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Influence of Extrusion of White Lupins (*Lupinus albus* L.) on the Apparent Metabolizable Energy and Ileal Nutrient Digestibility for Broilers

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Abstract: In the present study, the influence of extrusion on the composition and nutritive value of white lupins (*Lupinus albus* L.) was evaluated. The extrudate was produced by the extrusion cooking of white lupins at 22% moisture level and 140°C. Three treatment diets consisting of a corn-soy basal diet and two test diets containing raw and extruded lupin meals were formulated and, assayed in digestibility and balance trials using 4-week old broiler chickens. The test diets were formulated by substituting the raw and extruded lupin meals for 25% (w/w) of the basal diet. Apparent ileal nutrient digestibility was calculated using titanium oxide as the indigestible indicator and the Apparent Metabolizable Energy (AME) was determined using the classical total excreta collection method. Extrusion of white lupins had no effect ($p<0.05$) on the contents of crude protein, crude fat, ash and most amino acids. Soluble Non-Starch Polysaccharide (NSP) contents were increased ($p<0.05$) and the insoluble NSP contents were lowered ($p<0.05$) by extrusion. Increased soluble NSP contents were associated with reduced ($p<0.05$) ileal digestibility of fat and AME. Trypsin inhibitor activity was reduced ($p<0.05$) following extrusion, but this reduction had no effect ($p>0.05$) on the ileal digestibility of protein and amino acids. Overall, under the extrusion conditions employed in the present study, extrusion cooking adversely affected the AME but had no effect on the ileal digestibility of protein and amino acids in white lupins for broilers.

Key words: Broilers, extrusion, white lupins, apparent metabolizable energy, ileal digestibility, amino acids, non-starch polysaccharides

INTRODUCTION

Grain legumes, such as lupins (*Lupinus spp.*), are important protein sources in both human and animal nutrition. Of the more than 200 species of Lupinus, five species (*L. albus*, *L. angustifolius*, *L. luteus*, *L. mutabilis* and *L. polyphilu*) are suitable for cultivation as high protein crops (Gladstones, 1998). The feeding value of early cultivars of lupins to poultry was poor because of the presence of high concentrations of toxic and bitter alkaloids (Olver and Jonker, 1997; Olkowski *et al*., 2001). However, through recent developments in plant breeding programmes, lupin cultivars with almost zero alkaloid content have been developed and current lupin cultivars are essentially alkaloid-free (Cowling *et al*., 1998). The use of lupins in poultry diets nevertheless remains limited because of the presence of anti-nutritional factors which interfere with nutrient utilization resulting in poor animal performance. Of the various anti-nutritional factors present in grain legumes, protease inhibitors and Non-Starch Polysaccharides (NSP) are of particular interest, but these components are reduced or deactivated by thermal treatments.

Of the different thermal treatments, extrusion cooking has received more attention in recent years to improve the nutritional value of grain legumes. Extrusion cooking is a high-temperature, short-time process in which moistened, expansive, feed materials are subjected to mixing, shearing and heating under high pressure followed by forcing the extrudate through a die (Singh *et al*., 2007). During this process, the feed undergoes chemical reactions and molecular transformations which could be positive, if nutrient availability is enhanced or negative if nutrients are destroyed or altered to become resistant to digestion. Extrusion may influence the nature of feed components by changing physical (e.g. particle size), chemical (e.g. starch gelatinization, inactivation of anti nutrients) and nutritional (e.g. nutrient digestibility) properties (Bjorck and Asp, 1983; Camire, 2000). Extrusion has been to shown to have positive effects on the *in vivo* digestibility of fat (Danicke *et al*., 1998; Lichovnikova *et al*., 2004), amino acids (Lichovnikova *et al*., 2004) and starch (Alonso *et al*., 2000) in diets for poultry. These improvements have been attributed to the reduction of anti-nutritional factors, denaturation of native protein and gelatinization of starch.

The purpose of the study reported herein was to examine the effects of extrusion cooking on the

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composition, apparent ileal digestibility of fat, protein and amino acids and Apparent Metabolizable Energy (AME) of white lupins for broiler chickens.

MATERIALS AND METHODS

Processing: White lupin seeds, from an alkaloid-free cultivar (Kiev mutant), with hulls were ground in a hammer mill to pass through a 3 mm sieve and then extruded in a twin-screw co-rotating self wiping extruder (Clextral BC 21, Firminy Cedex, France) with length/diameter ratio of 25, screw speed up to 600 rpm and outer screw diameter of 25 mm. The screw configuration from feed section to die consisted of three zones with forward elements. The first zone had 4 elements (each 50mm length with 3 screw flights and 13 mm pitch), the second zone consisted 5 elements (each 50mm in length having 4 screw flights and 10 mm pitch) and the third zone had 5 elements (each 50 mm in length with 6 screw flights and 7 mm pitch). The total length of the screw was 700 mm with 14 elements in the three zones. The extruder was equipped with a bulk solids metering feeder (KTRON T20, Niederlenz, Switzerland). A round die (3.0 mm diameter), equipped with a cutting device set at 130 rpm, was used.

Extrusion of white lupins was performed at 22% moisture and 140°C. These processing conditions were selected since these were found to show the best nutritional properties for grain legumes in a previous *in vitro* evaluation in our laboratory (Nalle, 2009). The desired moisture levels were obtained by adding water prior to the extruder section by means of a pump. The water feed rate for obtaining the final moisture content of 22% was 0.75 kg/h. The optimum temperatures of the seven extruder sections from the feeder end were 50, 60, 70, 80, 100, 120 and 140°C. The extruded materials were then allowed to cool to room temperature.

The raw and extruded white lupins were ground in a hammer mill to pass through a 3 mm sieve and stored at 4°C until analysis. Four representative samples of raw and extruded materials were obtained and analysed for dry matter, starch, fat, ash, NSP, nitrogen, amino acids and trypsin inhibitor activity.

Experimental design: The experimental procedures were approved by the Massey University Animal Ethics Committee. Three treatment diets, consisting of a corn-soy basal diet (Table 1) and two test diets containing raw and extruded lupin meals, were assayed. The test diets were formulated by substituting the raw and extruded lupin meals for 25% (w/w) of the basal diet. All diets contained titanium dioxide (0.3%), as an indigestible marker to calculate the ileal digestibility.

Day-old male broilers (Ross 308) were raised in a floor pen and fed a commercial broiler starter diet (23% crude protein) till day 21. Feed and water were available at all times. The temperature was maintained at 32°C during the first week and gradually decreased to approximately 23°C by the end of the third week. Ventilation was controlled by a central ceiling extraction fan and wall inlet ducts. Birds received 20 hrs of fluorescent illumination per day. On day 21, 108 birds of uniform body weight were selected and randomly assigned to 18 cages (6 birds per cage). The birds were offered a commercial broiler finisher diet (18% crude protein) until the introduction of assay diets, in mash form, on day 28. On day 28, six replicate cages were randomly assigned to each assay diet.

Table 1: Composition (g/kg as fed) of the basal diet used in metabolizable energy and digestibility assays

Ingredient	
Corn	590.6
Soybean meal	351.8
Soybean oil	17.8
Dicalcium phosphate	21.7
Limestone	7.8
Salt	2.0
Sodium bicarbonate	2.3
Trace mineral-vitamin premix[1]	3.0
Titanium dioxide	3.0

[1]Provided per kg diet: Co, 0.3 mg; Cu, 5 mg; Fe, 25 mg; I, 1 mg; Mn, 125 mg; Zn, 60 mg; choline chloride, 638 mg; trans-retinol, 3.33 mg; cholecalciferol, 60 µg; dl-α-tocopheryl acetate, 60 mg; menadione, 4 mg; thiamin, 3.0 mg; riboflavin, 12 mg; niacin, 35 mg; calcium panthothenate, 12.8 mg; pyridoxine, 10 mg; cyanocobalalamin, 0.017 mg; folic acid 5.2 mg; biotin, 0.2 mg; antioxidant, 100 mg; molybdenum, 0.5 mg; selenium, 200 µg

The AME was determined using the classical total excreta collection method. Feed intake and excreta output were measured quantitatively per cage from day 32 for four consecutive days. The excreta from each cage were pooled, mixed, sub-sampled and freeze-dried. The dried excreta samples, together with samples of the diets, were subsequently ground to pass through 0.5-mm sieve and stored in airtight plastic containers for analysis of dry matter and gross energy.

On day 35, all birds were euthanized by an intracardial injection of sodium pentobarbitone solution and the contents of the lower half of the ileum were collected and processed as described by Ravindran *et al.* (2005). The diet and digesta samples were then analysed for dry matter, titanium dioxide, fat, nitrogen and amino acids.

Chemical analysis: All analyses were conducted in triplicates and the results are reported on a dry matter basis. The dry matter, crude fat and ash contents were determined according to AOAC (2005). Nitrogen content was determined by the combustion method (AOAC, 2005) using a CNS-2000 carbon, nitrogen and sulphur analyzer (LECO® Corporation, St. Joseph, Michigan, USA). The crude protein content of the samples was calculated by multiplying the nitrogen content by 6.25. Gross energy was determined using an adiabatic oxygen calorimeter (Gallenkamp Autobomb, London, UK) standardized with benzoic acid.

Starch content was measured using an assay kit (Megazyme, Boronia, VIC, Australia) based on the use of thermostable α-amylase and amyloglucosidase. Total, soluble and insoluble NSP were determined using an assay kit (Englyst Fiberzyme Kit GLC, Englyst Carbohydrate Services Limited, Cambridge, UK). Amino acid concentration was determined by high-performance liquid chromatography as described by Ravindran *et al.* (2009). Cystine and methionine were analysed as cysteic acid and methionine sulfone following oxidation with performic acid for 16 h at 0°C and neutralization with hydrobromic acid prior to hydrolysis. Tryptophan was not determined.

The titanium oxide content was measured using the colorimetric method described by Short *et al.* (1996). The procedure to determine trypsin inhibitor was that of Valdebouze *et al.* (1980). Trypsin inhibitor activity was expressed in units of Trypsin Inhibited (TIU) per milligram sample.

Calculations: The AME of test diets and lupins were calculated using the following formulas (Nalle *et al.*, 2010):

$$AME_{diet}\ (MJ/kg) = \frac{(Feed\ intake \times GE_{diet}) - (Excreta\ output \times GE_{excreta})}{Total\ feed\ intake}$$

$$AME_{lupin}\ (MJ/kg) = \frac{AME\ of\ lupin\ diet - (AME\ basal\ diet \times 0.75)}{0.25}$$

The Apparent Ileal Digestibility Coefficient (AIDC) of fat, crude protein and amino acids in the test diets and lupin samples were calculated, using titanium dioxide as the indigestible marker, as shown below (Nalle *et al.*, 2010):

$$AIDC\ of\ lupin\ diet = \frac{(Nutrient/Ti)d - (Nutrient/Ti)i}{(Nutrient/Ti)d}$$

$$AIDC\ of\ lupin = \frac{(AIDC\ of\ lupin\ diet \times Nutrient\ in\ lupin\ diet) - (AIDC\ of\ basal\ diet \times 0.75 \times Nutrient\ in\ basal\ diet)}{(0.25 \times Nutrient\ in\ lupins)}$$

Where, (Nutrient/Ti)d = ratio of nutrient and titanium in diet and (Nutrient//Ti)I = ratio of nutrient and titanium in ileal digesta.

Statistical analysis: Unpaired t-test was used to compare the nutritional value of raw and extruded white lupins (SAS, 1997). Differences were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

Nutrient composition: The results showed that extrusion of white lupins had no effect ($p<0.05$) on the contents of crude protein, crude fat and ash (Table 2). To the authors' knowledge, the present study is the first to examine the influence of extrusion on the nutritional value of white lupins and, as such, no comparable data are available. However, similar lack of effect of extrusion on the fat and ash contents of peas has been previously reported (Alonso *et al.*, 2001).

Table 2: The effect of extrusion the chemical composition (g/100 g dry matter) of white lupins[1]

	Raw, Unextruded	Extruded
Crude protein	36.87±0.76	37.66±0.89
Crude fat	12.95±0.61	13.59±0.82
Ash	3.68±0.20	3.56±0.11
Starch	ND[2]	ND[2]
Amino acids		
Indispensable		
Arginine	3.56±0.12[a]	3.21±0.11[b]
Histidine	9.12±0.28	8.66±0.35
Isoleucine	1.56±0.08	1.48±0.05
Leucine	2.98±0.11	3.17±0.17
Lysine	1.87±0.05	1.71±0.09
Methionine	0.38±0.01	0.37±0.04
Phenylalanine	1.75±0.04	1.66±0.09
Threonine	1.45±0.02	1.51±0.01
Valine	1.57±0.03	1.65±0.05
Dispensable		
Alanine	1.35±0.01	1.31±0.04
Aspartic acid	3.92±0.16	4.12±0.20
Cysteine	0.62±0.03[a]	0.41±0.04[b]
Glycine	1.44±0.01	1.49±0.04
Glutamic acid	6.56±0.06	6.40±0.20
Proline	1.23±0.04	1.21±0.06
Serine	1.55±0.06	1.36±0.09
Tyrosine	1.68±0.06	1.75±0.05
Non-starch polysaccharides		
Soluble	4.10±0.43[a]	7.40±0.70[b]
Insoluble	33.00±1.20[a]	30.50±0.58[b]
Total	37.10±1.50	37.90±1.45
TIA (TIU/mg)[3]	1.05±0.06[a]	0.30±0.09[b]

[a,b]Means in a row with different superscripts differ ($p<0.05$).
[1]Each value represents mean ± SE of four samples.
[2]Not detected.
[3]TIU, Trypsin inhibitor international units.
TIA = Trypsin Inhibitor Activity

One of the beneficial effects of extrusion is on starch gelatinization (Singh *et al.*, 2007), which increases the accessibility of starch to digestive enzymes and improves starch digestion. However, no starch was determined in white lupins, in contrast to other grain legumes such as field peas and faba beans (Petterson *et al.*, 1997; Nalle *et al.*, 2010, 2011a,b). The main carbohydrate reserves in white lupins were the NSP. White lupins contained 3.71% total NSP, which is in agreement with those reported by Kocher *et al.* (2000). Extrusion significantly ($p<0.05$) influenced the content of different NSP fractions in white lupins (Table 2). Soluble NSP contents were increased ($p<0.05$) and the insoluble NSP contents were lowered ($p<0.05$) by extrusion. Total NSP contents, however, were unaffected ($p>0.05$). The increase in soluble NSP with extrusion was in agreement with previous reports (Bjorck and Asp,

Table 3: The effect of extrusion on the apparent metabolizable energy (AME, MJ/kg dry matter) and Apparent Ileal Digestibility Coefficient (AIDC) of fat, protein and amino acids for broilers[1]

	Raw, Unextruded	Extruded
AME, MJ/kg DM	9.910±0.24[a]	7.780±0.40[b]
Apparent ileal digestibility		
Crude fat	0.942±0.007[a]	0.901±0.011[b]
Crude protein	0.870±0.013	0.852±0.025
Amino acids		
Indispensable		
Arginine	0.966±0.018	0.942±0.029
Histidine	0.821±0.042	0.835±0.022
Isoleucine	0.856±0.035	0.884±0.023
Leucine	0.878±0.022	0.869±0.031
Lysine	0.901±0.026	0.908±0.020
Methionine	0.789±0.036	0.768±0.026
Phenylalanine	0.922±0.040	0.909±0.024
Threonine	0.801±0.028	0.784±0.016
Valine	0.864±0.023	0.851±0.016
Dispensable		
Alanine	0.835±0.012	0.854±0.026
Aspartic acid	0.784±0.022	0.780±0.015
Cysteine	0.835±0.021	0.822±0.018
Glycine	0.874±0.015	0.855±0.019
Glutamic acid	0.841±0.034	0.830±0.022
Proline	0.845±0.022	0.831±0.029
Serine	0.868±0.025	0.881±0.031
Tyrosine	0.878±0.012	0.862±0.018

[a,b]Means in a row with different superscripts differ ($p<0.05$)

[1]Each value represents mean ± SE of four replicates

1983; Vasanthan *et al*., 2002) and can be attributed to the solubilisation of part of the insoluble NSP to soluble NSP.

Extrusion had no effect ($p>0.05$) on the contents of most amino acids. The exceptions were arginine and cysteine, the contents of which were lowered ($p<0.05$) by extrusion. No plausible reasons can be provided for the reduction in arginine concentrations, but the susceptibility of cystine to heat is well documented (Evans and McGinnes, 1948; Ravindran and Bryden, 1999).

The reduction ($p<0.05$) in trypsin inhibitor activity of white lupins following extrusion (Table 2) was an expected result and in agreement with previous research on other grain legumes (van der Poel, 1992; O'Doherty and Keady, 2001; Singh *et al*., 2007).

Apparent metabolizable energy: Extrusion cooking resulted in 21% reduction ($p<0.05$; 9.91 vs. 7.78 MJ/kg DM) in the AME of white lupins (Table 3). Similarly, Breytenbach (2005) found that the AME value of Australian sweet lupin (*Lupinus angustifolius*) decreased (8.61 vs. 7.52 MJ/kg) after extrusion with a single-screw extruder at a barrel jacket temperature of 120°C. The observed decrease was attributed to the increased bulkiness that occurred during expansion which lead to reduced feed and energy intakes. Our data, however, suggest that this detrimental effect on the AME may be related to the increased soluble NSP content in the lupin extrudate, The negative effects of soluble NSP on nutrient utilization is well documented and have been attributed to their viscous nature and interaction with gut microflora (Smits and Annison, 1996; Gabriel *et al*., 2006). The increase in gut viscosity caused by soluble NSP could also lead to lower gastric emptying rate of solids and liquids and transit time in the small intestine (Smits and Annison, 1996). Our hypothesis is supported by the lower ($p<0.05$) AIDC of fat in the lupin extrudate. It has been shown that the digestion of fat is affected more than other nutrients by NSP and digesta viscosity (Danicke *et al*., 1997).

Ileal nutrient digestibility: Extrusion had no effect on the AIDC of crude protein and amino acids in white lupins (Table 3). Based on the effect of extrusion on the trypsin inhibitor activity, however, it was expected that protein digestibility will be improved. A possible reason may be that the inhibitor activity in the raw, unextruded meal was low to have any negative effect on protein digestibility. It is also possible that any potential improvement after extrusion may have been negated by effects on protein aggregation (Carbonaro *et al*., 2005) and non-enzymatic browning-thermal cross-linking caused by Maillard reaction (Vasanthan *et al*., 2002).

Conclusion: In summary, under the extrusion conditions employed in the current study, extrusion cooking increased soluble NSP contents and lowered the ileal fat digestibility and AME of white lupin. Although the trypsin inhibitor activity was reduced, extrusion had no effect on the ileal protein and amino acid digestibility of white lupins for broiler chickens.

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