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Effects of legume seeds and processing treatment on growth, carcass traits and blood constituents of fattening lambs

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Abstract

This study aimed to evaluate the effects of faba bean, white lupin and pea seed when fed as protein supplements on growth performance, carcass characteristics and haematological characteristics of growing lambs. Forty-eight Gentile di Puglia male lambs, weaned at 38 ± 2 days old with an average initial bodyweight of 12.8 ± 0.5 kg, were divided into six homogenous groups. The six dietary treatments were RFB (diet containing raw faba bean seeds); EFB (diet containing extruded faba bean seeds); RL (diet containing raw lupin seeds); EL (diet containing extruded lupin seeds); RP (diet containing raw pea seeds); and EP (diet containing extruded pea seeds). Feeding lupin seeds reduced average daily feed intake compared with the other protein sources. Carcass conformation, loin weight as a percentage of half-carcass weight, and fat weight as a percentage of loin weight improved in the lambs that consumed both EP and RP diets. Extrusion reduced hide weight as a percentage of empty bodyweight and fat weight as a percentage of leg weight. The protein source had a significant effect on glucose and total cholesterol concentrations, albumin, α_1-globulin, β-globulin, and γ-globulin percentages and albumin-globulin ratios. The processing treatment decreased total cholesterol concentrations. Thus, feeding the various legumes did not affect growth performance, but only carcass characteristics; changes which may be due to the differences in feed intake. Extrusion also had minor effects, and further work is required to investigate the use of these protein sources, both raw and extruded.

Keywords: extrusion, faba bean, haematology, lupin, pea, protein source, slaughter data
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Introduction

Various species of home-grown grain legumes, such as pea, faba bean, and lupin, may represent strategically important alternatives to soybean. They are widely available in Mediterranean areas and increase the sustainability of crop–livestock systems by safeguarding soil fertility and reducing greenhouse gas emissions and use of nitrogen fertilizer (Bonanno *et al*., 2012; Calabrò *et al*., 2015). Recently, seed legumes have been regarded as alternatives to soybean as sources of protein in animal feeding owing to disputes about the use of genetically modified organisms (GMOs) (Calabrò *et al*., 2015). In addition, legume grains have attracted attention as alternative vegetable protein components for feedstuffs that are used in organic production of meat (Bonanno *et al*., 2012).

The use of a diet based largely on faba bean for fattening lambs resulted in growth and meat characteristics similar to the most frequently used diets containing soybean meal as the main source of protein (Caballero *et al*., 1992; Lanza *et al*., 1999; Lanza *et al*., 2011). Replacement of soybean meal with pea in lamb diets did not significantly affect growth and slaughter traits (Lanza *et al*., 2003, 2011; Scerra *et al*., 2011). However, some studies reported reduced animal performance with the use of lupin owing to the high rumen degradability of its protein, but the issue has been controversial (Kung *et al*.,1991; Murphy & McNiven, 1994; Stanford *et al*., 1996; Vicenti *et al*., 2009). In the authors' earlier studies, the use of diets that

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incorporated faba bean, lupin and pea, alone and in mixtures, as alternative protein sources to soybean in feeding for finishing lambs did not negatively affect the in vivo performances or the carcass yield and quality (Lestingi *et al*., 2015a, 2015b, 2016; Facciolongo *et al*., 2014, 2015). However, the authors continued with this research topic to determine the optimum percentages of these protein sources to include in feeds. Further, legumes such as peas, lupins, and faba beans require technological processing to reduce their nitrogen (N) degradability if they are to be used as the main protein source in highly productive diets for ruminants (INRA, 1988; Masoero *et al*., 2005; Zagorakis *et al*., 2015).

Modern nutrition science suggests an optimum 60 to 40 (ratio of rumen degradable protein to rumen undegradable protein (RDP : RUP) for production of ruminant livestock. With an effective protein degradability above 0.6, it is hard to sustain high total tract N digestibility (Milis & Liamadis, 2008; Zagorakis *et al*., 2015). Moreover, N degradability plays the major role in the amount of energy that is wasted in transforming blood ammonia (NH_3) into urea and environmental pollution with NH_3, both from urine and faeces (Miller & Baig, 2002). The high protein degradability of legume grains in the rumen has led to investigations into means to protect against fermentation and thus increase the undegraded dietary protein component that escapes the rumen. Attempts to increase the utilization of legumes have employed a wide range of processing techniques, such as soaking, boiling, autoclaving, radiating, cooking, roasting, dehulling, germinating, fermenting, supplementing with various chemicals and enzymes and utilizing extrusion cooking (Bishnoi & Khetarpaul, 1994; Alonso *et al*., 1998; Alonso *et al*., 2000).

The application of extrusion cooking to legume processing has developed quickly and can be regarded as a technology in its own right. Extrusion cooking has certain advantages, including versatility, high productivity, low operating costs, energy efficiency, and shorter cooking times. The temperatures reached by the feed during extrusion can be high (200 °C), but the residence time at this elevated temperature is short (5 to 10 seconds). This tends to maximize the beneficial effects (preservation of nutrients) of heating feeds, while minimizing the detrimental effects (destruction of nutrients) (Serrano, 1997).

The effect of extrusion of pea, lupin and faba bean seed in sheep and cows has been considered in several studies. Extrusion of these alternative legume grains is effective in reducing their crude protein degradation in the rumen and in increasing the supply of dietary protein to the small intestine (Benchaar *et al*., 1994; Aufrère *et al*., 2001; Masoero *et al*., 2005). However, there has been limited research on the effect of extrusion cooking on growth performance and carcass characteristic of fattening lambs.

Moreover, apart from the authors' previous studies (Lestingi *et al*., 2015b, 2016; Facciolongo *et al*., 2014), they are not aware of reports on the effect of the use of faba bean, white lupin, and pea seeds, raw or after an extrusion treatment, on lamb blood parameters, some of which are important indicators of the animal's nutritional state and may be useful in identifying metabolic imbalances or disorders. Therefore, the objective of this study was to evaluate the effects of faba bean, white lupin and pea seed, and their processing treatment, raw or extruded, on growth, carcass characteristics and blood parameters of growing lambs.

Material and methods

All procedures involving animals were conducted according to the Italian government guidelines (Directive 91/629/EEC, received in Italy by D.L. 533/92 and modified by D.L. 331/98).

The study was conducted on a farm in southern Italy (latitude: 41°5'54''24 N; longitude:16°46'43''68 E) at 620 m above sea level, during 7 weeks from November 2012 to January 2013. Forty-eight Gentile di Puglia male lambs, weaned at 38 ± 2 days old with an average initial bodyweight (BW) of 12.8 ± 0.5 kg (mean ± standard error) were divided into six homogenous groups for BW and age. They were housed in individual pens (1 m^2 per head) with continuous access to water. The temperature in the pens ranged from 7 °C to 15 °C. Lambs were assigned to one of six dietary treatments: RFB (diet containing raw faba bean seeds); EFB (diet containing extruded faba bean seeds); RL diet containing raw lupin seeds); EL (diet containing extruded lupin seeds); RP (diet containing raw pea seeds); and EP (diet containing extruded pea seeds). Local commercial pea (*Pisum sativum* L.), faba bean (*Vicia faba* L. var. *minor*) and lupin (*Lupinus albus* L.) seed were included in the diets at a rate of 250g/kg diet (on as fed basis). The chemical compositions, including the by-pass crude protein (CP) of the tested seeds, raw and extruded, are reported in Table1. Pea, faba bean and lupin seed were exposed to extrusion using the same industrial equipment used by Masoero *et al*. (2005), namely Anderson single-screw wet extruder with 300-350 Kg/h capacity and 120 A power absorption at Cortal Extrasoy (Vicenza, Italy). All pelleted total mixed rations (Table 2) were formulated to be isocaloric and isonitrogenous, and to meet the nutritional requirements of lambs (INRA, 1988).

Lambs were adapted to the ration for 10 days. Feed was offered daily at 08h00 at a rate of 110% of ad libitum intake, which was calculated by weighing back refusals weekly. Feed samples were taken weekly and stored at -20 °C until analysis. Straw was offered as a source of roughage. Straw intake was very low and was not recorded. Individual BW and feed intake were recorded weekly to calculate average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR).

Table 1 Chemical compositions of legume seeds (dry matter basis)

	Diets					
	Faba bean		Lupin		Pea	
	Raw	Extruded	Raw	Extruded	Raw	Extruded
Dry matter, %	88.29	87.24	90.12	90.23	88.11	89.15
Crude protein (CP), %	29.33	29.18	38.47	38.85	24.15	24.00
Ether extract, %	1.78	1.98	8.39	10.84	1.38	1.77
Ash, %	3.73	3.76	4.15	4.05	3.65	5.45
Neutral detergent fibre, %	31.28	22.93	20.13	20.95	18.45	15.51
Acid detergent fibre, %	12.86	14.68	12.87	13.18	9.87	7.09
Starch, %	37.03	38.19	12.48	8.89	49.58	47.15
CP enzymatic degradation (%)	81.67	51.05	95.01	91.06	87.20	74.95
Bypass CP (g/kg dry matter)	53.76	142.83	19.19	34.73	30.91	60.12

Samples of each legume seed and the pelleted total mixed rations were ground in a hammer mill with a 1-mm screen and analysed using these AOAC procedures (2004): dry matter (DM) (method 934.01), ether extract (EE) (method 920.39), ash (method 942.05), CP (method 954.01), crude fibre (CF) (method 945.18), acid detergent fibre (ADF) (method 973.18), and amylase-treated neutral detergent fibre (aNDF) (method 2002.04). Starch was determined according to AOAC procedure 996.11. Protein degradability was assessed in vitro as suggested by Krishnammoorty *et al*. (1983). The protein values PDIE (protein digested in the small intestine allowed by the energy), and PDIN)protein digested in the small intestine allowed by the nitrogen) was estimated using the equations proposed by INRA (1988).

At the end of the trial, blood was drawn from the jugular vein of each animal into a Vacutainer® (Becton Dickinson, Mississauga, ON, L5N 0B3, Canada). The blood samples were analysed to determine the energy (glucose, total cholesterol, triglycerides) and protein (creatinine, urea, total protein and respective electrophoresis fractions). After the blood samples were collected, and within 30 minutes of having been collected, the blood samples were centrifuged at 1600 x g for 15 minutes. The serum was divided into aliquots, transported at 4 °C to the laboratory, and frozen at -20 °C until analyses were performed. Serum concentration of parameters was assessed using Assel reagents and a SEAC photometer with interferential filters. Serum protein electrophoresis was performed on agarose gel according to the Helena BioSciences technique (Helena Biosciences, Gateshead, United Kingdom

At 97 ± 2 days old, after fasting for 12 hours, but with free access to water and with slaughter weight having been recorded, all lambs were slaughtered by exsanguination according to veterinary police rules (D.P.R. 320/54). After slaughter, the hot carcass, hide, head, pluck and full and empty gastro-intestinal tract (GIT) were weighed. Carcasses were then hung by the Achilles tendon, chilled at 4 °C (80–82% relative humidity) for 24 hours and reweighed. After chilling, carcasses were evaluated for conformation, using a 1–15 point scale (1 = poor, 15 = excellent) according to Colomer-Rocher *et al*. (1988), and fatness according to the EU scale (EEC Regulation 1278/94, 1994) for light lamb carcasses, which has 12 categories (1 = very scarce, 12 = important).

The weight of digestive content (full–empty GIT) was used to calculate the net dressing percentage (carcass weight after chilling/empty BW). The cold carcasses were then divided into two symmetrical sides. The right side was separated into cuts (neck, steak, brisket, shoulder, abdominal region, loin, leg) according to ASPA methods (1996). Loins and legs were dissected into tissue components (lean, separable fat and bone).

Statistical analysis was performed using the GLM procedure of SAS (SAS Institute Inc, Cary, NC, USA) with the linear model including protein sources (P) and seed treatment (T) effects, P × T interaction and experimental error. Least squares means (LSMEANS) were compared using the probability of difference option (PDIFF) in the LSMEANS statement of SAS. Significance was declared at P <0.05, while trends in the differences among means were declared at P <0.10.

Table 2 Ingredient and chemical composition the diets containing raw and extruded faba beans, lupin seeds and peas.

	Diets					
	Faba bean		Lupin		Pea	
	Raw	Extruded	Raw	Extruded	Raw	Extruded
Ingredient composition, % (as-fed basis)						
Corn	12.0	12.0	18.0	18.0	10.0	10.0
Oat	15.0	15.0	12.0	12.0	8.0	8.0
Barley	5.0	5.0	26.0	26.0	-	-
Dehydrated alfalfa 12	17.0	17.0	5.0	5.0	22.5	22.5
Straw	4.0	4.0	8.5	8.5	3.0	3.0
Molasses	1.7	1.7	1.7	1.7	1.7	1.7
Faba bean meal	25.0	-	-	-	-	-
Extruded faba bean	-	25.0	-	-	-	-
Lupin meal	-	-	25.0	-	-	-
Extruded lupin	-	-	-	25.0	-	-
Pea meal	-	-	-	-	25.0	-
Extruded pea	-	-	-	-	-	25.0
Wheat bran	15.5	15.5	-	-	25.0	25.0
Soy bean oil	1.0	1.0	-	-	1.0	1.0
Calcium carbonate	1.8	1.8	1.8	1.8	1.8	1.8
Dicalcium phosphate	1.0	1.0	1.0	1.0	1.0	1.0
Sodium chloride	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin mineral premix	0.5	0.5	0.5	0.5	0.5	0.5
Chemical composition (dry matter basis)						
Moisture, % (as fed)	13.4	13.7	13.1	13.7	13.8	13.5
Crude protein, %	17.1	16.6	17.2	17.4	16.3	16.8
Ether extract, %	4.3	4.0	4.4	4.3	4.2	4.8
Crude fibre, %	11.8	14.7	13.7	14.2	13.4	11.9
Ash, %	3.8	3.8	4.1	4.1	3.7	4.5
Neutral detergent fibre, %	30.4	29.0	25.5	19.8	31.2	29.6
Acid detergent fibre, %	15.4	19.2	13.3	13.9	15.5	14.7
Starch, %	26.4	26.6	35.2	36.3	27.8	27.3
PDIN[1] (g/kg dry matter)	105.0	110.5	113.5	115.6	97.6	99.9
PDIE[2] (g/kg dry matter)	93.5	110.4	88.5	90.7	90.2	95.3
Metabolizable energy (MJ/kg)	11.1	10.9	10.8	10.8	11.1	11.1

[1]PDIN: protein digested in the small intestine allowed by the nitrogen
[2]PDIE: protein digested in the small intestine allowed by the energy

Results and discussion

Feeding lupin seed significantly (P <0.01) reduced mean daily feed intake in relation to the other protein sources (Table 3). Lambs that were fed peas had greater (P <0.05) ADFI than lambs fed faba beans. These results are consistent with the authors' previous studies (Lestingi *et al*., 2015b; Lestingi *et al*., 2016).

As discussed in these works, some lupin components, in particular the alkaloids, may have exerted a negative effect on the ADFI, although the authors did not measure these components. The variety of sweet lupin that was used in the present study was Multitalia, an old Italian sweet variety, which is has not been selected for reduced alkaloid content to any great extent (Calabrò *et al*., 2015). This may have influenced palatability of the lupin seeds by giving them a bitter taste. The lambs that consumed lupin seed also tended (P <0.10) to have reduced ADG compared with lambs that were fed the other protein sources (Table 3).

Although the lambs that were fed the various protein sources had comparable EUROP classification scores for carcass fatness, pea and faba bean-fed lambs had on average 2.07 units higher (P <0.01) scores for conformation than lambs that received the lupin seed (Table 3). The minimal difference in carcass fat score among dietary groups could be attributed to the unique pattern of development of carcass fat depots. Lambs deposit most of the fat around the viscera and less of it in the carcass (Carrasco *et al*., 2009). On the other hand, the increased levels of carcass conformation with faba bean and pea allowances suggest that lambs responded to nutritional treatment by building up more muscle (Sheridan *et al*., 2003). The reduced feed intake in lupin-fed lambs probably decreased the availability of energy for deposition of muscle tissue, resulting in the subsequent reduction of carcass conformation. Slaughter BWs of lambs that consumed lupin seed were numerically lower than in lambs that received the other protein sources, although this effect was not significant.

Table 3 Effects of protein source and extrusion treatment on in vivo performances and slaughtering data (% of empty bodyweight) of fattening lambs

	Protein source (P)			Treatment (T)			*P*-value		
	Faba bean	Lupin	Pea	Raw	Extruded	MSE[1]	P	T	P × T
Initial bodyweight (kg)	12.51	12.04	12.25	12.21	12.32	1.661	0.723	0.830	0.782
Slaughter bodyweight (kg)	21.49	20.33	20.67	20.97	20.69	2.115	0.292	0.657	0.809
Average daily gain (kg/d)	0.194	0.166	0.188	0.185	0.180	0.036	0.077	0.575	0.502
Average daily feed intake (kg/d)	0.74^{Ab}	0.69^{B}	0.77^{Aa}	0.72	0.74	0.041	0.001	0.150	0.080
Feed conversion ratio (kg/kg)	3.91	4.41	4.21	4.13	4.23	1.028	0.428	0.738	0.446
Empty BW (kg)	19.31	17.93	18.30	18.71	18.32	1.898	0.114	0.484	0.917
Carcass conformation	12.0^{A}	9.94^{B}	12.03^{A}	11.77	10.87	2.215	0.008	0.110	0.972
Carcass fat score	6.75	6.56	6.87	6.96	6.50	1.338	0.713	0.140	0.626
Hide	13.92	14.13	14.14	13.56^{b}	14.57^{a}	1.521	0.902	0.026	0.266
Empty digestive tract	8.36	8.41	8.29	8.21	8.49	1.095	0.952	0.379	0.626
Omentun	0.80	0.69	0.80	0.84	0.68	0.401	0.668	0.184	0.478
Chilling loss	1.77	2.01	1.58	1.63	1.94	1.881	0.808	0.569	0.273
Head	4.61	4.72	4.95	4.81	4.72	0.480	0.134	0.533	0.885
Pluck	5.32	5.34	5.43	5.41	5.32	0.302	0.574	0.337	0.445
Net cold dressing percentage	48.80	51.43	49.52	50.43	49.40	3.906	0.156	0.364	0.449

[1] Mean square error
[A, B] row least squares means with different superscripts differ significantly at P <0.01
[a, b] row least squares means with different superscripts differ significantly at P <0.05

The percentage of hide on the empty bodyweight was the only slaughtering parameter that was affected (P <0.05) by processing treatment (Table 3). Lambs fed raw unprocessed seed showed lower percentages of hide compared with lambs fed extruded seeds. The hide included the wool and it could be speculated that the supply of intestinal amino acids, particularly the sulphur-containing amino acids (SAA), was improved by the extrusion process. Wool growth is a function mainly of the amount of amino acids that reach the intestine rather than energy supply (Rodehutscord *et al*., 1999). Furthermore, the amino acid pattern of the protein that reaches the intestine may affect wool growth, since SAA are first limiting in terms of wool protein synthesis (Rodehutscord *et al*., 1999). It has been reported that the ruminally degradable proportion of dietary protein becomes a critical factor for wool growth and, on isonitrogenous diets, wool growth responds well to less degradable proteins, particularly when they are high in SAA concentration (Rodehutscord *et al*., 1999).

Section data are reported in Table 4. Protein sources influenced (P <0.01) the percentages of loins on the half-carcass weight, which were higher (P <0.01) in lambs that consumed pea seed than in lambs that received the remaining protein sources. As discussed, the results could be ascribed to differences in feed intake.

No significant differences were found between groups for the other section parameters.

Table 4 Effects of protein source and extrusion treatment on section data (% of half carcass weight) of fattening lambs

	Protein source (P)			Treatment (T)			*P*-value		
	Faba bean	Lupin	Pea	Raw	Extruded	MSE[1]	P	T	P × T
Half carcass weight (kg)	4.45	4.36	4.31	4.43	4.31	0.612	0.813	0.492	0.755
Half carcass composition (%)									
Neck	9.56	9.16	9.12	9.41	9.15	0.676	0.137	0.199	0.119
Steaks	15.22	14.88	14.47	14.68	15.03	0.998	0.115	0.221	0.269
Brisket	11.58	11.41	11.22	11.24	11.57	0.734	0.390	0.128	0.738
Shoulder	16.81	17.11	16.59	16.95	16.72	0.683	0.110	0.268	0.394
Loin	8.27[B]	8.35[B]	8.94[A]	8.61	8.44	0.603	0.006	0.339	0.064
Abdominal region	4.86	4.91	5.03	4.94	4.92	0.506	0.616	0.901	0.287
Leg	31.79	32.18	32.32	32.03	32.17	0.838	0.193	0.570	0.663
Kidney fat	0.72	0.87	0.97	0.89	0.82	0.302	0.063	0.475	0.370
Kidney	0.80	0.77	0.86	0.83	0.79	0.148	0.251	0.314	0.264
Testicle	0.38	0.36	0.46	0.43	0.37	0.133	0.079	0.150	0.159

[1] Mean square error
[A, B] row least squares means with different superscripts differ significantly at P <0.01

Data on the dissection of legs and loins are shown in Table 5. Seed treatment affected (P <0.05) leg fat percentages and lean-to-fat ratios. It is well known that a number of components in legumes may exert a negative effect on the nutritional quality of their proteins. Protease inhibitors and lectins are among these components (Masoero *et al*., 2005). Protease inhibitors exert their antinutritional effect by reducing or preventing the digestion of nutrients and possibly impairing body metabolism, growth and health (Grant, 1999). Owing to their ability to bind to carbohydrate receptors of gut epithelial cells, lectins not only interfere with nutrient absorption but may also be taken up systemically and affect hormone balance and lipid and muscle metabolism (Grant, 1999). It could be speculated that the effect of extrusion on leg fat percentages was related to the inactivation of these antinutritional factors. Consistently with this speculation, Alonso *et al*. (2002) hypothesized reduced body lipid deposition in rats by feeding extruded pea diets.

Protein sources affected the percentages of lean (P <0.01) and fat (P <0.01) ratios, as well as lean to fat ratios (P <0.01) in loins. Lean percentages were lower in loins from pea-fed lambs than in loins from lupin-fed (P <0.01) or faba bean-fed (P <0.05) lambs. On the contrary, fat percentages were higher in loins from pea-fed lambs in comparison with loins from lupin-fed (P <0.01) or faba bean-fed (P <0.05) lambs. Consequently, lambs fed pea seeds had loins with lower lean-to-fat ratios than lambs fed lupin (P <0.01) or faba bean (P <0.05) seeds. These results could be related to the differences in ADFI, because the amount of carcass fat depots has been positively related to energy intake (Chestnutt, 1994; Carrasco *et al*., 2009).

The effects of protein source and processing treatment on blood constituents are presented in Table 6. The concentrations of the plasma metabolites in all the experimental animals were within the typical ranges reported for sheep (Abdel-Ghani *et al*., 2011; Lestingi *et al*., 2015b; Lestingi *et al*., 2016).

Table 5 Effects of protein source and extrusion treatment on composition of lamb leg and loin

	Protein source (P)			Treatment (T)			*P*-value		
	Faba bean	Lupin	Pea	Raw	Extruded	MSE[1]	P	T	P × T
Leg weight (kg)	1.43	1.43	1.40	1.41	1.42	0.207	0.872	0.890	0.480
Lean (% leg weight)	62.83	64.46	63.01	62.98	6.89	3.418	0.348	0.362	0.550
Fat (% leg weight)	10.88	10.70	11.54	11.67[a]	10.41[b]	2.037	0.474	0.037	0.236
Bone (% leg weight)	26.28	24.84	25.44	25.34	25.70	3.021	0.411	0.684	0.074
Lean/bone	2.46	2.64	2.45	2.50	2.56	0.384	0.375	0.605	0.183
Lean+fat/bone	2.89	3.07	2.94	2.96	2.97	0.419	0.436	0.942	0.093
Lean/ fat	5.97	6.32	5.69	5.55[b]	6.44[a]	1.360	0.431	0.028	0.610
Loin weight (kg)	0.37	0.37	0.39	0.39	0.37	0.060	0.503	0.273	0.551
Lean (% loin weight)	45.65[a]	46.73[A]	41.98[Bb]	45.29	44.29	4.208	0.007	0.413	0.485
Fat (% loin weight)	23.19[b]	22.19[B]	25.99[Aa]	23.55	24.03	3.949	0.006	0.680	0.967
Bone (% loin weight)	31.15	31.07	32.02	31.15	31.68	4.060	0.765	0.652	0.329
Lean/bone	1.47	1.54	1.36	1.47	1.44	0.283	0.204	0.739	0.321
Lean+fat/bone	2.22	2.27	2.20	2.24	2.23	0.425	0.887	0.932	0.364
Lean/ fat	2.04[a]	2.18[A]	1.65[Bb]	2.01	1.91	0.460	0.008	0.437	0.978

[1] Mean square error
[A, B] row least squares means with different superscripts differ significantly at *P* <0.01
[a, b] row least squares means with different superscripts differ significantly at *P* <0.05

Table 6 Effects of protein source and extrusion treatment on serum constituents of fattening lambs

	Protein source (P)			Treatment (T)			*P*-value		
	Faba bean	Lupin	Pea	Raw	Extruded	MSE[1]	P	T	P × T
Glucose (mg/dl)	74.61[b]	86.80[a]	79.58[ab]	82.03	78.63	10.715	0.013	0.346	0.052
Total cholesterol (mg/dl)	45.43[B]	46.59[B]	60.19[A]	57.54[A]	43.93[B]	11.843	0.001	0.001	0.067
Triglycerides (mg/dl)	23.30	25.01	24.77	25.22	23.50	6.176	0.724	0.366	0.801
Urea (mg/dl)	37.22	35.48	38.71	35.13	39.14	8.684	0.608	0.114	0.440
Creatinine (mg/dl)	1.18	1.15	1.23	1.23	1.15	0.312	0.726	0.383	0.986
Total protein (g/dl)	5.76	5.91	5.70	5.81	5.77	0.358	0.272	0.709	0.697
Albumin (%)	65.69[A]	63.03[B]	65.91[A]	64.79	64.97	1.737	0.001	0.748	0.393
α_1-globulin (%)	6.34[A]	7.21[B]	6.21[A]	6.56	6.62	0.742	0.001	0.763	0.691
α_2-globulin (%)	13.44	13.53	13.31	13.57	13.28	1.042	0.853	0.367	0.906
β-globulin (%)	3.83[a]	4.46[b]	4.43[b]	4.29	4.19	0.702	0.042	0.667	0.026
γ-globulin (%)	11.33[a]	11.69[A]	10.09[Bb]	10.70	11.37	1.262	0.003	0.090	0.345
Albumin/Globulin	1.88[A]	1.71[B]	1.94[A]	1.84	1.84	0.123	0.001	0.889	0.126

[1] Mean square error
[A, B] row least squares means with different superscripts differ significantly at *P* <0.01
[a, b] row least squares means with different superscripts differ significantly at *P* <0.05

Lambs that were fed lupin seeds had a greater concentration of plasma glucose than lambs fed faba bean seeds (*P* <0.05). Lambs that were fed peas were intermediate and not different from those fed either of the other seeds. The higher glucose concentrations in lambs fed lupin diets compared with those fed faba bean diets may be explained by the dietary inclusion rates of barley, which is rich in rapidly degradable starch in the rumen.

Feeding pea seed increased (P <0.01) total cholesterol concentration relative to the other two legume seeds. Decreased levels of total cholesterol in the blood of lambs that were fed on diets containing faba bean and lupin seed were observed in a previous study (Facciolongo *et al*., 2014). These two protein sources have been shown to have beneficial effects in animals and humans by reducing total serum cholesterol (Macarulla *et al*., 2001; Viveros *et al*., 2007). Their hypocholesterolaemic effect is confirmed in the present study.

Processing legume seed by extrusion exerted a diminishing effect (P <0.01) on plasma total cholesterol concentrations. This decrease may be related to some positive changes in rumen fermentation and population of microorganisms (bacteria and protozoa). The processing of legume seeds can also manipulate the sites of digestion of starch and of protein (Solanas *et al*., 2008). Extrusion disrupts the protein matrix surrounding starch granules, and results in the extensive gelatinization of the starch, rendering it more susceptible to enzymatic hydrolysis in the rumen (Offner *et al*., 2003). This consequently increases the energy that is available for microbial growth. Therefore, the extrusion in the conditions of this experiment may have resulted in starch gelatinization, thereby increasing its availability to enzymatic attack and consequently its fermentation in the rumen. Such a hypothesis is consistent with the results of Masoero *et al*. (2005) who observed increased starch digestibility in vitro of these extruded seeds (that is peas, faba beans, and lupins) compared with the untreated seeds. The higher degradability of starch in the rumen has been shown to change rumen fermentation patterns by increasing that production of short chain fatty acids, (particularly propionate), which has further been related to reduced synthesis of triglyceride and cholesterol in the liver cells, as well as to changes in the lipid profile of blood (Malekkhahi *et al*., 2015).

Lupin-fed lambs had reduced (P <0.01) percentages of serum albumin compared with lambs that were fed the other two protein sources, while they showed the opposite effect on α_1-globulin percentages (P <0.01). The significant effect of P × T interaction showed that extrusion increased the β-globulin content of faba beans, but reduced it for lupin seeds and peas (Table 7). Lambs that consumed raw faba bean seed had reduced (P <0.05) serum β-globulin concentration compared with lambs that received the other raw legume seeds. However, serum β-globulin concentrations did not differ among the legume seeds when they were fed to the lambs after being extruded.

Table 7 Effect of interaction between protein source and extrusion treatment on β-globulin in blood of fattening lambs (least squares mean ± standard error)

	Faba bean		Lupin		Pea	
	Raw	Extruded	Raw	Extruded	Raw	Extruded
β-globulin, %	$3.46^a \pm 0.26$	$4.20^b \pm 0.29$	$4.58^c \pm 0.26$	$4.34^b \pm 0.26$	$4.83^c \pm 0.26$	$4.04^b \pm 0.26$

[a, b, c] Least squares means with different superscripts differ significantly at P <0.05

Lambs that received the pea diet had reduced γ-globulin percentages compared with lambs that consumed the lupin (P <0.01) or faba bean (P <0.05) diets. The present results pertaining to serum protein fractions are in agreement with previous observations from lambs that received the same protein sources (Facciolongo *et al*., 2014). In particular, serum albumin levels were reduced by the addition of lupin seeds to the diet of chickens (Viveros *et al*., 2007). The higher α_1-globulin percentages observed in lambs that received lupin seeds compared with lambs that were fed with the other protein sources may be related to a cholesterol-reducing effect of lupin seed. The protein sources used in the current study have nutraceutical properties that can affect serum cholesterol, as mentioned above, although they do not all have the same efficacy, as shown in studies on rats (Macarulla *et al*., 2001; Sirtori *et al*., 2004). The lipid-lowering mechanism of these legume grains seems to be carried out through various mechanisms including their effect on the serum levels of high-density, very low density and low density lipoproteins, which are known to migrate in the fractions α_1, α_2, and β of serum protein electrophoresis, respectively (Kaneko, 1989). Similarly, it may be assumed that the lowest β-globulin percentages that were observed in lambs fed with raw faba bean rations, in comparison with the rations containing the other two raw seeds, could be associated with the cholesterol-lowering effect of faba bean seed. The lack of differences in lambs that were fed with extruded grain legumes might be associated with the cholesterol-lowering effect that is exerted by extrusion. The most significant *γ*-globulins are immunoglobulins (antibodies) and these are important protein for proper body functioning, specifically the immune system, being the antibodies that protect from the attack of pathogens. Recently, enzymatic protein hydrolysates of yellow pea seed have been shown to possess high antioxidant and antibacterial activities (Ndiaye *et al*., 2012). The relatively low *γ*-globulin percentages observed in pea-fed lambs support the speculation that pea proteins may have interfered with the immune system of the animals. Finally, A/G ratios were lower (*P* <0.01) in lambs from lupin group compared with lambs that were fed peas or faba beans. The A/G ratios obtained for the lupin-fed lambs are related to the differences previously observed in the individual fractions.

Conclusions

The use of pea seeds in diets for fattening lambs increased their feed intake compared with diets that contained lupin or faba bean as protein sources. However, this increase in feed intake did not result in a significant improvement in their growth. Carcass conformation, the percentage of loin in the half carcass weight, and the percentages of lean and fat in the loin were the only parameters that were affected by protein sources, probably because of the changes in feed intake. Various blood parameters were influenced by protein sources. This would be indicative of the interference that the legume seeds may have in the metabolism of nutrients and in maintaining a good state of health. Extrusion of the oils from these seeds had few effects, resulting only in differences in hide weight as a percentage of empty BW, the percentage of fat in the leg, and blood cholesterol level. The results would lead the authors to speculate on a relationship between consumption of extruded grain legumes and lipid and protein metabolism. Further work is required to investigate the use of these protein sources and their post-harvest processing, especially in terms of more recently selected lupine varieties and their effects on meat quality.

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Authors' Contributions

All the authors contributed from the onset of the study and approved of the final version.

Conflict of Interest Declaration

The authors have no conflict of interest to declare.

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