The effect of dietary pumpkin seed cake and extruded linseed on blood haemato-chemicals and milk quality in Alpine goats during early lactation

Željka Klir Šalavardić[1]*, Josip Novoselec[1],
Joaquín Miguel Castro-Montoya[2], Marcela Šperanda[1], Mislav Đidara[1],
Joachim Molkentin[3], Boro Mioč[4], Uta Dickhoefer[2], Zvonko Antunović[1]

[1]University of J. J. Strossmayer, Faculty of Agrobiotechnical Sciences Osijek, Department for Animal Production and
 Biotechnology, V. Preloga 1, Osijek, 31000, Croatia
[2]University of Hohenheim, Institute of Agricultural Sciences in the Tropics, Fruwirthstrasse 31, Stuttgart, 70593, Germany
[3]Max Rubner-Institute, Department of Safety and Quality of Milk and Fish Products, Hermann-Weigmann-Strasse 1,
 Kiel, 24103, Germany
[4]University of Zagreb, Faculty of Agriculture, Department of Animal Science and Technology, Svetosimunska cesta 25,
 Zagreb, 10000, Croatia
*Corresponding author: zeljka.klir@fazos.hr

Abstract

Due to the high content of crude protein and high concentration of unsaturated fatty acids, pumpkin seed cake (PSC) could be a good quality feedstuff for ruminants, while extruded linseed (ELS) has already shown some beneficial effect on fatty acids proportion of goat milk. Besides, lactation is the most demanding period when monitoring the metabolic condition of animal. Therefore, the aim of this study was to determine the effect of PSC and ELS replacing soybean meal in diets of dairy goats on blood haemato-chemical parameters and milk quality. A 28 lactating French Alpine dairy goats were monitored within 20 days in early lactation. Diet of each groups had a balanced content of energy and crude protein, and a slight difference in lipid content. Goats in control group (n = 9) received soybean meal and extruded soybean. In experimental groups soybean was partially replaced with ELS (n = 10) or completely with PSC (n = 9). Dietary treatments had no effect on haemato-chemical parameters, although PSC increased concentration of globulins in serum. PSC lowered linoleic acid compared to control. ELS increased proportion of α-linolenic acid, eicosapentaenoic acid, and total n-3 fatty acids in milk. Positive correlations were found between non-esterified fatty acids (NEFA) and ratio C18:1/C15:0, NEFA and ratio C17:0/C15:0, as well as β-hydroxybutyrate and ratio C17:0/C15:0. The replacement of soybean meal with PSC or partially with ELS is feasible with respect to haemato-chemical parameters of early lactating dairy goats, while ELS had much beneficial effect on fatty acids compared to PSC.

Key words: pumpkin seed cake; extruded linseed; dairy goats; haemato-chemicals; milk quality

Introduction

In Slovenia, Austria, Hungary and Croatia, pumpkin seeds (*Cucurbita pepo* L.) are used to produce edible oil and its by-products can be used in ruminant feeding. The nutritional value of pumpkin seed is based on high energy potential, due to high percentage of oil (40 % - 60 %; Bavec et al., 2007) and high crude protein content (30.8 %; Kim et al., 2012). Pumpkin seed cake is produced by pressing of hull-less seeds with screw press. This method of production preserves bioactive components, such as vitamins, provitamins, phytosterols, phospholipids and squalene, which are, together with some fatty acids, very important nutritional factors of pumpkin seed cake (Rabrenović et al., 2014). Due to the high content of digestible crude protein compared to soybean meal (496.9 vs. 396.0 g/kg) and high concentration of unsaturated fatty acids of which oleic (C18:1 n-9, OA) and linoleic (C18:2 n-6, LA) are predominant, pumpkin seed cake is a good quality feedstuff for goats (Zdunczyk et al., 1999). In livestock diets soybean meal was more frequently replaced by alternative sources of plant protein (Gugołek et al., 2017), due to its doubtful origin and quality (Hewlett and Azeez, 2008). Antunović et al. (2018) found that pumpkin seed cake can be used in lambs' feeding as a partial replacement for soybean meal in terms of blood parameters and carcass traits. Besides, the inclusion of pumpkin seed cake in ruminants´ diets has not been extensively researched yet.

On contrary, numerous studies reported a high nutritive value of extruded linseed added in concentrates mixtures for dairy goats regarding n-3 fatty acids in milk, especially α-linolenic acid (C18:3 n-3, ALA) (Klir et al., 2012; Nudda et al., 2013; Bernard et al., 2015; Klir et al., 2017). Production of milk that is low in medium-chain saturated fatty acids and high in odd and branched-chain fatty acids, C18:1 c9, and C18:2 c9t11 (CLA, conjugated linoleic fatty acid) and PUFA could have benefits on human health (Castro et al., 2019). Although, an excessive fat from linseed may have deleterious effects on digestive efficiency, milk fat and protein content (Doreau et al., 2015), while unsaturated fatty acids (UFA) in diets may lead to oxidative stress (Wang et al., 2010). One of the most important determinants of goats´ production efficiency is monitoring of herds' health status which is of fundamental importance for disease detections that impair goats' production (Rumosa-Gwaze et al., 2012). The beginning of lactation is the most demanding period in terms of energy balance status and also health management of herd (Walsh et al., 2011). High-yielding goats require high amounts of energy during early lactation leading animals to mobilize energy from their body reserves (Teh et al., 1994).

Therefore, the objective of the present study was to research if pumpkin seed cake in diets of early lactating dairy goats influences haematological and biochemical parameters in blood and milk quality as compared with diets containing soybean meal and diet where extruded linseed partially replaced soybean meal.

Materials and methods

Bioethics Committee for Research on Animals of Faculty of Agrobiotechnical Sciences Osijek established that the present research is carried out under the legal provisions according to Animal Protection Act of Croatia (NN 133/06, NN 37/13 and NN 125/13). Animal handling practices meets the European Union Directive 2010/63/UE about the protection of animals used for the experiment.

Experimental design, animals, and feeding

The research was conducted with 28 French Alpine dairy goats at family farm in Osijek-Baranya County (Croatia). All goats had kidded within a period of one week. After 7 days of adaptation period to experimental diets, animals were set to experiment (12th ± 3 days of lactation). Duration of the current experiment was 20 days, during early lactation, which was a part of larger experiment lasting 75 days (Klir et al., 2017). The sampling was conducted when goats were approximately in the 32nd ± 3 days of lactation. All goats were healthy and in adequate body condition (score 2.87 ± 0.51) according to Russel (1991) when the experiment started, and had an average age of 3.6 ± 1.73 years. Goats were machine milked twice per day, in the morning and the evening, and kept together in the barn. All goats were fed *ad libitum* with grass and red clover hay (60 % DM *Phleum pratense* and *Lolium*

multiflorium, and 40 % DM *Trifolium pratense*). In addition, each goat was supplemented with 1 kg/day of a concentrate mixture individually. Goats were fed in separated feeding troughs during milking, and had free access to fresh drinking water, while no refusals of concentrate were observed. Dietary treatments differed in the main source of crude protein and crude fat in the concentrate mixtures. Control group contained soybean meal (n = 9), while in ELS group 38.3 % of soybean was replaced with ELS (n = 10), and in PSC group soybean meal was completely replaced with PSC (n = 9; Table 1). The diet had an approximate hay to concentrate ratio of 70:30 (on DM basis).

TABLE 1. Ingredient composition of the concentrate mixtures used in diets for early lactating goats.

Ingredient (g/kg, as-fed)	Concentrate mixture		
	Control	ELS	PSC
Corn	429	408	459
Barley	80	80	90
Oat	100	100	135
Wheat flour	120	90	120
Extruded soybean meal	150	-	-
Extruded linseed	-	90	-
Pumpkin seed cake	-	-	160
Alfalfa dehydrated	-	40	-
Soybean meal (46 % crude protein)	85	157	-
Calcium carbonate	16	15	16
Monocalcium phosphate	5	5	5
Salt	4	4	4
Pellet binder	1	1	1
Mineral vitamin premix*	10	10	10

ELS - concentrate mixture with partial replacement of soybean meal with extruded linseed, PSC - concentrate mixture with complete replacement of soybean meal with pumpkin seed cake
*Mineral-vitamin premix: Fe (iron sulphate monohydrate) 4000 mg, Cu (copper sulphate pentahydrate) 800 mg, Mn (manganese oxide) 3500 mg, Zn (zinc sulphate monohydrate) 5000 mg, I (potassium iodide) 80 mg, Co (cobalt sulphate heptahydrate) 20 mg, Se (sodium selenite) 15 mg, Mg (magnesium oxide) 5000 mg; vitamin A 1 000 000 IU, vitamin D3 150 000 IU, vitamin E (α-tocopherol) 1500 mg, vitamin K3 (menadione sodium bisulfite) 50 mg, vitamin B1 (thiamine mononitrate) 100 mg, vitamin B2 200 mg, vitamin B6 200 mg, vitamin B12 1 mg, niacin 1000 mg, Ca-pantothenate 500 mg, choline chloride 10 000 mg

Analysis of feedstuffs

Composition of feed was determined with standard methods (AOAC, 2006). The crude protein concentrations were estimated from nitrogen content according to the Kjeldahl method with the steam distillation unit for Kjeldahl nitrogen (Behr, Germany). The crude fat concentrations were analysed using the Universal Extractions System B-811 (Buchi, Switzerland). Metabolizable energy (ME, MJ/kg DM) of feed samples were calculated from gas production after 24 h of *in vitro* incubation with Hohenheim gas test according to Menke and Steingass (1987). All plant samples were digested with 10 mL of a 5:1 mixture of HNO_3 and H_2O_2 at 180 °C for 60 min in microwave oven (CEM Mars 6, USA). The concentrations of mineral elements (Ca, P, Mg and Fe) in solutions of digested plant samples were determined by inductively coupled plasma mass spectrometer (ICP-MS, Agilent 7500a, Agilent Technologies Inc., California, USA). The value for N-free extract (NFE) was calculated as: NFE (g/kg DM) = 1000 - (crude protein + crude lipid + crude fibre + ash). Fatty acids in dietary ingredients were determined by gas chromatography following the methodology of State Office of Agricultural Chemistry in Baden Württemberg (LaChemie P23-5-008, V. 01).

Milk sampling and analysis

Two milk samples (2×100 mL) were taken on the 20th day of experiment at 07.00 am from each goat by hand during morning milking. Immediately after sampling 100 mL milk sample from each goat was transferred into bottles containing 0.3 mL of the preservative azidiol, cooled to 4 °C and set up for analysis. Chemical composition was analysed by infrared spectroscopy HRN ISO 9622:2001 (Croatian Standards Institute, 2001) method on the analyser MilkoScan FT 6000 (Foss Electric, Denmark). Somatic cell counts (SCC) were determined by fluoro-opto-electronic method HRN ISO 13366-2/Ispr.1:2007 (Croatian Standards Institute, 2007) with a Fossomatic 5000 analyser (Foss Electric, Denmark), while the bacterial colony forming units (CFU) were determined by the method of epifluorescence flow ISO 16297:2013 [IDF 161:2013] (ISO, 2013).

TABLE 2. Chemical composition and major fatty acid proportions of hay and concentrate mixtures used in the diets for early lactating goats

Parameter	Concentrate mixture			Hay
	Control	ELS	PSC	
DM (g/kg fresh matter)	876	874	873	925
Crude protein (g/kg DM)	162	162	163	110
Crude fibre (g/kg DM)	41.4	48.8	37.3	287
Crude ash (g/kg DM)	49.2	50.6	52.3	57.3
Crude fat (g/kg DM)	56.4	58.3	56.3	13.3
NFE (g/kg DM)	691	680	691	532
ME (MJ/kg DM)	13.2	13.0	13.2	8.0
Mineral composition (mg/kg DM)				
Ca	8949	8108	8548	1572
P	7100	6147	6818	1410
Mg	2114	1944	2096	480
Fe	321	278	294	92.8
Fatty acids proportion (g/100 g fatty acids)				
C16:0	10.7	9.43	12.9	29.8
C18:0	4.08	6.11	4.48	2.84
C18:1 *c*9	31.9	32.0	34.4	7.71
C18:2 n6	48.2	38.2	44.4	22.4
C18:3 n3	2.9	11.5	1.8	24.5
Saturated fatty acids	16.7	17.7	19.2	44.7
Monounsaturated fatty acids	32.1	32.6	34.6	8.4
Polyunsaturated fatty acids	51.2	49.7	46.2	46.9

ELS - concentrate mixture with partial replacement of soybean meal with extruded linseed, PSC - concentrate mixture with complete replacement of soybean meal with pumpkin seed cake, DM - dry matter, NFE - nitrogen free extract, ME - metabolizable energy

Another 100-mL milk sample from each goat was frozen at -80 °C and stored until fatty acid analysis. The extraction of milk fat was done following a three-steps-extraction with gravimetric reference method (ISO, 2010). Fatty acid methyl esters were obtained from the milk fat samples by transesterification according to ISO (2002a). The fatty acid composition was determined by gas-liquid chromatography (ISO, 2002b). Fatty acids were calculated as weight percentage (g/100 g fatty acids) and are presented as means of duplicates.

Sampling of blood and analysis

The blood was collected from the jugular vein (10 mL) into sterile vacuum tubes Venoject® (Sterile Terumo Europe, Leuven, Belgium) on day 20 after the morning feeding and milking. For haematology analysis, blood of goats was sampled into the sterile vacuum tubes containing ethylenediaminotetraacetic acid (EDTA) as the anticoagulant. Prior to analysis, whole blood was mixed with the Coulter mixer (Coulter electronics limited, Luton Bedfordshire, England). In whole blood, within 3 h after sampling, following haematological parameters were determined: number of leukocytes (WBC), erythrocytes (RBC), and the contents of haemoglobin (HGB), haematocrit (HCT), mean corpuscular volume (MCV), average haemoglobin content in erythrocytes (MCH) and mean haemoglobin concentration in erythrocytes (MCHC) on an automatic three differential haematology analyser (Sysmex PocH-100lv, Sysmex Europe GmbH, Hamburg, Germany).

For biochemical analysis, within 4 h after sampling, serum (supernatant) in vacuum tubes was separated by centrifugation (10 min) at 1609.92 g and frozen at -20 °C until analysis. Within blood serum the concentrations of the minerals (calcium, phosphorus-inorganic, magnesium and iron), concentrations of urea, glucose, total proteins, albumin, cholesterol (CHOL), high density lipoprotein (HDL), low density lipoprotein (LDL), triglyceride (TGC), β-hydroxybutyrate (BHB) and non-esterified fatty acids (NEFA) as well as alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gama-glutamyl-transferase (GGT) were determined. Globulin concentration was calculated as the difference between total protein and albumin. Analysis were obtained by Beckman Coulter (AU400, California, USA).

The activity of glutathione peroxidase (GPx) in serum was determined by Ransel® kits (Randox, UK) on an automatic analyser Beckman Coulter (AU400, California, USA) at a wavelength of 240 nm. The activity of total superoxide dismutase

(SOD) in serum was determined by using RANSOD® kits (Randox, UK) on an automatic analyser Beckman Coulter (AU400, California, USA) at a wavelength of 510 nm.

Statistical analysis

A General Linear Model (GLM) was applied to the data for milk performance and biochemical parameters, with diet treatment as fixed effect. Means were compared using the Tukey test and differences between the groups were declared significant at P<0.05. Values of SCC and CFU were logarithmically converted to a linear score with the aim to approximate normal distribution. Correlations between parameters of goats´ energy status (BHB and NEFA) and selected milk fatty acids and ratios were evaluated by Pearsons´ correlation with CORR procedure. Statistical analysis was performed using the statistical software SAS (2013, SAS Inst. Inc., Cary, NC. version 9.4).

Results and discussion

Milk yield and chemical composition of goat milk were not affected by the feeding treatment (Table 3). Similarly, the soybean replacement by PSC or partially by ELS did not result in any significant differences regarding the SCC and CFU. This indicated that PSC can be used as an alternative to soybean for the conditions of this study. Both PSC and ELS have a different fatty acids profile compared with soybean, but those changes in the diet did not affect either milk production. However, extruded linseeds addition in goats' diets lowered the milk yield, and increased the milk fat content in the study conducted by Chilliard et al. (2013).

The milk fatty acid profile has been changed when feeding goats with ELS and there were minor changes in PSC compared to Control (Table 4). The ELS and PSC diets did not influence the proportions of C6:0, C8:0 and C10:0, fatty acids responsible for the particular sensory properties of goats´ milk (Bessa et al., 2016) and health benefits providing better digestibility of goat milk (Zenebe et al., 2014). Bernard et al. (2015) found that SCFA, specifically C6:0, C8:0 and C10:0, were lowered in milk when feeding goats with 216 g/kg DM of extruded linseed in goats´ diets

TABLE 3. Milk yield and chemical composition and fatty acid proportions in milk of early lactating goats fed with diets containing extruded linseed (ELS) and pumpkin seed cake (PSC)

	Diets			SEM	P value
	Control	ELS	PSC		
Milk yield (kg)	1.93	1.24	1.57	0.127	0.08
Milk composition (g/100 g)					
Fat	3.43	4.05	3.81	0.199	0.46
Protein	3.01	3.22	2.98	0.080	0.40
Lactose	4.47	4.29	4.40	0.047	0.30
Non-fat-dry matter	8.50	8.52	8.39	0.102	0.86
Urea (mg/dL)	51.0	54.6	59.7	1.86	0.54
SSC (log. n./mL)	6.05	5.72	6.18	0.179	0.56
CFU (log. n./mL)	4.28	4.38	4.28	0.146	0.95

SEM - standard error of mean; SCC - somatic cells count, CFU - number of bacterial formed colonies; [a,b]Row means with different superscripts differ significantly at P<0.05

compared to control diets. The LCFA inhibit lipogenic enzymes of the mammary gland, leading to lowering of de novo synthesized fatty acid proportions (Chilliard et al., 2007). In the present study the proportion of ELS in the concentrate mixtures was probably not high enough to influence a lower proportion of these SCFA. Contrary, proportion of branched-chain fatty acids (BCFA) like C14:0 *iso*, and C15:0 *iso*, were significantly higher (P<0.05) when feeding goats with ELS compared to Control, while in PSC remained intermediate and unaffected. Supplementation of ruminants´ diet with different lipid source has relevant effect on ruminal bacteria community in goats (Cremonesi et al., 2018). Abuelfatah et al. (2016) reported that inclusion of linseed in the diet of goats at level of 10 or 20 g/100 g DM increases the molar proportion of acetate in rumen, which may be the reason for the increase of above stated milk fatty acids. Besides, Goetsch (2019) explained that numerous factors are responsible for microbial population in the rumen of dairy goats, including greater feed intake relative to body weight and shorter time of digesta residence in the rumen, and some other effects before treatment.

TABLE 4. Fatty acid proportions in milk of early lactating goats fed with diets containing extruded linseed (ELS) and pumpkin seed cake (PSC)

	Diets			SEM	P value		Diets			SEM	P value
	Control	ELS	PSC				Control	ELS	PSC		
C4:0	2.60	2.72	2.80	0.051	0.29	C18:1 c9 [3]	14.7	15.2	15.7	0.36	0.57
C5:0	0.03	0.03	0.04	0.001	0.13	C18:1 t15	0.30[ab]	0.33[a]	0.25[b]	0.012	0.02
C6:0	2.98	3.10	3.08	0.049	0.61	C18:1 c11	0.32	0.28	0.28	0.008	0.06
C7:0	0.05	0.06	0.06	0.003	0.48	C18:1 c12	0.43[a]	0.37[ab]	0.29[b]	0.019	0.01
C8:0	3.46	3.55	3.42	0.069	0.76	C18:1 c13	0.09	0.09	0.08	0.003	0.49
C9:0	0.11	0.12	0.12	0.006	0.53	C18:1 t16[4]	0.46	0.49	0.41	0.015	0.11
C10:0	11.5	11.6	11.3	0.23	0.83	C18:1 c15[5]	0.21[b]	0.27[a]	0.21[b]	0.008	<0.01
C10:1	0.19	0.21	0.19	0.006	0.36	C18 iso	0.04	0.04	0.04	0.002	0.84
C11:0	0.14	0.14	0.15	0.009	0.99	C18:2 n6	2.89[a]	1.87[b]	2.33[b]	0.111	<0.01
C12:0	5.02	4.43	4.42	0.185	0.37	C18:2 t9 t12[6]	0.38[ab]	0.44[a]	0.33[b]	0.014	<0.01
C12:1	0.09	0.07	0.07	0.005	0.22	C18:2 t8 c13	0.18[ab]	0.21[a]	0.17[b]	0.007	0.01
C13:0	0.10	0.10	0.11	0.004	0.92	C18:2 c9 t12	0.13[a]	0.11[ab]	0.10[b]	0.005	0.02
C13 iso	0.02	0.02	0.02	0.001	0.07	C18:2 t11 c15	0.22[b]	0.45[a]	0.19[b]	0.027	<0.01
C13 aiso	0.01	0.01	0.01	0.001	0.42	C18:2 c9 t11	0.95	1.02	0.79	0.056	0.25
C14:0	9.40	9.26	9.52	0.163	0.81	C18:3 n6	0.02	0.02	0.02	0.001	0.43
C14 iso	0.05[b]	0.07[a]	0.07[ab]	0.003	0.01	C18:3 n3	0.50[b]	0.79[a]	0.36[b]	0.042	<0.01
C14:1	0.09	0.10	0.09	0.003	0.91	C19:1	0.02	0.03	0.02	0.001	0.16
C15:0	0.68	0.77	0.75	0.018	0.12	C19:2	0.03	0.03	0.03	0.001	0.28
C15 iso	0.14[b]	0.18[a]	0.16[ab]	0.006	0.02	C20:0	0.21	0.20	0.21	0.005	0.95
C15 aiso	0.28	0.30	0.28	0.008	0.45	C20:1	0.06	0.05	0.05	0.001	0.07
C16:0	22.9	22.9	24.2	0.62	0.63	C20:2	0.02	0.02	0.02	0.001	0.08
C16 iso	0.17	0.20	0.20	0.008	0.13	C20:3	0.02	0.02	0.02	0.002	0.49
C16:1 t9	0.29	0.28	0.29	0.006	0.76	C20:4 n6	0.17	0.16	0.17	0.006	0.60
C16:1 c9	0.37	0.40	0.40	0.011	0.41	C20:5 n3	0.07[b]	0.09[a]	0.06[b]	0.003	<0.01
C17:0	0.44	0.46	0.46	0.008	0.62	C21:0	0.04	0.04	0.04	0.001	0.41
C17 iso	0.32	0.32	0.30	0.008	0.55	C22:0	0.06	0.06	0.05	0.003	0.34
C17 aiso	0.35	0.36	0.33	0.009	0.39	C22:1	0.01	0.01	0.01	0.001	0.17
C17:1	0.13	0.15	0.14	0.004	0.27	C22:5 n3	0.12[ab]	0.14[a]	0.09[b]	0.006	0.02
C18:0	10.0	10.0	10.2	0.31	0.94	C23:1	0.04	0.04	0.04	0.002	0.81
C18:1 t4	0.02	0.03	0.02	0.001	0.77	C24:0	0.01	0.01	0.01	0.001	0.11
C18:1 t5	0.03	0.03	0.03	0.001	0.64						
C18:1 t6[1]	0.35	0.31	0.31	0.013	0.33						
C18:1 t9	0.30	0.29	0.28	0.008	0.76						
C18:1 t11[2]	2.96	2.68	2.29	0.158	0.23						

[1]Contains C18:1 t7 + t8 as a minor components, [2]Contains C18:1 t10 as a minor component, [3]Contains C18:1 t12 + t13 + t14 as a minor components, [4]Contains C18:1 c14 as a minor component, [5]Contains C19:0 a minor component, [6]Contains C18:2 t9 t12 + t8 c12 + c9 t13 a minor components; SEM-standard error of mean; [a,b]Row means with different superscripts differ significantly at P < 0.05

Proportions of stearic (C18:0), OA, vaccenic (VA, C18:1 t11) acid and CLA remained unaffected by dietary treatments. Bernard et al. (2015) determined that addition of extruded linseed in combination with fish oil may be efficient in increasing VA and CLA proportions in milk. Although proportions of CLA, which is of the interest with respect to human health, were appropriate as compared to another studies, the amount of CLA consumed to improve human health still needs to be established (Hur et al., 2017).

In the present study ELS was abundant in ALA, which resulted in higher proportions of biohydrogenation metabolites, like C18:1 t15, C18:1 c15 and C18:2 t11 c15 compared to Control and PSC. Shingfield et al. (2010) reported that within putative pathway of ALA biohydrogenation C18:1 t15 and C18:1 c15 are produced as minor fatty acid metabolites, while in major ALA biohydrogenation pathway C18:2 t11 c15 is formed. Similar was reported in milk of ELS compared to Control and PSC of the present study.

Diets with PSC and ELS lowered LA compared to the Control, as a consequence of the higher LA concentration in the Control feed mixture containing soybean meal and extruded soybean (Table 1). This was reflected in lowered total n-6 fatty acids in ELS and PSC (Table 5). Proportion of ALA was the highest in ELS which resulted in higher ALA and total n-3 proportions, and lower LA/ALA ratio in milk of ELS goats compared to those of Control and PSC. The relationship between ALA intake and ALA proportion in milk is linear and transferred from diet to milk is close to 4.5 % (Doreau et al., 2015). According to the Commission Regulation EU (2010), a claim that food is a source of omega-3 fatty acids, may only be made when the product contains at least 0.3 g of ALA per 100 g, while food is high in omega-3 fatty acids when the product contains at least 0.6 g ALA per 100 g. As a consequence of greater ALA supply from the ELS diet proportion of EPA was higher compared to Control or PSC, as ALA is a precursor of EPA during the elongation.

The concentration of globulins was the highest when PSC was fed and the lowest for the Control, while the rest of haemato-chemical parameters in goats' blood resulted unchanged by the diets (Table 6). This observation could be due to high phenolic compounds in pumpkin seed cake of PSC diet

TABLE 5. Groups of fatty acids in milk from early lactating goats fed with diets containing extruded linseed (ELS) and pumpkin seed cake (PSC).

Fatty acids*	Diets			SEM	P value
	Control	ELS	PSC		
SCFA	21.0	21.4	21.0	0.32	0.83
MCFA	39.7	39.2	40.7	0.71	0.70
LCFA	37.6	37.5	36.8	0.78	0.90
SFA	71.2	71.1	72.3	0.60	0.63
BCFA	1.38	1.50	1.40	0.036	0.35
OCFA	1.83	1.98	1.95	0.039	0.29
∑C16:1 isomers	0.66	0.68	0.70	0.014	0.51
∑C18:1 isomers	2.51	2.47	2.17	0.075	0.14
∑C18:2 isomers	0.91[b]	1.21[a]	0.78[b]	0.048	<0.01
MUFA	22.1	22.4	22.2	0.45	0.96
UFA	27.8	27.8	26.8	0.55	0.73
PUFA	5.70[a]	5.36[ab]	4.68[b]	0.164	0.04
∑n-3	0.69[b]	1.02[a]	0.52[c]	0.044	<0.01
∑n-6	3.64[a]	2.64[b]	2.99[b]	0.116	<0.01
LA/ALA	5.88[a]	2.42[b]	6.63[a]	0.401	<0.01

*SCFA - short-chain fatty acids, MCFA - medium-chain fatty acids, LCFA - long-chain fatty acids, SFA - saturated fatty acids, BCFA - branched-chain fatty acids, OCFA - fatty acids with odd number of carbon atoms, MUFA - monounsaturated fatty acids, UFA - unsaturated fatty acids, PUFA - polyunsaturated fatty acids, LA - linoleic acid, ALA-α - linolenic acid; SEM - standard error of mean; [a,b]Row means with different superscripts differ significantly at P<0.05

TABLE 6. Haemato-chemical parameters from early lactating goats fed with diets containing extruded linseed (ELS) and pumpkin seed cake (PSC)

Parameter	Diets			SEM	P value
	Control	ELS	PSC		
*Haematological parameters**					
RBC (×10^{12} L)	12.1	11.0	11.5	0.32	0.39
WBC (×10^9 L)	8.36	9.27	9.09	0.53	0.78
HGB (g/L)	87.4	79.6	83.8	2.11	0.32
HCT (L/L)	0.33	0.30	0.39	0.02	0.37
MCH (pg)	7.21	7.33	7.33	0.08	0.80
MCV (fL)	27.27	28.17	28.17	0.47	0.69
MCHC (g/L)	265	261	261	2.38	0.79
*Biochemical parameters (mmol/L) **					
Urea	8.02	7.67	7.38	0.162	0.29
Proteins (g/L)	65.3	67.0	70.1	0.99	0.15
Albumin (g/L)	27.6	26.8	26.9	0.58	0.83
Globulin (g/L)	37.7[b]	40.2[ab]	43.2[a]	0.85	0.03
Glucose	3.65	3.62	3.38	0.087	0.42
Ca	2.10	2.21	2.12	0.030	0.31
P-inorganic	1.94	1.87	1.98	0.099	0.90
Mg	0.87	0.93	0.92	0.013	0.22
Fe (µmol/L)	20.9	22.2	24.0	0.91	0.41
Cholesterol	2.26	2.52	2.28	0.114	0.60
HDL	1.50	1.56	1.50	0.062	0.91
LDL	0.68	0.86	0.70	0.056	0.35
TGC	0.16	0.21	0.18	0.010	0.14
NEFA	0.05	0.05	0.05	0.003	0.88
BHB	0.51	0.55	0.50	0.019	0.53
GPx (U/L)	1515	1523	1534	39.3	0.98
SOD (U/mL)	0.19	0.17	0.10	0.024	0.28
AST (U/L)	92.2	108	96.4	3.70	0.17
ALT (U/L)	14.3	15.9	13.1	0.59	0.15
GGT (U/L)	41.6	51.6	44.9	2.41	0.22

*RBC - erythrocytes, WBC - number of leukocytes, HGB - contents of haemoglobin, HCT - haematocrit, MCH - average haemoglobin content in erythrocytes, MCV - mean corpuscular volume, MCHC - mean haemoglobin concentration in erythrocytes, HDL - high-density lipoprotein, LDL - low-density lipoprotein, TGC - triglycerides, NEFA - non-esterified fatty acids, BHB - β-hydroxybutyrate, GPx - glutathione peroxidase, SOD - superoxide dismutase, AST - aspartate aminotransferase, ALT - alanine aminotransferase, GGT - gamma-glutamyl transferase, SEM - standard error of mean; [a,b]Row means with different superscripts differ significantly at P<0.05

as reported by Peričin et al. (2009). Polyphenol rich fraction from food have been shown to result in a significant increase in the IgM- and IgG-plaque forming cell responses of blood leukocytes (Hikosaka et al., 2007). The addition of feedstuff rich in crude fat in ruminants´ feeding may affect the production traits and health of ruminants (Nudda et al., 2013). All values of haemato-chemical parameters were within values determined in lactating Alpine goats by Antunović et al. (2013 and 2017).

It is well known that ruminants are susceptible to oxidative stress during pregnancy and lactation (Bernabucci et al., 2005), resulting in physiological state of metabolic stress that may cause diseases by changing the metabolic status (Antunović et al., 2013). The concentration of BHB in blood (≥0.8 mmol/L) and changes of the body score index (≤2.0) are specific indicators for assessment and prevention of metabolic disorders of highly productive lactating goats (Binev et al., 2014). Jahani-Moghadam et al. (2015) did not determine significant differences in the glucose, NEFA, BHB and urea concentrations in blood of cows fed ELS. Likewise, the results of the current study indicate the positive energy balance in early lactating dairy goats fed with ELS or PSC.

The ELS or PSC did not influence the activity of enzymes of antioxidant status. Bernabucci et al. (2005) reported that oxidative status is associated with energy status, which means that higher concentrations of NEFA and BHB are associated with lower activity of antioxidative enzymes in plasma. Additionally, Wang et al. (2010) established lower activity of SOD in plasma of cows fed UFA rich diets compared to cows fed with diets added SFA, while GPx activity and BHB concentration did not differ between groups. Owing to similar concentration of UFA in diets (Table 2) and stable energy status (Table 6), differences in the activity of the SOD and GPx enzyme were missing, and the antioxidant status was preserved.

Negative correlation was determined between the NEFA and the total SCFA or certain SCFA (Table 7). Short chain fatty acids which are synthesized *de novo* in the mammary gland decrease when plasma NEFA concentration increases (Craninx et al., 2008). The variability of milk C18:0 and OA

concentration is linked to changes in the energy balance during the first 4 months of goats' lactation, since ruminant adipose tissue is very rich in palmitic, stearic, and OA (Chilliard et al., 2003). Established correlations result from the negative effect of LCFA mobilized from adipose tissue on *de novo* synthesized SCFA (Barber et al., 1997), as confirmed by present study on goats. Möller et al. (2018) determined the relationship between MCFA in hair and fat metabolism of cows. Dórea et al. (2017) found high sensitivities of milk C6:0, C8:0, C10:0 and C12:0 related to NEFA concentrations in cows' plasma. Accordingly, these authors determined threshold values to detect high NEFA concentrations in plasma, but when the proportion of these fatty acids are above these thresholds, the cow is classified as low concentrations of NEFA. In the present research a negative correlation was determined between the serum NEFA and C10:0 as well as C15:0 in goat milk while the other individual SCFA did not show any significant differences. Dórea et al. (2017) established that plasma NEFA concentrations may be predicted by linear regression models using rations C18:1 to C15:0 and C17:0 to C15:0 in cows´ milk. In the present study Pearson correlation showed positive correlation between both ratios in goat milk and serum NEFA, and only C17:0 to C15:0 ratio and BHB in serum. These correlations indicate low mobilization of lipids from adipose tissue in early lactating dairy goats in the current research.

Conclusion

The current findings indicate that the replacement of soybean meal with pumpkin seed cake (PSC) or partially with extruded linseed (ELS) is feasible with respect to haemato-chemical parameters of the early lactating dairy goats. Moreover, these indicate adequate health, preserved antioxidant and nutritional status when goats were fed with PSC or ELS. Partially substituting soybean meal with ELS enhanced goat milk with ALA and total n-3 fatty acid proportions while compared to conventional diets, soybean meal replaced by PSC lowered linoleic acid in milk. In further study it is necessary to determine antioxidant status of milk which will contribute to better understanding whether PSC is suitable for goats´ feeding regarding milk quality.

TABLE 7. Correlations between the selected milk fatty acids and parameters of energy status in the serum of early lactating goats

Fatty acids	BHB		NEFA	
	Pearson's (r)	P value	Pearson's (r)	P value
C6:0	0.444	0.11	-0.094	0.75
C8:0	0.375	0.19	-0.283	0.33
C10:0	-0.234	0.42	-0.768	<0.01
C12:0	-0.672	<0.01	-0.423	0.13
C14:0	-0.670	<0.01	-0.282	0.33
C15:0	-0.284	0.33	-0.584	0.03
C16:0	-0.263	0.36	-0.373	0.19
C17:0	0.374	0.19	0.410	0.15
C18:0	0.579	0.03	0.612	0.02
C18:1 n9	0.402	0.15	0.673	0.01
C18:1/C15:0	0.341	0.08	0.660	<0.01
C17:0/C15:0	0.462	0.01	0.701	<0.01
SCFA	0.073	0.80	-0.591	0.03
MCFA	-0.508	0.06	-0.478	0.08
LCFA	-0.450	0.11	-0.307	0.29

NEFA - non-esterified fatty acids, BHB - beta-hydroxybutyrate, SCFA - short-chain fatty acids, MCFA - medium-chain fatty acids, LCFA - long-chain fatty acids

Acknowledgements

Utjecaj pogače sjemenki bundeve i ekstrudiranog lana u obrocima na hematološko-biokemijske pokazatelje u krvi i na kvalitetu mlijeka alpske koze tijekom rane laktacije

Sažetak

Zbog visokog sadržaja sirovih bjelančevina i visoke koncentracije nezasićenih masnih kiselina, pogača sjemenki bundeve (PSC) potencijalno je kvalitetno krmivo za preživače, dok je ekstrudirani lan (ELS) u obrocima koza već pokazao blagotvoran utjecaj na kvalitetu kozjeg mlijeka. Laktacija je vrlo zahtjevno razdoblje za praćenje metaboličkog statusa životinja. Prema tome, cilj ovoga istraživanja bio je utvrditi utjecaj PSC i ELS na hematološko-biokemijske pokazatelje u krvi i na kvalitetu mlijeka, kao zamjena za soju u obrocima mliječnih koza. Istraživanje je provedeno s 28 koza pasmine francuska alpine koje su bile podijeljene u dvije pokusne skupine tijekom 20 dana u ranoj laktaciji. Obroci su bili izbalansirani u pogledu energije i sadržaja sirovih bjelančevina, uz neznatne razlike sadržaja lipida. Obroci kontrolne skupine (n = 9) sadržavali su sačmu i ekstrudiranu soju. U obrocima pokusnih skupina soja je djelomično zamijenjena s ELS (n = 10) ili u potpunosti zamijenjena s PSC (n = 9). Hematološko-biokemijski pokazatelji u krvi koza nisu rezultirali značajnijim promjenama pod utjecajem hranidbe, iako je obrok s PSC povećao koncentraciju globulina u serumu. PSC je rezultirao smanjenjem linolne kiseline (n-6) u odnosu na kontrolnu skupinu. ELS obroci povećali su koncentraciju α-linolenske, eikozapentaenske kiseline i koncentraciju ukupnih n-3 masnih kiselina u mlijeku. Utvrđene su pozitivne korelacije između ne-esterificiranih masnih kiselina (NEFA) i C18:1/C15:0, NEFA te C17:0/C15:0, kao i β-hidroksibutirata te C17:0/C15:0. Potpuna zamjena sačme i ekstrudirane soje s PSC ili djelomično s ELS opravdana je s obzirom na hematološko-biokemijske pokazatelje koza u ranoj laktaciji, dok je ELS imao blagotvoran utjecaj na masne kiseline u mlijeku u odnosu na PSC.

Ključne riječi: pogača sjemenki bundeve; ekstrudirani lan; mliječne koze; hemato-biokemijski pokazatelji; kvaliteta mlijeka

References

1. Abuelfatah, K., Zuki, A.B., Goh, Y., Sazili, A.Q., Abubakr, A. (2016): Effects of feeding whole linseed on ruminal fatty acid composition and microbial population in goats. *Animal Nutrition* 2, 323-328.
https://doi.org/10.1016/j.aninu.2016.10.004

2. Antunović, Z., Klir, Ž., Šperanda, M., Ćavar, S., Mioč, B., Novoselec, J. (2017): Production traits, blood metabolic profile and fatty acids of meat and tallow in response to the partial replacement of soybean meal with peas in organic lambs' feed. *Archives Animal Breeding* 60, 409-417.
https://doi.org/10.5194/aab-60-409-2017

3. Antunović, Z., Klir, Ž., Šperanda, M., Sičaja, V., Čolović, D., Mioč, B., Novoselec, J. Partial replacement of soybean meal with pumpkin seed cake in lamb diets (2018): Effects on carcass traits, haemato-chemical parameters and fatty acids in meat. *South African Journal of Animal Science* 48, 695-704.
http://dx.doi.org/10.4314/sajas.v48i4.11

4. Antunović, Z., Novoselec, J., Klir, Ž., Đidara, M. (2013): Hematological parameters in the Alpine goats during lactation. *Agriculture/Poljoprivreda* 19, 40-43.

5. Antunović, Z., Šperanda, M., Novoselec, J., Đidara, M., Mioč, B., Klir, Ž., Samac, D. (2017): Blood metabolic profile and acid-base balance of dairy goats and their kids during lactation. *Veterinarski Arhiv* 87, 43-55.
https://doi.org/10.24099/vet.arhiv.0780

6. AOAC. (2006): Official methods of analysis of AOAC International. *Association of Analytical Communities*, Arlington, Virginia, USA.

7. Barber, M.C., Clegg, R.A., Travers, M.T., Vernon, R.G. (1997): Lipid metabolism in the lactating mammary gland. *Biochimica et Biophysica Acta* 1347, 101-126.
https://doi.org/10.1016/s0005-2760(97)00079-9

8. Bavec, F., Grobelnik Mlakar, S., Rozman, C., Bavec, M. (2007): Oil Pumpkins: Niche for Organic Producers, in: *Issues in new crops and new uses*, edited by: Janick, J., Whipkey, A., ASHS Press, Alexandria, USA, 185-189.

9. Bernabucci, U., Ronchi, B., Lacetera, N., Nardone, A. (2005): Influence of body condition score on relationship between metabolic status and oxidative stress in periparturient dairy cows. *Journal of Dairy Science* 88, 2017-2026. https://doi.org/10.3168/jds.S0022-0302(05)72878-2

10. Bernard, L., Leroux, C., Rouel, J., Delavaud, C., Shingfield, K. J., Chilliard, Y. (2015): Effect of extruded linseeds alone or in combination with fish oil on intake, milk production, plasma metabolite concentrations and milk fatty acid composition in lactating goats. *Animal* 9, 810-821. https://doi.org/10.1017/S1751731114003048

11. Bessa, M.E., Pereira Rodarte, M., Otênio, M.H., Stringheta, P.C., de Oliveira, J.M., Barbosa, J.S., Oliveira Pinto, M.A. (2016): Sensory perception of the fermented goat milk: potential application of the DSC method. *Food Science and Technology* 36, 406-412. https://doi.org/10.1590/1678-457X.05315

12. Binev, R., Marutsova, V., Radev, V. (2014): Clinical and haematological studies on subclinical lactational ketosis in dairy goats. *Journal of Agricultural Science and Technology* 6, 427-430.

13. Castro, T., Martinez, D., Isabel, B., Cabezas, A., Jimeno, V. (2019): Vegetable oils rich in polyunsaturated fatty acids supplementation of dairy cows' diets: Effects on productive and reproductive performance. *Animals* 9, 205. https://doi.org/10.3390/ani9050205

14. Chilliard, Y., Ferlay, A., Rouel, J., Lamberet, G. (2003): A review of nutritional and physiological factors affecting goat milk lipid synthesis and lipolysis. *Journal of Dairy Science* 86, 1751-1770. https://doi.org/10.3168/jds.S0022-0302(03)73761-8

15. Chilliard, Y., Glasser, F., Ferlay, A., Bernard, L., Rouel, J., Doreau, M. (2007): Diet, rumen biohydrogenation and nutritional quality of cow and goat milk fat. *European Journal of Lipid Science and Technlogy* 109, 828-855, https://doi.org/10.1002/ejlt.200700080

16. Chilliard, Y., Rouel, J., Guillouet, P. (2013): Goat alpha-s1 casein genotype interacts with the effect of extruded linseed feeding on milk fat yield, fatty acid composition and post-milking lipolysis. *Animal Feed Science and Technology* 185, 140-149. https://doi.org/10.1016/j.anifeedsci.2013.08.002

17. Commission Regulation, EU (2010): No 116/2010 of 9 February 2010 amending Regulation (EC) No 1924/2006 of the European Parliament and of the Council with regard to the list of nutrition claims (Text with EEA relevance).

18. Craninx, M., Steen, A., Van Laar, H., Van Nespen, T., Martin-Tereso, J., De Baets, B., Fievez, V. (2008): Effect of lactation stage on the odd- and branched-chain milk fatty acids of dairy cattle under grazing and indoor conditions. *Journal of Dairy Science* 91, 2662–2677. https://doi.org/10.3168/jds.2007-0656

19. Cremonesi, P., Conte, G., Severgnini, M., Turri, F., Monni, A., Capra, E., Rapetti, L., Colombini, S., Chessa, S., Battelli, G., Alves, S. P., Mele, M., Castiglioni, B. (2018): Evaluation of the effects of different diets on microbiome diversity and fatty acid composition of rumen liquor in dairy goat. *Animal* 12, 1856-1866. https://doi.org/10.1017/S1751731117003433

20. Croatian Standards Institute (2001): HRN ISO 9622:2001-Whole milk - Determination of milkfat, protein and lactose content - Guidance on the operation of mid-infrared instruments (ISO 9622:1999), *Croatian Standards Institute*, Zagreb, Croatia

21. Croatian Standards Institute (2007): HRN ISO 13366-2/Ispr.1:2007 (ISO 13366-2:2006) Milk-Enumeration of somatic cells-Part 2: Guidance on the operation of fluoro-opto-electronic counters, *Croatian Standards Institute*, Zagreb, Croatia

22. Dórea, J.R.R., French, E.A.,Armentano, L.E. (2017): Use of milk fatty acids to estimate plasma nonesterified fatty acid concentrations as an indicator of animal energy balance. *Journal of Dairy Science* 100, 6164-6176. https://doi.org/10.3168/jds.2016-12466

23. Doreau, M., Ferlay, A (2015): Linseed: a valuable feedstuff for ruminants. Oilseeds and fats. *Crops and Lipids* 22(6), D611 https://doi.org/10.1051/ocl/2015042

24. Goetsch, A. L. (2019): Recent research of feeding practices and nutrition of lactating dairy goats. *Journal of Applied Animal Research* 47(1), 103-114. https://doi.org/10.1080/09712119.2019.1580585

25. Gugołek, A., Juśkiewicz, J., Strychalski, J., Zwoliński, C, Żary-Sikorska, E., Konstantynowicz, M. (2017): The effects of rapeseed meal and legume seeds as substitutes for soybean meal on productivity and gastrointestinal function in rabbits. *Archives of Animal Nutrition* 71, 311-326. https://doi.org/10.1080/1745039X.2017.1322796

26. Hewlett, K.L., Azeez, G.S.E. (2008): The economic impact of GM contamination incidentes on the organic sector, in: *Proceddings of the 16th IFOAM Organic World Congress "Cultivate the Future"*, Modena, Italy, June 18-20, http://orgprints.org/view/projects/conference.html

27. Hikosaka, K., El-Abasyk, M., Koyama, Y., Motobu, M., Koge, K., Isobe, T., Kang, C. B., Hayashidani, H., Onodera, T., Wang, P.C., Matsumura, M., Hirota, Y. (2007): Immunostimulating effects of the polyphenol-rich fraction of sugar cane (*Saccharum officinarum* L.) extract in chickens. *Phytotherapy Research* 21, 120-5. https://doi.org/10.1002/ptr.2033

28. Hur, S.J., Kim, H.S., Bahk, Y.Y., Park, Y. (2017): Overview of conjugated linoleic acid formation and accumulation in animal products. *Livestock Science* 195, 105-111. https://doi.org/10.1016/j.livsci.2016.11.016

29. International standard ISO (2010): 1211:2010(E) IDF 1:2010(E) Milk-Determination of fat content-Gravimetric method. *The International Organization for Standardization and International Dairy Federation*, Geneva, Switzerland.

30. International Standard ISO (2002a): 15884|IDF 182:2002 Milk fat - Preparation of fatty acid methyl esters. *The International Organization for Standardization and International Dairy Federation*, Geneva, Switzerland.

31. International Standard ISO (2013): ISO 16297:2013 [IDF 161:2013] Milk-bacterial count-Protocol for the evaluation of alternative methods. *The International Organization for Standardization and International Dairy Federation*, Geneva, Switzerland.

32. International Standard ISO (2002b): 15885|IDF 184:2002 Milk fat - Determination of the fatty acid composition by gas-liquid chromatography. *The International Organization for Standardization and International Dairy Federation,* Geneva, Switzerland.

33. Jahani-Moghadam, M., Mahjoubi, E., Dirandeh, E. (2015): Effect of linseed feeding on blood metabolites, indices of cystic follicles and productive and reproductive performance in fresh Holstein dairy cows. *Journal of Dairy Science* 98, 1828-1835.
https://doi.org/10.3168/jds.2014-8789

34. Kim, M.Y., Kim, E.J., Kim, Y.N., Choi, C., Lee, B.H. (2012): Comparison of the chemical compositions and nutritive values of various pumpkin (*Cucurbitaceae*) species and parts. *Nutrition Research and Practice* 6(1), 21-2.
https://doi.org/10.4162/nrp.2012.6.1.21

35. Klir, Ž., Antunović, Z., Novoselec, J. (2012): Influence of goats feeding on the fatty acids content in milk. *Mljekarstvo* 62 (4), 231-240.

36. Klir, Ž., Castro-Montoya, J.M., Novoselec, J., Molkentin, J., Domaćinović, M., Mioč, B., Dickhoefer, U., Antunović, Z. (2017): Influence of pumpkin seed cake and extruded linseed on milk production and milk fatty acid profile in Alpine goats. *Animal* 11, 1772-1778.
https://doi.org/10.1017/S175173111700060X

37. Menke, K.H., Steingass, H. (1987): Schätzung des energetischen Futterwerts aus der *in vitro* mit Pansensaft bestimmten Gasbildung und der chemischen Analyse. II. Regressionsgleichungen. Übers *Tierernährung* 15, 59-94.

38. Möller, R., Dannenberger, D., Nürnberg, G., Strucken, E.M., Gudrun, A., Brockmann, A. (2018): Relationship between the fatty acid profile of hair and energy availability of lactating primiparous cows. *Journal of Dairy Research* 1-8.
https://doi.org/10.1017/S0022029918000791

39. Nudda, A., Battacone, G., Atzori, A.S., Dimauro, C., Rassu, S.P.G., Nicolussi, P., Bonelli, P., Pulina, G. (2013): Effect of extruded linseed supplementation on blood metabolic profile and milk performance of Saanen goats. *Animal* 7, 1464-1471.
https://doi.org/10.1017/S1751731113000931

40. Peričin, D., Krimer, V., Trivić, S., Radulović, L. (2009): The distribution of phenolic acids in pumpkin´s hull-less seed, skin, oil cake meal, dehulled kernel and hull. *Food Chemistry* 113, 450-456.
https://doi.org/10.1016/j.foodchem.2008.07.079

41. Rabrenović, B.B., Dimić, E.B., Novaković, M.M., Tečević, V.V., Basić, Z.N. (2014): The most important bioactive components of cold pressed oil from different pumpkin (*Cucurbita pepo* L.) seeds. *Food Science and Technology* 55, 521-527.
https://doi.org/10.1016/j.lwt.2013.10.019

42. Rumosa-Gwaze, F., Chimonyo, M., Dzama, K. (2012): Effect of season and age on blood minerals, liver enzyme levels, and faecal egg counts in Nguni goats of South Africa. *Czech Journal of Animal Science* 57, 443-453.
https://doi.org/10.17221/6345-CJAS

43. Russel, A. (1991): Body condition scoring of sheep. Sheep and goat practice. Boden, E. (Ed.). Bailliere Tindall, Philadelphia, pp. 3.

44. SAS (2013): Statistical Analysis System (SAS/STAT program, Version 9.4). SAS institute, Cary, NC, USA.

45. Shingfield, K.J., Bernard, L., Leroux, C., Chilliard, Y. (2010): Role of trans fatty acids in the nutritional regulation of mammary lipogenesis in ruminants. *Animal* 4, 1140-1166.
https://doi.org/10.1017/S1751731110000510

46. Teh, T.H., Trung, L.T., Jia, Z.H., Gipson, T.A., Ogden, K.B., Sweeney, T.F. (1994): Varying amounts of rumen-inert fat for high producing goats in early lactation. *Journal of Dairy Science* 77, 253-258.
https://doi.org/10.3168/jds.S0022-0302(94)76948-4

47. Walsh, S.W., Williams, E.J., Evans, A.C.O. (2011): A review of the causes of poor fertility in high milk producing dairy cows. *Animal Reproduction Science* 123, 127-138.
https://doi.org/10.1016/j.anireprosci.2010.12.001

48. Wang, Y.M., Wang, J.H., Wang, C., Chen, B., Liu, J.X., Cao, H., Guo, F.C., Vazquez-Anon, M. (2010): Effect of different rumen-inert fatty acids supplemented with dietary antioxidant on performance and antioxidative status of early-lactation cows. *Journal of Dairy Science* 93, 3738-3745
https://doi.org/10.3168/jds.2010-3054

49. Zdunczyk, Z., Minakowski, D., Frejnagel, S., Flis, M. (1999): Comparative study of the chemical composition and nutritional value of pumpkin seed cake, soybean meal and casein. *Nahrung* 43, 392-395.
https://doi.org/10.1002/(SICI)1521-3803(19991201)43:6<392::AID-FOOD392>3.0.CO;2-2

50. Zenebe, T., Ahmed, N., Kabeta, T., Kebede, G. (2014): Review on medicinal and nutritional values of goat milk. *Academic Journal of Nutrition* 3, 30-39.
https://doi.org/10.5829/idosi.ajn.2014.3.3.93210