Asian-Aust. J. Anim. Sci.
Vol. 20, No. 8 : 1229 - 1235
August 2007

www.ajas.info

Effects of Processing Method on Performance and Nutrient Digestibility in Growing-finishing Pigs Fed Lupine Seeds

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ABSTRACT : Three trials were conducted to study the effect of processing method on performance, apparent nutrient and amino acid digestibilities in growing-finishing pigs fed lupine seeds. Ground, expanded or flaked lupine seed was prepared for the trials. In trial 1, a total of 72 growing barrows (Landrace×Large White×Duroc, initial BW of 54.22±5.87 kg) were allocated to three treatment diets containing 15% percent of differently processed lupine seed for 28 days. Each treatment had four replicates comprising 6 pigs each. In trial 2, a total of 48 finishing barrows (Landrace×Large White×Duroc, initial BW of 84.40±6.84 kg) were subjected to three treatments for 21 days. The experimental design was the same as trial 1 except for supplementation with 20% of lupine seed. Each treatment had four replicates comprising 4 pigs each. To evaluate the ileal digestibility of amino acids, a total of 9 barrows (Landrace×Large White ×Duroc), with an average initial BW of 41.07±1.98 kg, were fed with ground, expanded or flaked lupine for 7 days. Each pig was surgically equipped with a simple T-cannula fitted in the distal ileum. Amino acid composition and presence of anti-nutritional factors (ANFs) in processed lupine sources were also evaluated. The results showed that there was no significant difference ($p>0.05$) in lupine amino acid composition due to the different processing methods. Expanded and flaked lupine significantly decreased ($p<0.05$) the concentration of ANFs compared with ground lupine. There was no effect ($p>0.05$) on the growth performance in growing pigs. However, processing method had a significant effect ($p<0.05$) on average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) in finishing pigs. There was no effect ($p>0.05$) of the different processing methods on the digestibility of dry matter (DM), gross energy (GE), ether extract (EE), Calcium (Ca) and phosphorus (P), but expanded lupine could significantly increase ($p<0.05$) the apparent digestibility of CP in finishing pigs. Furthermore, expanded lupine had a higher ($p<0.05$) apparent ileal digestibility for most indispensable and dispensable amino acids compared with ground and flaked lupine. It was concluded that expanded lupine could be beneficial in improving lupine's quality and improve performance and nutrient utilization in growing-finishing pigs. (**Key Words :** Lupine, Processing Method, Performance, Digestibility, Growing-finishing Pigs)

INTRODUCTION

Lupine seeds have been cultivated as a grain crop for over 3,000 years (Henson and Stephens, 1958), primarily in the Mediterranean, parts of the Middle East, Australia, and South America. However, the extreme bitterness of the seed generally made lupine unsuitable for human and animal consumption without prior treatment to remove toxic alkaloids (King, 1981). After extensive plant breeding efforts, Australian sweet lupine (ASL), *Lupinus angustifolius*, has been developed since the early 1930s. Around 1.5 million tons of ASL is traded on world markets annually, with approximately 85% originating from Western Australia (Kwak et al., 2000).

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Received October 25, 2006, Accepted April 5, 2007

Lupine is recognized as a good nutritional source and an alternative to soybean meal due to its high concentration of energy and protein (Hove, 1974). The seeds contain 30 to 40% protein, which approaches the percentage in soybean meal. The concentration of amino acids is similar to that in typical legumes, whereas the content of arginine is higher than that for most other legumes.

Many studies have shown that lupine increases growth performance, improves milk production and enhances reproduction when used as partial or total replacement for soybean meal in ruminants (Moss et al., 1997), swine (Gdala et al., 1996; Kwak et al., 2000), and poultry (Perez-Maldonado et al., 1998). Generally, raw lupine seeds are ground and used as a feed ingredient. But lupine seeds contain a high amount of anti-nutritional factors (ANFs), which might also have a negative effect on feed utilization. Since using different processing methods can change the

Table 1. Formula and chemical composition of experimental diets

Ingredients (%)	Trials		
	Growing (1)	Finishing (2)	Ileal digestibility
Corn (ground)	54.47	47.86	-
Wheat (ground)	-	20.00	-
Lupine	15.00	20.00	46.81
Soybean meal	15.30	7.40	-
Rice bran	7.00	-	-
Animal fat	3.30	2.20	-
Molasses	2.00	-	-
Corn starch	-	-	33.00
Glucose	-	-	15.00
Soybean oil	-	-	1.45
Tricalcium phosphate	1.00	0.90	1.35
Limestone	0.95	0.70	1.35
Salt	0.30	0.30	0.30
Vitamin premix[1]	0.10	0.25	0.26
Mineral premix[2]	0.20	0.24	0.24
L-lysine HCL (78%)	0.15	0.10	-
DL-methionine (100%)	0.04	-	-
Olaquindox (100 g/kg)	0.10	-	0.10
Chlortetracycline (100 g/kg)	0.05	-	0.04
Choline chloride (25%)	0.04	0.05	0.10
Total	100.00	100.00	100.00
Chemical composition[3] (%)			
ME (kcal/kg)	3,300	3,300	3,600
Crude protein	17.00	16.05	14.20
Ether extract	7.02	6.03	6.30
Calcium	0.74	0.65	0.88
Phosphorus	0.58	0.51	0.63

[1] Supplied per kilogram of diet: vitamin A, 7,500 IU; vitamin D_3, 1,550 IU; vitamin E, 40 IU; vitamin K_3, 1.25 mg; thiamin, 1.25 mg; riboflavin, 7.5 mg; pantothenic acid, 12.5 mg; niacin, 50 mg; pyridoxine, 1.5 mg; biotin, 0.14 mg; folic acid, 2.5 mg; vitamin B_{12}, 0.025 mg.
[2] Supplied per kilogram of diet: Fe, 92.5 mg; Cu, 75 mg; Zn, 125 mg; Mn, 42.5 mg; Co, 0.25 mg; Se, 0.2 mg; I, 0.6 mg.
[3] Calculated values.

nutritive value of livestock feeds (Casiraghi et al., 1993; Pérez-Conesa et al., 2002; Ure et al., 2005), questions are raised regarding the application of such methods. The purpose of the present study was conducted to evaluate the effect of ground, expanded and flaked lupine on apparent nutrient digestibility and growth performance in growing and finishing pigs.

MATERIALS AND METHODS

Lupine preparation

Lupine seeds (*Lupinus angustifolius* L.), grown in Australia, were purchased from Yangju National Livestock Corporation, GyeongGi-Do, Korea. Three different processing methods were used prior to incorporation of lupine into compound feeds. Raw lupine was ground to pass through a 3-mm screen using a hammer mill (Buhler, Switzerland). For expansion, the barrel temperature of the expander (Mapado, Denmark) was 80°C, with 10 bar of steam pressure and a residence time of 20 s. For flaking, the lupine was subjected to 1.5 bar of pressure in the chamber (Joda, Japan) for 1 h, and then passed through a roller mill. The temperature of the lupine leaving the tempering chamber was about 100°C.

Experimental design and feeding management

Two feeding trials were conducted to evaluate the feeding values of the processed lupine seeds. In trial 1, a total of seventy-two barrows (Landrace×Large White× Duroc, average initial BW of 54.22±5.87 kg), were randomly divided into three groups containing 15% percent of different processed lupine seed. The diets were prepared to meet or exceed the recommendations of NRC (1998) containing about 0.94% of lysine. Each treatment group had four replicates comprising 6 pigs each. Treatments included ground, expanded and flaked lupine, administered for 28 days.

In trial 2, forty-eight barrows (Landrace×Large White×Duroc) were assigned randomly to one of the three dietary treatments (four pens/treatment, four pigs/pen) with 20% of lupine seed. The average initial BW was 84.40± 6.84 kg, and three treatment diets were fed for 21 days. The three diets differed in the method of processing lupine, including ground, expanded or flaked lupine.

To determine the ileal digestibility of amino acids in the processed lupine seeds, nine (Landrace×Large White× Duroc) barrows weighing 41.07±1.98 kg were used for an ileal digestibility study. Pigs were allocated to three equal groups, and fed differently processed lupine (ground, expanded or flaked) for 7 days. Each pig was surgically equipped with a simple T-cannula in the distal ileum, according to Walker et al. (1986).

For trial 1 and 2, all pigs were housed in a total-confinement, concrete-floor facility in 3.0×3.0 m pens with a self-feeder and nipple waterer. For the ileal digestibility trial, the pigs were individually penned in 1.2×1.5 m concrete slatted-floor pens with a self-feeder and nipple waterer. All experimental diets were prepared to meet or exceed the recommendations of NRC (1998). The compositions of the three diets are listed in Table 1. All pigs were given feed *ad libitum* and had free access to water. The three trials were conducted in an environmentally controlled (23±5°C) building.

Sample collection

In trials 1 and 2, body weight of pigs was recorded at the beginning and end of each trial, and average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR) were calculated at the conclusion of the feeding trial. In trial 2, in order to study the effect of different processing methods of lupine on apparent fecal

Table 2. Analyzed amino acid compositions of lupine with different processing methods (n = 3 per lupine)

Items (%)	Ground	Expanded	Flaked	SEM[1]
Indispensable amino acids				
Arg	0.81	0.77	0.83	0.05
His	0.37	0.33	0.36	0.05
Ile	0.52	0.51	0.49	0.04
Leu	1.10	1.17	1.06	0.07
Lys	0.50	0.45	0.47	0.04
Met	0.31	0.28	0.25	0.04
Phe	0.68	0.68	0.68	0.04
Thr	0.36	0.36	0.33	0.04
Val	0.58	0.54	0.57	0.05
Sub-total	5.23	5.09	5.04	0.26
Dispensable amino acids				
Ala	0.42	0.39	0.40	0.03
Asp	1.00	0.99	1.06	0.06
Cys	0.09	0.08	0.09	0.02
Glu	2.31	2.30	2.36	0.07
Gly	0.51	0.57	0.57	0.06
Pro	1.23	1.26	1.10	0.11
Ser	0.71	0.70	0.70	0.06
Tyr	0.30	0.29	0.31	0.06
Sub-total	6.57	6.58	6.59	0.22
Total	11.80	11.67	11.63	0.15

[1] Standard error of means.

nutrient digestibility, chromic oxide (0.25%) was included as an inert indicator in all of the diets. The pigs were fed these diets for 7 days. Fecal samples were freely collected to avoid microbial contamination from day 4 to day 7. The fecal samples were pooled and dried in a forced-air drying oven at 60°C for 72 h and ground with a 1 mm mesh Wiley mill for chemical analysis.

For ileal digestibility trial, ileal digesta were collected for 10 h on day 6 and day 7 with plastic bags. Bags were removed whenever they were filled with digesta, or at least once every 30 min, and immediately stored at -20°C to prevent microbial degradation of the amino acids in the digesta.

Chemical analysis

In preparation for scanning electron microscopy, the processed lupine samples were dried in increasing concentrations of acetone and then were subjected to a critical point drier for dehydration, and then the dehydrated samples were mounted on aluminum stubs, rendered conductive by sputter-coating with gold, and examined in a scanning electron microscope (SEM) at an accelerating voltage of 20 kV. The samples were examined at low magnifications of 1,000×, and representative photomicrographs were obtained.

The alkaloid and phytic acid concentrations in the lupine were determined as described by Cuadra et al. (1994). The trypsin inhibitor activity was determined following the procedures of Valdebouze et al. (1980). Tannin was analyzed according to Świech et al. (2004), and stachyose and raffinose were measured following Szarvas et al. (1999).

Table 3. Effects of different processing method on anti-nutritional factors in lupine (n = 3 per lupine)

Items	Ground	Expanded	Flaked	SEM[1]
Alkaloids (%)	0.03	0.01	0.02	0.01
Phytic acid (%)	0.58[a]	0.16[c]	0.44[b]	0.06
Trypsin inhibitor (mg/g)	0.24[a]	0.08[c]	0.18[b]	0.02
Stachyose (%)	1.81	1.77	1.78	0.03
Raffinose (%)	0.23[a]	0.19[b]	0.19[b]	0.01
Tannins (mg/g)	0.49[a]	0.29[c]	0.37[b]	0.03

[a, b, c] Values of the same row with different superscript differ ($p<0.05$).
[1] Standard error of means.

In trial 2, proximate analyses of the diets and fecal samples were carried out following the AOAC (1990) methods. Gross energy was measured by a bomb calorimeter (Model 1261, Parr Instrument Co., Molin, IL) and chromium with an automated spectrophotometer (Jasco V-550, Japan).

For amino acid analyses, ileal samples were thawed and pooled. A subsample was used for the chemical analyses. The samples were lyophilized, finely ground and hydrolyzed for 24 h at 110°C with 6 N HCL before analysis. The amino acid concentrations in the lupine and digesta samples were quantified using a High Performance Liquid Chromatography (Waters 486, USA). Sulfur containing amino acids was analyzed after cold performic acid oxidation (Moore, 1963) overnight before hydrolysis.

Statistical analyses

All the data were analyzed by ANOVA using the General Linear Model (GLM) procedure of SAS software (SAS Institute., Inc., Cary, NC) as a completely randomized design. The treatments were the main effects and the replicates were used as units. When significant differences were noted, the means were compared using Duncan's multiple range tests. The level of significance was accepted at $p<0.05$, unless otherwise noted.

RESULTS AND DISCUSSION

Lupine seed properties as affected by processing methods

The effects of different processed methods on lupine seeds are shown in Table 2, 3 and Figure 1.

The amino acid composition of lupine were not affected ($p>0.05$) by the processing methods used in this study (Table 2), which were similar to values from previous reports where there were no difference in amino acid composition between raw and extruded white lupine seeds (Cros et al., 1992; Aldo Prandini et al., 2005). However, processing method had a significant effect ($p<0.05$) on the anti-nutritional factors in lupine (Table 3). Expanded and flaked lupine significantly decreased ($p<0.05$) phytic acid.

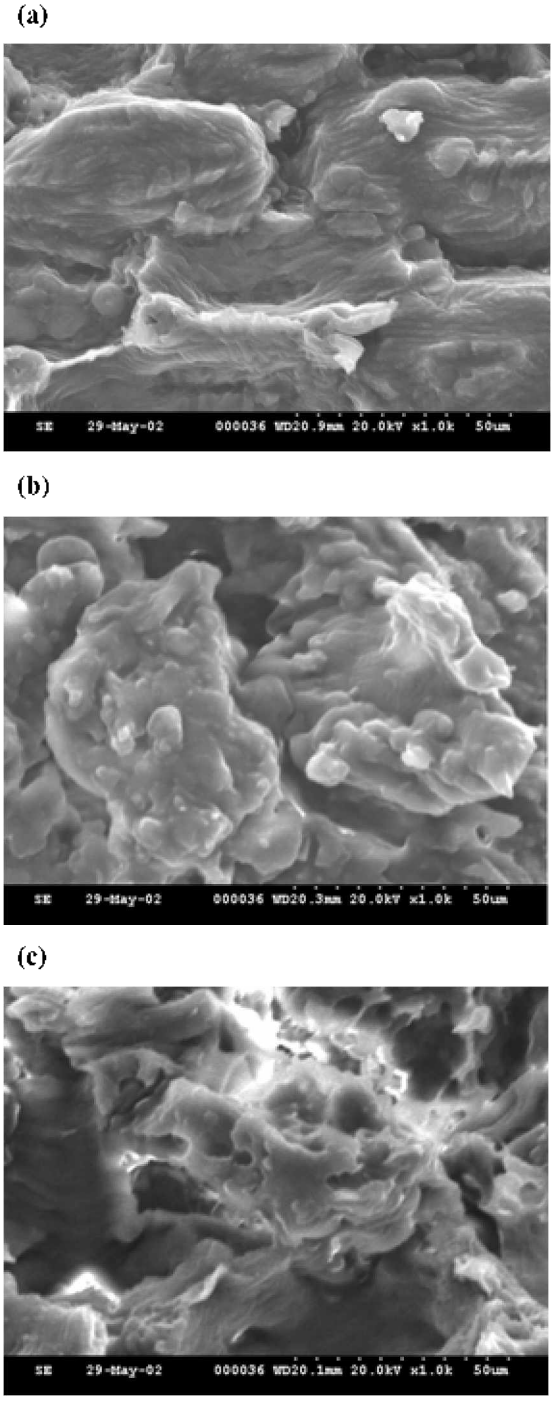

Figure 1. Scanning electron micrographs (1,000×) of lupine seed cotyledon cells affected by different processing methods (a): ground lupine, (b): expanded lupine, (c): flaked lupine.

trypsin inhibitor, raffinose and tannins compared with ground lupine. Expanded lupine significantly decreased ($p<0.05$) phytic acid, trypsin inhibitor and tannins compared with flaked lupine. The extent of inactivation of trypsin inhibitor activity was not in complete agreement with some previous results. Studies have shown that many ANFs are heat labile and are usually inactivated by proper heat treatment. Several forms of heat treatment have been documented to effectively eliminate many ANFs in soybean, pea and other legumes (Qin et al., 1996; Gilbert, 1998; Panda et al., 2006). Other authors (Alonso et al., 1998; Alonso et al., 2000a; Alonso et al., 2000b) have also shown that extrusion is the best method to abolish trypsin, chymotrypsin, alphaamilase inhibitors and haemoagglutinating activity. The results of these researches are in agreement with those of our study. However, Aldo Prandini et al. (2005) found extruded lupin seeds contained higher ANFs, especially trypsin inhibitor activity, when compared to raw lupin seeds. These inconsistent results may be due to different processing conditions and some other factors. Potential mechanism need to be further studied.

The scanning electron micrographs showed the effect of different processing methods on the structure of cotyledon cells in lupine. After expansion and flaking, the cotyledon cells of the lupine were changed not only in shape, but also in size. The cotyledon cells of ground lupine (Figure 1a) were compacted, well organized and oval shaped with a smaller diameter. The cell walls, appearing as parallel layers, were visible. By contrast, in the case of expanded and flaked lupine, the cotyledon cells (Figure 1b, c) formed three-dimensional network structures and were loosely arranged. The cotyledon cells in the case of both of expanded and flaked lupine showed a larger size compared with those in the ground lupine. The cells of the flaked lupine, meanwhile, exhibited different sizes of air vents (Figure 1c). These observations indicated that starch and protein were embedded and exhibited a rigid structure in the ground matrix (Lee and Kim, 1985). When lupine was heat-treated by expansion and flaked, the cell wall was destroyed and the protein mass was enlarged, few starch granules were present in the network structure due to gelatinization at such a temperature. Yuno-Ohta et al. (1992) and Li et al. (2007) reported that heat-induced sesame globulins become softer and starch becomes gelatinized, which is in agreement with our results.

Growth performance

The effects of processed lupine seeds on growth performance in growing-finishing pigs are shown in Table 4.

In trial 1, the effects of the different methods of processing lupine on ADG, ADFI and FCR showed no significant effect on performance ($p>0.05$) in growing pigs. However, in trial 2, expanded lupine significantly increased ADG and flaked lupine significantly increased ($p<0.05$) ADFI compared with ground treatment, respectively. Expanded and ground lupine treatments significantly decreased ($p<0.05$) FCR compared with flaked treatment.

Legumes are well known for containing a range of ANFs that have apparent adverse effects on the monogastric

Table 4. Effect of processed lupine seeds on growth performance in growing-finishing pigs[1]

Items	Ground	Expanded	Flaked	SEM[2]
Growing (Trial 1)				
ADG (g)	807	803	825	8.71
ADFI (g)	2,359	2,253	2,273	54.03
FCR	2.92	2.81	2.77	0.06
Finishing (Trial 2)				
ADG (g)	837[b]	884[a]	859[ab]	8.88
ADFI (g)	2,560[b]	2,693[ab]	2,812[a]	47.82
FCR	3.06[b]	3.05[b]	3.27[a]	0.05

[a,b] Values of the same row with different superscript differ ($p<0.05$).
[1] n = 4 per treatment in both trials.
[2] Standard error of means.

Table 5. Apparent fecal digestibility of experimental diets used in finishing pigs[1] (Trial 2)

Items (%)	Ground	Expanded	Flaked	SEM[2]
DM	80.54	81.92	78.50	0.78
GE	78.54	80.42	76.78	0.77
CP	78.89[ab]	79.98[a]	76.25[b]	0.72
EE	67.13	64.64	66.01	0.63
Ash	50.48[b]	49.96[b]	54.50[a]	0.92
Ca	46.73	45.20	44.99	0.48
P	38.49	41.95	40.93	0.72

[a,b] Values of the same row with different superscript differ ($p<0.05$).
[1] n = 4 per treatment.
[2] Standard error of means.

Table 6. Apparent ileal digestibility of amino acids in lupine meal diets[1]

Items (%)	Ground	Expanded	Flaked	SEM[2]
Indispensable amino acids				
Arg	83.18	82.37	86.37	0.98
His	64.69	65.85	64.35	0.81
Ile	75.16[ab]	78.51[a]	73.28[b]	1.05
Leu	80.81	82.43	80.83	0.73
Lys	79.17[b]	84.91[a]	80.83[ab]	1.12
Met	76.11[b]	82.82[a]	77.31[b]	1.28
Phe	73.17[b]	77.75[a]	75.20[ab]	0.91
Thr	65.91	63.41	61.64	1.03
Val	77.71[a]	76.98[a]	73.34[b]	0.82
Sub-mean	75.10	77.23	74.79	0.64
Dispensable amino acids				
Ala	63.97	70.39	65.44	1.32
Asp	75.89[b]	81.06[a]	74.15[b]	1.22
Cys	74.22[b]	83.19[a]	79.69[ab]	1.58
Glu	75.98[a]	76.81[a]	73.21[b]	0.64
Gly	72.38[b]	77.71[a]	71.16[b]	1.21
Pro	81.64[b]	85.69[a]	79.49[b]	1.08
Ser	75.82[b]	80.32[a]	72.06[c]	1.28
Tyr	64.33	64.66	67.26	0.92
Sub-mean	73.03[b]	77.48[a]	72.81[b]	0.91
Total-mean	74.07	77.35	73.80	0.76

[a,b,c] Values of the same row with different superscript differ ($p<0.05$).
[1] n = 3 per treatment.
[2] Standard error of means.

species ingesting them, subsequently causing a negative effect on performance. In our research, however, there was no palatability effect in growing pigs, whereas there was a significant effect in finishing pigs, which might be due to the low concentration of some ANFs. Many studies have reported that lupine inclusion levels of 20 to 37% showed no detrimental effects on the growth of growing pigs (Petterson, 2000), similar to our results for Trial 1. Kwak et al. (2000) replaced soybean meal in corn-based diets for finishing pigs with 15 or 30% inclusion of lupine, in which case they found no loss of performance. Batterham et al. (1986) showed that autoclaving lupine at 121°C for 5 min had no effect on pig growth in growing pigs, which was in agreement with our research.

Nutrient digestibility

For apparent fecal nutrient digestibility in finishing pigs (Trial 2), there was a small increment in the digestibility of dry matter, gross energy and phosphorus in expanded lupine compared with the other processing methods (Table 5), although there was no significant difference ($p>0.05$). The digestibility of crude protein in expanded lupine showed a significant increase compared with flaked treatment ($p<0.05$), but no difference with the ground treatment ($p>0.05$), which resulted in higher ADG in flaked treatment. Flaked lupine showed higher ($p<0.05$) digestibility of crude ash compared with ground and expanded treatments. Studies have shown that the nutritional value of lupine can be improved by the removal of the hull and that expansion or steam flaking can improve the digestibility of nutrients in many grain feeds (Fan et al., 1995; Wigan et al., 1995; Mariscal-Landín et al., 2002). Kung et al. (1991) reported that roasting lupine at 175°C resulted in a more than 40% decrease in ruminal *in situ* nitrogen disappearance after 12 h of incubation. Similar effects of extrusion on the degradation of crude protein in the rumen have been reported (Walhain et al., 1992). Fernández and Batterham (1995) found dehulled lupine could improve protein and amino acids utilization in growing pigs when compared to raw lupine seed. In our study, the extent of digestion of crude protein was affected by expansion, a result supported by the previous studies. The major proportion of protein found in lupine seeds is globulins, followed by albumins and small amounts of glutelins and prolamins (Varasundharosoth and Barnes, 1985; Petterson, 2000). Albumins and glutelins are sensitive to heat treatment, which renders them insoluble in water, thus increasing the absorption of protein in the small intestine (Duranti and Cerletti, 1979; Blethen et al., 1990).

Generally, expanded lupine significantly increased ($p<0.05$) the apparent ileal digestibility of indispensable, dispensable and total amino acid compositions (Table 6), which is in close agreement with previous studies of apparent ileal amino acid digestibility for lupine (van

Barneveld et al., 1995; van Barneveld, 1999; Dunshea et al., 2001). The apparent ileal digestibility of amino acids in expanded lupine tended to be higher than in ground and flaked lupine, perhaps due to the effects of non-starch polysaccharides and oligosaccharides contained in expanded lupine (Gdala et al., 1997). van Barneveld (1997) also discovered the negative effects of lupine non-starch polysaccharides and oligosaccharides on nutrient digestion. In our study, expanded lupine had lower concentrations of stachyose and raffinose than ground or flaked lupine, which might contribute to higher digestibility in an expanded lupine diet.

Overall, we concluded that expanded lupine could be beneficial in improving lupine's quality, nutrient utilization and growth performance, and could be an alternative protein source in growing and finishing pigs.

ACKNOWLEDGEMENT

The authors sincerely acknowledge for the partial financial support provided by the Institute of Animal Resources at Kangwon National University, Korea.

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