

# Alternative protein sources in lamb feeding 1. Effects on productive performances, carcass characteristics and energy and protein metabolism

*Anna Maria Facciolongo<sup>1</sup>, Giuseppe Rubino<sup>2</sup>, Antonia Zarrilli<sup>2</sup>, Arcangelo Vicenti<sup>3</sup>, Marco Ragni<sup>3</sup>, Francesco Toteda<sup>2</sup>*

<sup>1</sup>National Research Council, Institute of Plant Genetics, Bari, Italy, <sup>2</sup>Department of Emergency and Organ Transplantation, Section of Veterinary Clinics and Animal Production, University of Bari "Aldo Moro", Italy, <sup>3</sup>Department of Agro-Environmental and Territorial Sciences, University of Bari "Aldo Moro", Italy

**Summary.** The influence of protein sources alternative to soybean was evaluated for feeding "Gentile di Puglia" breed lambs. The lambs were fed pelleted diets containing alternatively: a) soybean b) faba bean; c) lupin or d) pea. Lambs fed the faba bean diet showed the greatest slaughter weight and a better carcass conformation; however, the carcasses were significantly fatter than those obtained from soybean fed lambs. The protein source did not affect the incidence of the different meat cuts of the half carcass with exception for the loin, that was higher in lambs fed with pea in comparison with those fed the lupin and soybean diets. The blood cholesterol level was lowered following feeding with faba bean as compared to the soybean and pea groups. In conclusion, the protein sources tested, with particular concern for faba bean, may effectively replace soybean in feeding for fattening lambs.

**Key words:** Lamb, protein sources, performance, carcass characteristics, blood parameters

«IMPIEGO DI FONTI PROTEICHE ALTERNATIVE PER AGNELLI ALL'INGRASSO 1. PERFORMANCE IN VIVO, CARATTERISTICHE DELLA CARCASSA ED INDICI DEL METABOLISMO ENERGETICO E PROTEICO»

**Riassunto.** È stata valutata l'influenza dell'uso di fonti proteiche alternative alla soia nella razione di agnelli Gentile di Puglia all'ingrasso. Gli agnelli sono stati alimentati con mangime pellettato contenente: a) soia; b) favino; c) lupino; d) pisello proteico. L'uso del favino ha fatto registrare il maggior peso alla macellazione ed una migliore conformazione della carcassa che, però, è risultata significativamente più grassa di quelle dei soggetti alimentati con la soia. La fonte proteica non condiziona l'incidenza dei vari tagli sulla mezzena ad eccezione della lombata che è più elevata nei soggetti alimentati con pisello anziché lupino e, soprattutto, soia. La soia ed il pisello proteico hanno determinato un aumento significativo del colesterolo totale mentre il lupino ne ha condizionato i livelli più bassi. In conclusione, tutte le fonti proteiche saggiate e soprattutto il favino possono efficacemente sostituire la soia nella razione di agnelli all'ingrasso.

**Parole chiave:** Agnelli, fonti proteiche, performance, caratteristiche della carcassa, parametri ematici

## Introduction

The worry of the public opinion about the widespread use of genetically modified (GMOs) feedstuffs in animal feeding, such as soybean, has led to focus on the

possibility to replace soybean with alternative legume grains able to satisfy animal dietary protein requirements. This alternative feeding strategy has also been encouraged by the agricultural policy of the EC that promotes the development of sustainable livestock farming (Regulation

EC 1804/1999). In the Mediterranean area there is a large diffusion of local legume crops that, in addition to the reduction of feed costs for livestock, may provide an additional benefit for the environment against soil nitrogen impoverishment and disruption of biological cycles of pests and plant diseases (1). In Italy there has been a growing interest in the development of GMO-free production chains by promoting the production of organic and local foods. Legume grains such as pea, faba bean and lupin may be easily cultivated in the Mediterranean area and may be used for animal feeding with particular regards to monogastrics (2).

Despite the high degradability and solubility of their proteins (3), it has been reported that legume grains may not completely replace soybean in the rations for ruminants (4), although several studies report the positive effects of supplementation with lupin, faba bean and pea on growth performances of lambs and calves (5, 6, 7, 8, 9,10).

Faba bean (*Vicia faba* var. *minor*) is a typical cultivation in South Italy areas that is economically feasible and requires limited crop investments; the seed contains 30% of protein (rich in lysine but lacking in sulfur amino acids and tryptophan) and 42% of starch (11, 12).

Sweet lupin (*Lupinus albus*), with particular concern for the *Multitalia* variety, is characterized by a low content in alkaloids and a high protein content that can be up to 35–40%, being very close to the soybean protein content (13). However, sweet lupin seeds contain anti-nutritional factors (14) which may limit its use.

Pea (*Pisum sativum*) is grown especially in the inland areas of South Italy; in rotation with other crops this cultivation is economically more competitive than other legumes grains (15). Compared to soybean (16) pea has a protein content ranging between 21 and 24% (13, 17), with an amino acid profile lacking of methionine but characterized by a good level of tryptophan, arginine and lysine.

The values and/or trend assessment of some blood parameters are important indicators of the animal nutritional state and welfare (18, 19) and may be useful to identify metabolic imbalances or disorders, such as those caused by incorrect feeding or subclinical disease (20), thus leading to intervene with potential corrective actions.

The total cholesterol serum concentration is related to animal growth processes (21), while the blood glucose level provides information on the animal nutritional status as observed in goats by Morand-Fehr et al. (22). The determination of the total protein and blood urea level is important for the evaluation of the animal protein metabolism (23, 24, 25).

The aim of this study was to evaluate the influence of dietary faba bean, lupin and pea, such as protein sources alternative to soybean, in rations for finishing lambs, on the productive and slaughtering performances as well as on some blood parameters. The effects on meat quality, meat chemical composition and fatty acid profile of intramuscular fat will be discussed in a follow-up study.

## Material and methods

The trial was conducted from November 2009 to January 2010, at a livestock farm situated in the province of Foggia (Italy) (41° N, 15° E, 620 m a.s.l.).

In accordance with the rules about animal welfare (97/2/EC directive, received in Italy by D. Lgs. 331/98), 32 male lambs, weaned at about  $42 \pm 2$  days of age, were randomly divided into four groups of eight animals each, homogeneous for age and initial live body weight, and fed with complete isoenergetic, isonitrogenous and isofibrose pellet feeds containing respectively: a) soybean (control group); b) faba bean; c) lupin or d) pea.

The pelleted feeds, whose composition is shown in Table 1, were administered *ad libitum* during the trial that lasted 7 weeks. Lambs were housed in individual pens (1 m<sup>2</sup>) where the average temperature ranged between 7 and 15°C. In order to evaluate the growth performances, lambs were weekly weighed and the amounts of feed administered and refusals were recorded in order to calculate the average daily weight gain and feed conversion ratio.

On the 1<sup>st</sup> and 7<sup>th</sup> week of the trial, blood samples were collected from the jugular vein by the Vacutainer® system from each animal in order to assess the energy (glucose, triglycerides, cholesterol) and protein (creatinine, urea, total protein and respective electrophoresis fractions) metabolism parameters. The serum samples collected in the farm were centrifuged at

**Table 1.** Ingredients and chemical composition (%) of the diets

	Soy bean	Faba bean	Lupin	Pea
Maize	12.0	12.0	18.0	10.0
Oats	18.0	15.0	12.0	8.0
Barley	12.0	5.0	26.0	-
Alfalfa	17.5	17.0	5.0	22.5
Straw	5.0	4.0	8.5	3.0
Molasses	1.7	1.7	1.7	1.7
Soy bean meal	12.0	-	-	-
Faba bean meal	-	25.0	-	-
Lupin meal	-	-	25.0	-
Pea meal	-	-	-	25.0
Middlings	17.0	15.5	-	25.0
Soy bean oil	1.0	1.0	-	1.0
Calcium carbonate	1.8	1.8	1.8	1.8
Dicalcium phosphate	1.0	1.0	1.0	1.0
Sodium chloride	0.5	0.5	0.5	0.5
Vitamin supplement	0.5	0.5	0.5	0.5
Total	100	100	100	100
Crude protein	16.88	17.07	17.2	16.32
Crude lipids	4.49	4.26	4.43	4.20
Crude fiber	13.02	11.78	13.73	13.45
NDF	29.37	30.45	25.53	31.21
ADF	15.23	15.40	13.32	15.53
ME (MJ/kg)	11.11	11.12	10.82	11.08

3000 rpm for 15', divided into aliquots and transported at 4 °C to the laboratory, where they were frozen at -20 °C until analyses were performed. Serum biochemical parameters were assessed by using Assel reagents and SEAC photometer endowed with interferential filters. Serum protein electrophoresis was performed on agarose gel according to the Helena BioSciences method.

At the end of the 7<sup>th</sup> week lambs were slaughtered, after 12 h of fasting and live weight assessment, according to the veterinary rules and in respect of animal welfare (D.P.R. 320/54). The skin and fleece along with the empty gastro-intestinal tract were weighed in order

to calculate the net warm dressing (carcass weight/net live weight) and net cold dressing (carcass weight after 24 h refrigeration/net live weight) percentage.

The carcasses were refrigerated at 4°C for 24 h. After chilling, carcasses were weighed and evaluated for conformation and fatness using a 1-15 points scale according to Dransfield et al. (26) on the basis of EU grid (European Commission, 1992; 1993).

After detachment of the head, shins, omentum and pluck, the carcasses were split into two symmetric halves along the midline. The right half-carcass was then sectioned into commercial meat cuts (neck, shoulder, ribs, brisket, bacon, loin, leg) (27); the pelvic limb (leg) and the lumbar region (loin) were further dissected in order to evaluate their tissue components (percentages of lean, separable fat and bone).

#### Statistical analysis

Data were analyzed using the GLM procedure of the SAS (28). The statistical analysis of blood indexes was carried out using the ANOVA model for repeated measurements, taking into consideration as main effects diet (D), lamb age (A) and their interaction (DxA). Means were compared by the Student's *t* test.

## Results and discussion

#### Growth trial and carcass characteristics

Lamb performance data and slaughtering traits are shown in Table 2. Lambs fed the faba bean diet showed the greatest slaughter weight (23.07 kg) with significant differences ( $P < 0.01$ ) compared to the soybean (19.93 kg) and lupin (20.30 kg) groups, adversely to the results reported for Barbaresca (5) and Comisana x Valle del Belice lambs (10) where no differences were found following soybean replacement with faba bean. The difference in slaughtering weight is related to the average daily weight gain recorded; lambs fed with faba bean showed a markedly higher ( $P < 0.05$ ) daily weight gain (0.21 kg/d) compared to the soybean group (0.16 kg/d). Feeding with soybean and lupin determined similar daily weight gains, in agreement with the results reported in studies conducted on

**Table2.** In vivo performances and slaughtering data (% of empty body weight)

Variable	Diet				SD <sup>1</sup> (DF=19)	F value
	Soy bean	Faba bean	Lupin	Pea		
Initial live weight (kg)	12.06	12.57	12.10	12.25	1.938	00.9
Final live weight (kg)	19.93B	23.07A	20.30B	21.65	1.954	3.41*
Weight gain (kg/day)	0.16b	0.21a	0.17	0.19	0.042	2.13
Feed conversion index (kg/kg)	4.71	3.67	5.05	4.39	1.39	1.14
Empty body weight (kg)	17.72b	20.35a	18.09	19.10	1.983	1.90
Hide	14.46	14.23	13.43	13.75	1.753	0.42
Empty digestive tract	8.76	8.32	8.51	7.74	1.014	1.11
Omentum	0.73	1.23	0.78	0.83	0.530	0.95
Head	5.21a	4.42b	4.69	4.68	0.606	1.70
Pluck	5.34	5.47	5.40	5.40	0.262	0.23
Net warm dressing percentage	53.15	50.21	52.19	52.03	3.582	0.64
Net cold dressing percentage	52.22	49.19	51.41	51.29	3.258	0.81
Chilling loss	1.73	2.00	1.47	1.36	0.872	0.75
Carcass conformation score	9.86 b	12.50 a	10.71	12.00	2.020	2.31
Carcass fatness score	6.00 b	7.50 a	6.86	6.50	1.240	1.66

<sup>1</sup> Standard deviation of mean; On row: A, B:  $P < 0.01$ ; a, b, \*:  $P < 0.05$ .

fattening calves (29, 30, 31). Adversely, other studies carried out in lambs (32) and calves (33) reported a lower weight gain following soybean replacement with lupin. In this study the pea diet slightly improved the daily weight gain compared to the lupin and soybean groups, in agreement with the results reported by other Authors for lambs of the same breed (6, 34). On the contrary Lanza et al. (10) found a slight reduction in lamb growth when pea was used in place of soybean and faba bean. The feed conversion rate was slightly better ( $P > 0.05$ ) in lambs fed the faba bean diet (3.67) while it was worsened in those fed with lupin and soybean (4.71 and 5.05, respectively), as also found by Yu et al. (35). In general agreement with our data, also other Authors did not find any differences following soybean meal replacement with pea (7, 10, 36).

Lambs fed the faba bean diet showed a better carcass conformation (12.50 *vs* 9.86;  $P < 0.05$ ) compared to the control group although the carcasses were significantly fatter (7.50 *vs* 6.00;  $P < 0.05$ ), in

contrast with the results reported by Lanza et al. (5) who found similar fatness scores between carcasses of lambs fed with field beans or soybeans. As for the pea diet, our data are quite comparable to those of other studies that report slightly fatter carcasses following feeding with pea (6) respect to soybean.

The incidence of skin, gastrointestinal tract, pluck and omentum was quite comparable among the groups despite the protein source administered; the incidence of the head was higher in subjects fed with soybean compared to the faba bean group (5.21 *vs* 4.42%;  $P < 0.05$ ). In agreement with other studies (6, 7) and adversely to the findings of Yu et al. (37) who used integrations with other legume seeds, the net warm and net cold dressing percentage were not affected by the protein source. However, the results of this trial are slightly higher than those reported for Awassi (38), Manchego (39), Sarda (40) and Comisana x Valle del Belice breed lambs (10), probably due to the influence of the slaughtering weight (38).

The chilling loss was similar in all the groups, while Lanza et al. (6) recorded a higher chilling loss for carcasses obtained by lambs fed pea instead of soybean.

#### Section and dissection data

The protein source didn't affect the incidence of the different meat cuts (Table 3) on the half carcass, except for the pea diet that provided a significantly higher incidence of the loin in comparison with the lupin and faba bean (9.26 vs 8.43-8.63%;  $P < 0.05$ ) diets, and mainly compared to the control diet containing soybean (28.8%;  $P < 0.01$ ).

Table 4 shows the results referring to the dissection data of the loin and leg. In agreement with previous findings reported by Lanza et al. (5, 6), diets containing soybean, faba bean or pea provided similar results in terms of the edible part (lean and fat) as well as for the incidence of the bone, whose growth rate seems to be influenced more by the animal age than by the feeding regimen (41-44).

In lambs fed with soybean and pea, the lean/fat and lean/bone ratios are similar to those reported by Lanza et al. (6), whereas they are lower compared to those found by Abdullah and Qudsieh (38) in Awassi lambs slaughtered at approximately the same weight of our study.

The tissue composition of the loin, however, showed differences only in the percentage of fat that was significantly higher ( $P < 0.05$ ) in lambs fed with pea (25.63%) compared to those receiving soybean; as a consequence the lean/fat ratio is also markedly lower (1.70 vs 2.40;  $P < 0.05$ ). The increased fat deposition following the pea diet requires further investigation, although Purroy et al. (36) have hypothesized that the greater deposition of internal fat may be due to the higher net energy content of pea for growth.

Despite the protein source administered, the loin contained a higher percentage of fat compared to the leg (Table 4), accordingly to the results found in a previous study in nursing lambs of the same genotype (45).

#### Blood parameters

Blood glucose levels increased significantly ( $P < 0.01$ ) with age in all groups (Table 5), as confirmed by the data of previous studies (46, 47). This may be probably due to an increased sympathetic activity and functionality of the anabolic metabolism (48). The values recorded at the end of the trial were higher in lambs fed soybean, with marked differences only compared to the faba bean group (91.98 vs 80.24 mg/dl;  $P < 0.05$ ). This increase may be due to the increase of

**Table 3.** Section data (% of half carcass)

Variable	Diet				SD (DF=19)	F value
	Soy bean	Faba bean	Lupin	Pea		
Half carcass (kg)	4.36	4.67	4.37	4.62	0.608	0.40
Neck	9.47	9.88	9.07	8.92	0.857	1.40
Steaks	14.70	15.27	14.19	14.53	1.078	0.95
Brisket	11.37	11.42	11.57	11.20	0.735	0.26
Shoulder	17.17	16.72	17.26	16.55	0.797	1.08
Loin	8.28B	8.43b	8.63b	9.26Aa	0.492	4.48*
Abdominal region	4.92	4.88	5.17	4.80	0.584	0.44
Leg	32.16	31.45	31.97	32.44	1.023	0.90
Kidney fat	0.73	0.74	0.98	1.03	0.340	1.27
Kidney	0.85	0.77	0.75	0.85	0.165	0.56
Testicle	0.35	0.43	0.42	0.42	0.146	0.37

On row: A, B:  $P < 0.01$ ; a, b, \*:  $P < 0.05$ .

**Table 4.** Dissection data (%)

Variable	Diet				SD (DF=19)	F value
	Soy bean	Faba bean	Lupin	Pea		
Leg (kg)	1.41	1.47	1.39	1.50	0.195	0.35
Lean	64.24	62.44	63.59	62.29	2.294	0.97
Fat	11.23	12.83	11.06	11.67	1.811	1.03
Bone	24.53	24.73	25.35	26.05	1.761	0.88
Lean/bone	2.62	2.54	2.53	2.40	0.247	0.81
Lean+fat/bone	3.08	3.06	2.97	2.85	1.744	0.79
Lean/ fat	5.82	4.97	6.01	5.43	1.076	0.99
Loin (kg)	0.37	0.39	0.38	0.43	0.064	0.95
Lean	46.82	45.01	46.45	43.06	4.914	0.72
Fat	20.54 <sup>b</sup>	23.67	22.64	25.63 <sup>a</sup>	4.286	1.46
Bone	32.64	31.32	30.91	31.32	4.324	0.18
Lean/bone	1.48	1.44	1.52	1.42	0.287	0.13
Lean+fat/bone	2.14	2.20	2.25	2.27	0.403	0.12
Lean/ fat	2.40 <sup>a</sup>	2.02	2.12	1.70 <sup>b</sup>	0.577	1.53

On row: a, b:  $P < 0.05$ .

live weight that the animals showed at the end of trial, in accordance with El-Barody et al. (46) who found a correspondence between the glucose increase and weight gain.

Total cholesterol blood levels (Table 5) were significantly influenced by feeding ( $P < 0.01$ ), and a significant ( $P < 0.01$ ) diet x age interaction was recorded.

The total cholesterol blood level did not change in the faba bean group, while a significant ( $P < 0.01$ ) increase was found for the soybean (57.54 vs 66.69 mg/dl) and pea (57.64 vs 73.53 g/dl) groups; this result confirms the observations reported by El-Barody et al. (46) for different genotypes. In contrast, lambs fed the lupin diet showed a significant decrease (58.64 vs 47.86 mg/dl;  $P < 0.01$ ) in blood total cholesterol levels with age. Since the level of this parameter is affected by feeding (49), the cholesterol-lowering action exerted by lupin found by Sirtori et al. (50) in rats and by Viveros et al. (51) in chickens may be confirmed also in the present study.

The blood concentration of triglycerides (Table 5) was not significantly affected by the diet and animal

age and ranged between 23.82 and 25.42 mg/dl at the beginning of the trial and between 24.53 and 25.78 mg/dl at the end of it.

Both blood urea and creatinine concentrations (Table 5) increased markedly ( $P < 0.01$ ) with animal age and were unaffected by the diet. The urea concentration reflects the changes of protein metabolism (23, 52) and the rise of its blood concentration during growth has also been reported in other studies (53-55). This may be related to the higher rumen degradability of protein and carbohydrates acquired by growing lambs (56-60) with age. The rise of creatinine blood levels during growth has been reported also by Abdel-Ghani et al. (53) for Sohagi lambs.

In general, the total proteins values (Table 6) were not affected by the diet but they significantly increased ( $P < 0.05$ ) with the animal age in agreement with the results reported for lambs of other genotypes (46, 53).

With regard to the protein fractions (Table 6), the albumin percentage was markedly affected ( $P < 0.05$ ) by the diet only in lambs fed with lupin, that at the end of the trial showed a lower value (63.25 vs 65.59-65.32

**Table 5.** Blood metabolites (mg/dl)

Variable	Age (days)	Diet				SD (DF = 56)	F value		
		Soy bean	Field bean	Lupin	Pea		Diet	Age	D x A
Glucose	42	48.40 **	41.98 **	44.12 **	45.84 **	10.667	2.08	223.3**	0.22
	90	91.98a	80.24 <sup>b</sup>	82.93	84.62				
Cholesterol	42	57.54 **	56.12	58.64 **	57.64 **	7.014	12.93**	1.41	12.7**
	90	66.69 <sup>A</sup>	50.20 <sup>B</sup>	47.86 <sup>B</sup>	73.53 <sup>A</sup>				
Triglycerides	42	25.37	24.69	23.82	25.42	6.404	0.01	0.03	0.15
	90	25.06	24.97	25.78	24.53				
Urea	42	15.84 **	16.02 **	15.62 **	14.29 **	6.407	0.95	204.6**	0.56
	90	35.43	37.09	31.27	37.02				
Creatinine	42	0.85 **	0.84 **	0.84 **	0.82 **	0.187	0.17	152.8**	1.57
	90	1.34	1.20	1.20	1.24				

On row: A, B:  $P < 0.01$ ; a, b:  $P < 0.05$ ; on column: \*\*,  $P < 0.01$ .

**Table 6.** Protein fraction

Variable	Age (days)	Diet				SD (DF = 56)	F value		
		Soy bean	Field bean	Lupin	Pea		Diet	Age	D x A
Total protein (g/dl)	42	5.60	5.57	5.56	5.45	0.531	0.98	6.43*	0.35
	90	6.11	5.76	5.97	5.66				
Albumin (%)	42	63.90	65.11	64.64	64.94	2.122	1.89	0.02	0.70
	90	64.17	65.59 <sup>a</sup>	63.25 <sup>b</sup>	65.32 <sup>a</sup>				
$\alpha$ 1-globulin (%)	42	5.67	5.86 <sup>a</sup>	5.58 **	5.14 <sup>b</sup> **	0.573	3.15*	32.70**	1.84
	90	6.05 <sup>B</sup>	6.44	6.98 <sup>Aa</sup>	6.21 <sup>b</sup>				
$\alpha$ 2-globulin (%)	42	13.28	13.99	13.35	13.39	1.377	0.48	0.63	1.03
	90	14.51	13.73	13.40	13.43				
$\beta$ -globulin (%)	42	4.85	4.54 *	4.56	4.77	0.824	3.45*	1.95	1.99
	90	3.89 <sup>b</sup>	3.53 <sup>B</sup>	4.89 <sup>A</sup>	4.95 <sup>Aa</sup>				
$\gamma$ -globulin (%)	42	12.23	10.50	11.87	11.76	1.708	2.23	1.09	1.09
	90	11.45	10.71	11.48	10.09				
Alb/glob	42	1.78	1.88	1.83	1.87	0.172	1.80	0.01	0.57
	90	1.81	1.91 <sup>a</sup>	1.73 <sup>b</sup>	1.89				

On row: A, B:  $P < 0.01$ ; a, b:  $P < 0.05$ ; on column: \*\*,  $P < 0.01$ ; \*,  $P < 0.05$ .

mg/dl) compared to lambs fed with faba bean and pea; likewise, also Viveros et al. (51) found a decrease of the serum albumin level in chickens fed with lupin.

The  $\alpha_2$  and  $\gamma$ -globulins were not influenced by age or by the feeding treatment;  $\beta$  fractions were affected only by the diet while the  $\alpha_1$  globulin concentration was influenced by the diet ( $P < 0.05$ ) and increased with age ( $P < 0.01$ ).

The albumin/globulin ratio was not markedly affected by lambs' age while at the end of the trial a significant difference between the faba bean and lupin groups (1.91 *vs* 1.73;  $P < 0.05$ ) was recorded.

It has been reported that the protein sources used in this study have nutraceutical properties (61) able to lower blood cholesterol, although not all with the same effectiveness. Research conducted on rats showed that both soybean and lupin lowered total serum cholesterol by 22.7% (50, 62), faba bean by 30% (63) and pea only by 14% (64). The anticholesterolemic action of these legume grains seems to be carried out through various mechanisms including their effect on the serum levels of HDL, VLDL and LDL lipoproteins, which are known to migrate respectively in the fractions  $\alpha_1$ ,  $\alpha_2$  and  $\beta$  of serum protein electrophoresis (65).

Based on the results of the present study, feeding with lupin showed to be very effective in lowering the cholesterol level maybe by affecting the HDL lipoproteins as shown by the significant increase of the  $\alpha_1$  protein fraction. This effect is confirmed also by the results reported by Sirtori et al. (65) who found that the administration of a total protein lupin extract to rats determined a significant increase in HDLs along with a decrease of LDLs that, however, was not observed in the present study.

Although soybean is supposed to exert the same action assumed for lupin (66), in this study feeding with soybean showed no meaningful changes on the  $\alpha$  and  $\beta$  fractions while it significantly increased the cholesterol levels.

In this study soy proteins lacked to show an hypocholesterolemic action; this result is in agreement with a previous research of Sirtori et al. (66), who hypothesized that the cholesterol lowering effect of soybean may be detected only in the presence of high cholesterol values which didn't occur in the lambs of the present study since the subjects were devoid of diseases

or metabolic disorders. Therefore, the cholesterolemic rise exerted by soybean is likely to depend on age (46) or on species-dependent actions (67).

Feeding based on faba bean determined cholesterol levels similar to those of the lupin diet; it induced a significant decrease of the  $\beta$ -globulins fraction, and therefore of LDL-lipoproteins, while no variation of the  $\alpha_1$  and  $\alpha_2$  fraction and, thus of the HDL and VLDL lipoproteins, occurred, accordingly to previous findings in hypercholesterolemic rats (63).

Although the diet containing pea determined a significant increase of  $\alpha_1$  globulins, and thus of HDL lipoproteins, the serum cholesterol level was significantly increased instead of being lowered. This could be due not only to the subjects' age but also to a reduced action of its components as reported in a study conducted on rats fed with five different legumes types in which pea showed a lower capacity in cholesterol reducing (64).

## Conclusions

The use of faba bean, lupin and pea, as alternative protein sources to soybean in feeding for finishing lambs, did not negatively affect neither the *in vivo* performances nor the carcass yield and quality. The blood parameters studied seemed to be affected more by the animal age than by the diet except for total cholesterol. The results obtained in this study confirm the greater cholesterol-lowering capacity exerted by faba bean and lupin compared to soybean and pea in agreement with previous results reported for monogastric animals. Therefore, our data lead us to conclude that soybean can be effectively replaced by these legumes that have the great advantage of being GMO-free besides being local products, easily cultivated in the Mediterranean area.

A follow up study has been conducted in order to analyse the effect of feeding with legume grains alternative to soybean on meat quality and fatty acid profile of lamb meat that will be the subject of a subsequent paper. It would be advisable, moreover, to evaluate the occurrence of a possible associative effect of protein sources along with the effect of applying technological treatments to the legume grains in order to improve their nutritive efficiency.



## References

1. Caballero R, Castile-La Mancha. A once traditional and integrated cereal-sheep farming system under change. *Amer J Alt Agric* 1999; 14: 188-192.
2. Salgado P, Freire JPB, Mourato M, Cabral F, Toullec R, Lallès JP. Comparative effects of different legume protein sources in weaned piglets: nutrient digestibility, intestinal morphology and digestive enzymes. *Livest Prod Sci* 2002; 74: 191-202.
3. Goelema JO, Smits A, Vaessen LM, Wemmers A. Effects of pressure toasting, expander treatment and pelleting on in vitro and in situ parameters of protein and starch in a mixture of broken peas, lupins and faba beans. *Anim Feed Sci Techn* 1999; 78: 109-126.
4. INRA. *Alimentation des bovines, ovins e caprins*. Paris: INRA, 1988.
5. Lanza M, Pennisi P, Priolo A. Faba bean as an alternative protein source in lamb diets: effects on growth and meat quality. *Zoot Nutr Anim* 1999; 25: 71-79.
6. Lanza M, Bella M, Priolo A, Fasone V. Peas (*Pisum sativum* L.) as an alternative protein source in lamb diets: growth performances, and carcass and meat quality. *Small Rum Res* 2003; 47: 63-68.
7. Loe ER, Bauer ML, Lardy GP, Caton JS, Berg PT. Field Pea (*Pisum sativum*) inclusion in corn-based lamb finishing diets. *Small Rum Res* 2004; 53: 39-45.
8. Cutrignelli MI, Piccolo G, Bovera F, et al. Effects of two protein sources and energy level of diet on the performance of young Marchigiana bulls. 1. Intra vitam performance and carcass quality. *Ital J Anim Sci* 2008; 7: 259-270.
9. Atti N, Mahouachi M. Effects of feeding system and nitrogen source on lamb growth, meat characteristics and fatty acid composition. *Meat Sci* 2009; 81: 344-348.
10. Lanza M, Fabbro C, Scerra M, et al. Lamb meat quality and intramuscular fatty acid composition as affected by concentrates including different legume seed. *Ital J Anim Sci* 2011; 10:e18: 87-94.
11. Martillotti F, Bartocci S, Verna M, Malossini F. *Composizione chimica e valore nutritivo di mangimi semplici*. Roma: MAF - ISZ, 1989.
12. Yu P, Goelema JO, Leury BJ, Tamminga S, Egan AR. An analysis of the nutritive value of heat processed legume seeds for animal production using the DVE/OEB model: a review. *Anim Feed Sci Techn* 2002; 99: 141-176.
13. Masoero F, Pulimeno AM, Rossi F. Effects of extrusion, expansion and toasting on the nutrition value of peas, faba beans and lupins. *Ital J Anim Sci* 2005; 4: 177-189.
14. Rotger A, Ferret A, Calsamiglia A, Manteca X. In situ degradability of seven plant protein supplements in heifers fed high concentrate diets with different forage to concentrate ratio. *Anim Feed Sci Techn* 2006; 125: 73-87.
15. Lombardo DM, Tuttobene R, Vagliasindi C. Il pisello proteaginoso (*Pisum sativum* L.) in alternativa alle leguminose tradizionali (The protein pea (*Pisum sativum* L.) as an alternative crop to traditional uses). *Riv Agron*, 1989; 23: 442-446.
16. Gatel F. Protein quality of legume seeds for non ruminant animals: a literature review. *Anim Feed Sci Tech* 1994; 45: 317-348.
17. NRC. *Nutrient requirements of dairy cattle*. Washington, DC: 6th ed. National Academy Press, 1989.
18. Mondal M, Prakash BS. Effects of long-term GH-releasing factor administration on patterns of GH and LH secretion in growing female buffaloes (*Bubalus bubalis*). *Reproduction* 2004; 127: 45-55.
19. Pavlík A, Jelínek P, Matějček M, Illek J. Blood plasma metabolic profile of Aberdeen Angus bull during postnatal ontogenesis. *Acta Vet BRNO* 2010; 79: 419-429.
20. Ravarotto L, Dalvit P, Parenti E, Bettio M, Barberio A, Marangon S. Studio di alcuni parametri biochimici ed ematologici nel vitellone di razza Charolais. *Sel Vet* 2000; Suppl: S233-S242.
21. Kumar GAR, Rottan PJS. Plasma thyroidal and adrenocortical hormones during different development stages in buffalo heifers. *Indian J Anim Sci* 1992; 62: 747-754.
22. Morand-Fehr P, Sauvant DB, Rouzean A. Parameters indicating nutritional status of goats. *Zootechnica* 1977; 19: 195-203.
23. Hammond AC. Update on BUN and MUN as a guide for protein supplementation in cattle. US Florida: Department of Agriculture, 2006.
24. Cabaraux JF, Dufrasne I, Istasse L, Hornick JL. Variation of plasma parameters and nitrogen metabolism in finishing Belgian Blue double-muscle cull females. *J Anim Phys and Anim Nutr* 2005; 89: 55-62.
25. Doornenbal H, Tong AKW, Murray NL. Reference values of blood parameters in beef cattle of different ages and stages of lactation. *Can J Vet Res* 1988; 52: 99-105.
26. Dransfield E, Nute GR, Hogg BW, Walters BR. Carcass and eating quality of ram, castrated ram and ewe lambs. *Anim Prod* 1990; 50: 291-299.
27. ASPA (Scientific Association of Animal Production). *Metodiche per la determinazione delle caratteristiche qualitative della carne (Procedures for Meat Quality Evaluation)*. Perugia, Italy: University of Perugia, 1996: 69-70.
28. SAS/STATM. *Guide for personal Computers*. Cary, NC, USA: Ed. SAS Institute Inc., Version 8.1, 1999/2000.
29. Emile JC, Huyghe C, Huguet L. Utilisation du lupin blanc doux pour l'alimentation des ruminants: resultants et perspectives. *Ann Zoot* 1991; 40: 31-44.
30. Moss AR, Givens DI, Grundy HF, Wheeler KPA. 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 Techn* 1997; 68: 11-23.
31. Vicenti A, Toteda F, Di Turi L, et al. Use of sweet lupin (*Lupinus Albus* L. var. Multitalia) in feeding for Podolian young bulls and influence on productive performances and meat quality traits. *Meat Sci* 2009; 82: 247-251.
32. Tracy VA, Barton BA, Anderson GW, Williams MS. Comparison of sweet white lupin seeds with soybean oil meal as a protein supplement for sheep. *J Anim Sci* 1988; 66 (Suppl. 1): 499.

33. Murphy SR, McNiven MA. Raw or roasted lupin supplementation of grass silage diets for beef steers. *Anim Feed Sci Techn* 1994; 46: 23-35.
34. Purroy A, Surra J. Empleo de guisantes y de habas en el pienso para cebo de corderos (Use of peas and beans in feeds for fattening lambs), *Arch Zoot*, 1990; 39: 59-66.
35. Yu P, Leury BJ, Sprague M, Egan AR. Effect of the DVE and OEB value changes of grain legumes (lupin and faba beans) after roasting on the performance of lambs fed a roughage-base diet. *Anim Feed Sci Techn* 2001; 94: 89-102.
36. Purroy A, Surra J, Munoz F, Morago E. Use of crops in the fattening diets for lambs. III. Pea seeds. *ITEA Prod Anim* 1992; 88A: 63-69.
37. Yu P, Sprague M, Egan AR, Castleman GH, Leury BJ. Comparison of raw and roasted narbon beans (*Vicia narbonensis*) on performance and meat sensory attributes of lambs fed a roughage-based diet. *Anim Feed Sci Techn* 2001; 92: 1-16.
38. Abdullah AY, Qudsieh RI. Carcass characteristics of Awassi ram lambs slaughtered at different weights. *Livest Sci* 2008; 117: 165-175.
39. Vergara H, Molina A, Gallego L. Influence of sex and slaughter weight on carcass and meat quality in light and medium weight lambs produced in intensive systems. *Meat Sci* 1999; 52: 221-226.
40. Rizzi L, Simioli M, Sardi L, Monetti PG. Carcass quality, meat chemical and fatty acid composition of lambs fed diets containing extruded soybeans and sunflower seeds. *Anim Feed Sci Techn* 2002; 97: 103-114.
41. Atti N, Ben Salem H, Priolo A. Effects of polyethylene glycol in concentrate or feed blocks on carcass composition and offal weight of Barbarine lambs fed *Acacia cyanophylla* Lindl. Foliage. *Anim Res* 2003; 52: 363-375.
42. Aziz NN, Murray DM, Ball RO. The effect of live weight gain and live weight loss on body composition of Merino wethers: dissected muscle, fat and bone. *J Anim Sci* 1992; 70: 1819-1823.
43. Kamalzadeh A, Koops WJ, Bruchem J, van Bangma GA, Tamminga S, Zwart D. Feed quality restriction and compensatory growth in growing sheep: development of body organs. *Small Rum Res* 1998; 29: 71-82.
44. Murray DN, Tulloh NM, Winter WH. Effects of three different growth rates on empty body weight, carcass weight and dissected carcass composition of cattle. *J Agr Sci* 1974; 82: 535-547.
45. Toteda F, Facciolongo A, Ragni M, Vicenti A. Effect of suckling type and PUFA use on productive performances, quanti-qualitative characteristics of meat and fatty acid profile in lamb. *Progr Nutr* 2011; 13: 125-134.
46. El-Barody MAA, Abdalla EB, El-Hakeam A. The changes in some blood metabolites associated with the physiological responses in sheep. *Livest Prod Sci* 2002; 75: 45-50.
47. Ponnampalam EN, Egan AR, Sinclair AJ, Leury BJ. Feed intake growth, plasma glucose and urea nitrogen concentration, and carcass traits of lambs fed isoenergetic amounts of canola meal, soybean meal and fish meal whit forage based diets. *Small Rum Res* 2005; 58: 245-252.
48. Ball AJ, Thompson JM, Hinch GN, Fennesy PE, Blakely AR. Feed requirements for maintenance of mature rams and ewes from lines selected for differences in body composition. *Proc New Zeal Soc Anim Prod* 1995; 55: 133-136.
49. Solomon MB, Lynch GP, Paroczay E, Norton S. Influence of rapeseed meal, whole rapeseed, and soybean meal on fatty acid composition and cholesterol content of muscle and adipose tissue from ram lambs. *J Anim Sci* 1991; 69: 4055-4061.
50. Sirtori CR, Lovati MR, Mansoni C, Castiglioni S, Duranti M, Magni C. Proteins of white lupin seed, a naturally isoflavone-poor legume, reduce cholesterolemia in rats and increase LDL receptor activity in HepG2 cells. *J Nutr* 2004; 134: 18-23.
51. Viveros A, Centeno C, Arija I, Brenes A. Cholesterol-lowering effects of dietary lupin (*Lupinus Albus* var. Multolupa) in chicken diets. *Poultry Sci* 2007; 86: 2631-2638.
52. Payne JM. Indicators of protein status. In: Payne J.M., *The metabolic profile test*. New York: Oxford University Press, 1987: 27-35.
53. Abdel-Ghani AA, Solouma GMA, Abd Elmoty AKI, Kasab AY, Soliman EB. Productive performance and blood metabolites as affected by protected protein in sheep. *Open J Anim Sci* 2011; 1 (2): 24-32.
54. Hornick J. L., Van Eenaeme C., Gauthier S., Baldwin P., Istasse L. Glucose, alpha-amino nitrogen, and amino acid exchange across the hindlimb in young double-muscled type bulls maintained at two growth rates. *Can J Anim Sci*, 1996; 76: 193-202.
55. Hornick JL, Van Eenaeme C, Diez M, Minet V, Istasse L. Different periods of feed restriction before compensatory growth in Belgian Blue bulls: II. Plasma metabolites and hormones. *J Anim Sci* 1998; 76: 260-271.
56. Grunwaldt EG, Guevara JC, Estévez OR, et al. Biochemical and haematological measurements in beef cattle in Mendoza plain rangelands (Argentina). *Trop Anim Health Prod* 2005; 37: 527-540.
57. Nazi S, Saeb M, Rowghani E, Kaveh K. The influences of thermal stress on serum biochemical parameters of Iranian fat-tailed sheep and their correlation with tri-iodothyronine (T3), thyroxine (T4) and cortisol concentrations. *Comp Clin Pathol* 2003; 12: 135-139.
58. Thrall MA, Baker C, Campell TW, et al. *Veterinary haematology and clinical chemistry*. Philadelphia: Lippincott Williams and Wilkins, 2004.
59. Godden SM, Lissemore KD, Keton DF, Leslie KE, Walton JS, Lumsden JH. Factors associated with milk urea concentrations in Ontario dairy cows. *J Dairy Sci* 2001; 84: 107-114.
60. Brown WF, Adjei MB. Urea and (or) feather meal supplementation for yearling steers grazing limpograss (*Hemarthria altissima* var. *Floralta*) pasture. *J Anim Sci* 2001; 79: 3170-3176.
61. Duranti M. Grain legume proteins and nutraceutical properties. *Fitoterapia* 2006; 77: 67-82.

62. Fukui K, Tachibana N, Wanezaki S, et al. Isoflavone-free soy protein prepared by column chromatography reduces plasma cholesterol in rats. *J Agr Food Chem* 2002; 50: 5717-5721.
63. Macarulla MT, Medina C, De Diego MA, et al. Effects of the whole seed and a protein isolate of faba bean (*Vicia faba*) on the cholesterol metabolism of hypercholesterolaemic rats. *Brit J Nutr* 2001; 85: 607-614.
64. Dabai FD, Walker AF, Sambrook IE, Welch VA, Owen RW, Abeyasekera S. Comparative effects on blood lipids and faecal steroids of five legume species incorporated into a semi-purified, hypercholesterolaemic rat diet. *Brit J Nutr* 1996; 75: 557-571.
65. Kaneko JJ. Serum proteins and the dysproteinemias. In Kaneko JJ: *Clinical biochemistry of domestic animals*. San Diego, USA: Academic Press Inc, 1989: 142-165.
66. Sirtori CR, Eberini I, Arnoldi A. Hypocholesterolaemic effects of soya proteins: results of recent studies are predictable from the Anderson meta-analysis data. *Brit J Nutr*, 2007; 97: 816-822.
67. Potter SM. Overview of proposed mechanism for the hypocholesterolemic effect of soy. *J Nutr* 1995; 125 (suppl. 3): 606-611.

Correspondence:

Prof. Francesco Toteda

Department of Emergency and Organ Transplantation, Section of Veterinary Clinics and Animal Production, University of Bari "Aldo Moro", Via G. Amendola, 165/A 70126, Bari, Italy

Tel. +39 080 544 2836

Fax. +39 080 544 2822

E-mail: francesco.toteda@uniba.it