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Extrusion of Peas (*Pisum sativum* L.): Effects on the Apparent Metabolisable Energy and Ileal Nutrient Digestibility of Broilers

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Abstract: Problem statement: The potential feeding values of grain legumes, such as peas (*Pisum sativum*), are limited because of the presence of anti-nutritional factors. In particular, protease inhibitors are of interest, but these can be readily destroyed by thermal treatments. In the present study, the influence of extrusion on the chemical composition and nutritive value of peas was evaluated. **Approach:** Two extrudates were produced by extruding the peas at two moisture levels (19 and 22%) and one temperature (140°C). Four treatment diets consisting of a corn-soy basal diet and three test diets containing raw and the two extruded pea meals were formulated and assayed in digestibility and balance trials using broiler chickens. The test diets were formulated by substituting the raw and extruded pea meals for 25% (w/w) of the basal diet. Ileal nutrient digestibility was calculated using titanium oxide as an indigestible indicator and the apparent metabolisable energy was determined using the classical total excreta collection method. **Results:** Extrusion had no effect ($P>0.05$) on the contents of crude protein and starch. Soluble non-starch polysaccharide contents were increased ($P<0.05$) and the contents of total and insoluble non-starch polysaccharide were lowered ($P<0.05$) by extrusion. As expected, trypsin inhibitor activity of peas was reduced ($P<0.05$) following extrusion Extrusion increased ($P < 0.05$) the apparent ileal starch digestibility, but had no effect ($P > 0.05$) on the apparent ileal protein digestibility and the apparent metabolisable energy of peas. **Conclusion:** Under the extrusion conditions employed in the present study, extrusion was not beneficial to improving the nutritive value of peas for broilers.

Key words: Extrusion cooking, nutrient digestibility, protein digestibility, starch digestibility, antinutritional factors, thermal treatments, *pisum sativum*

INTRODUCTION

Extrusion cooking is a high-temperature, short-time process in which moistened, expansive, starchy and/or proteinacious food materials are plasticised and cooked in a tube by a combination of moisture, pressure, temperature and mechanical shear, resulting in molecular transformation and chemical reactions (Havck and Huber, 1989; Castells *et al*., 2005). This technology has some unique positive features compared with other heat processes, because the material is subjected to intense mechanical shear. It is able to break the covalent bonds in biopolymers and the intense structural disruption and mixing facilitate the modification of functional properties of food ingredients and/or texturizing them (Asp and Bjorck, 1989; Carvalho and Mitchelle, 2000). In addition, the extrusion process denatures undesirable enzymes; inactivates some antinutritional factors (trypsin inhibitors, haemagglutinins, tannins and phytates); sterilises the finished product; and retains natural colours and flavours of foods (Fellows, 2000; Bhandari *et al*., 2001). The process has found numerous applications.

Extrusion cooking is a process where the feed is subjected to mixing, shearing and heating under high pressure before the extrudate is forced through a die. During this process, the feed may undergo reactions which could be beneficial, if nutrient availability is improved or detrimental 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 (Björk and Asp, 1983; Riaz, 2000; Purushotham *et al*., 2007; Domoney and Welham, 1992). In addition, appropriate processing temperature is critical for the elimination of heat-labile anti-nutritional factors found in legume

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seeds. Extrusion has been to shown to have positive effects on the *in vitro* digestibility of protein (Alonso *et al*., 2000a; 200b; El-Hady and Habiba, 2002) and 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*., 2000b) in diets for poultry. These improvements have been attributed to due to the reduction of anti-nutritional factors, denaturation of native protein and gelatinisation of starch.

Grain legumes, such as peas (*Pisum sativum*), are widely available in many parts of the world. These legumes are important protein sources in both human and animal nutrition (Nalle *et al*., 2010). However, their use in poultry feed industry remains limited because of the presence of anti-nutritional factors which interfere with nutrient utilisation resulting in poor animal performance. Of the various anti-nutritional factors present in grain legumes, protease inhibitors are of particular interest, but these components are readily destroyed by thermal treatments. The purpose of the study reported herein was to examine the effects of extrusion cooking on the chemical composition, ileal digestibility of protein and starch and apparent metabolisable energy (AME) of peas for broiler chickens

MATERIALS AND METHODS

Processing: Round seeded peas, purchased from a commercial supplier, 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 sections with forward elements. The first section 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 three zones. The extruder was equipped with a bulk solids metering feeder (KTRON T20, Switzerland). A round die (3.0 mm diameter), equipped with a cutting device set at 130rpm, was used.

Extrusion of peas was performed at two moisture levels (19 and 22%) and one temperature (140°C). These processing conditions were selected since these were found to show the best nutritional properties in a previous *in vitro* evaluation in our laboratory. 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 19% was 0.50, while 0.75 kg h^{-1} was used to achieve 22% final moisture content. The optimum temperatures of the seven extruder sections from the feeder end were 50, 60, 70, 80, 100, 100 and 140°C. The extruded materials were then allowed to cool to room temperature. The raw and extruded peas were ground in a hammer mill to pass through a 3 mm sieve.

Experimental design: The experimental procedures were approved by the Massey University Animal Ethics Committee. Four treatment diets consisting of a corn-soy basal diet (Table 1) and three test diets containing raw and extruded pea meals were assayed. The test diets were formulated by substituting the raw and extruded pea meals for 25% (w/w) of the basal diet. All diets contained titanium oxide (3 g kg^{-1}), as an indigestible marker as an indigestible marker to calculate the nutrient digestibility.

Day-old male broilers (Ross 308) were raised in floor pens and fed a commercial broiler starter diet (23 % crude protein) till day 21. Feed and water were available at all times. On day 21, 64 birds of uniform body weight were selected and randomly assigned to 16 cages (4 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, four replicate cages were randomly assigned to each assay diet.

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.

Table 1: Composition (g 100^{-1} g as fed) of the basal diet used in metabolisable energy and digestibility assays

Ingredient	
Corn	594.6
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

[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

On day 35, all birds were euthanised 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; 2009). The diet and digesta samples were then analysed for dry matter, titanium oxide, starch and nitrogen.

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 *et al*. (2010). Nitrogen content was determined by the combustion method (AOAC *et al*., 2010) 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 non-starch polysaccharides (NSP) were determined using an assay kit (Englyst Fiberzyme Kit GLC, Englyst Carbohydrate Services Limited, Cambridge, UK). The titanium oxide content was measured using the colorimetric method described by Short *et al.* (1996). The procedure to determine trypsin inhibitor. Trypsin inhibitor activity was expressed in units of trypsin inhibited (TIU) per milligram sample.

Calculations: The AME values of the test diets and pea samples were calculated using the following formulas:

$$\text{AME}_{\text{diet}}(\text{MJ kg}^{-1}) = \frac{(\text{Feed intake} \times \text{GE}_{\text{diet}}) - (\text{Excreta output} \times \text{GE}_{\text{extrecta}})}{\text{Total feed intake}}$$

$$\text{AME}_{\text{peas}}(\text{MJ kg}^{-1}) = \frac{\text{AME of pea diet} - (\text{AME basal diet} \times 0.75)}{0.25}$$

The apparent ileal digestibility coefficient (AIDC) of nitrogen and starch in the test diets and pea samples were calculated, using titanium oxide as the indigestible marker, as shown below:

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

$$\text{AIDC of peas} = \frac{(\text{AIDC of pea diet} \times \text{Nutrient in pea diet}) - (\text{AIDC of basal diet} \times 0.75 \times \text{nutrient in the of basal diet})}{0.25 \times \text{Nutrient in peas}}$$

Where:

(Nutrient/Ti)d = Ratio of nutrient and titanium in diet

(Nutrient//Ti)i = Ratio of nutrient and titanium in ileal digesta

Statistical analysis: The data were analysed by one-way analysis of variance using the General Linear Model procedure of SAS with cage as the experimental unit. Differences were considered to be significant at $P < 0.05$ and significant differences between means were separated by the Fisher's Least Significant Difference test.

RESULTS AND DISCUSSION

The results showed that extrusion cooking of peas had no effect ($P<0.05$) on the contents of crude protein, crude fat and ash of the extrudates (Table 2). The lack of effect of extrusion on the fat and ash contents of peas is in agreement with the findings of Alonso *et al*. (1998). In contrast, Diaz *et al*. (2006) reported that the fat and ash contents of peas were increased by 61 and 4%, respectively, following extrusion. The observed discrepancy is likely to be due to the differences in the extruder type used. In the present study, a twin-screw extruder type was used, whilst in the study by Diaz *et al*. (2006), a single-screw extruder type was used. As reported by Björk and Asp (1983), the type of extruder is a critical factor which will affect the degree of modification in nutritional properties. Extrusion conditions are also important, but it was difficult to compare the effects of this aspect, because Diaz *et al*. (2006) did not describe the conditions used in their study.

Extrusion significantly ($p<0.05$) influenced the NSP contents of peas (Table 2). Soluble NSP contents were increased ($p<0.05$) and the contents of total and insoluble NSP were lowered ($p<0.05$) by extrusion. The increase in soluble NSP with extrusion was in agreement with previous studies (Vasanthan *et al*., 2002) and this may be attributed to the conversion of part of the insoluble NSP to soluble NSP.

Table 2: The effect of extrusion the chemical composition (g/100 g dry matter) of peas

	Raw, Unextruded	Extruded, 19% moisture, 140°C	Extruded, 22% moisture, 140°C	Pooled SEM	Probability
Crude protein	23.00	22.60	22.90	0.880	NS
Crude fat	2.50	2.50	2.60	0.210	NS
Ash	3.10	3.10	3.10	0.160	NS
Starch	46.50	46.00	46.10	1.120	NS
Non starch polysaccharides					
Soluble	2.30[a]	2.50[b]	2.80[c]	0.160	**
Insoluble	17.70[a]	17.20[b]	16.60[c]	0.170	*
Total	20.00[a]	19.70[b]	19.40[c]	0.080	*
Trypsin inhibitor activity (TIU/mg)	0.23[a]	0.25[a]	0.19[b]	0.004	*

[a,b]: Means in a row with different superscripts differ ($p<0.05$); S: Not Significant ($p>0.05$); *: Significant at $p<0.05$; **: Significant at $p<0.01$

Table 3: The effect of extrusion on the apparent metabolisable energy (AME, MJ/kg dry matter) and Apparent Ileal Digestibility Coefficient (AIDC) of protein and starch of peas for broilers

	Raw, Unextruded	Extruded, 19% moisture, 140°C	Extruded, 22% moisture, 140°C	Pooled SEM	Probability
AME	11.7	11.1	11.1	0.45	NS
AIDC of protein	0.828	0.838	0.803	0.0912	NS
AIDC of starch	0.865[a]	1.015[b]	0.986[b]	0.0321	**

[a,b]: Means in a row with different superscripts differ ($p<0.05$); NS: Not Significant ($p>0.05$); **: Significant at $p<0.01$; [1]: Each value represents the mean of four replicates (4 birds/replicate)

In this evaluation, extrusion cooking had no effect ($p<0.05$) on the starch content (Table 2). This finding is in disagreement with those of Diaz *et al*. (2006) who showed a decrease in the starch content of peas extruded with a single-screw extruder. This variability was probably due to the difference in methodology, especially the type of extruder used. Perez-Navarrete *et al*. (2006) also reported a decrease in the starch content of extruded products and attributed this observation to the formation of indigestible starch, which makes it difficult to be extracted by enzymes used in the analysis of starch.

The reduction in trypsin inhibitor activity of peas following extrusion (Table 2) was an expected result and in agreement with previous research (van der Poel, 1992; O'Doherty and Keady, 2001; Diaz *et al*., 2006). In the study by Van der Poel (1992), the trypsin inhibitor activity of pea cultivars (round- and wrinkle seeded peas) were reduced by extrusion at different processing temperatures (106-140°C) and moisture contents (14-33%). However, the degree of inactivation was dependent on the processing conditions and the cultivar used. The inactivation of trypsin inhibitor activity in round-seeded peas was almost complete under the different processing conditions investigated, whereas the trypsin inhibitor activity in wrinkle-seeded pea was inactivated only at the highest temperature.

Ileal nutrient digestibility: Extrusion had no effect on the AIDC of protein in peas (Table 3). It is possible that the lack of improvement in the protein digestibility of pea protein after heat treatment could be due to protein aggregation (Meng *et al*., 2002; Carbonaro *et al*., 2005) and non-enzymatic browning-thermal cross-linking caused by Maillard reaction (Vasanthan *et al*., 2002).

The improvements observed in ileal starch digestibility in pea extrudates (Table 3) was as expected. The improvement of starch digestibility after extrusion was likely to be due to gelatinisation which increases the accessibility of starch to digestive enzymes. Native granule starch, which consists predominantly of α-glucan in the form of amylose and amylopectin, is hydrolysed slowly by α-amylase and amyloglucosidase compared with gelatinised starch in processed foods. When native starches are heated in excess water, the crystalline structure is disrupted and water molecules form hydrogen bonds to the exposed hydroxyl groups of amylose and amylopectin (Ratnayake *et al*., 2002; Tester *et al*., 2004). This causes an increase in granule swelling and solubility. Granule structure is completely lost, making the starch to be completely digested by starch-hydrolysing enzymes.

Curiously, ileal starch digestibility in the 19% 140°C treatment was calculated to exceed 100%. This error may be due to the method of determination employed. The substitution method was used in the present study in order to calculate ileal digestibility. This method assumes that there is no interaction between the basal diet and the test ingredient (Ravindran and Bryden, 1999; Lemme *et al*., 2004), but which may not be the case.

Apparent metabolisable energy: In the present study, extrusion cooking had no effect on the AME of peas (Table 3). In contrast, Breytenbach (2005) reported that the AME value of Australian sweet lupin decreased (8.61and 7.52 MJ kg^{-1}) 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 leads to reduced feed and energy intakes. In contrast, Prinsloo (1993, cited by Breytenbach, 2005) demonstrated that the AME value of Australian sweet lupins for adult cockerels was increased by 11.5% (6.71 and 7.58 MJ kg^{-1}) by extrusion with a barrel temperature of 120°C. These findings suggest that the extent of changes in the nutritional value of a feed ingredient via extrusion was dependent on the type of extruder, processing condition, type of ingredient and class of chicken.

It is noteworthy that although the ileal starch digestibility of peas markedly increased after extrusion, the AME values were unaffected. It is difficult to provide a reason for these observations. Starch digestibility was determined at the ileal level, whereas the AME was determined at the excreta level and this may provide some explanation. It is well documented that microbial activity in the hindgut of chickens has a marked influence on the utilisation of nutrients (Ravindran *et al*., 1999).

CONCLUSION

In summary, under the extrusion conditions employed in the current study, extrusion cooking had no effect on the protein and fat contents of peas, but resulted in increased soluble NSP contents. Extrusion had no effect on the ileal protein digestibility of peas for broiler chickens, but increased the ileal starch digestibility of peas. However, improvements in starch digestion failed to translate into any beneficial effect in terms of AME in peas for broilers. It is concluded that, under the conditions of the present study, extrusion cooking was not beneficial to improve the nutritive value of peas for broilers.

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