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Pea seeds (*Pisum sativum*), faba beans (*Vicia faba* var. *minor*) and lupin seeds (*Lupinus albus* var. *multitalia*) as protein sources in broiler diets: effect of extrusion on growth performance

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ABSTRACT

The effect of extrusion of pea seeds (*Pisum sativum*) (PS), faba bean (*Vicia faba*, variety *minor*) (FB) and lupin seeds (*Lupinus albus*, variety *multitalia*) (LS) on broiler performance were evaluated. Four hundred sixty two 1d-old Ross male chicks, Marek vaccinated, were randomly assigned to seven dietary treatments (3 pens per treatment/22 birds per pen). Chicks were floor housed, ad libitum fed isocaloric and isonitrogenous diets and had free access to water. Artificial light was provided 10 h/d. The bulk of the base diet (control diet) was corn (48.8%, 53.7% and 57%), solvent-extracted soybean meal (42.8%, 37.3% and 33.4%), corn oil (4.4%, 5.2% and 6.3%), plus synthetic amino acids, minerals, trace minerals and vitamins, respectively for the 1-10d-old, 11-28d-old and 29 to 42d-old growing periods. The amounts of PS, FB and LS used on an as fed basis were: PS and extruded PS (EPS): 353 (1-10d-old), 356 (11-28d-old) and 350 (29-42d-old) g/kg; FB and extruded FB (EFB): 479 (1-10d-old), 497 (11-28d-old) and 500 (29-42d old) g/kg; LS and extruded LS (ELS): 360 (1-10d-old) and 300 (11-42d-old) g/kg. High levels of pea (350 g/kg) and faba bean (500 g/kg) did not show negative effects on body weight gain (BWG) and bird feed intake compared to control. Lupin at the 300 g/kg level reduced ($P < 0.05$) the BWG during the finishing period (22 to 42 d), however the effect disappeared over the whole experimental period (1-42 d) compared to the control group. The ELS group had a lower ($P < 0.01$) feed intake compared to the control group and to the LS group. The feed conversion rate (FCR) was similar among groups for the whole experimental period; however during the grower period the FCR was higher ($P < 0.05$) for the PS, FB and EFB groups compared to the control group. Birds consuming the PS diet had a reduced ($P < 0.05$) eviscerated carcass yield compared to the control group. The breast meat percent yield was higher ($P < 0.01$) for birds consuming the FB and EFB diets compared to the control group. There were no statistical differences in percent yield of the leg quarters and in blood parameters.

Key Words: Broilers, Pea, Faba bean, Lupin, Extrusion.

RIASSUNTO

PISELLO, FAVA E LUPINO IN DIETE PER BROILERS: EFFETTI DELL'ESTRUSIONE SULLE PERFORMANCE DI CRESCITA

*Lo studio ha valutato l'effetto dell'estrusione di Pisello (*Pisum sativum*) (PS), Fava (*Vicia faba*, varietà *minor*) (FB) e Lupino (*Lupinus albus*, varietà *multitalia*) (LS) sulle performance di crescita dei broilers. 462 pulcini maschi Ross di 1 un* *giorno di vita, vaccinati Marek, sono stati randomizzati a 7 trattamenti (3 box per trattamento/22 animali per box). I pulcini erano allevati a terra, alimentati ad libitum con diete isocaloriche ed isoproteiche ed avevamo libero accesso all'acqua di bevanda. Agli animali veniva fornita una luce artificiale per 10 h/d. La dieta di base (dieta di controllo, CTR) era costituita da, rispettivamente per i periodi da 1-10, 11-28 e 29-42 giorni di età: mais (48,8%, 53,7% e 57%), farina di estrazione di soia (42,8%, 37,3% e 33,4%), olio di mais (4,4%, 5,2% e 6,3%), aminoacidi di sintesi, minerali e vitamine. Le quantità di PS, FB ed LS utilizzate come tal quale sono state: PS e PS estruso (EPS): 353 (1-10 giorni), 356 (11-28 giorni) e 350 (29-42 giorni) g/kg; FB e FB estrusa (EFB): 479 (1-10 giorni), 497 (11-28 giorni) e 500 (29-42 giorni) g/kg; LS e LS estruso (ELS) 360 (1-10 giorni) e 300 (11-42 giorni) g/kg. L'impiego di elevati livelli di pisello (350 g/kg) e fava (500 g/kg) nella dieta non ha determinato effetti negativi sugli incrementi medi giornalieri (BWG) e sul consumo di alimento rispetto al gruppo di controllo. Il lupino (300 g/kg) ha determinato una riduzione (P < 0,05) del BWG nel periodo di finissaggio, mentre nessuna differenza significativa veniva osservata su tutto il periodo sperimentale rispetto al gruppo di controllo. L'ingestione di alimento è risultato inferiore (P< 0,01) nel gruppo ELS rispetto al gruppo di controllo ed al gruppo LS. L'indice di conversione (FCR) è risultato analogo tra i gruppi per l'intero periodo sperimentale, mentre il FCR è aumentato (P < 0,05) nel periodo di crescita nei gruppi PS, FB ed EFB rispetto al gruppo di controllo. I soggetti alimentati con PS hanno evidenziato una resa di macellazione inferiore (P < 0,05) rispetto al controllo. La resa in petto è risultata più elevata (P < 0,01) nei soggetti alimentati con FB e EFB rispetto al controllo. Nessuna differenza è stata riscontrata sulla resa in cosce e sui parametri ematici analizzati.*

Parole chiave: *Broiler, Pisello, Fava, Lupino, Estrusione.*

Introduction

Research on vegetable-based protein sources has grown as a result of the European Union ban on the inclusion of meat and bone meal in diets of agricultural livestock. This together with recent concern over genetically modified soybeans, the protein source of choice for monogastric diets, has sparked research into the identification of some alternative protein sources. The concern over antinutritional factors (protease inhibitors, lectins, phenolic compounds, saponins, etc.) in possible protein sources, like pea seeds (*Pisum sativum*) faba beans (*Vicia faba* var. *minor*) and lupin seeds (*Lupinus albus* var. *multitalia*), is minimal since genetic improvements of these ingredients make for products with minimal risk (Bond and Duc, 1993; Gatel, 1993; Castell *et al.*, 1996; Rubio *et al.*, 2003). The primary concern with these vegetable-based protein sources is related to their content of non-starch-polysacharides (NSP). The alfa-galactoside linkages in these polysaccharides are not broken down for digestion in the gut of monogastric animals (Evans *et al.*, 1993; Gdala and Buraczewska, 1996, 1997; Perez-Maldonado *et al.*, 1999; Kocher *et al.*, 2000).

These negative effects of high NSP containing protein sources can however be minimized by several methods. One of the most utilized and most studied is the utilization of specific enzymes (Gilbert *et al.*, 1999; Kocher *et al.*, 2000; Steenfeldt *et al.*, 2003; Cowieson *et al.*, 2003). Another effective method is by the optimization of their particle size with technological feed processing procedures (Lacassagne *et al.*, 1991; Gatel, 1993; Daveby *et al.*, 1998; Farrell *et al.*, 1999; Alonso *et al.*, 2001). For example, pelleting or extrusion has been shown to improve the starch and protein digestibility in faba bean, peas and lupin (Carré *et al.*, 1987; Carré *et al.*, 1991; Alonso *et al.*, 1998; Farrell *et al.*, 1999; Alonso *et al.*, 2000a; Alonso *et al.*, 2000b).

The objective of this study was to evaluate the effect of different levels of raw or extruded peas, faba beans or lupin seeds in partial substitution of soybean meal and other starch sources in broiler diets.

Material and methods

Four hundred sixty two 1d-old Marek vaccinated ROSS male chicks were obtained from a commercial hatchery (Dal Verme Camillo e Filippo, Torre degli Alberi, Pavia, Italy). Dietary treatments (seven treatments) were randomly assigned to twenty one pens (22 birds per pen with 3 pens/treatment). Chicks were floor housed (0.09 m^2/bird) in two controlled environment rooms (24°C), *ad libitum* fed isocaloric and isonitrogenous diets and had free access to water. Artificial light was provided 10 h/d. Fluorescent lights with ultraviolet filters was provided 24 h/d for the first

Table 1. Ingredients (g/kg as fed basis) and chemical composition of diets fed from 1 to 10 d-old.

			Pea seeds		Faba beans		Lupin seeds	
		CTR	PS	EPS	FB	EFB	LS	ELS
Ingredients:								
Corn meal		488	298	298	251	251	452	452
Soybean meal		428	258	258	180	180	88	88
Pea seeds		-	353	353	-	-	-	-
Faba beans		-	-	-	479	479	-	-
Lupin seeds		-	-	-	-	-	360	360
Corn oil		44	50	50	45	45	50	50
L-Lysine hydrochloride		1.1	-	-	1.3	1.3	5.0	5.0
DL-Methionine		1.8	2.8	2.8	3.5	3.5	3.5	3.5
L-Threonine		-	0.3	0.3	0.7	0.7	1.1	1.1
L-Tryptophan		-	0.5	0.5	0.8	0.8	1.3	1.3
Calcium carbonate		4.9	1.4	1.4	0.5	0.5	-	-
Dicalcium phosphate		23.3	27.1	27.1	28.7	28.7	30.1	30.1
Sodium chloride		2.8	3.0	3.0	2.3	2.3	1.4	1.4
Sodium bicarbonate		1.5	0.8	0.8	1.6	1.6	3.0	3.0
Premix [1]		5	5	5	5	5	5	5
Composition by analysis:								
Crude protein		230	225	209	221	228	217	217
Ether extract		75	70	74	76	68	76	74
Crude fiber		30	34	36	53	47	50	46
Ash		57	59	58	56	57	52	52
Starch		356	364	372	357	354	357	357
Total sugars		40	39	31	35	37	37	35
Composition by calculation:								
ME	kcal/kg	2975	3013	3022	2986	2987	3014	3017

[1] Content for kg of premix: sodium lasalocid 18,000 mg; vitamin A 2,700,000 U; vitamin D_3 950,000 U; vitamin E 13,000 mg; vitamin B_1 480 mg; vitamin B_2 1575 mg; vitamin B_6 1380 mg; D-phantotenic acid 4800 mg; vitamin H 60 mg; vitamin K_3 510 mg; vitamin PP 14,800 mg; vitamin B_{12} 5 mg; Folic acid 580 mg; Co 40 mg; Fe 9800 mg; I 390 mg; Mn (oxide) 12,200 mg; Mn (sulphate) 12,200 mg; Cu 3900 mg; Se 48 mg; Zn (oxide) 9800 mg; Zn (sulphate) 9800 mg.

CTR: control; PS: raw pea seeds; EPS: extruded pea seeds; FB: raw faba beans; EFB: extruded faba beans; LS: raw lupin seeds; ELS: extruded lupin seeds.

14d in the experiment. Birds were raised according to the European Union (European Commission, 1986) and Italian (Gazzetta Ufficiale, 1992) directives on animal welfare for experimental and other scientific purposes. Diets were formulated according to the ROSS breeder's requirements for starter (1-10d-old), growing (11-28d-old) and finishing (29-42d-old) periods. The bulk of the base diet (control diet - CTR) was corn (48.8%, 53.7% and 57%), solvent-extracted soybean meal (42.8%, 37.3% and 33.4%), corn oil (4.4%, 5.2% and 6.3%), plus synthetic amino acids, minerals, trace minerals and vitamins, respectively for the 1-10d-old, 11-28d-old and 29 to 42d-old growing periods

Table 2. Ingredients (g/kg as fed basis) and chemical composition of diets fed from 11 to 28 d-old.

		Pea seeds		Faba beans		Lupin seeds	
	CTR	PS	EPS	FB	EFB	LS	ELS
Ingredients:							
Corn meal	537	307	307	251	251	483	483
Soybean meal	373	230	230	146	146	111	111
Pea	-	356	356	-	-	-	-
Faba beans	-	-	-	497	497	-	-
Lupin	-	-	-	-	-	300	300
Corn oil	52	67	67	64	64	61	61
L-Lysine hydrochloride	2.0	0.4	0.4	1.4	1.4	4.6	4.6
DL-Methionine	2.4	3.1	3.1	3.8	3.8	3.7	3.7
L-Threonine	0.4	0.6	0.6	0.9	0.9	1.2	1.2
L-Tryptophan	-	-	-	0.3	0.3	0.9	0.9
Calcium carbonate	9.1	10.0	10.0	8.0	8.0	8.0	8.0
Dicalcium phosphate	14.7	15.0	15.0	18.0	18.0	17.0	17.0
Sodium chloride	2.5	2.0	2.0	2.0	2.0	1.0	1.0
Sodium bicarbonate	1.9	3.0	3.0	3.0	3.0	4.0	4.0
Premix[1]	5	5	5	5	5	5	5
Composition by analysis:							
Crude protein	202	204	204	204	208	195	195
Ether extract	93	83	93	85	87	104	111
Crude fiber	32	38	41	57	51	39	37
Ash	56	59	57	57	57	49	51
Starch	337	408	391	398	392	375	358
Total sugars	40	39	31	38	37	37	35
Composition by calculation:							
ME kcal/kg	3175	3187	3172	3161	3159	3186	3163

[1] Content for kg of premix: sodium lasalocid 18,000 mg; vitamin A 2,700,000 U; vitamin D3 950,000 U; vitamin E 13,000 mg; vitamin B_1 480 mg; vitamin B_2 1575 mg; vitamin B_6 1380 mg; D-phantotenic acid 4800 mg; vitamin H 60 mg; vitamin K_3 510 mg; vitamin PP 14,800 mg; vitamin B_{12} 5 mg; Folic acid 580 mg; Co 40 mg; Fe 9800 mg; I 390 mg; Mn (oxide) 12,200 mg; Mn (sulphate) 12,200 mg; Cu 3900 mg; Se 48 mg; Zn (oxide) 9800 mg; Zn (sulphate) 9800 mg.

CTR: control; PS: raw pea seeds; EPS: extruded pea seeds; FB: raw faba beans; EFB: extruded faba beans; LS: raw lupin seeds; ELS: extruded lupin seeds.

(Tables 1, 2 and 3). The pea seeds (PS), faba beans (FB) and Lupin seeds (LS), either raw or extruded (Anderson single-screw wet extruder with 300-350 kg h^{-1} capacity, 120A power absorption; Cortal Extrasory, Vicenza, Italy), entered diets in substitution of the soybean meal and corn according to the cost optimization in diet formulation. The amount of PS, FB and LS used as fed basis were: PS and extruded PS (EPS): 353 (1-10d-old); 356 (11-28 d-old) and 350 (29-42-d old) g/kg; FB and extruded FB (EFB): 479 (1-28 d-old) and 500 (29-42-d old) g/kg; LS and extruded LS (ELS): 360 (1-10d-old) and 300 (11-42d-old) g/kg.

The PS, EPS, FB, EFB, LS and ELS and experimental diets were characterized for protein, crude lipids, total fiber, total sugar and ash contents

Table 3. Ingredients (g/kg as fed basis) and chemical composition of diets fed from 29 to 42d-old.

		Pea seeds		Faba beans		Lupin seeds	
	CTR	PS	EPS	FB	EFB	LS	ELS
Ingredients:							
Corn meal	570	378	378	291	291	536	536
Soybean meal	334	172	172	109	109	64	64
Pea	-	350	350	-	-	-	-
Faba beans	-	-	-	500	500	-	-
Lupin	-	-	-	-	-	300	300
Corn oil	63	64	64	65	65	61	61
L-Lysine hydrochloride	0.6	0.1	0.1	0.1	0.1	3.3	3.3
DL-Methionine	1.7	3.0	3.0	3.2	3.2	3.0	3.0
L-Threonine	-	0.4	0.4	0.3	0.3	0.5	0.5
L-Tryptophan	-	0.3	0.3	0.2	0.2	0.6	0.6
Calcium carbonate	3.9	1.4	1.4	4.1	4.1	-	-
Dicalcium phosphate	20.5	23.2	23.2	20.2	20.2	25.2	25.2
Sodium chloride	3.6	2.0	2.0	2.0	2.0	2.0	2.0
Sodium bicarbonate	0.3	3.0	3.0	3.0	3.0	2.3	2.3
Premix[1]	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Composition by analysis:							
Crude protein	190	189	187	187	185	188	182
Ether extract	66	86	84	89	89	79	74
Crude fiber	29	29	38	58	50	45	43
Ash	54	54	53	51	49	43	48
Starch	473	428	433	435	436	456	464
Total sugars	35	48	45	36	33	31	33
Composition by calculation:							
ME (kcal/kg)	3235	3262	3249	3253	3250	3256	3232

[1] *Content for kg of premix: sodium lasalocid 18,000 mg; vitamin A 2,700,000 U; vitamin D_3 950,000 U; vitamin E 13,000 mg; vitamin B_1 480 mg; vitamin B_2 1575 mg; vitamin B_6 1380 mg; D-phantotenic acid 4800 mg; vitamin H 60 mg; vitamin K_3 510 mg; vitamin PP 14,800 mg; vitamin B_{12} 5 mg; Folic acid 580 mg; Co 40 mg; Fe 9,800 mg; I 390 mg; Mn (oxide) 12,200 mg; Mn (sulphate) 12,200 mg; Cu 3900 mg; Se 48 mg; Zn (oxide) 9800 mg; Zn (sulphate) 9800 mg.*

CTR: control; PS: raw pea seeds; EPS: extruded pea seeds; FB: raw faba beans; EFB: extruded faba beans; LS: raw lupin seeds; ELS: extruded lupin seeds.

(Table 4) (Martillotti *et al.*, 1987), and for ADF and NDF (Van Soest *et al.*, 1991). The starch content was measured by polarimetric method as described by Martillotti *et al.* (1987). Amino acids were measured (Table 5) with an amino acids analyser (Carlo Erba 3A29) according to published methods (Moore, 1963; Eggum, 1968; Moore *et al.*, 1980). The methionine content was determined after oxidation with performic acid. Feeds were analyzed for total phenols and tannins, fractioned by adsorption chromatography according to Carmona *et al.* (1991), daidzein and genistein were determined by gas chromatography-mass spectrometry (Liggins *et al.*, 1998) and by reverse-phase HPLC (Franke *et al.*, 1994). The antitripsin activity was analyzed by the method described by Smith *et al.* (1980). The *in*

Table 4. Chemical composition (g/kg as fed basis) of the grain legumes.

Parameter	Soybean meal	Pea seeds PS	Pea seeds EPS	Faba beans FB	Faba beans EFB	Lupin seeds LS	Lupin seeds ELS
Dry matter	885.9	880.1	861.8	883.1	872.6	911.2	912.3
Crude protein	451	212.5	210.3	259	254.6	350.5	354.4
Ether extract	13	12.3	31.5	16.1	17.5	77.4	99.8
Crude fiber	-	68.2	41	77.7	82.8	95.8	96.1
Ash	60.7	32.6	33.9	33.8	33.7	38.1	37.3
Starch	-	436.6	413	327.2	333.7	114.1	81.5
Total sugars	92.6	38.4	45.1	40.3	57.6	65.6	67.6
Neutral Detergent Fiber	179.3	162.8	105.7	276.4	200.4	183.7	192.4
Acid Detergent Fiber	66.7	87.5	57.6	114.2	128.4	118	120.6

PS: raw pea seeds; EPS: extruded pea seeds; FB: raw faba beans; EFB: extruded faba beans; LS: raw lupin seeds; ELS: extruded lupin seeds.

vitro alpha-amylase starch digestibility was determined using 1 mm screen flours (25 mg/mL of 0.2 M phosphate buffer, pH 6.9 at 37°C) after amylolysis with microbial alpha-amylase suspension at 37°C for 2 h according to the method of Mercier and Guilbot (1974). After 0, 30, 90 and 120 min. of incubation 3 mL of suspension were transferred in a 27 mL mixture of ethanol-acetone to block the enzyme activity. This mixture was then boiled for 8 min. with 2 mL of phenol and 5 mL of concentrated sulfuric acid. After cooling, the absorbance of the filtered solution was measured at 490 nm with glucose used as a standard. The *in vitro* amyloglucosidase starch digestibility was determined in 1 mm screen flours (1 g in 2.5 mL distilled water, 2,5 mL of buffer - pH 4.8 and 5 mL amyloglucosidase 1%) after suspension at 42°C for 1 h according to the method of Casper *et al.* (1990). Pens were weighted at days 1, 21 and 42 and feed intake was monitored. The weight gain, feed intake and feed efficiency were calculated. Bird mortality was recorded daily and dead birds were removed and weight was recorded. At 42d old, after being weighted, two broilers per pen were randomly selected and litium-heparinized blood samples were obtained by wing puncture and centrifuged at 3000 rpm for 20 min. The plasma was collected and stored at -20°C until analyzed for urea, total proteins, bilirubin, albumin, aspartate amino transferase, alanine amino transferase and gamma-glutamyl transferase according to Bertoni *et al.* (1998). At 42d-old birds were processed in a commercial processing plant (Avicola Valtidone S.r.l., 29015 Castel San Giovanni, Piacenza, Italy), eviscerated and weight were recorded. One bird per pen was randomly selected and part weights were recorded for breast meat (*pectoralis major*) and leg quarter (drumstick plus thigh).

Statistical analysis was carried out using the general mixed model procedure of SAS (2001). The statistical model included effects of treatment, room and treatment by room interaction with the experimental unit being the pen. Least square means for treatments were compared using the Student-t test with statistical significance declared at $P < 0.05$.

Results and discussion

The antitrypsin activity, and the estrogenic molecules (genistein and daizein) were relatively low (Table 6) for peas, faba beans and lupin seeds. Extrusion tended to reduce poliphenol concentrations and antitrypsin activity in peas and fava

Table 5. Amino acids content of the grain legumes (g/kg as fed basis).

Amino acid	Soybean meal	Pea seeds	Faba beans	Lupin seeds
Alanine	19.8	8.9	10.6	11.6
Arginine	34.6	14.2	23.1	35
Aspartic Acid	48.9	26.5	24.7	41.6
Cystine	6.7	3	3.3	5
Glutamic acid	85.6	34.6	42.9	80.7
Glycine	19.1	8.5	10.6	13.6
Histidine	11.9	3.8	6.3	8.2
Isoleucine	22.7	8.7	11.1	16.3
Leucine	35.2	14.1	18.8	26.1
Lysine	28.4	13	15.9	14.9
Methinonine	6.5	1.8	2.1	2.4
Phenylalanine	23.5	9.5	10.9	14.7
Proline	24.5	8.5	11.8	15
Serine	25.4	11.3	13.2	21.8
Threonine	18	8.1	9	13
Tyrosine	17.3	6.2	7.5	16
Valine	22.8	9.1	12.2	13.8
Tryptophan	6.1	1.8	2.3	2.1

Table 6. Antinutritional content in protein sources.

Analysis		Soybean meal	PS	EPS	FB	EFB	LS	ELS
Tannins,	mg/g	0.39	0.14	0.34	0.47	0.42	0.61	0.81
Polyphenols,	"	2.09	11.19	7.54	2.49	1.07	6.39	2.68
Genisteine,	ppm	0.7	0	0	0	0	0	0.1
Daidzeine,	"	1.6	0.1	0.1	0.1	0.2	0	0.2
Trypsin inhibiting activity		1.3	0.89	0.4	0.78	0.3	0.72	1.4

PS: raw pea seeds; EPS: extruded pea seeds; FB: raw faba beans; EFB: extruded faba beans; LS: raw lupin seeds; ELS: extruded lupin seeds

Table 7. *In vitro* starch digestibility (%) for raw and extruded grain legumes.

	PS	EPS	FB	EFB	LS	ELS
Alpha-amylase digestibility:						
0 min.	13.88	9.2	18.38	17.83	100	100
30 min.	5.94	66.98	4.70	53.72	23.32	79.83
90 min.	11.89	82.56	8.94	96.80	41.98	93.28
120 min.	11.96	86.85	10.42	91.50	48.51	100
Amyloglucosidase digestibility	11.8	85.37	11.39	85.05	54.48	100

PS: raw pea seeds; EPS: extruded pea seeds; FB: raw faba beans; EFB: extruded faba beans; LS: raw lupin seeds; ELS: extruded lupin seeds.

Table 8. Influence of alternative protein sources on the average feed intake (FI), average body weight gain (BWG) and feed conversion rate (FCR) of broiler chickens.

Parameter		CTR	PS	EPS	FB	EFB	LS	ELS	SEM	P[1]
FI, 1-21d	g/d	50.7	48.2	51.1	51.4	51.1	50.4	48.2	1.04	0.204
FI, 1-42d	"	147.7^{ad}	159.1^{c}	156.6^{ac}	154.0^{ac}	152.0^{adc}	144.3^{d}	132.0^{b}	2.66	0.005
FI, 22-42d	"	98.9^{ac}	103.5^{a}	103.5^{a}	102.4^{a}	100.1^{ac}	97.4^{c}	90.1^{b}	1.43	0.007
BWG, 1-21d	"	38.4	34.6	38.4	36.5	37.1	36.9	36.6	0.87	0.179
BWG, 1-42d	"	71.9^{acd}	76.6^{a}	76.6^{a}	69.8^{cd}	74.3^{ac}	66.9^{bd}	62.9^{b}	1.63	0.006
BWG, 22-42d	"	55.1^{ad}	55.6^{ad}	57.5^{d}	53.1^{ab}	55.7^{ad}	51.9^{bc}	49.7^{c}	1.08	0.033
FCR, 1-21d		1.32^{a}	1.40^{bc}	1.34^{ac}	1.42^{b}	1.41^{b}	1.37^{a}	1.32^{a}	0.02	0.028
FCR, 1-42d		2.10^{a}	2.08^{a}	2.05^{a}	2.22^{b}	2.08^{a}	2.16^{ab}	2.10^{a}	0.03	0.043
FCR, 22-42d		1.81	1.87	1.81	1.94	1.84	1.88	1.81	0.03	0.13

[1] P of the model.
Within a row, means without a common superscript letter differ ($P < 0.05$).
PS: raw pea seeds; EPS: extruded pea seeds; FB: raw faba beans; EFB: extruded faba beans;
LS: raw lupin seeds; ELS: extruded lupin seeds.
CTR: control.

Table 9. Influence of alternative protein sources on eviscerated carcass, breast muscle and leg quarter percentages of broiler chickens.

Parameter	CTR	PS	EPS	FB	EFB	LS	ELS	SEM	P[1]
Eviscerated carcass	82.7[ac]	73.8[b]	76.6[bc]	82.5[ac]	85.0[ad]	79.3[ce]	80.0[cde]	1.42	0.043
Breast	18.3[acd]	18.7[bd]	18.7[bd]	19.4[b]	19.6[b]	17.6[c]	18.1[cd]	0.32	0.001
Leg quarter	28.8	29.7	29.1	28.3	29.4	28.9	26.5	1.85	0.95

[1] P of the model.
Within a row, means without a common superscript letter differ (P < 0.05).
PS: raw pea seeds; EPS: extruded pea seeds; FB: raw faba beans; EFB: extruded faba beans;
LS: raw lupin seeds; ELS: extruded lupin seeds.
CTR: control.

Table 10. Influence of alternative protein sources on blood parameters of broilers chickens.

Parameter		CTR	PS	EPS	FB	EFB	LS	ELS	SEM	P[1]
Urea	mmol/L	0.64	0.79	0.7	0.66	0.76	0.86	0.85	0.07	0.37
Total protein	g/L	30	29.25	29	30.63	29.63	30.5	27.4	1.02	0.08
Bilirubin	mmol/L	5.29	5.49	5.64	7.86	6.01	7.33	4.83	1.46	0.75
Albumin	g/L	14.38	14.5	14.38	14	14.38	15.13	14.13	0.47	0.6
Aspartate amino transferase	U/L	292.3	478.9	411	307.5	257.8	436.4	326.9	85.3	0.53
Alanine aminotransferase	"	4.38	6.38	6.25	5.38	4.75	6.13	5.13	0.88	0.77
Gamma-Glutamyltransferase	"	38.13	44.38	41	44.25	38.5	43.13	43.88	2.88	0.6

[1] P of the model.
PS: raw pea seeds; EPS: extruded pea seeds; FB: raw faba beans; EFB: extruded faba beans; LS: raw lupin seeds; ELS: extruded lupin seeds.
CTR: control.

bean (Table 6). Alonso *et al.* (2000a) reported that extrusion was effective in reducing trypsin, chymotrypsin, alpha-amilase inhibitors and haemoagglutinating activity in peas (*Pisum sativum* L.) and kidney beans (*Phaseolus vulgaris* L.). In our study, both peas and faba beans *in vitro* alpha-amylase digestibility (after 120 min. of incubation) (Table 7) increased almost 100% after extrusion. Amyloglucosidase digestibility was similarly increased for peas and faba beans after extrusion. Alonso *et al.* (2000a, 2000b) found a similar effect of extrusion when they reported that extrusion was more effective in improving faba and pea protein and starch digestibility than dehulling, soaking or germination.

The mortality during the entire experiment was very low (3.6 %), and was not related to the experimental diets. The effects of experimental diets on weight gain, feed intake and the feed conversion rate (FCR) are showed in Table 8. High levels of peas (350 g/kg) and faba (500 g/kg) did not show negative effects on bird weight gain. Lupin at the 300 g/kg level reduced ($P < 0.05$) the weight gain during the finishing (22 to 42 d) and grower periods (1 to 21 d) but did not affect weight gain when the entire experimental period (1-42 d) was analyzed. Similarly, high levels of faba beans or peas did not affect feed intake. Extrusion of the lupin seeds reduced feed intake ($P < 0.01$) in comparison to the control group and to the non-extruded lupin diet. Feed conversion rate was not significantly affected by any of the experimental treatments for the full experimental period. During the grower period the FCR was higher ($P < 0.05$) for faba bean diets independent of extrusion. Although the feed analysis showed better quality in the extruded feeds (i.e. less antinutritional factors) and a better *in vitro* starch and protein digestibility, as compared to raw products, no mayor effects on bird performance were observed over the entire experimental period between extruded and raw protein source treatment. Indeed, Moschini *et al.* (2005) showed that 350 g/kg of raw peas and 500 g/kg of raw faba beans did not have any negative effects on body weight gain and FCR during the grower period (1-21). Our results help support the concept that utilization of level higher than 150 g/kg of lupin pea has a negative effect on broiler performance (Olver and Jonker, 1997; Farrell *et al.*, 1999). To the best of our knowledge there are no previous reports on the effects of extrusion of these legumes on broiler performance. There are however several studies that evaluate the effects of pelleting. Carré *et al.* (1987) saw an increase of 1.8-4.6% in apparent metabolisable energy for peas and of 3.5 and 5.4% respectively for protein and starch digestibility. Similarly, Lacassagne *et al.* (1991) saw the same effect of pelleting on protein and starch digestibility of faba beans. Carcass measurements are shown in Table 9. Birds consuming non-extruded peas had lower dressing percentage than the control group ($P < 0.05$). Breast meat percent yield was higher ($P < 0.01$) for birds consuming the faba bean diets independent of processing. There were no statistical differences in percent yield of the leg quarters. These values are in accordance to Quarantelli and Bonomi (1991). Serum chemical values are presented in Table 10. No statistical significance was observed for any of the parameters measured as was expected.

Conclusions

Data obtained in this experiment is supportive of the utilization of peas (*Pisum sativum*), faba beans (*Vicia faba* var. *minor*) and lupin seeds (*Lupinus albus* var. *multitalia*) in partial substitution of soybean as an effective protein source. Lupin seeds in general were not as effective as the other two protein sources. Partial improvement was seen with extrusion of the faba beans and peas. This improvement attributed to the extrusion process however, was not seen for the lupin seeds based diets.

The utilization of these alternative proteins sources in broilers diets, without any negative effects in general performance, offers a viable protein option to help counteract the current constraints of soybean meal.

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