beans 70%, T-2 – basal diet plus extruded rapeseeds 70% and faba beans 30%, *p*-value contrast: C-0 *vs*. T-1 – control group 0 *vs*. trial group T-1, C-0 *vs* T-2 – control group 0 *vs*. trial group T-2, T-1 *vs* T-2 – trial group T-1 *vs* trial group T-2, SEM – standard error of the mean, N – nitrogen, NH_3-N – ammonia nitrogen

The effect of mixtures of extruded rapeseeds and faba beans on the digestibility *in vitro*

The *in vitro* digestion results are presented in Table 5. No significant difference was found between the groups. As the data show, the digestion of all tested feeds varied from 92% to 93.8%.

Table 5

Effect of mixture of extruded rapeseeds and faba beans on the *in vitro* dry matter digestibility

Feed	C-0		T-1		T-2	
	mean	SEM	mean	SEM	mean	SEM
Day 0						
Crude rapeseed	92.6	0.19	92.1	0.21	93.1	0.45
Crude faba beans	92.0	0.17	92.4	0.18	92.8	0.19
Extruded rapeseeds 30% and extruded faba beans 70%	92.6	0.23	92.1	0.24	93.5	0.17
Extruded rapeseeds 70% and extruded faba beans 30%	92.2	0.13	92.7	0.14	93.8	0.18
Day 90						
Crude rapeseed	92.08	0.88	92.47	0.08	93.16	0.18
Crude faba beans	92.4	0.14	92.63	0.16	93.21	0.09
Extruded rapeseeds 30% and extruded faba beans 70%	92.08	0.09	92.51	0.13	92.26	0.07
Extruded rapeseeds 70% and extruded faba beans 30%	92.7	0.21	92.09	0.11	92.9	0.12

C-0 – control (basal diet), T-1 – basal diet plus extruded rapeseeds 30% and extruded faba beans 70%, T-2 – basal diet plus extruded rapeseeds 70% and extruded faba beans 30%, SEM – standard error of the mean

DISCUSSION

Many European countries use rapeseed products as an excellent source of high-quality protein and energy (Soltan et al. 2009). Improving the physical structure and nutritional value of feed can be achieved by the hydrothermal process of extrusion, which uses a combination of pressure, moisture and heat (Chichlowski et al. 2005). An extruder is also a technical device in which the feed is processed, extruded, and cooked under the consistent increase of pressure; it is also expanded due to a sudden pressure drop (Moghaddam et al. 2016). Lock, Shingfield (2004) noticed the impact of thermal processing on degradation of protein and starch of corn (Crépon et al. 2010). Boots et al. (2013) stated that process of extrusion is required to ensure an adequate digestion of the protein (Moghaddam et al. 2016) and starch contained in beans. The objective of this study was to evaluate the effect of adding different proportions of extruded rapeseeds and faba beans to dairy cows' rations.

Milk production and composition

Heat treatment affects individual milk constituents differently when comparing various feedstuffs. Some scientists (Huhtanen et al. 2011) did not find a difference in milk yield using extruded and not extruded ingredients in cow's diet. Other scientists state that extrusion of ingredients has advantageous effects on milk yield (Stoffel et al. 2015). Our study also revealed beneficial effects on milk yield during the experiment (T-1 3.97% per day, T-2 3.21% per day). Although milk yield was not found to have increased for all groups, the milk fat content decreased in cows fed extruded diets compared to those fed non-extruded diets. There are different opinions about extrusion and fat content: some studies investigated the effects of extrusion on milk fat content and have reported increase (Prestløkken, Harstad 2001), decrease (McGregor et al. 2007), or no changes (Chen et al. 2007, Fallahi et al. 2013) in milk fat content when extruded diets were fed to dairy cows compared to those fed non-extruded diets. In our study, milk fat and yield contents differ statistically significantly ($P<0.01$). Heat treatment of protein rich feedstuffs such as faba beans has resulted in an increase in milk fat content, milk fat yield and milk lactose yield (Prestløkken, Harstad 2001), although the milk fat and lactose yield in some circumstances was a direct result of increased milk yield. Faba beans contain relatively high levels of protein and energy, which make them useful within feeding of dairy cows; they also have a higher soluble protein fraction than rapeseeds (Ramos--Morales et al. 2010). The values of Ca content in milk obtained in our experiment were lower than those reported by Barłowska et al. (2013).

Fatty acid composition

Milk fat contains a high proportion (70-75%) saturated fatty acids (SFA), followed by quite high percentages of monounsaturated fatty acids (MUFA; 20-25%) and polyunsaturated fatty acids (PUFA; 5%) – Johansson et al. (2015). The profile of milk fatty acids may also be modified if the animals are fed with heat-treated fat rich feed materials. According to Neves et al. (2009) and McGregor et al. (2007), the suppression of milk fat can be the effect of increased unprotected fat in the rumen depressing fermentation or it may be a result of increased milk fat depression by polyunsaturated fatty acids (PUFA). Lock et al. (2004) and Johansson et al. (2015) states that trans-10 C18:1 and trans-10 cis-12 conjugated linoleic acid (CLA) content in the milk is correlated with milk fat depression (MFD). Bauman and Lock (2006) also establishes that these trans C18 fatty acids can accumulate in milk and can be produced in the rumen. Neves et al. (2009) and McGregor et al. (2007) found that feeding extruded rapeseed increased C18:1 in the milk, indicating incomplete ruminal bio hydrogenation and greater post ruminal flow of this bio hydrogenation intermediate, which would influence mammary fat synthesis. Enhanced consumption of dairy products may be caused by increasing the concentration of PUFA in milk, which may also be beneficial to public health. The data in our experiment showed that PUFA and MUFA were higher in groups B and C than in the control group (A). Increasing the ratio of SFA (especially C12:0, C14:0, C16:0) is associated with the risk of cardiovascular diseases and higher concentrations of total and low-density lipoprotein (LDL) cholesterol (Soyeurt, Gengler 2008). Our experiment proved that adding mixtures of extruded rapeseeds and faba beans to dairy cows' diet decreased the SFA concentration (T-1 – 3.77%, T-2 – 4.58%). Using rapeseed products to a diet of dairy cows changes the milk fatty acid profile as lipids of rapeseed are highly unsaturated, with oleic and linoleic acids being the main ingredients (Kudrna, Marounek 2006). Focant et al. (1998) and Crépon et al. (2010) reported that extruded rapeseeds in the diet increased the concentration of long-chain FA (mainly C18:0, C18:1, C18:2) in milk and decreased the concentration of palmitic acid (C16:0), which is consistent with our results (C18:0, C18:1 increased and C16:0 decreased at the end of the experiment).

The rumen fluid and in vitro digestibility parameters

It was found that extruded ingredients increased dietary ungradable protein and decreased the NH_3-N in the rumen (Bertipaglia et al. 2010). However, Soltan et al. (2009) and Prestløkken and Harstad (2001) have reported a decrease in ruminal pH when feeding extruded ingredients and an increased total ruminal VFA concentration. During our experiment, the ruminal pH values decreased at the end of the experiment and the total ruminal VFA concentration increased for both experimental groups at the end of the experiment. The total protozoa count in the rumen content generally increases with the addition of extruded supplements diet. In our experiment, the amount of protozoa and enterococci increased statistically significantly ($P<0.05$) at the end of the experiment. The *in vitro* digestion methods attempt to stimulate the natural ruminal digestive processes (Pardío et al. 2012). In our study, the course of *in vitro* digestion was similar in all groups and was not statistically significantly affected by the experimental factors.

CONCLUSION

Using mixtures of extruded rapeseeds and extruded faba beans (mixed in different proportions: 30% vs 70% and 70% vs 30%) in our experiment showed statistically significant results on milk yield, fat, protein and lactose content and fat acids composition ($P<0.05$) although it had no effect on rumen fluid parameters or *in vitro* digestion ($P>0.05$).

Conflicts of interest

The authors declare no conflict of interest.

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