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Partial replacement of soybean meal with pumpkin seed cake in lamb diets: Effects on carcass traits, haemato-chemical parameters and fatty acids in meat

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Abstract

The composition of lamb diets has an effect on production traits and meat quality, especially fatty acid proportions. Recently, in organic farming, soybean meal has frequently been replaced with feedstuffs that are rich in protein. The aim of the present study was to determine the effects of partial replacement of soybean meal with pumpkin seed cake on carcass traits, biochemical parameters and fatty acids of lamb meat produced in organic farming. The research was carried out on 70-day-old lambs of the Merinolandschaf breed. Thirty-six lambs were grouped by gender, and allotted to three treatment groups, which were given one of the three diets: control diet with no pumpkin seed cake; a diet in which 10% of soybean meal was replaced with 10% pumpkin seed cake; and a diet in which 15% of soybean meal was replaced with 15% pumpkin seed cake. The experimental feeding period was 30 days. Hay and water were provided *ad libitum*. Differential blood tests and haematological parameters were determined, and the concentrations of minerals and biochemical parameters, and enzyme activity were ascertained in blood serum. Carcass traits and lamb meat colour did not differ among dietary treatments. Significant differences were observed in the concentrations of some biochemical parameters, which indicated good energy and protein balance, and changes in fat metabolism that did not impair antioxidant status. Compared with the control, the concentration of linoleic acid (C18:2 n-6) was higher in diets containing 10% and 15% of pumpkin seed cake replacements. The results indicated that partial replacement of soybean meal with 10% or 15% of pumpkin seed cake could be implemented in lamb feeding in organic farming without major changes in carcass traits, haemato-chemical parameters and the fatty acid profile in meat.

Keywords: Blood parameters, meat quality, Merinolandschaf lambs, organic farming

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Introduction

Organic livestock farming should comply with high animal welfare standards and meet animal behavioural needs. Animal health management should be based on disease prevention. Feedstuffs for livestock should be organically produced, preferably on the holding itself or on neighbouring organic farms (Official Journal of the European Union (OJ), 2007).

The success of organic sheep farming depends exclusively on the quality of feedstuffs used, which influences the production and quality of products produced (Rahmann, 2009). Well-balanced diets with added protein sources organically produced, improve the production and health of sheep (Sarhan, 2011). Along with monitoring the production process, it is necessary to supervise the haemato-chemical parameters of animals in organic production systems (Antunović *et al*., 2017a). In organic farming, as many organic feedstuffs as possible should be produced locally. Since the use of genetically modified material is prohibited, soybean and its by-products, usually a major source of protein in animal diets, have been prohibited in animal diets in organic farming systems because of their doubtful quality and origin (Hewlett &

Azeez, 2008). Therefore, as alternatives to soybean products, other organically produced feedstuffs and by-products that are rich in protein have to be used in animal diets (Alves *et al.*, 2016, Antunović *et al.*, 2017b). Enishi *et al.* (2004) reported that pumpkin is a valuable feedstuff for ruminants because of its high content of total digestible nutrients, digestible energy and metabolizable energy. Pumpkin seed is also an excellent source of protein, minerals, vitamins and unsaturated fatty acids (Juranovic *et al.*, 2003; Siegmund & Murkovic, 2004; Glew *et al.*, 2006). Zdunczyk *et al.* (1999) pointed out that pumpkin seed cake contains more crude protein than soybean meal (598 g/kg versus 474.2 g/kg, respectively). According to Murković *et al.* (1996), the lipids in pumpkin seed contains 35.6–60.8% linoleic acid (LA) (C18:2 n-6), 21.0–46.9% oleic acid (OA) (C18:1 n-9), 9.5–14.59% palmitic acid (C16:0) and 3.1–7.4% stearic acid (C18:0) with a favourable ratio of polyunsaturated (PUFA) to saturated fatty acids (SFA). Pumpkin seed cake also improves the palatability of concentrates for ruminants.

Ruminant meat has long been a sought-after source of nutrients as an integral part of healthy and balanced human diets (Adeyemi *et al.*, 2016). Numerous research have established the influence of feeding on the fatty acid profile in lamb's meat when feeding lambs with diets rich in PUFA (Demirel *et al.*, 2006; Elmore *et al.*, 2005; Ponnampalam *et al.*, 2001). Pumpkin seed cake is a rich source of protein and fat, but there is apparently no research on the inclusion of pumpkin seed cake in sheep and lamb diets in organic farming systems. In dairy goat diets, a complete replacement of soybean meal with pumpkin seed cake showed no adverse effects on milk production or on the milk fatty acid profile (Klir *et al.*, 2017). Therefore, the objective of the present study was to determine whether soybean meal could be replaced with pumpkin seed cake in slaughter lamb production, and to assess the effects of this replacement on carcass traits, haemato-chemical parameters and the fatty acid profile of lamb's meat produced under organic farming conditions.

Material and Methods

The research was carried out on a certified organic family farm in Osijek-Baranya County (Croatia), which has reared sheep organically for 10 years. The research involved 36 weaned Merinolandschaf lambs, 12 animals in three treatment groups, with an average bodyweight of 25.9 ± 1.27 kg. The lambs were 70 days old, with an equal sex ratio in each group (50%♀: 50%♂) and were healthy and in adequate condition. The lambs were fed a mixture of organic feedstuffs. A mineral premix (Panto Mineral L84) approved for use in organic farming of sheep and lambs was used. Lambs were offered hay and water *ad libitum*. Table 1 presents the ingredients of the isonitrogenous and isoenergetic feed mixtures and the clover-grass hay which contained cocksfoot (*Dactylis glomerata* L.*)*, timothy grass (*Phleum pratense* L.), meadow fescue (*Festuca pratensis Huds*.), white clover (*Trifolium repens* L.*)* and Italian ryegrass (*Lolium multiflorum* L.). Soybean meal, which is rich in protein, was used in the diet for the control group. In the first experimental group pumpkin seed cake partially replaced 10% of the soybean meal. In the second experimental group pumpkin seed cake replaced 15% of the soybean meal. All feed mixtures were formulated to obtain similar energy and protein contents of the diets among groups (Table 1). The experiment lasted 30 days and was conducted during the winter season. Rearing and feeding of the lambs was conducted according to the Council Regulation (EC) No. 834/2007 on Organic Production and Labelling of Organic Products with Regard to Organic Production, Labelling and Control (Official Journal of the European Union (OJ), 2007). The Committee for Animal Welfare of the Faculty of Agriculture in Osijek approved that the research was carried out according to the legal provisions of the Animal Protection Act 2017 (Official Gazette No. 133, 2006; No. 37, 2013; No. 125, 2013).

The lambs were weighed at the beginning and end of the investigation. Feed was offered at the same time each day. Feed refusals were recorded to calculate voluntary feed intake, which was measured in the control and in the two experimental groups to be 0.898, 0.819 and 0.835 kg of feed mixture, respectively, and weight gains were 0.224, 0.282, and 0.227 kg, respectively.

The chemical composition of the feed mixtures and hay is presented in Table 1. Crude protein content of feed samples was determined with the Kjeldahl method (Pearson, 1976), while ether extract content was established according to method described in Onwuka (2005). Crude fibre content was carried out by the Weende method (Offor *et al.*, 2014) and neutral detergent fibre (NDF), as described by Foss (2001).

Five lambs (3♂ : 2♀) per group were selected to be similar in live bodyweight (Table 2). Samples of the meat (*musculus semimembranosus*) and carcass parameters were taken immediately after slaughtering and exsanguination on the farm. The *m. semimembranosus* from the hind leg and part of subcutaneous adipose tissue were removed. Meat samples were frozen at -80 °C. The skin was removed and the abdominal organs (rumen, stomach, spleen, intestine, and liver) and the thoracic cavity content (trachea with lungs and heart) were removed. Internal organs, skin, lower parts of the legs and carcass were weighed. On the Croatian market, lamb is usually sold as whole carcass with head and kidneys, and therefore these parts were not separated from the carcass or weighed separately. After that, a standard measure of lamb carcass

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development (linear measure) was determined, namely carcass length (*os pubis-atlas* and *os pubis*-first rib), the length of the hind legs (*tuber calcanei-tuberculum ossis Ischia*) and the circumference of the leg (at the widest point). The carcass colour was determined in the *m. semimembranosus* 45 minutes after slaughtering. Values of colour was measured according to the CIE L* a* b* system (CIE, 1976) with a Minolta chroma meter CR-410 (Minolta Camera Co., Ltd., Japan). Triplicate readings were made for each sample and average values were recorded.

Table 1 Ingredients and chemical composition of the concentrate mixtures and the hay for the lambs

Component	Group			Clover-grass hay
	T0	T10	T15	
Ingredient composition (%)				
Corn	30.80	12.00	1.65	
Oat	8.40	10.20	8.40	
Barley	18.00	27.00	33.00	
Triticale	19.80	28.90	35.45	
Soybean meal	20.00	8.90	3.50	
Pumpkin seed cake	0.00	10.00	15.00	
Mineral premix*	3.00	3.00	3.00	
Chemical composition (g/kg)				
Dry matter	877.50	884.70	891.50	890.60
Crude protein	172.10	177.70	179.00	149.70
Ether extract	18.80	31.70	43.30	13.30
Crude fibre	38.60	41.40	38.80	259.00
Ash	67.20	61.10	66.50	86.40
NDF	98.60	157.00	145.80	510.80
NET	581.30	572.80	563.50	382.70
Fatty acids (g/100 g)				
C14:0	0.15	0.19	0.24	0.37
C16:0	16.74	16.81	16.26	26.52
C16:1	0.24	0.28	0.26	1.67
C18:0	2.99	4.09	5.16	0.52
C18:1	24.94	29.64	32.42	4.57
C18:2 n6 (LA)	50.58	45.87	43.41	17.90
C18:3 n3 (ALA)	3.46	2.28	1.60	25.55
C20:0	0.44	0.35	0.38	21.98
C20:1 n9	0.46	0.51	0.25	0.91
SFA	20.32	21.44	22.04	49.39
PUFA	54.04	48.15	45.01	43.45

T0: control, T10: 10% replacement of soybean meal with pumpkin seed cake, T15: 15% replacement of soybean meal with pumpkin seed cake, NDF: neutral detergent fibre; NET: net energy; LA: linoleic acid; ALA: alpha-linolenic acid; SFA (saturated fatty acids): C10:0, C12:0, C13:0, C14:0, C16:0, C18:0, C20:0, C21:0, C23:0, C24:0
PUFA (polyunsaturated fatty acids): C18:2 c9 t11, C18:2, C18:3, C20:2, C20:3, C20:4, C22:2, C20:5, C22:6
*Mineral premix: 18% Ca, 5% P, 9,5% Na, 2,00% Mg, 400.000 IU vitamin A, 40.000 IU vitamin D, 500 mg vitamin E, 4.000 mg Zn, 2.000 mg Mn, 60 mg I, 10 mg Co, 50 mg Se

The fat phase for fatty acid (FA) analysis was extracted from the *m. semimembranosus* samples using the Folch method as recommended for isolation of total lipids, especially from animal tissues (Folch *et al.*, 1957). Fatty acid methyl esters were prepared from the extracted lipids by the transmethylation method,

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which uses 14% wt. boron trifluoride/methanol solution (Čolović *et al.*, 2012). Samples were analysed with a gas chromatographer (GC; Agilent 7890A system Agilent Technologies, CA, USA) with a flame ionization detector (GC-FID), and auto-injection module for liquid, equipped with a fused silica capillary column (Supelco SP-2560 capillary GC column 100 m x 0.25 mm, d 0.20 µm). Helium was used as a carrier gas (purity 99.9997 vol %, flow rate 1.5 mL/min, and pressure 1.092 bar). A total of 1 µL of sample was injected in split regime with a ratio of 30 : 1. The regimen was: Initial temperature 140 °C, hold time 5 min, heating rate 3 °C/min, final temperature 240 °C, and final temperature hold time 10 min. Nitrogen was used as a makeup gas. The FA peaks were identified by comparing retention times with those of standards from Supelco 37 component FA methyl ester mix and with data from the internal data library, based on previous experiments and FA methyl ester determination on gas chromatography–mass spectrometry (GC-MS). The results were expressed as a mass of individual FAs or FA group (g) in 100 g of total FAs or as relative mass contents from two replicates.

Blood was collected from the jugular vein (10 mL) in sterile vacuum tubes (Venoject®, Sterile Terumo, Leuven, Belgium) in the morning at the end of the study (30th day) from all experimental animals. After that, serum was separated by centrifugation (10 min) at 3000 revolutions/min and placed in the Olympus AU640 analyser. In blood serum, the concentrations of minerals (calcium, phosphorus-inorganic and iron) were determined, and those of biochemical parameters: urea, glucose, total proteins, albumin, cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglyceride, β-hydroxybutyrate (BHB) and non-esterified fatty acids (NEFA), and the activity of alanine aminotransferase (ALT, EC 2.6.1.2) and aspartate aminotransferase (AST. EC 2.6.1.1) enzymes were measured using Olympus System reagents (OSR) (Olympus Diagnostic GmbH, Lismeehan, Ireland). Globulin content was calculated as the difference between total protein and albumin. Haematological parameters: number of leukocytes (WBC), erythrocytes (RBC) and thrombocyte (PLT), and the contents of haemoglobin, haematocrit, mean corpuscular volume (MCV), average haemoglobin content in erythrocytes (MCH) and mean haemoglobin concentration in erythrocytes (MCHC) in whole blood of sheep were determined on an automatic three differential haematology analyser (Sysmex PocH-100Iv, Sysmex Europe GmbH, Hamburg, Germany). A differential blood test was carried out with a microscope using the prepared blood smears coloured according to Pappenheim staining. The activity of glutathione peroxidase (GPx, EC 1.11.1.9) in the serum was determined using a Ransel® kit (Randox, UK) on an automatic Olympus AU 400 (Olympus, Japan) analyser on a wavelength of 240 nm. The principle of the reaction is based on the oxidation of glutathione with cumene hydroperoxide, with a catalytic activity of GPx. The resulting oxidized form of glutathione was immediately converted to a reduced form with the presence of glutathione reductase and nicotinamide adenine dinucleotide phosphate (NADPH) as an oxygen acceptor. Thereby, NADPH, on receiving oxygen, turned to the oxidized form NADP. The activity of total superoxide dismutase (SOD, EC 1.15.1.1) in serum was determined with a Ransod® kit (Randox, UK) on an automatic analyser (Olympus AU 400, Olympus, Japan) at a wavelength of 510 nm. The method is based on the generation of superoxide radicals from xanthine by xanthine oxidase, which react with 2-(4-iodophenyl)-3-(4nitrofenil) 5-phenyltetrazole chloride to form formazan red coloration.

The normality of the data was checked with the Shapiro-Wilk test. Parametric distributed data (haemato-chemical parameters) were presented as mean and standard deviation, and analysed with the ANOVA procedure, using feeding treatment as a fixed effect. Mean values were compared with the Tukey test and the differences between the groups were declared significant at P <0.05. Non-parametric data (carcass traits, colour, and FAs of meat) were presented as median, minimal and maximal values. These data were analysed with the Kruskal-Wallis H test. When P <0.05, the result was considered statistically significant. All data were analysed with statistical software SAS 9.4® (SAS Institute Inc., 2002–2012).

Results

Replacing soybean meal with 10% or 15% of pumpkin seed cake did not influence (P >0.05) the carcass traits of the lambs. Colour parameters of *m. semimembranosus* did not differ between groups (Table 2). Table 3 indicates no differences (P >0.05) in haematological parameters of lambs in T10 and T15.

Changes in concentrations of NEFA, BHB, glucose, cholesterol, urea and albumin in serum were recorded (P <0.05) by analysing the biochemical parameters (Table 4). Compared with the control group, serum of T15 had a lower concentration of cholesterol. Higher (P <0.05) glucose and albumin, and lower (P <0.05) NEFA and BHB concentrations were measured in the serum of lambs fed T10 and T15 compared with the control group. The activity of GPx did not differ (P >0.05) among groups, while the activity of SOD was higher (P <0.05) in groups fed 15% pumpkin seed cake than in the other groups.

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Table 2 Median (min-max) values of lambs´ carcass traits and colour parameters of *musculus semimembranosus*

Parameter	Group			SE	*P*-value	
	T0	T10	T15		T0/T10	T0/T15
Carcass traits						
Slaughter weight (kg)	32.00 (30.50–32.50)	32.00 (30.00–34.50)	31.00 (30.00–37.50)	0.51	0.596	0.338
Carcass length 1 (cm)	80.00 (76.00–84.00)	83.00 (78.00–86.00)	82.00 (80.00–92.00)	1.00	0.292	0.209
Carcass length 2 (cm)	69.00 (63.00–73.00)	71.00 (66.00–73.00)	67.00 (66.00–70.00	0.85	0.340	0.833
Hind leg circumference (cm)	37.00 (36.00–39.00	36.50 (36.00–39.00)	37.00 (35.00–39.00)	0.34	0.830	0.916
Hind leg length (cm)	33.00 (30.00–36.00)	35.00 (34.00–37.00)	34.00 (33.00–37.00)	0.49	0.073	0.167
Hot carcass weight (kg)	16.50 (16.00–17.00)	16.00 (15.50–17.00)	16.00 (16.00–20.00)	0.28	0.381	0.910
Dressing (%)	52.31 (50.77 -53.13)	50.00 (48.44–53.33)	53.33 (51.61–54.84)	0.47	0.116	0.056
Organs weight* (kg)	1.55 (1.49–1.70)	1.58 (1.52–1.70)	1.60 (1.51–1.70)	0.02	0.750	0.667
Forestomach and intestines (kg)	8.10 (7.50–9.50)	8.10 (7.90–8.40)	8.00 (7.50–9.80)	0.16	0.916	0.916
Skin and lower legs (kg)	3.93 (3.70–5.10)	4.20 (4.00–4.92)	4.30 (4.00–4.90)	0.11	0.465	0.465
Colour parameters of m. semimembranosus						
Lightness (L*)	41.50 (40.06–47.90)	41.43 (39.97–47.96)	42.97 (35.48–44.23)	0.90	0.807	0.807
Redness (a*)	20.44 (19.64–21.18)	20.74 (20.19–21.21)	20.47 (18.33–22.08)	0.23	0.462	0.807
Yellowness (b*)	2.14 (1.60–2.85)	2.85 (2.20–4.05)	2.47 (0.52–2.96)	0.24	0.086	0.807

T0: control, T10: 10% replacement of soybean meal with pumpkin seed cake, T15: 15% replacement of soybean meal with pumpkin seed cake, carcass length 1: *os pubis-atlas*, carcass length 2: *os pubis*-first rib; *weight of lungs, heart, liver and spleen; SD-standard deviation, SEM-standard error of mean.

Table 3 Mean (± SD) haematological parameters and distribution of leukocytes in the whole blood of lambs

Parameters	Group			SEM	*P*-value	Reference values*
	T0	T10	T15			
WBC (x 10^9 L^{-1})	11.44 ± 2.68	11.71 ± 3.76	11.82 ± 4.27	0.59	0.967	5.1–15.9
RBC (x 10^{12} L^{-1})	7.25 ± 1.92	7.36 ± 2.18	7.68 ± 1.58	0.31	0.848	9.2–13.0
Haemoglobin, gL^{-1}	87.75 ± 14.99	88.75 ± 18.71	96.67 ± 13.86	2.67	0.338	105.0–137.0
Haematocrit, %	0.34 ± 0.05	0.35 ± 0.07	0.38 ± 0.05	0.01	0.227	0.28–0.39
MCV, fl	47.90 ± 6.29	49.28 ± 6.60	50.47 ± 6.29	1.05	0.621	28.0–35.0
MCH, pg	12.38 ± 1.24	12.40 ± 1.64	12.80 ± 1.51	0.24	0.732	10.0–13.0
MCHC, gL^{-1}	259.75 ± 17.09	251.67 ± 6.07	254.00 ± 9.96	2.01	0.248	332.0–392.0
PLT (x 10^9 L^{-1})	445.25 ± 243.39	400.67 ± 253.47	575.33 ± 216.09	40.85	0.196	426.0–1142.0
Distribution of leukocytes, %						
Lymphocytes	70.50 ± 10.75	60.45 ± 5.70	66.17 ± 13.07	1.85	0.085	50.0–70.0
Neutrophils seg.	28.50 ± 11.18	38.00 ± 6.26	32.25 ± 12.62	1.84	0.101	10.0–50.0
Eosinophils	1.00 ± 0.85	1.09 ± 1.04	1.33 ± 1.13	0.17	0.729	1.0–8.0
Monocytes	ND	0.46 ± 0.68	ND	0.07	0.395	0.0–4.0
Basophils	ND	ND	0.08 ± 0.28	0.03	0.063	0.0–1.0

T0: control, T10: 10% replacement of soybean meal with pumpkin seed cake, T15: 15% replacement of soybean meal with pumpkin seed cake; ND: not determined; SD: standard deviation, SEM: standard error of mean, WBC: number of leukocytes, RBC: erythrocytes, MCV: mean corpuscular volume, MCH: average haemoglobin content in erythrocytes, MCHC: mean haemoglobin concentration in erythrocytes, PLT: thrombocytes
*Latimer & Prasse (2003) for sheep

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Table 4 Mean (± SD) serum biochemical parameters and certain enzyme activities of lambs

Parameters, mmol L^{-1}	Group			SEM	*P*-value	Reference values*
	T0	T10	T15			
Glucose	4.33^{a} ± 0.51	4.78^{b} ± 0.44	4.82^{b} ± 0.47	0.08	0.029	2.7–4.8
Cholesterol	1.10^{a} ± 0.33	1.02^{ab} ± 0.23	0.87^{b} ± 0.12	0.04	0.028	1.04–1.67
HDL-cholesterol	0.62 ± 0.08	0.67 ± 0.20	0.69 ± 0.13	0.02	0.542	0.68–0.97[1]
LDL-cholesterol	0.30 ± 0.39	0.20 ± 0.10	0.13 ± 0.07	0.03	0.206	0.10–0.50[1]
Triglycerides	0.30 ±0.08	0.29 ± 0.06	0.27 ± 0.06	0.01	0.593	0.20–0.40[1]
NEFA	0.07^{a} ± 0.04	0.04^{b} ± 0.02	0.04^{b} ± 0.06	0.01	0.007	ND
Urea	6.95^{a} ± 1.70	6.48^{ab} ± 1.01	5.81^{b} ± 1.05	0.22	0.039	5.00–9.10
Total protein, g L^{-1}	62.99 ± 4.35	64.60 ± 4.59	66.52 ± 4.50	0.77	0.171	51.00–64.00
Albumin, g L^{-1}	27.93^{a} ± 1.23	28.26^{ab} ± 1.88	29.69^{b} ± 2.06	0.31	0.045	30.00–37.00
Globulin, g L^{-1}	35.07 ± 3.88	36.34 ± 3.97	36.83 ± 3.67	0.63	0.095	19.00–30.00
BHB	0.36^{a} ± 0.10	0.28^{b} ± 0.09	0.31^{b} ± 0.09	0.02	0.045	0.20–0.70
Calcium	2.30 ± 0.17	2.43 ± 0.10	2.39 ± 0.15	0.03	0.106	2.45–2.92
Phosphorus-inorg.	2.30 ± 0.21	2.25 ± 0.32	2.22 ± 0.31	0.05	0.773	1.88–3.34
Iron, µmol L^{-1}	23.87 ± 6.62	23.52 ± 5.98	26.20 ± 5.44	1.00	0.503	17.1–33.3[1]
ALT, U L^{-1}	11.72 ± 4.14	11.20 ± 3.87	14.22 ± 5.11	0.75	0.213	8.0-20.0[2]
AST, U L^{-1}	92.98 ± 26.84	89.95 ± 12.15	81.21 ± 17.46	3.33	0.277	83.0–140.0
GPx, U L^{-1}	432.06 ± 110.1	454.24 ± 93.94	464.12 ± 65.56	15.01	0.694	>600[3]
SOD, U mL^{-1}	0.12^{a} ± 0.07	0.21^{a} ± 0.10	0.27^{b} ± 0.13	0.02	0.002	0.184[4]

T0: control, T10: 10% replacement of soybean meal with pumpkin seed cake, T15: 15% replacement of soybean meal with pumpkin seed cake; HDL: high-density lipoprotein, LDL: low-density lipoprotein, NEFA: non-esterified fatty acids, BHB: beta-hydroxybutyrate, ALT: alanine aminotransferase, AST: aspartate aminotransferase, GPx: glutathione peroxidase, SOD: superoxide dismutase; SD-standard deviation, SEM: standard error of mean, ND: not determined; *Lepherd *et al.* (2009)
[1]Antunović *et al.* (2010) determined in lambs during the research
[2]Kaneko *et al.* (2008) for sheep
[3]Pavlata *et al.* (2012)-value determined in µkat/L of whole blood of sheep
[4]Maan *et al.* (2013)-value determined in sheep
[a,b] Rows means with different superscripts differ significantly at $P < 0.05$.

Table 5 presents the FA concentrations in the meat of lambs fed with 10% or 15% pumpkin seed cake compared with the control group. Median values of C16:1 were lower ($P < 0.05$) in both experimental groups compared with the control. Replacing soybean meal with 10% and 15% pumpkin seed cake in the mixture of lambs led to a higher concentration of LA in meat than in the control group. The elevated content of LA led to an increased ratio between n-6 and n-3 in both experimental groups, compared with the control group. Medians of OA, conjugated linoleic acid (CLA), α-linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) concentrations in lamb meat did not differ in T10 and T15 compared with control. The concentration of total SFA, monounsaturated fatty acids (MUFA) and PUFA did not differ when feeding lambs the diets containing 10% or 15% pumpkin seed cake.

Discussion

Replacing 10% and 15% soybean meal with pumpkin seed cake resulted in carcass traits similar to that in the control group, which means that the higher crude protein and ether extract contents (Table 1) of the experimental diets did not affect carcass traits. In research by Santos *et al.* (2013), lambs were fed diets which contained sunflower cake, peanut cake or soybean cake, all with a high ether extract content, and carcass traits were not affected. Also, experimental diets in the present study did not influence the colour parameters of the *m. semimembranosus*. These parameters were adequate for lambs of this age, as reported also by Držaić *et al*. (2016).

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Table 5 Median (min-max) value of fatty acid concentrations in *musculus semimembranosus* of lambs

Fatty acids (g/100 g total fatty acids)	Group			SE	*P*-values	
	T0	T10	T15		T0/T10	T0/T15
C10:0	0.19 (0.02–0.34)	0.23 (0.14–0.31)	0.25 (0.21–0.30)	0.02	0.917	0.602
C12:0	0.41 (0.23–0.70)	0.48 (0.31–0.70)	0.48 (0.42–0.67)	0.04	0.465	0.347
C14:0	5.18 (3.23–6.24)	5.52 (4.45–6.00)	5.42 (3.66–6.66)	0.27	0.465	0.175
C16:0	22.99 (20.73–23.40)	23.25 (22.78–23.70)	23.67 (21.61–24.11)	0.24	0.175	0.347
C16:1	1.97 (1.67–2.06)	1.61 (1.56–1.81)	1.77 (1.46–1.82)	0.05	0.028	0.047
C18:0	24.71 (24.16–38.50)	27.92 (25.14–33.10)	25.37 (21.35–33.63)	1.21	0.347	0.602
C18:1 t9	4.18 (2.89–5.57)	4.91 (0.57–5.81)	4.74 (4.42–6.02)	0.35	0.465	0.251
C18:1 c9 (OA)	34.23 (25.29–36.10)	30.33 (28.10–31.89)	31.42 (28.02–34.63)	0.75	0.175	0.347
C18:2 c9t11 (CLA)	0.29 (0.09–0.47)	0.25 (0.21–0.32)	0.30 (0.11–0.31)	0.03	0.462	0.754
C18:2 n6 (LA)	3.16 (2.72–3.58)	3.90 (3.43–5.38)	4.05 (3.66–4.40)	0.17	0.028	0.009
C18:3 n3 (ALA)	0.66 (0.49–1.55)	0.61 (0.52–0.69)	0.65 (0.57–0.70)	0.06	0.602	0.754
C23:0	0.16 (0.14–0.21)	0.20 (0.13–0.30)	0.14 (0.11–0.66)	0.04	0.327	0.462
C20:4 n6 (AA)	0.03 (0.02–0.03)	0.04 (0.02–0.05)	0.03 (0.02–0.03)	0.004	0.248	0.643
C24:0	0.02 (0.01–0.03)	0.03 (0.01–0.03)	0.01 (0.01–0.08)	0.005	0.476	0.476
C20:5 n3 (EPA)	0.04 (0.03–0.04)	0.04 (0.02–0.04)	0.02 (0.02–0.06)	0.006	0.721	0.384
C22:6 n3 (DHA)	0.02 (0.01–0.03)	0.03 (0.02–0.06)	0.02 (0.01–0.06)	0.005	0.245	0.772
LA/ALA	4.93 (2.31–5.64)	6.62 (5.18–8.83)	6.18 (5.62–7.66)	0.39	0.028	0.016
SFA	56.21 (53.25–63.19)	57.43 (55.41–62.28)	55.39 (53.94–60.35)	0.83	0.465	0.754
PUFA	4.15 (3.61–5.60)	4.69 (4.43–6.29)	5.06 (4.76–5.42)	0.18	0.117	0.117
MUFA	39.62 (32.36–42.37)	37.63 (32.40–39.32)	38.89 (34.28–41.29)	0.83	0.117	0.602
UFA	43.77 (36.82–46.75)	42.53 (37.72–44.01)	43.95 (39.63–46.04)	0.82	0.465	0.754

T0: control, T10: 10% replacement of soybean meal with pumpkin seed cake, T15: 15% replacement of soybean meal with pumpkin seed cake; ND: not determined; OA: oleic acid; CLA: conjugated linoleic acid; LA: linoleic acid; ALA: alpha-linolenic acid; AA: arachidonic acid; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; SFA (saturated fatty acids): C10:0, C12:0, C13:0, C14:0, C16:0, C18:0, C20:0, C23:0, C24:0; PUFA (polyunsaturated fatty acids): C18:2 c9 t11, C18:2, C18:3, , C20:3, C20:4, C20:5, C22:6; MUFA (monounsaturated fatty acids): C16:1, C18:1 t9, C18:1 c9; UFA (unsaturated fatty acids): sum of MUFA and PUFA; SEM: standard error of mean

The T15 diet in the current study promoted lower (P <0.05) concentrations of cholesterol in serum, emphasizing the positive influence of pumpkin seed cake on fat metabolism. However, pumpkin seeds contain high concentrations of phytosterols (Patel, 2013), which may decrease both total serum cholesterol and LDL-cholesterol in humans by inhibiting the absorption of dietary cholesterol (Piironen *et al.*, 2000). In the literature, there is no study that investigated the use of pumpkin seed cake in the feeding of sheep and lambs. Results obtained in the current study could therefore not be compared with other investigations. However, adding pumpkin oil to the diets of broiler chickens lowered (P <0.05) cholesterol and triglyceride concentrations in plasma (Hajati *et al*., 2011). Makni *et al.* (2010) carried out a study on rats with alloxan-induced diabetes and added linseed and pumpkin seeds to the feed to determine lower concentrations of cholesterol, triglycerides, LDL- and enhanced HDL-cholesterol, which is known to play an important role in the transportation of cholesterol from peripheral cells to the liver by a pathway termed reverse cholesterol transport, thus regarded as a cardio protective. Higher concentrations of glucose and albumin, as well as lower NEFA and BHB concentrations in the serum of lambs fed with pumpkin seed cake emphasize adequate energy status, since high levels of NEFA and glucose concentration are indicators of lipid metabolism and fatty acid oxidation (Wathes *et al.*, 2009). Lower haemoglobin content and red blood cells were determined in the blood of lambs fed pumpkin seed cake replacement compared with reference values for sheep, which might be related to the age of the lambs. Bornez *et al*. (2009) found that haemoglobin level and erythrocytes increased with the ageing of lambs. Based on the results of haemato-chemical parameters, it is evident that the health status of lambs was stable in the experimental groups. Higher activity of SOD in the group fed T15 was determined in the present study, while

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activity of GPx did not differ. Replacement with 10% of pumpkin seed cake did not lead to differences in SOD and GPX activity. Vazguez-Anon *et al.* (2008) stated that SOD activity in the plasma of dairy cows depends on the antioxidant level in the diets, but the effect was dependent on the type of fat in the diets, with the highest activity being observed when oxidized fat was added. Makni *et al.* (2008) indicated that feeding a mixture that was rich in flax and pumpkin seed, when adequately supplemented in standard diets, affected the higher activities of antioxidant enzymes, such as SOD, catalase, and GPx, and reduced the glutathione level in plasma and liver of hypercholesterolemic rats. In the present study, the higher activity of SOD in T15 was probably due to PUFA in the diets, which was also observed in meat, although this was not significant. The first line of defence against oxygen-derived free radicals occurs when SOD catalyses the dismutation of the superoxide anion into hydrogen peroxide (Dursun *et al*., 2006). The lipoperoxidation process, influenced by PUFA, increased SOD activity, although GPx activity was not influenced by feeding treatment, indicating stable antioxidant status of the experimental groups. The activity of GPx in serum was according to the values ascertained in whole blood of sheep by Pavlata *at al.* (2012). The GPx activity determined in bovine plasma/serum constituted only 0.5–2.0% of total activity in whole bovine blood (Ortman, 1999). Besides fatty acids, such results could be influenced by phenolic acids in pumpkin seed cake (Peričin *et al.*, 2009), which increase the activities of hepatic antioxidant enzymes, such as SOD and GPx, as found in rats by Yeh & Yen (2006). Since SOD and GPx activities were not lowered compared with the control group in the present study, the antioxidant status was preserved. These observations indicate that the stable antioxidant status of lambs was adequate owing to proper antioxidant levels in the diets and the desirable energy condition of the lambs.

The fatty acid concentrations in *m. semimembranosus* of lambs in T10 and T15 are presented in Table 5. Fatty acid concentration was established only in *m. semimembranosus,* which could be a limitation of the present study, since the fatty acid profile may differ among muscle types, as reported by Popova (2007), for instance between *m. longissimus lumborum* and *m. semimembranosus*. On the Croatian market, lamb is usually sold as a whole carcass. For this reason only the *semimembranosus* muscle was taken to avoid damaging the carcass. Thus, samples of the fatty acid profile of other muscles could not be obtained in the current experimental setup. Replacing soybean meal with 10% and 15% pumpkin seed cake in the mixture led to higher median value of LA concentrations in m. *semimembranosus*. LA is contained in pumpkin seed oil at a level of 35.6–60.8% (Murković *et al.*, 1996) and in the pumpkin seed cake mixture (Table 1). Although the control diet was rich in LA as well, the highest content of LA was obtained in the T10 and T15 treatments. Actually, owing to the hydrogenation by rumen microbes, most PUFAs are converted to SFA in the rumen and only a small percentage of PUFA escapes ruminal conversions (Wood *et al.*, 2008). According to the present study, probably more LA escaped the effect of the rumen when using T10 and T15. Elevated LA content led to a higher ratio between n-6 and n-3, when compared with the control group, which was not favourable, because the recommendation for n-6/n-3 ratio in human diet should be lower than 4. The n-6/n-3 ratio is required to maintain the balance of inflammatory and anti-inflammatory properties (Simopoulos, 2008). Simopoulos (2002) reviewed that humans evolved on a diet with a ratio of n-6 to n-3 essential fatty acids of approximately 1, whereas that ratio increased in Western diets (15/1–16.7/1), which is unfavourable. In the present study the inclusion of 10% and 15% pumpkin seed cake in the diets did not lower the contents of other important fatty acids in the *m. semimembranosus*, such as OA and CLA, and n-3 fatty acids, such as ALA, EPA and DHA, which are generally regarded as being beneficial to human health (Scollan *et al.*, 2005). According to the European Parliament and Council (2006), food is a good source of n-3 fatty acids if the product contains at least 0.3 g ALA per 100 g and the sum of EPA and DHA is at least 40 mg/100 g. When compared with the control diet in the present study, this suggests that pumpkin seed cake may be a good source of MUFA and PUFA when feeding lambs, although, owing to the high content of LA, the ratio between LA and ALA could be higher. In the present research levels of pumpkin seed cake replacing soybean meal at lower than 10% and higher than 15% were not considered. Thus, it is not known how these levels would affect production traits, meat quality and fatty acid proportions in lamb meat and is worth further investigations.

Conclusion

Partial replacement of soybean meal with pumpkin seed cake promoted adequate carcass characteristics and is feasible with respect to changes in haemato-chemical parameters of lambs' blood in organic farming. Replacing soybean meal with 10% and 15% of pumpkin seed cake had less favourable effects owing to higher contents of LA and a higher LA/ALA ratio in *m. semimembranosus*. Thus, pumpkin seed cake could be used in lamb feed as a replacement for soybean meal in terms of the haemato-chemical parameters and carcass traits. In future research it is necessary to obtain more levels of replacement with pumpkin seed cake and to research its antioxidant potential in lamb feed.

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Author´s contributions

ZA designed the experiment and wrote the paper. JN and ZK collected samples, analysed results, and prepared the paper for submission. MS carried out the biochemical analyses. DC prepared samples and analysed the fatty acid profile. VS collected samples and BM participated in the interpretation of the results.

Conflict of interest declaration

The authors declare that they have no conflicts of interest.

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