Raw and extruded pea (*Pisum sativum*) and lupin (*Lupinus albus* var. *Multitalia*) seeds as protein sources in weaned piglets' diets: effect on growth rate and blood parameters

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Paper received March 18, 2005; accepted May 26, 2005

ABSTRACT

The 42 days trial was carried out using 140 piglets weaned at 28 days of age. The piglets were allocated according to weight and sex to the 5 dietary treatments with 7 replicates for each treatments (4 pens x 4 castrated males and 3 pens x 4 females). The piglets were fed according to the following experimental design: 1) control diet (CTR) with soybean meal (SBM) 44% c.p. as protein source; 2) CRT diets with 200 g/kg of raw pea (*Pisum sativum*) (RP); 3) CTR diet with 200 g/kg extruded pea (EP); 4) CRT diet with 170 g/kg raw lupin (*Lupinus albus* var. *Multitalia*) (RL); 5) CTR diet with 170 g/kg of extruded lupin (EL). During the trial, animals were weighed at 0 - 21 and 42 days from the start of the trial. Feed intake was monitored and feed conversion ratio was calculated for the periods 0-21 d and 22-42 d. At the end of the trial, blood samples were taken for 14 animals for each dietary treatment (2 animals per replicate) and analysed for total protein, urea and liver activity (ALT, AST and ALP parameters). Average daily weight gain and feed intake did not differ according to dietary treatments whereas during the total experimental period (0-42 d), feed conversion ratio was higher for EP *vs* CTR diet (2.35 *vs* 2.09, respectively; P <0.05). The growth rate for diets with extruded protein sources compared with diets containing the raw ingredients did not differ. Feed conversion ratio for the RP was numerically higher than for the EP (2.35 *vs* 2.16 and 2.76 *vs* 2.32, respectively during 22-42 d and 0-42 d periods). Blood parameters did not show significant difference among dietary treatments except for higher total protein for CTR diet *vs* RL diet, EL and RP (67.3 *vs* 62.2, 62.8 and 63.6 g/l, respectively; P<0.05) and urea that resulted the highest with CTR diet *vs* RL and RL (4.7 *vs* 3.7 and 3.8 mmol/l respectively; P<0.05).

Key Words: Pig, Pea, Lupin, Protein sources, Extrusion.

RIASSUNTO

IMPIEGO DI PISELLO E LUPINO CRUDI O ESTRUSI COME FONTI PROTEICHE IN DIETE PER SUINETTI SVEZZATI: EFFETTI SULLE PERFORMANCE DI CRESCITA E SUI PARAMETRI EMATICI

La prova, della durata di 42 giorni, è stata condotta usando 140 suinetti svezzati di circa 28 giorni d'età. Gli animali sono stati distribuiti in base al peso ed al sesso in 7 box di 4 animali ciascuno (4 box di maschi castrati e 3 di femmine per ciascun replicato) in 5 tesi. Gli animali sono stati alimentati secondo il seguente schema sperimentale: 1) dieta di controllo (CTR) con farina di estrazione di soia (FES) come fonte proteica al 44% di PG; 2) dieta CRT con 200 g/kg di pisel-

*lo (*Pisum sativum*) crudo (RP); 3) dieta CTR con 200 g/kg di pisello estruso (EP); 4) dieta CTR con 170 g/kg di lupino (*Lupinus albus *var.* Multitalia*) crudo (RL); 5) dieta CTR con 170 g/kg di lupino estruso (EL). Durante la prova sono stati effettuati controlli ponderali a 0 - 21 e 42 giorni; si è controllato inoltre il consumo alimentare per il calcolo dell'indice di conversione. Al termine della prova è stato effettuato un prelievo ematico su 14 soggetti per ciascuna tesi (2 soggetti per ciascun replicato di ciascuna tesi). I campioni di sangue sono stati analizzati per contenuto di proteine totali, urea e parametri della funzionalità epatica (ALT, AST e ALP). L'incremento giornaliero medio e l'ingestione media giornaliera non hanno mostrato differenze statisticamente significative tra i diversi trattamenti durante il periodo sperimentale (0-42 d). L'indice di conversione è risultato più elevato nelle tesi con EP rispetto a CTR (rispettivamente 2,35* vs *2,09; P <0,05). La somministrazione della dieta EL non ha determinato variazioni nelle performances dei suinetti, mentre la somministrazione della dieta EP ha determinato un peggioramento numerico dell'indice di conversione nei periodi 0-42 d e 22-42 d (2,35* vs *2,16 e 2,76* vs *2,32, rispettivamente per la dieta contenente EP e quella con RP). I parametri ematici valutati non hanno evidenziato differenze significative fra le tesi a confronto ad eccezione delle proteine totali che sono risultate più elevate con la dieta CTR rispetto a quelle con RL, EL e RP (rispettivamente 67,3* vs *62,2, 62,8 e 63,6; P<0,05) e per l'urea che risulta superiore nella dieta CTR vs RL e EL (4,7* vs *3,7 e 3,8 mmol/l rispettivamente; P<0,05).*

Parole chiave: *Suino, Pisello, Lupino, Fonti proteiche, Estrusione.*

Introduction

In the past, the nutrient requirements of monogastric animals were met using meals of animal origin and soybean meal (SBM). The ban on using meat and meat and bone meal, following the BSE emergency, together with the growing market price of SBM totally imported from non-European markets, and consumer and institutional scepticism regarding Genetically Modified Organism (GMO) feed ingredients used in animal nutrition, have lead to growing interest in the using of legumes like pea, lupin, faba beans etc. as alternative protein sources to SBM. Much research has been conducted on these raw materials to evaluate their feeding value, the level of use, anti-nutritional factors content and the growing performances of animals. In nutrition and feeding strategies regarding pigs, many studies have been recently conducted on pea (Bertrand *et al.*, 1980; Gatel and Grosjean, 1990; Jacyno *et al.*, 1992; Perez J.M. and Bourdon D., 1992; Castell *et al.*, 1996; Alonso *et al.*, 2000a; Alonso *et al.*, 2001; Mariscal-Landin *et al.*, 2002), lupin (Fernandez and Batterham, 1995; McNiven and Castell, 1995; Gdala *et al.*, 1996; Noblet and Bourdon, 1997; King *et al.*, 2000; Salgado *et al.*, 2002; Ferguson *et al.*, 2003; Cherriere *et al.*, 2003; Froidmont *et al.*, 2003) faba bean (Jacyno *et al.*, 1992; Flis *et al.*, 1999; Alonso *et al.*, 2000b; Mariscal-Landin *et al.*, 2002; Salgado *et al.*, 2002; Berger *et al.*, 2003).

The inclusion of these alternative protein sources as ingredients in animal diets have to be carefully evaluated because in some cases negative effects have been noted on the growth rate, mainly in young animals. The decreased rate can be attributed to the presence of anti-nutritional factors such as tannins, lectins, protease inhibitors, non-starch-polysaccharides (NSP) and alkaloids in varying amounts according to species or cultivar (Gatel and Grosjean, 1990; Gatel 1994; Castell *et al.*, 1996; Gdala *et al.*, 1996). Negative effects can be minimised by choosing suitable quantities to include in the diet for these ingredients (McNiven and Castell, 1995) or using technological treatments such as flacking, expansion, extrusion, micronization etc. (Gatel, 1994; Alonso *et al.*, 2000a; Alonso *et al.*, 2000b; Alonso *et al.*, 2001; Mariscal-Landin *et al.*, 2002).

The effects of technological treatments seems to vary with the growing period of pigs; indeed, while the poor effects of pelleting, autoclaving, flaking and extrusion of pea have been noted in growing-finishing pigs (Gatel, 1994), a significant increase in the apparent digestibility of pea proteins due to extrusion treatment has been demonstrated in weaned pigs (Bengala Freire *et al.*, 1991). Some authors have studied the effects of pea extrusion (Alonso *et al.*, 1998; Alonso *et al.*, 2000a) on the level of Non Starch Polysaccharide (NSP) and anti-nutritional factors on starch digestibility, protein and energy, while Mariscal-Landin *et al.* (2002) have demonstrated higher apparent digestibility of pea amino acids, mainly tryptophan and cystin, as a result of extrusion treatment.

The aim of this study was to verify the possibility of using high levels of inclusion of pea seeds (*Pisum sativum*) or lupin seeds (*Lupinus albus* var. *multitalia*) as the main protein source in the diet of weaned pigs and the effect of extrusion treatment on performance.

Material and methods

Animals and housing

The study was carried out in the CERZOO facility (S. Bonico, PC, Italy) with (LW x L) x D female and castrated male commercial piglets. Animals were weighed and selected out of groups and vaccinated for typical disease prevention. The experimental design compares five different diets fed to pigs housed in five blocks with 7 replicates for each experimental diet, as follows: after a pre-experimental period of 19 days, 140 animals (10.4 ± 2.01 kg l.w.) were randomly distributed into 5 homogeneous groups of 28 animals each (16 castrated males and 12 females per dietary treatments), in order to achieve maximum homogeneity within each group and minimum differences between all trial groups. The 28 piglets for each dietary regime were divided into 7 pens (replicates), each pen containing 4 piglets of the same sex. The diets were assigned to the pens in a complete randomised block design using the Randomized Procedure of SAS software (1999) release 8.0. Animals were housed in five rooms of the same facility. Each room was divided in 6 pens of 1 m^2 each. The temperature was regulated by a thermostatically controlled system. The rooms were ventilated with an automatic system regulated for temperature and humidity. Animals had continuous access to feed and water for *ad libitum* consumption, with one feeder and an automatic drinker for each pen. The isonitrogenous and isoenergetic meal diets were formulated according to INRA (1989) requirements and provided for 42 consecutive days. At 21 and 42 days from the start of the study feed intake (FI) and the feed conversion ratio (FCR) were calculated. According to the CERZOO procedure, during the pre-experimental period the animals were fed medicated feed containing chlortetracycline (1000 mg/kg of a.p.) and spiramicyn (400 mg/kg of a.p.).

Dietary treatments

The experimental diets were produced in the CERZOO facility with a horizontal mixer (500-kg capacity) mixing a basal diet (table 3) with the protein sources (table 1) to be tested. The following experimental diets were compared: 1) Control (CTR): basal diet (84%), SBM (16%); 2) Pea (*Pisum sativum*) (RP): basal diet (72.99%), RP (20%), SBM (7%) and 0.01% amino acids supplement; 3) Extruded pea (EP): basal diet (72.99%), EP (20%), SBM (7%) and 0.01% amino acids supplement 4) Lupin (*Lupinus albus* var. *multitalia*) (RL): basal diet (82.75%), RL (17%) and 0.25% amino acids supplement; 5) Extruded lupin (EL): basal diet (82.75%), EL (17%) and 0.25% amino acids supplement.

Protein sources, basal, control and experimental diets were sampled before the beginning of the trial and during the experimental periods, at the start of the first (0-21 d) and second (22-42 d) growing phase. Samples were analysed for crude protein, ether extract, crude fibre, ash and total sugars according to ASPA (1980) and Martillotti *et al.* (1987); for ADF and NDF (Van Soest *et al.*, 1991), and for starch according to the polarimetric method (AOAC, 2000). The digestible and net energy were calculated according to Morgan and Whittemore (1982) and Noblet and Bourdon (1997) equation respectively. Amino acids were determined using the amino acid analyser (Carlo Erba 3A29) (Moore, 1963; Eggum, 1968; Moore *et al.*, 1980). Tannins and polifenol were analysed using spectrophotometric analysis according to Carmona *et al.* (1991); levels of genistein and daidzein using HPLC and spectrophotometric analysis according to Liggins *et al.* (1998) and Franke *et al.* (1994); antitripsic activity using the colorimetric methods (Smith *et al.*, 1980).

The chemical characteristics of SBM, pea and lupin seeds are reported in table 1, their anti-nutritional factors contents are reported in table 2 and the composition and the chemical characteristics of the basal, control and experimental diets are reported in tables 3 and 4 respectively.

Measurements

Animals were individually weighted at the beginning of the trial, and after 21 and 42 days. On the same days FI per pen was also recorded to calculate FCR and the average daily gain (ADG) during the three experimental periods (0-21; 22-42 e 0-42 days of trial) for each replicate.

Table 1. Analytical characteristics of protein sources (g/kg feed).

		Pea seeds		Lupin seeds	
	Soya Bean	Raw	Extruded	Raw	Extruded
Chemical composition:					
Dry Matter	885.9	880.1	861.8	911.2	912.3
Crude Protein	451.0	212.5	210.3	350.5	354.4
Ether Extract	13.0	12.3	31.5	77.4	99.8
Crude Fiber	62.1	68.2	41.0	95.8	96.1
Ash	60.7	32.6	33.9	38.1	37.3
Starch	59.6	436.6	413.0	114.1	81.5
Total Sugar	92.6	38.4	45.1	65.6	67.6
NDF	179.3	162.8	105.7	183.7	192.4
ADF	66.7	87.5	57.6	118.0	120.6
Amino Acids:					
Aspartic Acid	48.9	26.5		41.6	
Threonine	18.0	8.1		13.0	
Serine	25.4	11.3		21.8	
Glutamic Acid	85.6	34.6		80.7	
Proline	24.5	8.5		15.0	
Glycine	19.1	8.5		13.6	
Alanine	19.8	8.9		11.6	
Valine	22.8	9.1		13.8	
Methionine	6.5	1.8		2.4	
Isoleucine	22.7	8.7		16.3	
Leucine	35.2	14.1		26.1	
Tyrosine	17.3	6.2		16.0	
Phenylalanine	23.5	9.5		14.7	
Histidine	11.9	3.8		8.2	
Lysine	28.4	3.0		14.9	
Arginine	34.6	14.2		35.0	
Tryptophan	6.1	1.8		2.1	
Cystine	6.7	3.0		5.0	

At the end of the trial blood samples were taken from two animals from each replicate (14 samples per regime, 70 samples total). Blood samples were taken (jugular vein) from 6 hour fasted animals following the Vacutainer® method with lithium epharine as anticoagulant. Samples were immediately centrifuged and plasma frozen (-20°C). Plasma samples were analysed for urea, total proteins, alanine aminotransferase (ALT), aspartate aminotransferase (AST) alcaline phosphatase (ALP) and total bilirubin according to Bertoni *et al.* (1998).

Statistical analysis

The data for live weight, feed intake, ADG and FCR were statistically processed to determine the differences among protein sources. Animals were individually weighted but the pen was the experimental unit. Statistical analysis was performed according to the GLM (General Linear Model) procedure of SAS Institute software package (1999), release 8.0, with protein sources as independent variables in a two-way analysis of variance within a randomised complete bock design, between random error (between rooms variation) as the error

Table 2. Antinutritional content in protein sources.

			Pea seeds		Lupin seeds	
		Soya Bean	Raw	Extruded	Raw	Extruded
Tannins	mg/g	0.39	0.14	0.34	0.61	0.81
Polyphenols	"	2.09	11.19	7.54	6.39	2.68
Genisteine	ppm	0.70	0.00	0.00	0.00	0.10
Daidzeine	"	1.60	0.10	0.10	0.00	0.20
Trypsin Inhib.Actv.	mg/g	1.30	0.89	0.40	0.72	1.40

Table 3. Composition and analytical characteristics of the basal diet.

Ingredients:		
Corn meal	%	34.1
Barley meal	"	28.0
Wheat meal	"	22.5
Soya oil	"	1.00
Soybean meal 44%	"	9.61
Limestone	"	1.48
Dicalcium phosphate	"	1.65
Sodium chloride	"	0.670
Methionine DL	"	0.076
Lysine HCL 50%	"	0.450
Threonine L	"	0.026
Tryptophan L	"	0.038
Flavour	"	0.042
Premix[1]	"	0.358
Chemical composition:		
Dry matter	%	89.07
Crude protein	"	13.61
Ether extract	"	5.51
Crude fiber	"	3.61
Ash	"	5.28
Starch	"	50.27
Digestible energy	Kcal/kg	3423
Net energy	"	2590
Lys	%	1.10
Thr	"	0.53
Meth+Cys	"	0.55
Try	"	0.18

[1] Composition for kg feed: vit. A 15.500 U; vit D3 1.800 U; vit E acetate 35 mg; Cu 145 mg; Se 0.18 mg; flavofosfolipol 21 mg.

term. The treatment means were compared using Student's "t" test. Animals LW at the start of the study was used as covariata. Statements of statistical significance were based upon P<0.05

Results and discussion

Protein sources and diet characteristics

The characteristics of the protein source used and the experimental diets are reported respectively in tables 1 and 2. The composition and the analytical characteristics of the basal and experimental diets are reported respectively in tables 3 and 4. The anti-nutritional factor content in pea seeds and lupin seeds was very low (table 2) and the extrogenic activity factor content (genisteine and daidzeine) were lower *vs* SBM. Extrusion treatment determined a reduction of the polyphenol content both in pea and in lupin while the antitripsic activity diminished only in pea and was unexpectedly higher in lupin. Other authors (Alonso *et al.*, 1998; Alonso *et al.* 2000a; Alonso *et al.* 2000b) have shown that extrusion is the best method to abolish trypsin, chymotrypsin, alpha-amilase inhibitors and haemoagglutinating activity. In trial with rats (Alonso *et al.* 2000a), ADG was higher when animals were fed extruded pea diet than raw pea; FCR and protein efficiency ratio values greatly increased. Therefore according to authors the extrusion treatment did significantly improve the nutritional quality of pea.

Productive performance

The effect of experimental diets on ADG, FI and FCR are shown in table 5. The inclusion of high levels of pea (200 g/kg) and lupin (170 g/kg)

Table 4. Composition and analytical characteristics of the experimental diets.

			Pea		Lupin	
		Control diet	Raw	Extruded	Raw	Extruded
Ingredients:						
Basal diet	%	84.00	72.99	72.99	82.75	82.75
Lupin	"	-	-	-	17.00	-
Lupin extruded	"	-	-	-	-	17.00
Pea	"	-	20.00	-	-	-
Pea extruded	"	-	-	20.00	-	-
Soybean meal 44%	"	16.00	7.00	7.00	-	-
Methionine DL	"	-	0.01	0.01	0.02	0.02
Lysine HCL	"	-	-	-	0.18	0.18
Threonine L	"	-	-	-	0.02	0.02
Tryptophan L	"	-	-	-	0.02	0.02
Chemical composition:						
Crude protein	%	17.29	17.06	17.38	17.06	17.28
Ether extract	"	4.77	5.92	4.88	5.51	5.62
Crude fiber	"	3.30	3.51	3.80	4.25	4.34
Ash	"	5.22	4.82	5.08	4.88	4.94
Starch	"	42.49	44.61	42.91	38.90	40.29
Digestible energy	Kcal/kg	3411	3409	3411	3426	3425
Net energy	"	2569	2574	2573	2586	2587
Lys	%	0.88	0.86	0.90	0.89	0.87
Thr	"	0.71	0.68	0.65	0.65	0.68
Meth+Cys	"	0.64	0.59	0.63	0.60	0.59
Try	"	0.22	0.21	0.22	0.20	0.21

did not determine significant negative effects on ADG and FI, while FCR during the all experimental period (0-42 d) was higher for extruded pea *vs* control (2.35 *vs* 2.09, respectively; $P<0.05$). Extrusion treatment did not modify animal performance, although a numerically lower FCR for extruded *vs* raw pea diets in 0-42d (2.35 *vs* 2.16) and 22-42d (2.76 *vs* 2.32) periods was recorded. No examined parameters showed a sex/treatment interaction. Results confirm the possibility of using protein sources alternative to SBM in weaned pigs' diets, without significant reducing effects on animal performance. McNiven and Castell (1995), utilizing increasing levels of lupin (up to 100 g/kg) in the diet of pigs (from 10 to 20 kg l.w.) showed positive effects on ADG and FCR, while higher levels determine a decrease in performance; this appeared to be due to a reduction of feed intake, rather than a direct effect on amino acid composition or alkaloid content of diets, since feed efficiency was not affected by the level of inclusion in the diet (McNiven and Castell, 1995). Gdala *et al.* (1996) did not observe these negative effects using different species of lupin seed meal (*Lupinus luteus, L. albus, L. angustifolius*) at the inclusion level ranging from 310 to 410 g/kg, which totally substitutes the SBM; only pigs given the *L. albus* diet had a higher FCR. Also Fernandez and Batterham (1995) founded a positive effect on ADG and on FCR using 408 g/kg of *L. angustifolius* substituting SBM in growing pigs' diets from 20 to 45 kg l.w.. In a study with younger animals (pigs from 12 to 25 kg l.w.) and using *L. angustifolius* and *L. albus*, Cherriere *et al.* (2003) recommended to limit the inclusion in the diet at 10% of both lupin species. Jacyno *et al.* (1992) in a

Table 5. Performance of pigs.

Periods		Dietary treatments					
			Pea		Lupin		
		Control	Raw	Extruded	Raw	Extruded	SE
Average daily gain:							
0-21 d	g/d	317	297	314	292	294	15.19
0-42 d	"	383	370	354	345	355	14.03
22-42 d	"	451	448	395	399	417	20.17
Feed intake:							
0-21 d	g/d	593	589	609	590	580	22.38
0-42 d	"	811	812	840	790	763	31.03
22-42 d	"	1029	1035	1072	990	946	47.58
Feed conversion ratio:							
0-21 d		1.88	2.00	1.95	2.04	1.98	0.05
0-42 d		2.09[a]	2.16[ab]	2.35[b]	2.27[ab]	2.13[a]	0.06
22-42 d		2.30	2.32	2.76	2.50	2.28	0.12

[a, b]: *P<0.05*

Table 6. Blood parameters: effect of treatment.

Parameter		Dietary treatments					
			Pea		Lupin		
		Control	Raw	Extruded	Raw	Extruded	SE
Urea	mmol/l	4.7[b]	4.1[ab]	4.2[ab]	3.7[a]	3.8[a]	0.27
Total protein	g/l	67.3[b]	63.6[a]	64.0[ab]	62.2[a]	62.8[a]	1.20
ALT	U/l	51.5	52.7	53.4	51.4	557.5	2.65
AST	"	50.7	57.9	47.7	59.9	50.7	5.17
ALP	"	150.7	148.3	163.6	154.1	177.1	10.01
Total Bilirubin	μmol/l	4.1	4.9	3.7	5.2	4.8	0.62

[a, b]: *P<0.05*

trial on growing pigs fed on a diet containing 120 g/kg of lupin or 200 g/kg of pea, showed positive effects of pea on growth performance and on FCR, while lupin determined negative effects on both parameters. Poor information is available on the anti-nutritional content of pea; Castell *et al.* (1996) recommend maximum inclusion of pea of 100, 200 and 350 g/kg respectively in starter, grower and finisher diets. In a trial with piglets from 6 weeks of age, Bertrand *et al.* (1980) demonstrated that pea could be used at a maximum level of inclusion of 15-20% as partial substitute of SBM. Few data are available on the effect of the extrusion treatment of pea and lupin on pigs' growth performance. Some authors (Alonso *et al.*, 2000a and 2000b) found that extrusion was the most effective treatment for improving pea protein and starch digestibility when compared with dehulling, soaking and germination. The positive effect of extrusion on starch digestibility has been demonstrated by Masoero *et al.* (2005) in a recent work; nevertheless, despite these positive effects, the growth rate of pigs does not seem to be positively modified with extruded pea and lupin.

Blood parameters

Data regarding blood parameters are reported in table 6. No significant differences have been noted among dietary treatments for different parameters except for total protein that resulted the highest with CRT diet *vs* RP, RL and EL (67.3 *vs* 63.6, 62.2 and 62.8 g/l respectively P <0.05) and urea that resulted the highest with CRT diet *vs* raw and extruded lupin (4.7 *vs* 3.7 and 3.8 mmol/l respectively; P<0.05). Very few data are available in literature about the effects of pea, lupin and faba on blood parameters. According to Barneveld *et al.* (1995) the *Pisum sativum* heated to 150 or 165 degrees for 15 minutes decreased serum protein and serum urea concentrations in growing pigs but in our study no difference was found between RP *vs* EP or EL. Martinez *et al.* (1995) found that the inclusion of raw pea as the source of protein in the diet of growing rats induces a reduction in plasma glucose, triglycerides and protein. In a study on piglets fed diets containing 45% raw or extruded pea, Bengala-Freire *et al.* (1991) showed a marked increase in postprandial serum glucose and insulin concentration in piglets fed on extruded *vs* raw pea.

The blood parameters ALT, AST and ALP demonstrated that diets containing pea and lupin did not determine negative effects on liver functionality because the values were similar to the control diet.

Conclusions

In conclusion, considering our results and those found in the literature, pea (*Pisum sativum*) and lupin (*Lupinus albus* var. *multitalia*) seeds represent very interesting alternative protein sources to SBM in pigs' diets. In many trials these feedstuffs have been utilized at high level as total or partial substitutes of SBM without determining decreased growing performance; nevertheless, because of some negative effects due to anti-nutritional factors contained mainly in lupin and in the case of a complete lack of analysis of anti-nutritional factors, levels of inclusion in growing pigs' diets should not be more than 100-150 g/kg for lupin or 150-200 g/kg for pea. Data from the literature show that extrusion treatment enhances the nutritional characteristics such as starch and protein digestibility of alternative protein sources and reduces the anti-nutritional factor levels. Nevertheless, our results demonstrate that extrusion has not positive influence to the growing performances of pigs compared to the raw seeds.

Work supported by the ALBIO project Safe feeds for improving animal welfare and food from animal products (D.M. MiPAF n. 441/7303/01).

REFERENCES

ALONSO, R., GRANT, G., DEWEY, P., MARZO, F., 2000a. Nutritional assessment *in vitro* and *in vivo* of raw and extruded peas (*Pisum sativum* L.). J. Agr. Food Chem. 48:2286-2290.

ALONSO, R., AGUIRRE, A., MARZO, F., 2000b. Effects of extrusion and traditional processing methods on antinutrients and *in vitro* digestibility of protein and starch in faba and kidney beans. Anim. Food Chem. 68:159-165.

ALONSO, R., ORUE, E., MARZO, F., 1998. Effects of extrusion and conventional processing methods on protein and antinutritional factor contents in pea seeds. Food Chem. 63:505-512.

ALONSO, R., RUBIO, L.A., MUZQUIZ, M., MARZO, F., 2001. The effect of extrusion cooking on mineral bioavailability in pea and kidney bean seed meals. Anim. Feed Sci. Technol. 94:1-13.

AOAC, 2000. Official Methods of Analysis 17th ed. Association of Official Analytical Chemists, Washington, DC, USA.

ASPA, 1980. Commissione Valutazione Alimenti. Valutazione nutrizionale degli alimenti di interesse zootecnico. Analisi chimica. Zoot. Nutr. Anim. 6:19-34.

BARNEVELD, R.J. VAN, BATTERHAM, E.S., SKINGLE, D.C., NORTON, B.W., 1995. The effect of heat on amino acids for growing pigs. 4. Nitrogen balance and urine, serum and plasma composition of growing pigs fed on raw or heat-treated field peas (*Pisum sativum*). Brit. J. Nutr. 73:259-273.

BENGALA FREIRE, J., AUMAITRE, A., PEINIAU, J., 1991. Effects of feeding raw and extruded peas on ileal digestibility, pancreatic enzymes and plasma glucose and insulin in early weaned pigs. J. Anim. Physiol. An. N. 65:154-164.

BERGER, J.D., ROBERTSON, L.D., COCKS, P.S., 2003. Agricultural potential of Mediterranean grain and forage legumes. 2. Anti-nutritional factor concentrations in the genus *Vicia*. Genet. Resour. Crop Ev. 50:201-212.

BERTONI, G., TREVISI, E., CALAMARI, L., LOMBARDELLI, R., 1998. Additional energy and protein supplementation of dairy cows in early lactation: milk yield, metabolic-endocrine status and reproductive performances. Zoot. Nutr. Anim. 24:7-29.

BERTRAND, G., PEREZ, J.M., QUEMERE, P., 1980. Utilization du pois proteagineux par le porcelet sevré precocement: influence de la natura de la céréale. Journées Rech. Porcine en France. 12:215-226.

CARMONA, A., SEIDL, D.S., JAFFE, W.G., 1991. Comparison of extraction methods and assay procedures for the determination of the apparent tannin content of common beans. J. Sci. Food Agr. 56: 291-301.

CASTELL, A.G., GUENTER, W., IGBASAN, F.A., 1996. Nutritive value of peas for non-ruminant diets. Anim. Feed Sci. Technol. 60:209-227.

CHERRIÈRE, K., ALBAR, J., NOBLET, J., SKIBA, F., GRANIER, R., PEYRONNET, C. 2003. Utilization du lupin bleu (*Lupinus angustifolius*) et du lupin blanc (*Lupinus albus*) part les porcelets en post sevrage. Journées Rech. Porcine en France. 35:97-104.

EGGUM, B.O., 1968. A description of the method used at the National Institute of Animal Science. Acta Agric. Scand. 18:127-131.

FERGUSON, N.S., GOUS, R.M., IJI, P.A., 2003. Determining the source of anti-nutritional factor(s) found in two species of lupin (*L. albus* and *L. angustifolius*) fed to growing pigs. Livest. Prod. Sci. 84:83-91.

FERNANDEZ, J.A., BATTERHAM, E.S., 1995. The nutritive value of lupin-seed and dehulled lupin-seed meals as protein sources for growing pigs as evaluated by different techniques. Anim. Feed Sci. Technol. 53:279-296.

FLIS, M., SOBOTKA, W., PURWIN, C., ZDUNCZYK, Z., 1999. Nutritional value of diets containing field bean (*Vicia faba* L.) seeds with high or low proanthocyanidin levels for pig. J. Anim. Feed Manag. 8:171-180.

FRANKE, A.A., CUSTER, L.J., CERNA, C.M., NARALA, K.K., 1994. Quantitation of phytoestrogens in legumes by HPLC. J. Agr. Food Chem. 42:1905-1913.

FROIDMONT, E., SCHOELING, O., DELIÈGE, F., WATHELET, B., WAVREILLE, J., BARTIAUX-THILL, N., 2003. Influence de la substitution du torteau de soja par des graines de lupin, avec ou sans complément d'α-galactosidase, sur la digestibilité des régimes et de la rétention azotée du porc en croissance. Journées Rech. Porcine en France. 35:105-112.

GATEL, F., 1994. Protein quality of legume seeds for non-ruminant animals: a literature review. Anim. Feed Sci. Technol. 45:317-348.

GATEL, F., GROSJEAN, F., 1990. Composition and nutritive value of peas for pigs: a review of European results. Livest. Prod. Sci. 26:155-175.

GDALA, J., JANSMAN, A.J.M., VAN LEEUWEN, P., HUISMAN, J., VERSTEGEN, M.W.A., 1996. Lupins (*L. luteus*, *L. albus* and *L. angustifolius*) as a protein source for young pigs. Anim. Feed Sci. Technol. 62:239-249.

INRA, 1989. L'alimentation des animaux monogastriques: porc, lapin, volailles. 2nd ed. INRA, Paris, France.

JACYNO, E., CZARNECKI, R., OWSIANNY, J., WEJKSZA, D., PALUSINSKI, J., 1992. The effect of seeds of yellow lupine and pea as a source of protein in feeding pigs on their growth rate. World Rev. Anim. Prod. 27(4):11-14.

KING, R.H., DUNSHEA, F.R., MORRISH, L., EASON, P.J., VAN BARNEVELD, R.J., MULLAN, B.P., CAMPBELL, R.G., 2000. The energy value of *Lupinus angustifolius* and *Lupinus albus* for growing pigs. Anim. Feed Sci. Technol. 83:17-30.

LIGGINS, J., BLUCK, L.J.C., COWARD, W.A., BINGHAM, S.A., 1998. Extraction and quantification of daidzein and genistein in food. Anal. Biochem. 264:1-7.

MARISCAL-LANDÌN, G., LEBRETON, Y., SÈVE, B., 2002. Apparent and standardised true ileal digestibility of protein and amino acids from faba bean, lupin and pea, provided as whole seeds, dehulled or extruded in pig diets. Anim. Feed Sci. Technol. 97:183-198.

MARTILLOTTI, F., ANTONGIOVANNI, M., RIZZI, L., SANTI, E., BITTANTE, G., 1987. Metodi di analisi per la valutazione degli alimenti di interesse zootecnico. CNR-IPRA, Roma, Italy.

MARTINEZ, J.A., MARCOS, R., MACARULLA, M.T., LARRALDE, J., 1995. Growth, hormonal status and protein turnover in rats fed on a diet containing peas (*Pisum sativum* L.) as the source of protein. Plant Foods Hum. Nutr. 47:211-220.

MASOERO, F., PULIMENO, A.M., ROSSI, F., 2005. Effect of extrusion, espansion and toasting on the nutritional value of peas, faba beans and lupins. Ital. J. Anim. Sci. 4:177-189.

MCNIVEN, M.A., CASTELL, A.G., 1995. Replacement of soybean meal with lupinseed (*Lupinus albus*) in pig starter diets. Anim. Feed Sci. Technol. 52:333-338.

MOORE, S., 1963. On the determination of Cystine as Cysteic acid. J. Biol. Chem. 238:235-238.

MOORE, S., SPACKMAN, D.H., STEIN, W.H., 1980. Official methods of Analysis. 13th ed. AOAC, Arlington, VA, USA.

MORGAN, C.A., WHITTEMORE, C.T., 1982. Energy evaluation of feeds and compounded diets for pigs - A review. Anim. Feed Sci. Technol. 7:387-400.

NOBLET, J., BOURDON, D., 1997. Valeur énergétique comparée de onze matières premières chez le porc en croissance et la truie adulte. Journées Rech. Porcine en France. 29:221-226.

PEREZ, J.M., BOURDON, D., 1992. Energy and protein value of peas for pigs: synthesis of French result. pp 489-490 in Proc. 1st Europ. Conf. on Grain Legumes, Angers, France.

SALGADO, P., FREIRE, J.P.B., MOURATO, M., CABRAL, F., TOULLEC, R., LALLÈS, J.P., 2002. Comparative effects of different legume protein sources in weaned piglets: nutrient digestibility, intestinal morphology and digestive enzymes. Livest. Prod. Sci. 74:191-202.

SAS, 1999. SAS/STAT User's Guide (Release 8.0). SAS Inst. Inc. Cary, NC, USA.

SMITH, C., MEGEN, W.V., TWAALFHOVEN, L., HITCHCOCK, C., 1980. The determination of trypsin inhibitor levels in foodstuffs. J. Sci. Food Agr. 31:341-350.

VAN SOEST, P.J., ROBERTSON, J.B., LEWIS B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and non starch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583-3598.