



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

Zvonko Antunović¹, Željka Klir¹, Marcela Šperanda¹, Suzana Ćavar², Boro Mioč³, and Josip Novoselec¹

¹Department for Animal Husbandry, Faculty of Agriculture,
University of J.J. Strossmayer, Vladimira Preloga 1, Osijek, Croatia

²Institute of Public Health Osijek-Baranja County, Drinska 8, Osijek, Croatia

³Department of Animal Science and Technology, Faculty of Agriculture,
University of Zagreb, Svetošimunska cesta 25, Zagreb, Croatia

Correspondence to: Klir Željka (zklir@pfos.hr)

Received: 1 August 2017 – Revised: 12 October 2017 – Accepted: 18 October 2017 – Published: 23 November 2017

Abstract. The aim of this research was to investigate the production traits, blood metabolic profile and fatty acids of meat and tallow in response to the partial replacement of soybean meal with peas in lambs' feed. The research was conducted on 30 Merinolandschaf lambs of 90 days' age over 30 days. Lambs were fed with feed mixture ($1000 \text{ g day}^{-1} \text{ lamb}^{-1}$). In the control group protein supplement was soybean meal (SC), while in the experimental groups soybean meal was partially replaced with 13 % peas (P13) and 26 % peas (P26). In the haematological parameters of lambs' blood, concentrations of minerals (Ca, P, Mg and Fe) and biochemical parameters (urea, glucose, total protein, albumin, globulins, cholesterol, HDL, LDL, triglyceride, β -hydroxybutyrate and non-esterified fatty acids) as well as enzyme activity (ALT, AST, ALP, GGT and CK) were determined. After slaughter, carcass development was measured. Samples of m. semimembranosus and tallow were taken in which concentrations of fatty acids were analysed. Values of meat pH and colour were taken 45 min 24 h post mortem, and water-holding capacity was calculated. By analysing the production properties of lamb, we found that slaughtering characteristics of lamb carcasses, haematological and most of the biochemical indicators did not differ. Urea concentrations were reduced in the blood of lambs in P13 and P26. Concentration of C18:2 n-6 increased in tallow of lambs of group SC compared to group P26 of lambs. The above-mentioned results indicate the possibility of partial replacement of soybean meal with peas in lambs' diets in organic farming without changes in production.

1 Introduction

Interest and demand for organic products in the market, as well as for the sheep products, is constantly growing. Therefore, the number of sheep and family farms dealing with organic sheep farming is increasing. According to the Eurostat (2016) report in the European Union countries there were 10 250 742 ha of organic land area in 2014, with an increase of 59.5 % from 2005 to 2014, and the total number of organic producers was 257 525. In the above area, 4 366 042 reared sheep were counted. In Croatia, there was a significant increase in registered organically farmed sheep in period 2006–

2015, from 3952 to 23 774 sheep (Croatian Ministry of Agriculture). Meeting the protein needs in rations of farm animals is most commonly done by including soybean and its by-products. Due to the increasing pollution of soy with mould and mycotoxins, and due to the fact that significant amounts of soy are of GMO origin (Bonanno et al., 2012), which is prohibited in organic farming (European Union, 2007), alternative solutions are being sought. As a successful alternative in organic ruminant production, peas can be used (Tufarelli et al., 2012; Antunović et al., 2013, 2016). Giger-Reverdin et al. (2015) pointed out that pea seeds can be a relevant

source of protein for ruminants. Pea grain (*Pisum sativum* subsp. *sativum*) is a high-protein feed (22–34 %) whose protein majority consists of globulins and albumins (70–95 %) and has a good balance of essential amino acids. Pea grain, besides protein, is also a good source of starch (over 40 % of dry matter) whose value, in addition to protein, makes it a high-quality and economical source of protein and energy in the rations of animals (Jezierny et al., 2010). Pea grain contains good levels of unsaturated fatty acids (12.7 % oleic acid, 35.3 % linoleic acid and 5.4 % linolenic acid; Grela et al., 1995). In today's field pea cultivars, the content of anti-nutritional substances is very low and its use as grain without adverse effects on productivity and animal health (Corbett, 1997) is possible.

In previous research focusing on complete or partial replacement of soybean meal with peas in rations of lambs, there were no significant differences in the production properties and slaughtering quality of lamb carcasses (Lanza et al., 2003; Colonna et al., 2014; Facciolongo et al., 2014 and 2015). There are just a few studies that have examined the blood metabolic profile of lambs fed with rations where soybean meal has been replaced with peas (Lestingi et al., 2016). It is known that the metabolic profile of animals' blood is a good indicator of the nutritional status and welfare and can be used to detect metabolic imbalance or disorders, particularly those resulting from errors made in nutrition (Whitney et al., 2009; Antunović et al., 2010a). Lamb meat is an important source of beneficial fatty acids, especially of long-chain fatty acids from the n-3 group (Nudda et al., 2011). Numerous investigations have determined a significant influence of feeding on fatty acid profile and lamb meat, wherein the supplements and diets rich in polyunsaturated fatty acids (PUFAs) lead to modelling of fatty acid profile (Demirel et al., 2006; Ponnampalam et al., 2001).

According to these findings, we postulate that partial replacement of soybean meal with peas in rations of lambs will not have any negative effect on the blood metabolic profile or certain production traits. Therefore, the aim of the research was to investigate the effect of partial replacement of soybean meal with peas in rations of lambs in organic farming on growth, carcass quality, blood metabolic profile and fatty acids of meat and tallow.

2 Material and methods

2.1 Production traits, sampling and analysis

The research was conducted in Osijek-Baranja County, Croatia, on organic sheep farm 50 km from Osijek (45°20'05" N, 18°18'59" E). The research included 30 Merinolandschaf lambs in growth, after weaning. Lambs were 90 days old, evenly divided by gender (50 % female:50 % male) and in good health. Lambs were fed with feed mixture (1000 g day⁻¹ lamb⁻¹) composed of organic feedstuffs. The mineral premix used (Panto Mineral L84) is also certified for

Table 1. Ingredient and chemical composition of the feed mixture and meadow hay for lambs' feed.

Ingredient (%)	Group			Hay
	SC	P13	P26	
Ingredient composition				
Corn	30.8	22.8	14.8	
Oat	8.4	8.4	8.4	
Barley	18.0	18.0	18.0	
Triticale	19.8	19.8	19.8	
Soybean meal	20.0	15.0	10	
Pea	0	13.0	26.0	
Mineral premix*	3.0	3.0	3.0	
Chemical composition (g kg ⁻¹ DM)				
Dry matter	861.7	864.5	867.3	931.6
Crude proteins	169.1	168.9	166.7	91.3
Ether extract	23.4	30.1	30.4	14.19
Crude fibre	38.5	41.5	38.9	287.1
Ash	67.0	65.2	66.6	67.0
NDF	12.77	13.38	12.76	510.6
NEM, MJ kg ⁻¹	771.35	770.02	768.65	2.35
Fatty acids (g (100 g) ⁻¹)				
C12:0	nd	nd	nd	0.40
C13:0	nd	nd	nd	0.27
C14:0	0.23	0.22	0.26	1.24
C14:1 (cis-9)	nd	nd	nd	0.10
C15:0	nd	0.07	0.11	0.61
C15:1 (cis-10)	nd	nd	nd	0.00
C16:0	19.60	18.25	18.83	34.36
C16:1 (cis-9)	0.22	0.22	0.20	0.70
C17:0	0.12	0.12	0.15	0.66
C17:1 (cis-10)	0.00	nd	nd	0.32
C18:0	2.97	2.76	2.88	3.99
C18:1 (trans-9)	nd	nd	nd	0.16
C18:1 (cis-9)	22.73	24.08	24.80	6.40
C18:2 (cis-9,12)	49.17	49.17	47.22	16.43
C20:0	0.37	0.39	0.40	3.33
C18:3 (cis-6,9,12)	nd	nd	nd	0.26
C20:1 (cis-11)	0.47	0.48	0.54	0.17
C18:3 (cis-9,12,15)	3.39	3.43	3.74	15.52
C21:0	nd	nd	nd	0.63
C20:2 (cis-11,14)	nd	nd	nd	0.57
C22:0	0.33	0.30	0.27	4.21
C20:3 (cis-8,11,14)	nd	nd	0.00	0.58
C22:1 (cis-13)	nd	0.12	0.13	nd
C20:3 (cis-11,14,17)	nd	nd	nd	3.50
C20:4 (cis-5,8,11,14)	0.10	0.08	0.09	1.43
C22:2 (cis-13,16)	nd	nd	nd	0.29
C24:0	0.30	0.32	0.29	3.47
C20:5 (cis-5,8,11,14,17)	nd	nd	nd	0.16
C24:1 (cis-15)	nd	nd	0.10	0.23
SFA	23.93	22.44	23.18	53.18
PUFA	52.66	52.68	51.05	38.74
MUFA	23.42	24.89	25.77	8.08
UFA	76.07	77.56	76.82	46.82

* 18 % Ca, 5 % P, 9.5 % Na, 2.00 % Mg, 400 000 IU vitamin A, 40 000 IU vitamin D, 500 mg vitamin E, 4000 mg Zn, 2000 mg Mn, 60 mg I, 10 mg Co, 50 mg Se. NDF – neutral detergent fibre; NET – net energy; SFA – saturated fatty acids; PUFA – polyunsaturated fatty acids; UFA – unsaturated fatty acids; MUFA – monounsaturated fatty acids; nd – not determined; DM – dry matter.

use in organic sheep and lamb production. Lambs consumed hay and water ad libitum. Ingredient and chemical compositions of feed mixture and meadow hay are shown in Table 1. The crude protein content of feed samples was determined using the Kjeldahl method (Pearson, 1976), while ether extract was determined according to the method described in Onwuka (2005). The crude fibre content was determined using the Weende method (Offor et al., 2014) and neutral detergent fibre (NDF) described by Foss (2001). In the control group (SC) soybean meal was used as feed rich in protein while in the experimental group soybean meal has been partially replaced with peas; in the first experimental group with 13 % peas (P13) in the second experimental group with 26 % peas (P26). The experiment lasted for 30 days. Rearing and feeding of lambs were according to Council Regulation (EC) no. 834/2007 on organic production (European Union, 2007). Animals used in this study were maintained in facilities approved by the Croatian Association for Accreditation of Laboratory Animal Care, and in accordance with current regulations and standards issued by the Croatian Ministry of Agriculture.

Weighing and taking the body condition score of lambs (Russel, 1991) was carried out at the beginning (day 0) and end of the research (day 30). After that, daily gains of lambs were calculated.

2.2 Analysis of lambs' blood metabolic profile

The blood was collected from the jugular vein (10 mL) into sterile vacuum tubes (Venoject[®], Sterile Terumo Europe, Leuven, Belgium), at the end of research (day 30). After that, serum was separated by centrifugation (10 min) at 1609.92 g and placed into an Olympus AU640. Within blood serum the concentrations of the minerals (calcium, inorganic phosphorus and iron), concentrations of the 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, NEFAs) as well as enzyme activity (alanine aminotransferase, ALT; aspartate aminotransferase, AST) were all found with Olympus System Reagents (OSR), manufactured and distributed by Olympus Diagnostica GmbH (Irish branch), Lismeehan, Ireland. Globulin content was calculated as the difference between total protein and albumin. The analytical method of each parameter and the corresponding quality laboratory assay are reported in Table 2.

Determination of haematological parameters (leukocytes, WBC; erythrocytes, RBC; thrombocytes, PLT; content of haemoglobin, HGB; haematocrit, HCT; mean corpuscular volume, MCV; the average haemoglobin content in erythrocytes, MCH; and mean haemoglobin concentration in erythrocytes, MCHC) in whole blood of lambs was carried out on a Sysmex pochH-100iV automatic three-part differential haematology analyser. The blood analysis was performed in

the Laboratory for Small Ruminants and Non-ruminants as well as Laboratory for Animal Nutrition and Physiology of Animals at the Faculty of Agriculture in Osijek (Croatia).

2.3 Analysis of carcass and meat quality

Before slaughter, lambs were weighed on an automatic animal scale. After slaughter and exsanguination, the lambs' skin is peeled off the carcasses, and abdominal (forestomach, stomach, spleen, intestine and liver) and thoracic (trachea with the lungs and heart) cavity organs are then removed. Immediately afterwards, the internal organs, skin, lower parts of the legs and the carcasses themselves were weighed. After that, standard development measures (linear measure) of lamb carcasses were taken: the length of the carcass (carcass length 1 – os pubis to atlas; carcass length 2 – os pubis to first rib; and the carcass length 3 – os pubis to last rib), the circumference of carcass (at scapula) and the length of the hind legs (tuber calcanei to tubercle ossis ischia) and hind leg circumference (the widest part).

Samples of lamb meat (m. semimembranosus) and tallow (peritoneum) as well as carcass parameters were taken from five lambs from each group immediately after slaughtering and exsanguination, while pH₁ values and colour were taken 45 min post mortem and pH₂ 24 h after slaughter and cooling. The pH was measured with a Mettler Toledo contact pH meter, while the colour of meat was measured with a Minolta Chromameter CR-410 portable instrument (Minolta Camera Co. Ltd. Japan) according to the standard CIE L*a*b* colour system (CIE, 1976). After that, dressing percentage was calculated (weight before slaughter – carcass weight/100). Water-holding capacity was measured by the method of Sierra (1973). Hue angle was calculated according to the formula Eq. (1) and chroma using the formula Eq. (2):

$$H^* = \tan^{-1}(b^*/a^*), \quad (1)$$

$$C^* = \sqrt{(a^{*2} + b^{*2})}. \quad (2)$$

2.4 Fatty acid analysis

In order to remove fat from the meat or tallow, it was necessary to cook a specific mass of meat or tallow with 25 % HCl. After that it was necessary to shake the sample with organic solvents – ethanol, ether, and petroleum ether – and allow the solution to stand in order to separate the layers. An aliquot of supernatant was evaporated and dried in an oven until constant weight (Trajković et al., 1983). Preparation of fatty acid methyl esters was performed by means of gas chromatography according to standard HRN EN ISO 12966-2:2011. Conditions that were used and the method of determination followed standard HRN EN ISO 12966-1:2015. The samples were analysed by gas chromatograph (7890B, Agilent Technologies, USA).

The atherogenicity index (AI) and thrombogenic index (TI) were calculated according to Ulbricht and South-

Table 2. Analytical methods for blood parameters and quality laboratory assays.

Analytical method	Intra-assay variation (CV%)	Inter-assay variation (CV%)
Enzymatic colorimetric method test for the quantitative determination of NEFA	4.81	4.32
Enzymatic method test for the quantitative determination of D-3 hydroxybutyrate	3.78	5.25
Photometric colour test for the quantitative determination of albumin	1.17	1.55
Enzymatic colour test for the quantitative determination of cholesterol	0.72	1.45
Enzymatic colour test for the quantitative determination of HDL cholesterol	0.85	1.92
Enzymatic UV test (hexokinase method) for the quantitative determination of glucose	0.54	0.97
Photometric colour test for the quantitative determination of total protein	0.51	0.70
Enzymatic colour test for the quantitative determination of triglyceride	0.72	1.03
Kinetic UV test for the quantitative determination of urea	1.1	1.93
Photometric colour test for the quantitative determination of total calcium	0.65	0.95
Photometric UV test for the quantitative determination of inorganic phosphorus	0.63	1.33
Photometric colour test for the quantitative determination of iron	0.66	1.77
Photometric colour test for the quantitative determination of magnesium	0.75	1.29
Kinetic colour test for the quantitative determination of alkaline phosphatase, EC 3.1.3.1 (ALP)	0.56	0.99
Kinetic UV test for the quantitative determination of alanine aminotransferase, EC 2.6.1.2 (ALT)	1.43	1.78
Kinetic UV test for the quantitative determination of aspartate aminotransferase, EC 2.6.1.1 (AST)	0.84	1.35
Kinetic UV test for the quantitative determination of creatine kinase, EC 2.7.3.2 (CK)	1.00	3.20
Kinetic colour test for the quantitative determination of γ -glutamyltransferase, EC 2.3.2.2 (GGT)	1.01	1.13

gate (1991) as specified in Eqs. (3) and (4):

$$AI = [(12 : 0 + 4(14 : 0) + 16 : 0)] / [(n-6 + n-3) \times PUFA + 18 : 1 + \Sigma MUFA], \quad (3)$$

$$TI = (14 : 0 + 16 : 0 + 18 : 0) / [(0.5 \times 18 : 1) + 0.5 \times (\Sigma MUFA) + 0.5 \times (PUFA n-6) + 3 \times (PUFA n-3) + (PUFA n-3/PUFA n-6)]. \quad (4)$$

Nutritive values (NV) of meat lipids were calculated according to Solomon et al. (1991) as follows in Eq. (5):

$$NV = (C18 : 0 + cis-9C18 : 1) / C16 : 0. \quad (5)$$

2.5 Statistical analysis

The mean values of growth, carcass quality, blood metabolic profile and fatty acids of meat and tallow were measured using the MEANS procedure. Data were analysed by means of ANOVA, using feeding treatment as a fixed effect. Mean values were compared using Tukey's test and differences between the groups were declared significant at $p < 0.05$ or less. All data were analysed with SAS 9.4[®] statistical software (SAS Institute Inc., 2002–2012).

3 Results and discussion

From analysis of the production traits of lambs (Table 3), it is evident that there were no significant differences ($p > 0.05$) regarding nutrition. In the organic breeding of lambs in Italy no significant differences in production traits were found when feeding lambs with feed mixture in which soybean

meal was replaced with peas as well (Bonanno et al., 2012). Similar results in studies with lambs where soybean meal was completely or partially replaced with peas were obtained by Facciolongo et al. (2014), Colonna et al. (2014), Bonanno et al. (2012) and Lanza et al. (2011).

Lambs' blood metabolic profile indicators were within reference values in all three groups (Lepherd et al., 2009; Kaneko et al., 2008; Antunović et al., 2010b), which indicates the quality of their nutrition, which is also shown in the obtained production traits and indices of body condition score (Table 3). The cholesterol concentrations were slightly lower compared to reference values. There were no significant differences ($p > 0.05$) in either haematological or in biochemical indicators in the blood of lambs, except for the urea concentrations, which were significantly reduced in the blood of P13 and P26 lambs (Table 4). This was due to a decreased degradability of pea protein leading to a decreased ammonia level in the rumen and urea level in blood. Similar results were obtained with dairy cows (Tufarelli et al., 2012). Facciolongo et al. (2014), by replacing soybean meal with peas in feed mixture for lambs, also found a decrease in blood urea, but the differences were not significant (15.84 to 14.29 mg dL⁻¹). Total cholesterol concentration was lower in all groups compared to reference values. It is known that type of dietary protein, especially lysine-to-arginine ratio and the sulfur-containing amino acids (methionine and cysteine), has been considered a factor influencing the cholesterol serum concentration in animals (Kritchevsky et al., 1982; Kurowska and Carroll, 1994).

From analysis of slaughtering characteristics, development measures and physical properties of lamb carcasses and meat, it is evident that there were no significant differences ($p > 0.05$) depending on the feed treatment (Tables 5–7).

Table 3. Production traits of lambs.

Indicators	Measuring time (days)	Groups (mean)			SE	<i>p</i> values
		SC	P13	P26		
Body mass, kg	1	25.95	25.97	25.72	0.39	0.96
	30	34.04	34.00	33.81	0.61	0.98
Daily gain, g	1–30	269.67	267.65	269.66	12.58	0.99
Feed conversion ratio, g DM/g gain	1–30	3.20	3.23	3.22	–	–
BCS	1	3.45	3.77	3.45	0.08	0.18
	30	3.88	3.85	3.86	0.05	0.97

BCS – body condition score; DM – dry matter; SC – control group; P13 – soybean meal was partially replaced with 13 % peas; P26 – soybean meal was partially replaced with 26 % peas.

Table 4. Blood metabolic profile in lambs.

Indicator	Groups (mean)			SE	<i>p</i> value	Ref. values ^c
	SC	P13	P26			
Haematological parameters						
WBC × 10 ⁹ L	11.75	10.58	10.89	0.72	0.80	5.10–15.90
RBC × 10 ¹² L	9.79	9.88	9.20	0.49	0.75	9.20–13.00
PLT × 10 ⁹ L	685.10	457.50	526.10	40.64	0.06	426.00–1142.00
HGB (g L ⁻¹)	105.20	107.50	105.50	2.15	0.77	105.00–137.00
HEM, L L ⁻¹	0.34	0.40	0.39	0.01	0.07	0.28–0.47
MCV, fL	40.45	41.27	41.39	1.29	0.65	28–41
MCH, pg	12.01	10.70	11.32	0.32	0.25	10–13
MCHC, g L ⁻¹ L	305.60	259.90	262.00	9.07	0.06	332–392
Biochemical parameters (mmol L ⁻¹)						
Mg	0.99	0.86	0.85	0.04	0.19	0.91–1.31
Fe μmol/L	29.14	29.01	30.53	1.33	0.88	29.70–39.70 ^d
P	2.71	2.35	2.55	0.07	0.12	1.88–3.34
Ca	2.44	2.37	2.49	0.03	0.27	2.42–2.92
GUK	4.15	4.19	4.30	0.15	0.92	2.70–4.80
UREA	7.38 ^a	6.25 ^b	6.15 ^b	0.22	0.04	5.00–9.10
PROT, g L ⁻¹	58.24	58.58	59.48	0.79	0.81	51.00–64.00
ALB, g L ⁻¹	27.92	27.44	28.80	0.34	0.26	30.00–7.00
GLOB, g L ⁻¹	30.32	31.14	30.68	0.66	0.89	19.00–30.00
CHOL	0.99	0.94	0.96	0.04	0.86	1.35–1.97 ^d
TGC	0.51	0.43	0.47	0.11	0.07	0.00–2.00 ^d
HDL	0.63	0.62	0.61	0.02	0.91	0.68–0.97 ^e
LDL	0.17	0.13	0.20	0.02	0.50	0.10–0.50 ^e
NEFA	0.04	0.05	0.04	0.003	0.76	< 0.4
BHBA	0.51	0.45	0.43	0.03	0.41	0.2–0.7
AST, U L ⁻¹	92.53	85.72	94.68	4.04	0.66	83.00–140.00
ALT, U L ⁻¹	12.86	11.49	14.52	0.72	0.24	6.00–20.00 ^d
ALP, U L ⁻¹	274.31	256.63	303.98	18.07	0.57	–
GGT, U L ⁻¹	74.51	65.00	64.17	2.43	0.16	56.00–110.00
CK, U L ⁻¹	180.98	184.07	182.40	13.63	0.44	180.00–454.00

^{a,b} Values in rows with different letters differ significantly ($p < 0.05$); ^c Lephherd et al. (2009); ^d Kaneko et al. (2008); ^e Antunović et al. (2010b). SC – control group; P13 – soybean meal was partially replaced with 13 % peas; P26 – soybean meal was partially replaced with 26 % peas.

Table 5. Slaughtering characteristics of lamb carcasses.

Indicators, kg	Groups (mean)			SE	<i>p</i> value
	SC	P13	P26		
Pre-slaughter mass	33.33	33.39	34.73	0.55	0.53
Carcass mass	16.47	16.94	17.81	0.34	0.26
Entrail mass*	1.52	1.41	1.49	0.03	0.20
Skin and leg mass	4.25	4.22	4.31	0.09	0.93
Forestomach and intestine mass	9.02	8.42	8.54	0.21	0.48
Dressing percentage, %	49.45	50.62	51.32	0.50	0.50

* Lungs, trachea, heart and liver. SC – control group; P13 – soybean meal was partially replaced with 13 % peas; P26 – soybean meal was partially replaced with 26 % peas.

Table 6. Development measures of lamb carcasses.

Measure, cm	Groups (mean)			SE	<i>p</i> value
	SC	P13	P26		
Carcass length 1	66.00	65.00	66.33	0.57	0.66
Carcass length 2	44.33	45.80	47.67	0.62	0.07
Carcass length 3	19.50	20.60	20.17	0.29	0.32
Carcass circumference	64.83	65.20	67.50	0.53	0.07
Hind leg length	30.67	30.60	30.67	0.35	0.99
Hind leg circumference	36.83	36.20	39.33	0.65	0.11

Carcass length 1 – os pubis to atlas; carcass length 2 – os pubis to first rib; carcass length 3 – os pubis to last rib; SC – control group; P13 – soybean meal was partially replaced with 13 % peas; P26 – soybean meal was partially replaced with 26 % peas.

Table 7. Physical properties of lamb carcasses.

Indicator	Groups (mean)			SE	<i>P</i> value
	SC	P13	P26		
pH ₁	6.70	6.59	6.75	0.04	0.26
pH ₂	5.70	5.71	5.72	0.02	0.96
WHC (%)	16.87	17.43	19.04	0.49	0.06
Colour					
Lightness	38.26	42.07	41.85	1.94	0.69
Redness	19.86	20.20	18.36	0.47	0.24
Yellowness	2.35	2.23	1.61	0.19	0.22
Hue angle	6.73	6.13	5.87	0.46	0.24
Chroma	20.01	20.34	18.45	0.48	0.23

WHC – water-holding capacity; SC – control group; P13 – soybean meal was partially replaced with 13 % of peas; P26 – soybean meal was partially replaced with 26 % of peas.

It is evident that a higher water-holding capacity was established in the meat of lambs that had consumed feed mixture where soybean meal has been partially replaced with peas, but the differences were not significant ($p = 0.06$). Bonanno et al. (2012) also did not establish changes in the lambs' slaughtering characteristics in organic lamb breeding where lambs were fed with feed mixture with addition

of soybean meal or peas. Similar results were obtained by Lanza et al. (2003) in a study where peas were used as an alternative source of protein in nutrition of lambs (18 and 39 % in dry matter), compared to lambs fed with soybean meal diet. The above authors found significantly higher levels of drip losses in meat of lambs that had consumed the feed mixture with peas compared to the one with soybean meal (1.6 and 1.2 : 0.8 %). Facciolongo et al. (2015) and Colonna et al. (2014) also did not determine significant changes in the physical characteristics of carcass and meat in lambs fed with rations where soybean meal was replaced by peas.

The concentration of fatty acids in meat and tallow of lambs' carcasses was not significant, except for the concentration of linoleic acid (LA; C18:2 n-6), which was significantly higher in tallow of lambs of group SC compared to group P26 (Table 8). Higher concentrations of LA in tallow of group SC in comparison with P13 and statistically higher in comparison with group P26 were probably due to the presence of a higher content of corn in meal. By analysing the mentioned results, we found that a trend of increasing concentration of cis-9 C18:1 and C21:0, and a decreasing trend of C15:0 in groups P13 and P26 compared to SC may be seen. Also, an increasing trend of concentration of trans-9,12 C18:2; LA; cis-6,9,12 C18:3; linolenic acid (ALA; C18:3 n-3); cis-8, 11, 14 C20:3; C23:0; cis-5, 8, 11, 14, 17 C20:5; and PUFAs was established, as well as a decreasing trend of C16:0; cis-9 C16:1; C17:0; C18:0; and MUFAs, in group P13 compared to P26 and SC, but the differences were not significant. Colonna et al. (2014) did not find significant differences in the content of fatty acids in the meat of lambs where the soybean meal mixtures were replaced with peas (40 % peas in feed mixture) in m. longissimus dorsi (MLD) and m. semimembranosus. The authors also pointed out that meat from ruminants is only moderately influenced by the diet because of the hydrogenating action of the microbial consortium in the rumen (Colonna et al., 2014). Similar results in organic farming at nutrition of lambs with feed mixtures with different protein sources (soybean and pea) were obtained by Bonanno et al. (2012), who also did not determine significant differences in the concentrations of fatty acids in the MLD

Table 8. Concentration of fatty acids (g (100 g)⁻¹ fatty acids), atherogenic and thrombogenic indices in lambs' meat and tallow.

Fatty acids	Group						SE		p values	
	SC		P13		P26		meat	tallow	meat	tallow
	meat	tallow	meat	tallow	meat	tallow				
Fatty acids (g (100 g) ⁻¹)										
C10:0	0.14	0.26	0.16	0.28	0.13	0.27	0.02	0.02	0.911	0.968
C12:0	0.33	0.54	0.30	0.50	0.34	0.51	0.03	0.04	0.264	0.992
C14:0	3.61	5.65	3.61	5.56	4.07	5.47	0.23	0.28	0.577	0.867
C14:1 (cis-9)	0.23	0.12	0.23	0.11	0.27	0.14	0.02	0.01	0.845	0.386
C15:0	0.51	0.73	0.43	0.66	0.47	0.64	0.03	0.03	0.514	0.588
C16:0	22.89	22.61	21.89	23.22	23.24	23.51	0.36	0.30	0.529	0.758
C16:1 (cis-9)	2.27	1.29	2.16	1.16	2.45	1.41	0.10	0.07	0.557	0.379
C17:0	1.23	1.70	1.11	1.69	1.15	1.65	0.04	0.06	0.754	0.962
C17:1 (cis-10)	0.95	0.63	0.91	0.57	0.90	0.66	0.04	0.03	0.634	0.613
C18:0	13.06	21.72	12.17	23.20	12.83	20.96	0.40	1.09	0.633	0.516
C18:1 (trans-9)	2.95	5.46	2.42	4.32	2.19	3.87	0.16	0.29	0.244	0.150
C18:1 (cis-9)	39.62	34.65	39.69	34.35	41.98	36.79	0.86	0.88	0.457	0.183
C18:2 (trans-9,12)	0.42	0.08	0.52	0.09	0.41	0.23	0.02	0.02	0.064	0.056
C18:2 (cis-9,12)	6.94	3.10 ^a	7.42	2.51 ^{ab}	5.00	2.31 ^b	0.56	0.13	0.399	0.005
C20:0	0.08	0.19	0.07	0.17	0.05	0.15	0.009	0.01	0.321	0.533
C18:3 (cis-6,9,12)	0.05	0.01	0.11	0.02	0.05	0.02	0.01	0.004	0.266	0.931
C20:1 (cis-11)	0.15	0.13	0.16	0.10	0.15	0.13	0.01	0.004	0.971	0.077
C18:3 (cis-9,12,15)	0.51	0.36	0.91	0.49	0.53	0.38	0.07	0.03	0.071	0.123
C21:0	0.30	0.53	0.41	0.71	0.42	0.57	0.07	0.04	0.934	0.199
C20:2 (cis-11,14)	0.21	0.08	0.17	0.13	0.12	0.08	0.03	0.02	0.767	0.595
C22:0	0.35	0.05	nd	0.04	0.32	0.06	0.03	0.01	0.870	0.437
C20:3 (cis-8,11,14)	0.23	nd	0.36	0.01	0.15	0.003	0.04	0.002	0.307	0.299
C20:4 (cis-5,8,11,14)	0.18	nd	nd	nd	0.04	0.01	0.05	0.003	0.558	0.434
C23:0	1.96	0.09	2.85	0.08	1.86	0.15	0.30	0.02	0.625	0.655
C22:2 (cis-13,16)	nd	nd	0.27	nd	nd	nd	0.08	nd	0.506	nd
C24:0	0.07	nd	0.05	nd	0.13	nd	0.02	0.05	0.651	0.216
C20:5 (cis-5,8,11,14,17)	0.38	nd	0.65	nd	0.38	0.02	0.07	0.004	0.460	0.474
C24:1 (cis-15)	0.14	nd	0.12	nd	0.07	nd	0.02	nd	0.708	nd
C22:6 (cis-4,7,10,13,16,19)	0.17	nd	0.43	0.01	0.28	0.01	0.05	0.002	0.245	0.474
SFA	44.54	54.10	43.47	56.13	45.02	53.96	0.61	0.91	0.492	0.383
UFA	55.46	45.91	56.53	43.87	54.98	46.04	0.61	0.91	0.492	0.383
PUFA	9.16	3.63	10.83	3.25	6.97	3.05	0.76	0.12	0.349	0.284
MUFA	46.30	42.28	45.70	40.62	48.01	43.00	0.96	0.91	0.612	0.072
AI	0.68	1.00	0.65	1.05	0.73	1.02	0.03	0.03	0.543	0.861
TI	0.87	1.18	0.77	1.23	0.91	1.22	0.04	0.03	0.264	0.815
NV	2.32	2.53	2.37	2.48	2.38	2.46	0.05	0.06	0.937	0.973

SFA – saturated fatty acids; UFA – unsaturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids; AI – atherogenic index; TI – thrombogenic index, NV – nutritive value; nd – not determined; ^{a,b} values in rows with different letters differ significantly ($p < 0.05$); nd – not determined; SC – control group; P13 – soybean meal was partially replaced with 13 % of peas; P26 – soybean meal was partially replaced with 26 % of peas.

intramuscular fat. By analysing the nutritive values of meat lipids we found no significant differences between groups. Nutritive values of meat lipids were within values of 2 and 3, also reported by Facciolongo et al. (2015). The above justifies partial replacement of soybean meal with peas in feed mixtures for the lambs in organic farming.

4 Conclusions

Based on the results from our research, considering the realized production and carcass traits of lambs and development measures and physical properties of carcasses, as well as blood metabolic profile and fatty acids in meat and tallow, it can be concluded that partial replacement of soybean meal with peas in diets from weaned lambs can be used in organic production.

Data availability. The original data are available upon request from the corresponding author.

Competing interests. The authors declare that they have no conflict of interest.

Acknowledgements. The research required for the present study was the part of the VIP project financed by the Ministry of Agriculture, Republic of Croatia.

Edited by: Manfred Mielenz

Reviewed by: two anonymous referees

References

- Antunović, Z., Novoselec, J., Šperanda, M., Domaćinović, M., and Djidara, M.: Monitoring nutritional status of lambs in organic breeding, *Krmiva*, 52, 27–34, 2010a.
- Antunović, Z., Novoselec, J., Senčić, Đ., Šperanda, M., Steiner, Z., and Samac, D.: Production traits and blood biochemical parameters in organic lamb production. 45th Croatian and 5th International Symposium on Agriculture, 15–19 February 2010, Opatija, Croatia, 1014–1018, 2010b.
- Antunović, Z., Novoselec, J., Klir, Ž., and Đidara, M.: Inclusion of peas as protein sources in the diet of dairy goats in organic breeding, *Maced. J. Anim. Sci.*, 3, 63–67, 2013.
- Antunović, Z., Novoselec, J., Sičaja, V., and Klir, Ž.: Pea (*Pisum sativum* L.) – an alternative source of protein in diets of sheep in organic farming, *Krmiva*, 58, 79–85, 2016.
- Bonanno, A., Tornambe, G., Di Grigoli, A., Genna, V., Bellina, V., Di Miceli, G., and Giambalvo, D.: Effect of legume grains as a source of dietary protein on the quality of organic lamb meat, *J. Sci. Food Agr.*, 92, 2870–2875, <https://doi.org/10.1002/jsfa.5616>, 2012.
- CIE (Commission Internationale de l'Éclairage): Colorimetry, Official Recommendations of the International Commission on Illumination. Publication CIE No. 15 (E-1.3.1), Bureau Central de la CIE, Paris, France, 1976.
- Colonna, M. A., Giannico, F., Marsico, G., Vonghia, G., Ragni, M., and Jambrenghi, A. C.: Effect of pea (*Pisum sativum* L.) as alternative to soybean meal on the productive performance and meat quality traits of Merino crossbred lamb type, *Prog. Nutr.*, 16, 39–51, 2014.
- Corbett, R. R.: Peas as a protein and energy source for ruminants, *Adv. Dairy Technol.*, 9, 213–247, 1997.
- Demirel, G., Ozpınar, H., Nazli, B., and Keser, O.: Fatty acids of lamb meat from two breeds fed different forage: concentrate ratio, *Meat Sci.*, 72, 229–235, <https://doi.org/10.1016/j.meatsci.2005.07.006>, 2006.
- European Union (EU): Council Regulation (EC) No. 834/2007 on organic production and labeling of organic products with regard to organic production, labeling and control. Official Journal of the EU 2007, available at: <http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex:32007R0834> (last access: 17 November 2017), 2007.
- Eurostat 2016: Area under organic farming, available at: <http://ec.europa.eu/eurostat/web/agriculture/>, last access: 13 March 2017.
- Facciolongo, A. M., Rubino, G., Zarrilli, A., Vicenti, A., Ragni, A., and Toteda, F.: Alternative protein sources in lamb feeding 1. Effects on productive performances, carcass characteristics and energy and protein metabolism, *Prog. Nutr.*, 16, 105–115, 2014.
- Facciolongo, A. M., De Marzo, D., Ragni, A., Lestingi, A., and Toteda, F.: Use alternative protein sources for finishing lambs. 2. Effects on chemical and physical characteristics and fatty acid composition of meat, *Prog. Nutr.*, 17, 165–173, 2015.
- Foss: Determination of acid detergent fibre (ADF) and acid detergent lignin in feed and forage using FiberCap™ 2021/2023 in agreement with EN ISO 13906:2008, Foss Tecator AB, FOSS, Hilleroed, Denmark, Höganäs, Sweden, 2001.
- Giger-Reverdini, S., Maaroufi, C., Peyronnet, C., and Sauvant, D.: Effects of particle size and dietary nitrogen content on the nutritive value of pea-based in mid-lactation goats, *Anim. Feed Sci. Tech.*, 210, 56–65, <https://doi.org/10.1016/j.anifeedsci.2015.09.013>, 2015.
- Grela, E. R. and Günter, K. D.: Fatty acids composition and tocopherol content of some legume seeds, *Anim. Feed Sci. Technol.*, 52, 325–331, [https://doi.org/10.1016/0377-8401\(94\)00733-P](https://doi.org/10.1016/0377-8401(94)00733-P), 1995.
- Jezierny, D., Mosenthin, R., and Bauer, E.: The use grain legumes as a protein source in pig nutrition: a review, *Anim. Feed Sci. Tech.*, 157, 111–128, <https://doi.org/10.1016/j.anifeedsci.2010.03.001>, 2010.
- Kaneko, J. J., Harvey, J. W., Bruss, M. L.: *Clinical Biochemistry of Domestic Animals*, 6th Edn., Elsevier/Academic Press, Amsterdam, the Netherlands, p. 931, 2008.
- Kritchevsky, D., Tepper, S. A., Czarnecki, S. K., and Klurfeld, D. M.: Atherogenicity of animal and vegetable protein: influence of the lysine to arginine ratio, *Atherosclerosis*, 41, 429–431, 1982.
- Kurowska, E. M. and Carroll, K. K.: Hypercholesterolemic responses in rabbits to selected groups of dietary essential amino acids, *J. Nutr.*, 124, 364–370, 1994.
- Lanza, M., Bella, M., Priolo, A., and Fasone, V.: Peas (*Pisum sativum* L.) as an alternative protein source in lamb diets: growth performances, and carcass and meat quality, *Small Ruminant Res.*, 47, 63–68, [https://doi.org/10.1016/S0921-4488\(02\)00244-4](https://doi.org/10.1016/S0921-4488(02)00244-4), 2003.
- Lanza, M., Fabro, C., Scerra, M., Bella, M., Pagano, R., Brogna, D. M. R., and Pennisi, P.: Lamb meat quality and intramuscular fatty acid composition as affected by concentrates Peas (*Pisum sativum* L.) as an alternative protein source in lamb diets: growth performances, and carcass and meat quality, *Small Ruminant Res.*, 47, 63–68, [https://doi.org/10.1016/S0921-4488\(02\)00244-4](https://doi.org/10.1016/S0921-4488(02)00244-4), 2011.
- Lepherd, M. L., Canfield, P. J., Hunt, G. B., and Bosward, K. L.: Haematological, biochemical and selected acute phase protein reference intervals for weaned female Merino lambs, *Aust. Vet. J.*, 87, 5–11, <https://doi.org/10.1111/j.1751-0813.2008.00382.x>, 2009.
- Lestingi, A., Facciolongo, A. M., Caputi Jambrenghi, A., Ragni, M., and Toteda, F.: The use of peas and sweet lupin seeds alone or in association for fattening lambs: Effect on performance, blood parameters and meat quality, *Small Ruminant Res.*, 143, 15–23, <https://doi.org/10.1016/j.smallrumres.2016.08.006>, 2016.

- Nudda, A., McGuire, M. K., Battacone, G., Manca, M. G., Boe, R., and Pulina, G.: Documentation of fatty acid profiles in lamb meat and lamb-based infant foods, *J. Food Sci.*, 76, 43–47, <https://doi.org/10.1111/j.1750-3841.2010.02027.x>, 2011.
- Offor, I. F., Ehiri, R. C., and Njoku, C. N.: Proximate nutritional analysis and heavy metal composition of dried Moringa Oleifera leaves from Oshiri Onicha L.G.A, Ebonyi State, Nigeria, *J. Environ Sci. Toxicol. Food Technol.*, 8, 57–62, 2014.
- Onwuka, G. I.: *Food Analysis and Instrumentation: Theory and Practice*, Naphthali Prints, Lagos, Nigeria, 2005.
- Pearson, D. A.: *The Chemical Analysis of Foods*, Churchill Livingstone, Edinburgh, UK, 6–25, 1976.
- Ponnampalam, E. N., Sinclair, A. J., Egan, A. R., Blakeley, S. J., and Leury, B. J.: Effect of diets containing n-3 fatty acids on muscle long-chain n-3 fatty acid content in lambs fed low- and medium-quality roughage diets, *J. Anim. Sci.*, 79, 698–706, <https://doi.org/10.2527/2001.793698x>, 2001.
- Russel, A.: Body condition scoring of sheep, in: *Sheep and Goat Practice*, edited by: Boden, E., Bailliere Tindall, Philadelphia, USA, 3 pp., 1991.
- SAS Institute Inc.: *SAS® Proprietary Software 9.4.*, Cary, NC, USA, 2002–2012.
- Sierra, I.: Contributions to the study of the Belgian White × Landrace cross: productive characters, carcass quality and meat quality, *Rev. Inst. Econom. Prod. Ebro.*, 16, 43–48, 1973.
- Solomon, M. B., Lynch, G. P., Paroczay, E., and Norton, S.: Influence of rapeseed meal, whole rapeseed, and soybean meal on fatty acid composition and cholesterol content of muscle and adipose tissue from ram lambs, *J. Anim. Sci.*, 69, 4055–4061, 1991.
- Trajković, J., Mirić, M., Baras, J., and Šiler, S.: *Analize Životnih Namirnica*, Tehnološko-metalurški Fakultet, Beograd, Serbia, 786 pp., 1983.
- Tufarelli, V., Naz, S., Khan, R. U., Mazzei, D., and Laudadio, V.: Milk quality, manufacturing properties and blood biochemical profile from dairy cows fed peas (*Pisum sativum* L.) as dietary protein supplement, *Arch. Tierzucht*, 55, 132–139, 2012.
- Ulbricht, T. L. V. and Southgate, D. A. T.: Coronary heart-disease 7 dietary factors, *Lancet*, 338, 985–992, 1991.
- Whitney, T. R., Waldron, D. F., and Willingham, T. D.: Evaluating nutritional status of Dorper and Rambouillet ewes in range sheep production, *Sheep Goat Res. J.*, 24, 10–16, 2009.