

Journal homepage: http://www.sasas.co.za/journals



South African Journal of Animal Science 2018, 48 (No. 3)

Effects of replacing soybean meal with alternative sources of protein on nutrient digestibility and energy value of sheep diets

K. Zagorakis^{1#}, D. Liamadis¹, Ch. Milis², V. Dotas¹ & D. Dotas¹

¹ Department of Animal Nutrition, Aristotle University of Thessaloniki, 54124, Macedonia, Greece ² Ministry of Rural Development and Foods, Feedstuffs' Analysis and Control Laboratory in Thessaloniki, Thermi 57001, Greece

(Received 23 August 2016; Accepted 26 January 2018; First published online 16 February 2018)

Copyright resides with the authors in terms of the Creative Commons Attribution 4.0 South African Licence. See: http://creativecommons.org/licenses/by/4.0/za Condition of use: The user may copy, distribute, transmit and adapt the work, but must recognise the authors and the South African Journal of Animal Science.

Abstract

The study determined the potential of eight locally produced protein sources to replace soybean meal (SBM) in the diet of sheep. Three *in vivo* digestibility trials were conducted using a Latin square (3x3, 4x4 and 4x4) experimental design with castrated Chios rams. The authors estimated nutrient digestibility coefficients and energy value of diets with soybean meal (control), lupin seeds (LS), rapeseed meal (RSM), sunflower meal (SFM), fababean seeds (FBS), vetch seeds (VS), pea seeds (PS), flaxseeds (FS), and chickpea seeds (CS). The results showed that the SBM, RSM, and PS diets had similar nutrient digestibility and energy value. The SFM diet had lower organic matter (OM) digestibility than the SBM diet. The FS and SFM diets had similar crude protein (CP) digestibility to the SBM diet. Additionally, FS, VS, and SBM diets had similar energy value. Furthermore, SBM, SFM, VS and FS diets had similar dry matter (DM), neutral detergent fibre (NDF), hemicelluloses, and cellulose digestibility coefficients. Additionally, SBM, LS, and CS diets had similar energy value. This study reveals that diets with RSM, PS, FS, and LS, compared with diets with SBM, did not have adverse effects on nutrient digestibility and energy value. These results tend to support the idea that some locally available protein sources seem to have the potential to replace SBM in sheep diets.

Keywords: Dietary supplementation, nutrition, nutrient digestibility, protein, ruminants [#] Corresponding author: konstantinoszagorakis@yahoo.gr

Introduction

The rapid growth in human population has increased the demand for proteins from animals. Soybean meal (SBM) has been established as the main protein source for animal nutrition. However, high prices, because of the increased costs of production and transportation, and fluctuation in production, have necessitated the search for alternative locally produced protein sources for feeding livestock. Moreover, customers are aware of the usage of genetically modified SBM, although there is no clear scientific evidence for its association with health problems (Domingo & Bordonada, 2011). These challenges were recognized by international bodies such as the European Union, which encourage self-reliance via the support of cultivation and production of protein supplements from locally produced ingredients.

The interest in legume seeds as a source of protein in ruminant feeding has increased, mainly because of the positive ecological functions in crop rotation of these plants. Moreover, SBM could be replaced in animal diets by many other feeds and by-products, such as oilseeds and oilseed residues. However, these two categories of feeds have different CP and ether extract (EE) content. According to Fiorentini *et al.* (2013), lipid metabolism is highly variable in the rumen and total tract owing to factors such as the nature and concentration of lipids in the diet, the types of chemical and physical treatments applied to the feeds, and their characteristics, and the forage to concentrate ratio in the diet. Additionally, genetic selection for better crops normally focuses on better crop yield, with limited interest in their nutritional value. It should not be forgotten that local climate may affect the nutritional value of crops. Other factors include

processing methods. For these reasons, continuous evaluation of the nutritional value of animal feeds should be carried out for each production area. With this in mind, the eight most promising protein sources were selected for comparison with SBM in terms of digestibility of diet nutrients. The protein sources included two meals (SFM and RSM) and six untreated ground seeds (LS, FBS, VS, PS, linseed or FS, and CS). Most of these feeds used to be cultivated extensively in past decades in Europe and the wider Mediterranean area.

There are many types of SFM, depending on the variety of sunflower seeds and the method of processing. Molina *et al.* (2003) reported that SFM could be a good supplement to low degradable protein feedstuffs. Additionally, the increased interest in biodiesel has renewed the demand for oils from rapeseed. The RSM is one of the main protein sources used in animal feeding, and has been used successfully as a protein feed in dairy cow diets (Mulrooney *et al.*, 2009).

The PS and FBS are interesting crops to grow in cool temperate climate conditions, as they can partly replace imported protein sources owing to their relatively high CP content (Larsen *et al.*, 2009). Additionally, LS can successfully replace soybean meal in dairy cow diets (Froidmont & Bartiaux-Thill, 2004). Compared with PS, LS contains more nitrogen (N) and EE, and therefore qualifies as a high-quality feedstuff for ruminants (Froidmont & Bartiaux-Thill, 2004).

The chickpea seed is cultivated principally as a legume crop. According to the literature, it could replace SBM in lamb fattening diets (Hadjipanayiotou, 2002). Furthermore, increasing FS inclusion in cow diets did not affect digestibility or milk yield (Martin *et al.*, 2016), while, according to Schroeder *et al.* (2014), feeding steers with FS did not affect CP total tract digestibility. The VS is a multipurpose, cool season, annual legume grown for livestock feed and soil fertility improvement in Mediterranean environments. The hay and grain could be used as sources of protein in ruminant diets (Gul *et al.*, 2008).

The objectives of this study were to determine the effects of eight alternatives to SBM protein sources on apparent total tract nutrient digestibility and energy value of sheep diets. Since no single study has ever compared these N supplements in terms of energy value and nutrient digestibility of diets, the main goal of this study was to evaluate the capability of these protein sources to totally replace SBM in sheep diets and to provide crucial information that could be used to make this replacement feasible.

Materials and methods

The management and care of animals were approved by the protocol of Aristotle University of Thessaloniki, Greece. Three experiments were carried out. An *in vivo* digestibility trial (Trial 1) was conducted with three castrated rams, aged 8 to 9 months and weighing 42±1 kg, using three diets in a 3x3 Latin square experimental design. The three diets were isocaloric and were formulated to meet the maintenance energy requirements of rams (Table 1). The tested feeds were SBM, RSM and PS. All the seeds were ground into meal. Experimental diets were fed in two equal amounts at 08:00 and 17:00 each day (Tables 1 and 2). Each of the three periods consisted of 8 days' adaptation and 7 days of sample collection. Water was freely accessible through individual drinkers. Faeces and urine were collected using the total collection for seven days for each treatment, weighed at approximately 08:00 each day, and composted by treatment and ram. Samples were stored at a temperature of 2–3 °C until all the samples for that collection period had been taken. Diet samples were taken for laboratory analysis by grab sampling as the feed allowances were being weighted. Diets were made up of alfalfa hay (450 g/day), ground corn grain (390 g/day) and 145 g/day SBM (SBM diet), 170 g/day RSM (RSM diet) and 145 g/day PS (PS diet).

The same procedure was used for the second digestibility trial (Trial 2). This experiment used rams aged 15 to 16 months, with a live bodyweight of 58±1kg. The experimental design was a 4x4 design. Diets corresponded to maintenance requirements for energy with protein sources being SBM, SFM, VS or FS (Tables 1 and 2). Diets were made of alfalfa hay (580 g/day), ground corn grain (470 g/day), and 170 g/day FS (FS diet), 245 g/day SFM (SFM diet), 200 g/day VS (VS diet) and 200 g/day SBM (SBM diet).

The same procedure was used for the third digestibility trial (Trial 3). This time the experiment used rams aged 20 to 21 months with a live bodyweight of 61±1kg. The tested feeds were SBM, LS, FBS, and CS (Tables 1 and 2). Diets were made up of alfalfa hay (580 g/day), ground corn grain (490 g/day) and 205 g/day SBM (SBM diet), 190 g/day LS (LS diet), 215 g/day FBS (FBS diet) and 215 g/day CS (CS diet).

Feed and composite faecal samples were ground to pass through a 1-mm screen (AOAC, 2006; method 950.02B). Dry matter (DM) was determined by drying in an oven (AOAC, 2006; method 934.01). Ash was determined by ignition in a muffle furnace (AOAC, 2006; method 942.05). The CP was measured as Kjeldahl N x 6.25 (AOAC, 2006; method 984.13). Ether extract (EE) was determined according to AOAC (2006; method 920.39). Acid detergent fibre (ADF) and acid detergent lignin (ADL) were determined using AOAC (2006; method 973.18) and NDF using AOAC (2006; official 2002.04). The NDF was assayed without a heat stable amylase and expressed inclusive of residual ash. Additionally, ADF was expressed inclusive of residual ash. The hemicellulose value was measured as the difference between NDF and ADF, and the

analyses were sequential on the same sample. Gross energy (GE) was measured with an adiabatic bomb calorimeter (Parr Instrument Co., 1970).

Diet	First digestibility trial ¹			Second digestibility trial ²			Third digestibility trial ³				
Diet	SBM	RSM	PS	FS	SFM	VS	SBM	SBM	LS	FBS	CS
Composition %											
Alfalfa hay	43.9	42.9	43.9	46.0	43.4	45.0	45.0	44.1	44.6	43.8	43.8
Corn grain	38.0	37.1	38.0	37.3	35.2	36.4	36.4	37.3	37.7	37.0	37.0
SBM	14.1	0	0	0	0	0	15.5	15.6	0	0	0
PS	0	0	14.1	0	0	0	0	0	0	0	0
RSM	0	16.2	0	0	0	0	0	0	0	0	0
FS	0	0	0	13.5	0	0	0	0	0	0	0
SFM	0	0	0	0	18.4	0	0	0	0	0	0
VS	0	0	0	0	0	15.5	0	0	0	0	0
LS	0	0	0	0	0	0	0	0	14.6	0	0
FBS	0	0	0	0	0	0	0	0	0	16.2	0
CS	0	0	0	0	0	0	0	0	0	0	16.2
Salt	1.0	1.0	1.0	0.8	0.7	0.8	0.8	0.8	0.8	0.8	0.8
Ca(H ₂ PO ₄) ₂	1.5	1.4	1.5	1.2	1.1	1.2	1.2	1.1	1.2	1.1	1.1
Premix	1.5	1.4	1.5	1.2	1.1	1.2	1.2	1.1	1.2	1.1	1.1
Sum	100	100	100	100	100	100	100	100	100	100	100
Chemical analysis (g	g/kg DM)										
Organic matter	942	942	948	946	943	948	944	945	949	949	950
Crude protein	210	188	175	150	166	158	189	189	171	160	147
Ether extract	22	29	20	64	21	23	24	25	32	22	29
NDF	369	404	371	381	385	378	335	362	385	394	361
ADF	187	207	185	203	206	162	164	184	198	191	177
Nutritional value ⁴											
ME (MJ/kg DM) ⁵	11.1	10.8	11.1	11.5	10.6	11.1	11.1	11.1	11.2	11.1	11.0
PDI (g/kg DM)⁵	127.5	120.3	105.6	103.8	112.9	109.3	129.3	129.6	112.1	109.0	104.7
DMI (g/day) ⁵	848	873	848	1055	1126	1074	1077	1099	1087	1105	1107

Table 1 Composition (%), chemical analysis (g/kg DM) and nutritional value (ME, PDI and DMI)⁵ of the experimental diets

¹ Diets were made of alfalfa hay (450 g/day), ground corn grain (390 g/day) and 145 g/day SBM (SBM diet), 170 g/day RSM (RSM diet) and 145 g/day PS (PS diet) ² Diets were made of alfalfa hay (590 g/day), ground corn grain (470 g/day) and 145 g/day SBM (SBM diet), 170 g/day

² Diets were made of alfalfa hay (580 g/day), ground corn grain (470 g/day) and 170 g/day FS (FS diet), 245 g/day SFM (SFM diet), 200 g/day VS (VS diet) and 200 g/day SBM (SBM diet)

³ Diets were made of alfalfa hay (580 g/day), ground corn grain (490 g/day) and 205 g/day SBM (SBM diet), 190 g/day LS (LS diet), 215 g/day FBS (FBS diet) and 215 g/day CS (CS diet)

⁴ME and PDI values calculated according to INRA (1988)

⁵ ME: metabolizable energy; PDI: Protein Digestible Intestine; DMI: Dry Matter Intake; NDF: Neutral Detergent Fibre; ADF: Acid Detergent Fibre

Urinary N content (UN %) was measured according to the Kjeldahl method (AOAC, 1990). Urinary energy (UE) was calculated using the equation UE (kcal/g) = 0.027 + 0.119 (UN%) (Street *et al.*, 1964). According to Blaxter & Clapperton (1965), gaseous energy (G) is broadly proportional to the apparent digestibility of the diet and at a maintenance level of nutrition can be calculated by the following equation:

100G/I = 3.67 + 6.22 (I-F)/I

I is the intake of energy

F is the faecal energy

Additionally, digestible energy (DE) was calculated as DE = GE-F and ME by the difference ME=DE-UE-G. For all the methods, measurements were made in triplicate and standards were included in each run of each method. Digestibility of nutrient coefficients and energy value were analysed statistically using S-Plus (2001). Significance was declared at P < 0.05.

Table 2 Chemical composition (g/kg DM) of protein supplements

Composition (g/kg DM)RSMPSFSSFMVSLSFBSCSDry matter (g/kg)898880910920870890870880Organic matter926964945930960964960966Crude protein318257251300287382299220Ether extract639302918741052NDF416202456452427288344155ADF209663122985113710325Hemicelluloses207136144154376151241130Cellulose845321215325666324										
Organic matter926964945930960964960966Crude protein318257251300287382299220Ether extract639302918741052NDF416202456452427288344155ADF209663122985113710325Hemicelluloses207136144154376151241130	Composition (g/kg DM)	RSM	PS	FS	SFM	VS	LS	FBS	CS	SBM
Organic matter926964945930960964960966Crude protein318257251300287382299220Ether extract639302918741052NDF416202456452427288344155ADF209663122985113710325Hemicelluloses207136144154376151241130	Dry matter (g/kg)	898	880	910	920	870	890	870	880	886
Ether extract639302918741052NDF416202456452427288344155ADF209663122985113710325Hemicelluloses207136144154376151241130	, (6 6,									934
NDF416202456452427288344155ADF209663122985113710325Hemicelluloses207136144154376151241130	Crude protein	318	257	251	300	287	382	299	220	478
ADF209663122985113710325Hemicelluloses207136144154376151241130	Ether extract	63	9	302	9	18	74	10	52	25
Hemicelluloses 207 136 144 154 376 151 241 130	NDF	416	202	456	452	427	288	344	155	152
	ADF	209	66	312	298	51	137	103	25	59
Cellulose 84 53 212 153 25 66 63 24	Hemicelluloses	207	136	144	154	376	151	241	130	93
	Cellulose	84	53	212	153	25	66	63	24	29

RSM: rapeseed meal; PS: ground pea seed; FS: ground linseed; SFM: sunflower meal; VS: ground vetch seed; LS: ground lupin seed; FBS: ground faba bean seed; CS: ground chickpea seed; SBM: soybean meal; NDF: Neutral Detergent Fibre; ADF: Acid Detergent Fibre

Results

The results from Trial 1 are given in Table 3. Statistical analysis revealed that the SBM, RSM and PS diets had no differences (P > 0.05) in terms of DM, OM, CP, EE, NDF, ADF, hemicelluloses, cellulose DE/GE, ME/GE and ME/DE parameters.

Table 3 Mean values of nutrient digestibility and energy value (expressed as DE/GE, ME/GE and ME/DE)¹ of sheep diets: Trial 1

ltem (%)	Diet A (SBM)	Diet B (RSM)	Diet C (PS)	SEM	Р
Dry matter	71.9	67.8	70.5	0.778	NS
Organic matter	75.6	71.8	74.3	0.681	NS
Crude protein	74.5	67.3	69.1	1.645	NS
Ether extract	70.3	73.3	63.8	1.710	NS
NDF	55.5	51.4	52.0	1.764	NS
ADF	53.0	45.7	47.9	1.369	NS
Hemicelluloses	58.2	57.5	56.1	2.960	NS
Cellulose	68.1	63.2	68.7	3.862	NS
DE/GE	71.2	67.0	69.2	0.846	NS
ME/GE	61.3	57.3	59.3	1.050	NS
ME/DE	86.1	85.5	85.6	0.502	NS

SBM: soybean meal; RSM: rapeseed meal; PS: ground pea seed

¹ DE/GE: Digestible Energy/Gross Energy; ME/GE: Metabolizable Energy/Gross Energy; ME/DE: Metabolizable Energy/Digestible Energy

NS: not significant;

The results from Trial 2 are given in Table 4. Statistical analysis showed that the SFM diet is not similar to the SBM diet in terms of OM and DE/GE parameters. Also, the SFM diet is not similar to the FS diet in terms of DE/GE parameters. Additionally, the VS diet is not similar to the SBM diet in terms of CP parameter. Furthermore, the FS diet is not similar to the SFM, VS, and SBM diets in terms of EE parameter. Additionally, the FS diet is not similar to the SFM and VS diets in terms of ADF parameter.

The results from Trial 3 are given in Table 5. Statistical analysis revealed that the SBM diet is not similar to the FBS diet in terms of CP, DE/GE, and ME/GE parameters. Also, the SBM diet is not similar to the CS diet in terms of the CP parameter. Additionally, the CS diet is not similar to the FBS diet in terms of the EE parameter. Furthermore, the LS diet is not similar to the FBS diet in terms of the ADF parameter.

Item (%)	Diet A (FS)	Diet B (SFM)	Diet C (VS)	Diet D (SBM)	SEM	Р
Dry matter	67.2	64.4	67.2	68.9	1.058	NS
Organic matter	70.9 ^{ab}	67.9 ^b	71.3 ^{ab}	72.8 ^a	0.964	*
Crude protein	66.2 ^{ab}	67.0 ^{ab}	63.9 ^b	70.2 ^a	1.294	*
Ether extract	85.5 ^a	69.1 ^b	72.2 ^b	73.1 ^b	1.317	*
NDF	53.1	43.4	51.1	48.7	2.263	NS
ADF	46.9 ^a	34.2 ^b	33.0 ^b	39.6 ^{ab}	2.418	*
Hemicelluloses	60.1	54.0	64.7	57.5	2.433	NS
Cellulose	38.1	38.7	35.7	40.3	2.037	NS
DE/GE	67.3 ^a	62.7 ^b	66.0 ^{ab}	68.0 ^a	1.039	*
ME/GE	55.5	50.6	53.3	55.7	1.359	NS
ME/DE	82.5	80.5	80.9	81.8	1.758	NS

Table 4 Mean values of nutrient digestibility and energy value (expressed as DE/GE, ME/GE and ME/DE)¹ of sheep diets: Trial 2

^{a, b} Means in the same row with different superscript are different at *P* <0.05; NS: not significant; * Treatment effect; FS: ground linseed; SFM: sunflower meal; VS: ground vetch seed; SBM: soybean meal

¹ DE/GE: Digestible Energy/Gross Energy; ME/GE: Metabolizable Energy/Gross Energy; ME/DE: Metabolizable Energy/Digestible Energy

Table 5 Mean values of nutrient digestibility and energy value (expressed as DE/GE, ME/GE and ME/DE) ¹	of
sheep diets: Trial 3	

ltem (%)	Diet A (SBM)	Diet B (LS)	Diet C (FBS)	Diet D (CS)	SEM	Р
Dry matter	70.9	70.0	68.7	70.3	0.925	NS
Organic matter	73.6	72.6	71.6	72.9	0.896	NS
Crude protein	72.1 ^a	71.7 ^{ab}	69.0 ^b	68.0 ^b	1.017	*
Ether extract	76.7 ^{ab}	78.5 ^{ab}	75.3 ^b	79.8 ^a	1.122	*
NDF	53.1	55.0	52.0	50.7	1.689	NS
ADF	41.7 ^{ab}	45.8 ^a	38.3 ^b	39.9 ^{ab}	1.893	*
Hemicelluloses	64.9	64.7	64.8	61.0	1.396	NS
Cellulose	51.5	54.5	50.1	50.7	1.824	NS
DE/GE	69.7 ^a	68.6 ^{ab}	66.9 ^b	68.7 ^{ab}	0.617	*
ME/GE	59.3 ^a	58.7 ^{ab}	57.0 ^b	58.4 ^{ab}	0.664	*
ME/DE	85.1	85.5	85.2	85.0	0.417	NS

^{a, b} Means in the same row with different superscript are different at *P* <0.05; NS: not significant; *Treatment effect; SBM: soybean meal; LS: ground lupin seed; FBS: ground fababean seed; CS: ground chickpea seed

¹ DE/GE: Digestible Energy/Gross Energy; ME/GE: Metabolizable Energy/Gross Energy; ME/DE: Metabolizable Energy/Digestible Energy

Discussion

The results of Trial 1 suggest that the nutritional value of sheep diets would not be negatively affected by replacing 100% SBM with RSM and PS. Similarly, Maxin et al. (2013) reported that RSM could replace SBM in dairy cow diets, while Petit et al. (1997) reported that PS and SBM have similar DE. Additionally, Gilbery et al. (2007) reported that replacing RSM with PS or CS in cow diets did not affect CP digestibility. It seems that RSM and PS could totally replace SBM in sheep diets, despite the significant differences in their chemical composition (CP, NDF and ADF). Additionally, the present study revealed that OM digestibility of the RSM diet was 71.8%, a value similar to that of 73.2% reported by Eghbali et al. (2011) for the same diet. Furthermore, Górka et al. (2015) reported that replacing RSM and barley grain with high-lipid (9%) byproduct pellets in heifer diets affected total tract nutrient digestibility negatively. This is not inconsistent with the current results because in the present study, replacing SBM with RSM or FS had no effect on diet digestibility coefficients, probably because lipid metabolism is highly variable owing to many factors, such as the nature and concentration of lipids in the diet (Fiorentini et al., 2013). Furthermore, in line with the current results, Hentz et al. (2012) reported that feeding wethers with increasing levels of RSM (up to15 g/kg of bodyweight) had no effect on diet NDF digestibility. On the other hand, Gilbery et al. (2007) reported that replacing RSM with PS positively affected the diet's ADF digestibility, which is in contrast with the current results, possibly because the animals in the current study were fed at maintenance level, and the digestibility of nutrients did not compromise nutrient demand at this level. As a result, differences in nutrient requirements (maintenance vs growth vs reproduction vs wool growth) might influence the digestibility coefficients of the diets in different ways.

Eweedah *et al.* (1996) reported similar CP and ADF, and lower OM digestibility values for lamb diets with SFM or SBM. These findings are in agreement with the current results. Furthermore, Irshaid *et al.* (2003) reported that replacing SBM with increasing levels of SFM (up to100%) in lamb diets had no effect on the DM, OM, CP, NDF, and ADF digestibility of the diets. Additionally, it was reported in a previous study (Richardson *et al.*, 1981), that replacing SBM with SFM did not affect DM, CP, and ADF digestibility of the diets. Data of the present study indicate the same effects. Furthermore, according to Molina *et al.* (2003), the addition of increasing amounts of SFM improved the in vitro DM and OM digestibility of olive cake and olive leaves. It seems that SFM diets could replace SBM in ruminant diets, with limited negative effects on the digestibility coefficients of the most nutritious components (excluding OM digestibility) and on their energy value (excluding digestibility of energy). The chemical composition of commercial flours varies widely, which also affects their nutritional value.

Similar energy values of the VS diet compared with the SFM, SBM and FS diets, but lower CP and similar DM, OM, EE, NDF and ADF digestibility in comparison with the SBM diet in Trial 2, are consistent with results reported by Seifdavati *et al.* (2012). Replacing SBM with VS affected the CP digestibility of the diets negatively. Although these two diets were isocaloric, the VS diet had a higher energy to protein ratio – the VS diet had lower CP content – which negatively affected the nitrogen sufficiency in the rumen, resulting in a possible increase in rumen fermentable protein to meet the needs of microorganisms, thereby reducing the efficiency of CP utilization. Furthermore, it was confirmed in the present study that the digestibility of NDF is not affected by the protein source of the diet, which is consistent with the findings of Seifdavati *et al.* (2012). Additionally, this study confirmed the results of Ohsihita *et al.* (1997) that the addition of SBM in ruminant diets has no effect on digestibility of hemicelluloses.

According to the literature, the addition of FS to steer and cow diets had limited effects on digestibility and milk yield (Schroeder et al., 2014; Martin et al., 2016). Furthermore, Gonthier et al. (2004) reported that the addition of FS to cow diets tended to increase their DM, OM, CP, NDF, and GE digestibility. These reports are in line with the current findings. Additionally, the FS diet showed higher EE digestibility than the SFM, VS and SBM diets, probably because of the superior EE content of FS. In line with the results of the present study, Bernard (1990) reported that diets with high EE content had high EE digestibility coefficients. The high EE digestibility of the FS diet was observed concurrently with a higher percentage of endogenous EE in the faeces of the animals fed with diets of low EE content (SFM, VS, and SBM diets). This might result in recording lower apparent total tract digestibility than it is, because of the high proportion of endogenous EE in the faeces, in comparison with EE derived from the feed. The results of the present study indicate that FS did not negatively affect the energy value and nutrient digestibility of sheep diets. These findings are significant and support the central idea of this study that sheep diets with different chemical composition exhibit similar nutritional value. Furthermore, the authors found that replacing FS with SFM or VS affected the ADF digestibility of diets negatively, suggesting that FS is a better ingredient than SFM or VS. This effect may be related to the high ether extract content of FS, which overlaps the feed, and prevents microorganisms from digesting ADF. Seifdavati et al. (2012) reported that replacing SBM with VS in lamb diets had a negative effect on ADF digestibility. Furthermore, Eweedah et al. (1996) reported that digestibility of ADF remained unaffected when SFM replaced SBM in diets. These results are partially in agreement with the current results, probably owing to differences in the chemical composition of SFM (Irshaid *et al.*, 2003).

Results of Trial 3 tend to support the notion that CS and FBS seem to have the potential to replace SBM. Similarly, Tufarelli *et al.* (2012) reported that FBS could replace SBM in dairy cow diets. Furthermore, Mustafa *et al.* (2000) reported that replacing SBM with CS did not affect the digestibility coefficients of nutritious components of diets. Additionally, Abreu & Bruno-Soares (1998) reported that CS, PS, and VS showed similar OM digestibility, but higher OM digestibility, when compared with LS. This variation in results could be attributed to differences in chemical composition in these feeds. Furthermore, consistent with the current result, Gebru *et al.* (2015) reported that the inclusion of white LS in sheep diets improved total DM and nutrient intake, nutrient digestibility, average daily gain, and feed conversion efficiency, and could be recommended not only for maintenance, but also for optimum performance of ruminants. Earlier, Moss *et al.* (1997) reported that feeding cattle with increasing levels of LS improved the DE of the diets. Therefore, it would seem that LS is a good protein and energy source (equal to SBM) for sheep diets, even without further processing except for grinding, with similar energy value and nutrient digestibility coefficients to SBM.

Finally, taking into account that no single study has ever compared all these N supplements in terms of diets' nutrient digestibility and energy value, the authors can say that RSM, PS, FS, and LS could be used as alternatives to SBM protein sources to form sheep diets with different chemical composition, but similar nutritional value. Additionally, SFM, CS, FBS, and VS seem to have the potential to replace SBM in sheep diets. These feeds (SFM, CS, FBS, and VS) could be used in diets of moderately productive sheep. Further investigation is needed to examine the effects of these protein sources and their treated forms on the characteristics of digestion, energy value, growth performance, and milk production of sheep in different feeding conditions.

Conclusions

This study revealed that diets with RSM, PS, FS, and LS compared with diets with SBM have no adverse effect on energy value and nutrient digestibility coefficients. These results support the idea that some locally available protein sources could replace SBM in sheep diets.

Acknowledgements

The authors wish to thank State Scholarships Foundation of Greece for the scholarship (0060821658.001.071 - 16.89) provided to the first author.

Authors' Contributions

Conception, design of experiments and supervision of project by DL and DD. KZ conducted the experiments and data analysis. All authors participated in interpretation of results and writing and integration of the manuscript. Critical revision and final approval of version to be published was also done by all authors.

Conflict of Interest Declaration

None of the authors has any conflict of interest to declare.

References

Abreu, J.M.F. & Bruno-Soares, A.M., 1998. Chemical composition, organic matter digestibility and gas production of nine legume grains. Anim. Feed Sci. Technol. 70, 49-57.

- AOAC, 1990. Association of Official Analytical Chemists International. Official methods of analysis, 15th edition. Arlington, Virginia, USA.
- AOAC, 2006. Association of Official Analytical Chemists, International. Official methods of analysis. 18th edition. Current through revision 1, Maryland, USA.
- Bernard, J.K., 1990. Effect of raw or roasted whole soybeans on digestibility of dietary nutrients and milk production of lactating dairy cows. J. Dairy Sci. 73, 3231-3236.
- Blaxter, K.L. & Clapperton, J.L., 1965. Prediction of the amount of methane produced by ruminants. Br. J. Nutr. 19, 511-522.
- Domingo, J.L. & Bordonada, J.G., 2011. A literature review on the safety assessment of genetically modified plants. Environ. Int. 37, 734-742.
- Eghbali, M., Kafilzadeh, F., Hozhabri, F., Afshar, S. & Kazemi-Bonchenari, M., 2011. Treating canola meal changes in situ degradation, nutrient apparent digestibility, and protein fractions in sheep. Small Rumin. Res. 96, 136-139.
- Eweedah, N., Varhegyi, J., Gundel, J. & Rozsa L., 1996. A note on the use of some oilseeds in compound feeds for lamb: digestibility, rumen fermentation, nutritive value and degradability. Nutr. Abstr. Rev. Ser. B. 68, 216-223.
- Fiorentini, G., Messana, J.D., Dian, P.H., Reis, R.A., Canesin, R.C., Pires, A.V. & Berchielli, T.T., 2013. Digestibility, fermentation and rumen microbiota of crossbred heifers fed diets with different soybean oil availabilities in the rumen. Anim. Feed Sci. Technol. 181, 26-34.
- Froidmont, E. & Bartiaux-Thill, N., 2004. Suitability of lupin and pea seeds as a substitute for soybean meal in highproducing dairy cow feed. Anim. Res. 53, 475-487.
- Gebru, T., Firew, T., Yeshambel, M., Solomon, M. & Atsushi, T., 2015. Effects of different forms of white lupin (Lupinus

albus) grain supplementation on feed intake, digestibility, growth performance and carcass characteristics of Washera sheep fed Rhodes grass *(Chloris gayana)* hay-based diets. Trop. Anim. Health and Prod. 47 (8), 1581-1590.

- Gilbery, T.C., Lardy, G.P., Soto-Navarro, S.A., Bauer, M.L. & Anderson, V.L., 2007. Effect of field peas, chickpeas, and lentils on rumen fermentation, digestion, microbial protein synthesis, and feedlot performance in receiving diets for beef cattle. J. Anim. Sci. 85, 3045-3053.
- Gonthier, C., Mustafa, A.F., Berthiaume, R., Petit, H.V., Martineau, R. & Ouellet, D.R., 2004. Effects of feeding micronized and extruded flaxseed on ruminal fermentation and nutrient utilization by dairy cows. J. Dairy Sci. 87, 1854-1863.
- Górka, P., Castillo-Lopez, E., Joy, F., Chibisa, G.E., McKinnon, J.J. & Penner G.B., 2015. Effect of including high-lipid by-product pellets in substitution for barley grain and canola meal in finishing diets for beef cattle on ruminal fermentation and nutrient digestibility. J. Anim. Sci. 93 (10), 4891-4902.
- Gul, M., Yoruk, M.A., Macit, M., Esenbuga, N., Karaoglu, M., Aksakal, V. & Aksu, M.I., 2008. The effects of diets containing different levels of common vetch (*Vicia sativa*) seed on fattening performance, carcass and meat quality characteristics of Awassi male lambs. J. Sci. Food Agric. 85, 1439-1443.
- Hadjipanayiotou, M., 2002. Replacement of soybean meal and barley grain by chickpeas in lamb and kid fattening diets. Anim. Feed Sci. Technol. 96, 103-109.
- Hentz, F., Kozloski, G.V., Orlandi, T., Avila, S.C., Castagnino, P.S., Stefanello, C.M. & Pacheco, G.F.E.,2012. Intake and digestion by wethers fed a tropical grass-based diet supplemented with increasing levels of canola meal. Livest. Sci. 147, 89-95.
- INRA, 1988. Alimentation des bovins, ovins et caprins. INRA, Paris.
- Irshaid, R.H., Harb, M.Y. & Titi, H.H., 2003. Replacing soybean meal with sunflower seed meal in the ration of Awassi ewes and lambs. Small Rumin. Res. 50, 109-116.
- Larsen, M., Lund, P., Weisbjerg, M.R. & Hvelplund, T., 2009. Digestion site of starch from cereals and legumes in lactating dairy cows. Anim. Feed Sci. Tech. 153, 236-248.
- Martin, C., Ferlay, A., Mosoni, P., Rochette, Y., Chilliard, Y. & Doreau, M., 2016. Increasing linseed supply in dairy cow diets based on hay or corn silage: Effect on enteric methane emission, rumen microbial fermentation, and digestion. J. Dairy Sci.99 (5), 3445-3456.
- Maxin, G., Ouellet, D.R. & Lapierre, H., 2013. Ruminal degradability of dry matter, crude protein, and amino acids in soybean meal, canola meal, corn, and wheat dried distiller grains. J. Dairy Sci. 96 (8), 5151-5160.
- Molina Alcaide, E., Yáñez Ruiz, D.R., Moumen, A. & Martin Garcia, A.I., 2003. Ruminal degradability and *in vitro* intestinal digestibility of sunflower meal and *in vitro* digestibility of olive by-products supplemented with urea or sunflower meal. Comparison between goats and sheep. Anim. Feed Sci. Technol. 110, 3-15.
- Moss, A.R., Givens, D.I., Grundy, E.L.F. & Wheeler, K.P.A., 1997. The nutritive value for ruminants of lupin seeds from determinate plants and their replacement of soya bean meal in diets for young growing cattle. Anim. Feed Sci. Technol. 68,11-23.
- Mulrooney, C.N., Schingoethe, D.J., Kalscheur, K.F. & Hippen, A.R., 2009. Canola meal replacing distillers grains with solubles for lactating dairy cows. J. Dairy Sci. 92, 5669-5676.
- Mustafa, A.F., Thacker, P.A., McKinnon, J.J., Christensen, D.A.& Racz, V.J., 2000. Nutritional value of feed grade chickpeas for ruminants and pigs. J. Sci. Food 86, 134-139.
- Ohshita, T., Kyuma, T. & Kondo, T., 1997. Effects of soybean meal supplement on digestibilities, chewing time and passage rates of digesta in wether fed timothy (*Phleum pratense L.*) hay. J. Jpn. Society Grassland Sci. 67, 65-73.
- Parr Instrument Co., 1970. Manual No. 142. Instructions for 1241 and 1242 Adiabatic Calorimeters. Parr Instrument Co., Moline, Illinois.
- Petit, H., Rioux, R. & Ouellet, D.R., 1997. Milk production and intake of lactating cows fed raw or extruded peas. J. Dairy Sci. 80, 3377-3385.
- Richardson, R.C., Beville, N.R., Ratcliff, K.R. & Albin, C.R., 1981. Sunflower meal as a protein supplement for growing ruminants. J. Anim. Sci. 53 (3), 557-563.
- S-Plus, 2001. User's manual. TIBCO Software Inc., Texas, USA.
- Schroeder, J.W., Bauer, M.L. & Bork, N.R., 2014. Effect of flaxseed physical form on digestibility of lactation diets fed to Holstein steers. J. Dairy Sci. 97 (9), 5718-5728.
- Seifdavati, J., Taghizadeh, A., Janmohammadi, H., Seyd, A.R. & Sadegh, A., 2012. In vivo digestibility and in vitro intestinal digestibility of three legume seeds. Int. J. Agric. Res. Rev. 2 (5), 630-638.
- Street, J.C., Butcher, J.E. & Harris, L.E., 1964. Estimating urine energy from urine nitrogen. J. Anim. Sci. 23, 1039-1041.
- Tufarelli, V., Khan, R.U. & Laudadio, V., 2012. Evaluating the suitability of field beans as a substitute for soybean meal in earlylactating dairy cow: Production and metabolic responses. J. Anim. Sci. 83, 136-140.