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Effect of *Lupinus albus* as protein supplement on yield, constituents, clotting properties and fatty acid composition in ewes' milk

F. Masucci [a,*], A. Di Francia [a], R. Romano [b], M.T. Maresca di Serracapriola [a], G. Lambiase [b], M.L. Varricchio [a], V. Proto [a]

[a] *Dipartimento di Scienze Zootecniche ed Ispezione degli Alimenti, Facoltà di Agraria Università degli Studi di Napoli Federico II, via Università 133, 80055 Portici, Napoli, Italy*
[b] *Dipartimento di Scienze degli Alimenti, Facoltà di Agraria Università degli Studi di Napoli Federico II, via Università 133, 80055 Portici, Napoli, Italy*

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Abstract

The effect of feeding lupin seeds (*Lupinus albus* L.) as an alternative protein source in ewe diets was investigated. Two groups of 18 Sarda ewes were fed two different isonitrogenous diets: with lupin (L) seed, given after 12 h soaking, or soybean meal (SBM) as the main protein source. DMI, variations of body weight and milk production were unaffected by the treatment. Although not statistically significant, in the group fed L diet the production of milk fat and protein was higher. Clotting properties of milk were similar for the two treatments, probably due to the small differences in the milk protein contents. The fatty acid profile of milk was affected by treatment with a larger content of short (14.19 wt% versus 12.26 wt%)- and medium (49.37 wt% versus 47.76 wt%)-chain fatty acids in milk from ewes fed the L diet. CLA content was unaffected by treatment. Triglyceride content of fat from the two diets reflects the milk fatty acid composition. Indeed, milk from L diet showed a higher level of medium-chain triglycerides, which are of particular interest to consumers with concerns over health and heart disease. The inclusion of lupin seed in the diet of lactation ewes can be a means of achieving a more desirable triglyceride profile in milk fat. Milk with enhanced nutritive quality may promote wider market penetration of sheep dairy products.

Keywords: Sheep; Milk protein; Milk fatty acids; Lupin seeds; Soybean meal

1. Introduction

The agro-ecosystems of inland areas of southern Italy are mainly mountainous and are characterised by low-fertility soils, long cold winters and limited rain-

* Corresponding author. Tel.: +39 081 2539307; fax: +39 081 7762886.
E-mail address: masucci@unina.it (F. Masucci).

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fall, mainly concentrated in early spring and autumn. Because of the natural conditions, fodder crops – mainly oats, barley and meadow hay – are grown and the focus is on animal husbandry, mainly sheep.

Most sheep farms are based on extensive grazing or mixed cereals and livestock on small holdings. Sheep dairy-farming is becoming more intensive and more specialised with increases in average herd size and average yield per ewe. The lack of low cost local feed resources, especially protein supplements, limits further intensification of sheep farming.

The main protein source is generally soybean meal, which is imported, as it is agronomically suitable only in limited areas and is costly. In contrast, white sweet lupin (*L. albus* L.) is suited to the climate and agronomic conditions of inland areas of southern Italy and is a potential alternative high protein crop. Indeed, lupin is one of the few grain legumes that comes close to soybean in seed protein content, averaging between 38 and 42% as against 24–28% for beans and peas (Hymowitz, 1990; Milford and Shield, 1996).

In the last few years, new autumn-sown cultivars have been selected in Italy with very low alkaloid content, high over-winter survival rates and good seed yields (Fagnano and Postiglione, 1994). In particular, during a 5-year research period the Multitalia variety, with an indeterminate growth habit, showed an average seed production of 4.5 t^{-1} ha and as well as good protein production (1–1.5 t^{-1} ha) (Postiglione, 1994). Moreover, in the Mediterranean environment the autumn sowing of this variety allows the optimal use of rainfall on the low fertility soils (Fagnano and Bozzini, 2001).

However, the high ruminal degradability of protein from lupin may lead to decreased milk yield and milk protein percentage, but this issue is still controversial (Guillaime et al., 1987; Singh et al., 1995; Bayourthe et al., 1998). Many researchers suggest heat treatment of lupins to decrease solubility and ruminal degradability of lupin protein (Emile et al., 1991; May et al., 1993; Robinson and McNiven, 1993; Murphy and McNiven, 1994; Zaman et al., 1995). However, the treatment is too expensive, considering the economic conditions of sheep farming in southern Italy. Moreover, because ewes' milk is used almost entirely for processing into cheese, the composition of lupin must be evaluated not only for nutritive quality, but also for technological quality in cheese making.

The fatty acid profile in lupin seeds is featured by a high proportion of unsaturated fatty acids and a low $n-6$:$n-3$ fatty acid ratio (Singh et al., 1995; Moss et al., 2001). Transfer of these characteristics into animal lipids consumed by humans is likely to have human health benefits (Department of Health, 1994). The aim of this study was to describe and analyse performance in production, chemical composition, fat characteristics and clotting properties of milk from ewes fed two different diets with untreated lupin seeds or soybean meal as the main protein source.

2. Materials and methods

2.1. Protein sources

Quantities of soybean meal (SBM) and lupin seeds sufficient for the entire study were procured in a single batch. The SBM (44% CP) was commercially produced by solvent extraction. The lupins belonged to the species *L. albus* (cv. Multitalia) and were grown at the Torre Lama experimental farm in southern Italy. The SBM was fed to the animals as received, whereas lupins were fed as unprocessed whole seeds after soaking overnight.

2.2. Production study

The experiment was carried out at the dairy sheep farm Fontana, located near Melfi, Basilicata, in southern Italy, from March to May 2001. Thirty-six multiparous Sarda ewes in early lactation were divided into two groups, balanced for parity (on average 3.6), litter size (1.6), days in milk (73), milk production and composition (measured 1 week before the beginning of the experiment) and body weight (BW). Ewes in the control group averaged 41.5 ± 3.7 kg BW, 1978 ± 273 g milk/day, 4.4 ± 0.46% milk fat and 4.8 ± 0.5% milk protein. Ewes in the treated group averaged 41.4 ± 3.2 kg BW, 2022 ± 307 g milk/day, 4.5 ± 0.65% milk fat and 4.7 ± 0.26% milk protein. Ewes were housed on straw litter in two pens of 18 ewes each, and had free access to water. The groups were fed complete mixed diets, formulated in order to have the same CP and energy content. The main source of protein was SBM for the control group (SBM diet), whereas it was completely replaced by lupin seeds in

Table 1
Ingredients of experimental diets (% as fed)

	L diet[a]	SBM diet[a]
Meadow hay[b]	25.6	25.6
Maize silage	12.9	12.9
Barley malt	6.5	6.5
Maize meal	12.9	12.9
Concentrate[c]	6.5	6.5
Oat seeds	6.5	8.1
Ensiled artichoke leaves	19.4	19.4
Lupin seeds	9.7	–
Soybean meal	–	8.1

[a] Main protein source of L diet: lupin seeds; SBM diet: soybean meal.

[b] Characterised by CP 12.5% DM, NDF 73.1% DM, ADL 10.2% DM, NEL 6.89 MJ/kg DM and consisting of *Festuca* spp., *Dactylis* spp., *Trifolium* spp., *Phleum* spp., *Poa* spp., *Lolium* spp. and *Bromus* spp. Meadow age: 3 years.

[c] Based on: wheat flour shorts, wheat middlings, soybean meal, maize meal, sunflower meal, soybean hulls, maize germs, beet molasses, lucerne hay, calcium carbonate and palm oil.

order to obtain the other treatment (L diet). Ewes were offered diets (Table 1) twice a day after milking.

The total group feeds offered and refused were measured daily to determine DM intake. Feed and refusal samples were collected weekly throughout the trial, dried in a forced air oven at 65 °C, ground through a 1-mm screen and stored until further analyses. Weekly individual milk production was recorded and milk samples, alternatively from evening and morning milking, were collected from each ewe. The animals were weighed once a week before feeding. The experimental period was 8 weeks long.

2.3. Analytical methods

All feedstuffs were analysed for DM, ash and CP using recommended standard procedures (AOAC, 1995). Neutral detergent fibre (NDF), ADF and acid detergent lignin (ADL) were determined by the method of Van Soest et al. (1991). Starch content in the feed was measured after acid hydrolysis and polarimetric detection (Martillotti et al., 1987). Energy values of feedstuffs (NEL) were calculated according to INRA (1988). Soluble protein (SP), NDF insoluble protein (NDF IP) and ADF insoluble protein (ADF IP) of all feedstuffs were determined, using standardisation and recommendations published by Licitra et al. (1996). All analyses were carried out at least in duplicate.

Individual milk samples were analysed for fat, protein, lactose, non-fat solid (Milkoscan 605, Foss Electric, Sweden), urea (CL 10, Eurochem) content and somatic cell count (SCC; Fossomatic 250, Foss Electric, Sweden). The total bacterial count was determined on pooled milk from each group. Milk production was standardised to 6.5% of fat and 5.8% of protein (FPCM) according to Pulina et al. (1989).

Analysis of fatty acids was carried out on individual milk samples collected every 2 weeks. Gas-chromatographic analyses were performed by means of trans-estherification reaction: 1 ml hexane and 0.2 ml KOH (2N) were added to 50 ml lipid extract. After a 2 min centrifugation at 2000 rpm it was possible to collect the hexane phase, which was immediately injected into a Dani GC-FID, mod. 8521A, with the following parameters: stationary phase 50% cyanopropyl methyl silicone; column 50 m, 0.25 mm i.d., 0.25 μm f.t. (Quadrex 007-23). The operating parameters were: helium as carrier gas, 2 ml/min flow, split ratio 1/60 (v/v), FID temperature 260 °C. The PTV temperature program was: 50 °C for 10 s; 400 °C/min up to 260 °C; 260 °C for 3 min. The oven temperature program was: 50 °C for 3 min; 7 °C/min up to 230 °C; 230 °C for 10 min. Peak identification was performed injecting pure FAME standards, under the same chromatographic conditions; calculation of correction factors and normalisation of the results were necessary for quantitative analysis.

The gas-chromatographic analysis of triglycerides was performed by injecting a 5% solution of anhydrous fat into columns with the following characteristics: 65% diphenyl–35% dimethyl polysiloxane (rtx-65 TG) 30 m; 0.25 mm i.d.; 0.1 μm f.t. The PTV temperature program was: 60 °C for 10 s; 400 °C/min up to 360 °C; 360 °C for 7 min. The oven temperature program was: 250 °C for 20 min; 7 °C/min up to 360 °C; 360 °C for 7 min.

Coagulation parameters were determined by means of a Formagraph on 10 ml of milk, at 35 °C, with the addition of 0.2 ml of a rennet solution according to the methods proposed by ASPA, the Scientific Association of Animal Production (1995). The technical time of analysis was 30 min and the following parameters were measured: clotting time, curd firming time and curd firmness. Measurements were performed at the milk natural pH, and were duplicated.

2.4. Calculations

The SSC and total bacterial count were logarithmically transformed. Results of analytical examinations were analysed by ANOVA using the general linear model procedure of SAS (1990). The sheep were used as the experimental unit. Data were analysed with analyses of variance for repeated measures with diet as a non-repeated factors and week of observation (1, . . ., 8) and diet *x* week of observation as repeated factors.

For renneting characteristics of milk, statistical analysis was performed considering the diet and the week as constant factors and FPCM yield, fat, protein and lactose percentages and pH as covariate factors.

Least square means were compared by *t*-test at the significance level of 0.05.

3. Results and discussions

The results of the laboratory analyses of feeds and diets are shown in Table 2. In general, the chemical composition of lupin seeds does not agree with the values reported previously for *L. albus*, as the cultivar Multitalia shows a marked tendency for CP to be higher and EE to be lower than in other varieties (Guillaime et al., 1987; Benchaar et al., 1994; Singh et al., 1995; Moss et al., 2001). The nutritional characteristics of the two diets were similar regarding major components (CP, starch, fibre and calculated energy densities). With regard to protein partition, the L diet showed a higher percentage of soluble protein, as a consequence of the higher content in lupin seeds.

Table 2
Chemical composition of lupin seeds, soybean meal and experimental diets

	Lupin seeds	SBM	L diet[a]	SBM diet[a]
Ash (% DM)	7.6	4.3	7.2	7.5
Crude protein (% DM)	41.4	48.4	16.2	16.2
Ether extract (% DM)	7.6	0.94	3.1	2.4
NDF (% DM)	21.2	14.3	47.7	47.2
ADF (% DM)	16.5	8.7	29.1	28.2
ADL (% DM)	0.7	0.6	4.9	4.9
NSC[b] (% DM)	26.4	30.6	30.0	31.0
Starch (% NSC)	18.5	ND	63.1	62.0
SP[c] (% CP)	59.0	8.2	34.6	18.2
NDF IP[d] (% CP)	4.1	2.1	26.2	26.9
ADF IP[e] (% CP)	2.4	4.5	6.3	5.7
NEL[f] (MJ/kg DM)	8.03	7.75	6.25	6.21

[a] Main protein source of L diet: lupin seeds; SBM diet: soybean meal.
[b] NSC, non-structural carbohydrate.
[c] SP, soluble protein.
[d] NDF IP, NDF insoluble protein.
[e] ADF IP, ADF insoluble protein.
[f] Net energy for lactation (NEL) estimated from the analysis of the ingredients and INRA (1988).

The results of fatty acid analyses are given in Table 3. In agreement with others (Robinson and McNiven, 1993; Singh et al., 1995; Moss et al., 2001), the C18 unsaturated fatty acids were the most frequent acids in both protein sources. The predominant fatty acids in lupins were C18:1 and, to a lesser degree, C18:2 and C18:3, which accounted for 69.7% of total fatty acids; in SBM, as in most oilseeds, C18:2 was the most commonly found fatty acid and the total C18 unsaturated fatty acids accounted for 73.2%. In general, both feeds showed high proportions of unsaturated fatty acids, with the higher value being found for SBM.

Replacing the SBM by lupin seeds in the diet did not influence total DM intake (2.1 and 2.2 kg DM/day, respectively, for diet L and SBM) or the increases in body weight during the trial (1.4 and 1.0 kg).

Prior to treatment, milk production and composition were similar for the two groups and in line with the values reported for Sarda ewes bred in Italy. Only milk fat percentages were noticeably lower due to the early stage of lactation of the animals during the trial (on average 73 days).

The isonitrogenous substitution of ground raw lupin seeds for SBM in the diets did not substantially affect milk yield (Table 4). The comparison of results with previous works must be done with caution because studies have been conducted on cows. To our knowledge, the present study is the first to focus on the effect of feeding lupin seeds on ewes' milk production. Anyway some general considerations can be done. Our result conflicts with Guillaime et al. (1987), who reported a tendency for decreased milk production in cows receiving lupin in substitution of SBM, but agrees with Singh et al. (1995), who did not observe differences. In the Guillaime study, cows fed lupins consumed less DM than the cows fed SBM and this could explain the productivity reduction; moreover, in the research the lupin seeds were finely ground and this could have resulted in increased ruminal degradability of lupin seeds (Singh et al., 1995).

Table 3
Fatty acid profile of lupin seeds, soybean meal (SBM) and milk (wt%)

	Feeds		Milk		S.E.	Significance
	Lupin seeds	SBM	L diet[a]	SBM diet[a]		
C4	–	–	2.5	2.45	0.06	
C6	–	–	1.4	1.37	0.08	
C8	–	–	1.92	1.24	0.04	***
C10	–	–	8.37	7.2	0.14	***
C12	–	–	4.9	4.64	0.290	
C14:0	0.17	–	13.3	13.93	0.20	*
C14:1	–	–	0.55	0.33	0.03	***
C15	–	–	1.3	0.75	0.09	***
C16:0	11.4	13.1	27.5	26.47	0.39	*
C16:1	0.61	–	1.82	1.64	0.08	
C17	–	–	0.53	0.34	0.03	***
C18:0	3.36	4.52	8.31	8.87	0.20	*
C18:1 c	36.33	17.1	19.8	21.9	0.23	***
C18:1 t	–	–	2.52	2.26	0.08	*
C18:2	18.34	52.76	2.5	3.6	0.03	***
CLA	–	–	0.92	0.98		
C18:3	12.76	8.15	1.29	1.18	0.08	
C20:0	1.67	0.4	0.28	0.25	0.02	
C20:1	0.5	0.45	0.28	0.17	0.04	*
C22:0	0.7	0.82				
Unsaturated/saturated	4.09	3.91	0.41	0.45		

[a] Main protein source of L diet: lupin seeds; SBM diet: soybean meal.
* $P < 0.05$.
*** $P < 0.001$.

Percentages of milk fat and protein tended to be lower in sheep fed L diet than in those receiving SBM, but the differences between the two groups were not statistically significant; milk fat and protein yields, instead, were numerically higher but not significantly different from the SBM group (Table 4). These opposite patterns could be explained by a dilution effect of milk solids secreted due to the slightly higher milk produc-

Table 4
Milk yield and composition for ewes offered experimental diets

	L diet[a]	SBM diet[a]	S.E.	Significance
Production				
Milk (g/day)	1481	1412	82.9	W
FPCM[b] (g/day)	1219	1265	74.5	W
Protein (g/day)	72.4	72.0	6.2	W
Fat (g/day)	74.0	71.8	4.4	W
Lactose (g/day)	73.0	67.8	6.2	W
Composition				
Protein (%)	4.93	5.14	0.661	W
Fat (%)	5.07	5.23	0.89	W
Lactose (%)	4.90	4.77	0.046	W
pH	6.83	6.82	0.26	W
Total bacterial count (log 10)	5.32	5.34	0.01	W
SCC (log 10)	4.96	4.98	0.13	W
Urea (mg/dl)	45.5	46.4	1.77	W

[a] Main protein source of L diet: lupin seeds; SBM diet: soybean meal. W, effect of week of observation.
[b] FPCM, fat (%) and protein (%) corrected milk.

Table 5
Clotting properties of milk for ewes offered experimental diets

	L diet[a]	SBM diet[a]	S.E.	Significance
Rennet clotting time (min)	10.30	10.65	0.74	L, W, Y, pH
Curd firming time 20 (min)	1.8	1.9	0.09	L, F
Curd firming time 30 (min)	2.9	3.0	0.14	L, W, Y, F
Curd firming time 40 (min)	4.4	4.4	0.22	L, W, P
Curd firmness 10 (mm)	55.7	55.7	0.84	L, W, Y, P
Curd firmness 20 (mm)	47.6	51.1	1.39	L, W, Y, pH, D

[a] Main protein source of L diet: lupin seeds; SBM diet: soybean meal. L, effect of lactose percentage; W, effect of week of observation; Y, effect of fat (%) and protein (%) correct yield; pH, effect of pH; F, effect of fat percentage; P, effect of protein percentage; D, effect of diet.

tion observed for L diet. However, a plot of CP content versus week of treatment showed a higher protein percentage during the whole trial for ewes consuming the SBM diet. The reduction in milk protein percentage, previously observed by others (Guillaime et al., 1987; May et al., 1993; Robinson and McNiven, 1993; Singh et al., 1995), can be explained by the fact that SBM showed a lower SP content than lupin (8.2 versus 59.0), and a lower protein effective degradability (0.62% versus 0.95%, Verité et al., 1987). Consequently, the L diet would have had a high N degradability that may have resulted in a lower use of N in the rumen and less production of microbial protein.

No differences were noted in the level of milk urea, although the higher SP content of the L diet could have affected this parameter.

Milk pH was almost identical in the two treatments, as were the majority of clotting properties (Table 5). Only curd firmness at 20 min was slightly lower for the L diet. The main factors affecting rheological characteristics were lactose percentage, FPCM yield and week of treatment.

The lack of effect of dietary treatment on clotting proprieties is partly due to the small differences in the milk protein concentration of samples on which the rheological measurements were carried out. Milk protein concentration is an essential factor of the variation in firming time and in curd firmness (Remeuf et al., 1991; Martin and Coulon, 1995). When the change in diet type is accompanied by a major change in milk protein concentration, its clotting ability is also modified (Grandison et al., 1984). In our study, the SBM diet, associated with slightly higher protein concentration in milk, did not show a greater ability to coagulate than the other.

The fatty acid profile of milk fat was modified by substituting SBM with lupins (Table 3). Although the portions of C4 and C6 were unaffected by treatment, C8, C10 and the medium-chain fatty acids (especially C14:1 and C15) were statistically higher in milk from ewes fed L diet. By contrast, proportions of long-chain ($\geq$17 carbons) fatty acids were higher in milk from the SBM diet, especially as regards C18:0, C18:1 and C18:2. Furthermore, milk from L diet, in comparison with that from the SBM diet, showed higher percentages of saturated fatty acid and a lower unsaturated/saturated ratio. Finally, although CLA content was numerically slightly higher in milk from ewes receiving the SBM diet, the differences were not statistically significant.

Overall results seem to indicate, that fat from lupin seeds underwent lipolysis and bio-hydrogenation in the rumen, and that in mammary tissues ex novo synthesis of short-chain fatty acids occurred. These results are in contrast with those of Robinson and McNiven (1993) and Singh et al. (1995), who reported reduced proportions of the medium-chain saturated fatty acids, and an increment in total C18 fatty acids in lactating cows receiving lupins probably due to dietary transfer of C18:1 and C18:2 fatty acids to milk in lupin-fed cows.

There are two possible reasons for the lack of protection of lupin fat in the rumen. The first is, that lupins were administered as unprocessed whole seeds after overnight soaking, which may have made the fat contained within more fully accessible to microorganisms than after coarse grinding. Moreover, unlike cows, sheep grind seeds: chewing could have partially removed the integument protection of the inner fat (Murphy et al., 1987; White et al., 1987; Keele

Table 6
Triglyceride composition of lupin seeds, soybean meal and milk (wt%)

	Feeds		Milk		S.E.	Significance
	Lupin seeds	SBM	L diet[a]	SBM diet[a]		
C26			0.73	0.48	0.05	**
C28			2.37	1.32	0.16	***
C30			3.17	1.8	0.13	***
C32			4.56	3.63	0.12	***
C34			6.80	6.23	0.130	**
C36			11.51	10.37	0.08	***
C38			13.15	12.25	0.10	***
C42			9.49	9.26	0.090	*
C44			8.58	7.99	0.1	***
C46	1.8	4.2	6.92	6.92	0.06	
C48	6.0	6.0	5.75	8.56	0.2	***
C50	2.5	5.0	5.89	7.76	0.16	***
C52	15.8	20.0	4.9	7.38	0.13	***
C54	37.7	55.1	2.26	4.45	0.08	***
C56	16.31	9.0				
C58	14.14	0.5				
C60	5.7	–				
Cholesterol			0.35	0.28	0.009	***

[a] Main protein source of L diet: lupin seeds; SBM diet: soybean meal.
* $P<0.05$.
** $P<0.01$.
*** $P<0.001$.

et al., 1989). The second is that the newly synthesised short-chain fatty acids successfully competed with the C18:1 for the sn-3 position in milk triglyceride synthesis (Hawke and Taylor, 1983).

The triglyceride composition of milk fat from ewes receiving the two diets is given in Table 6. The substitution of SBM with lupin seed significantly increased triglycerides from C26 to C44 and decreased triglycerides C48, C50, C52 and C54. Cholesterol content was greater for sheep receiving L diet. Other authors also highlighted the influence of diet on triglyceride composition of milk fat (Banks et al., 1989; Precht, 1991; DePeters et al., 2001). The higher proportion of short- and medium-chain fatty acid and the lower content of the long-chain fatty acid associated with use of lupin seed would explain some of the changes in carbon number for the triglyceride structure.

The larger level of medium-chain triglycerides (MCT) observed in milk fat from L diet is of particular interest for human nutrition. MTC differ from long-chain triglycerides, which have fatty acids of >12 carbons, in that they are absorbed directly into the portal circulation and transported to the liver for rapid oxidation and do not follow the general lipid transport pathway through the lymphatic system and into the prostaglandin metabolism (Babayan and Rosenau, 1991). MCT can provide energy to the consumer without contributing to laying down fat in the body, and this property makes MCT beneficial for many health conditions including coronary bypass, premature infant feeding, childhood epilepsy and cystic fibrosis (Bach and Babayan, 1982; Babayan, 1987). MTC is supplied in greater amounts from sheep's milk than from cow's milk although it can be greatly affected by feeding regimes (Haenlein, 2001). In confirmation of this assertion, the milk from both groups showed MTC levels larger than 25 wt%. Overall results indicate a better triglyceride profile for milk from L diet, except for cholesterol content.

4. Conclusions

Isonitrogenous substitution of SBM with lupins in the diet did not substantially affect milk production or milk characteristics. The clotting properties were not

influenced by the diet. Therefore, despite the low protein quality of untreated lupin seeds, replacing dietary N from soybean meal with lupin seeds is unlikely to have a major effect on the performance of lactating ewes. However, the high solubility of protein must be balanced in rations.

Research confirms that the triglyceride composition in the sheep's milk has considerable nutritional value in human diets as regard the MTC content that is of particular interest to consumers with concerns over health and heart disease. The valuable nutritional property of milk fat obtained by using lupin seed could add weight to the promotion of dairy sheep products as "functional food". Therefore, sheep's milk/cheese, produced by using lupin seed with their enriched concentration of MCT, could have considerable market potential.

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