Influence of processing of full fat soya beans included in diets for piglets. I. Performance

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Abstract

The programme reported examined the effect of different processing conditions for full fat soya beans (FFSB) during micronization (two steam inlets, being full or low steam—(FS or LS) × two temperatures, being high cook and low cook (HC and LC); Trial 1) and extrusion (four exit temperatures of 70, 90, 110 and 170 °C; Trial 2) on performance in post-weaned piglets over the live weight range 10–27 kg fed diets containing 300 g processed FFSB/kg.

Trypsin inhibitor activity (TIA, mg pure trypsin inhibited/g of sample) ranged from 16.0, 5.2, 4.1 and 2.0 for LSLC, LSHC, FSLC and FSHC, respectively (Trial 1) and from 24.1, 16.8, 6.4 and 2.9 with increasing extrusion temperature (Trial 2).

In Trial 1, piglets offered the FS FFSB diets had significantly higher daily live weight gain (DLWG, 652 versus 487 g/day, \( P < 0.001 \)), consumed significantly more feed (feed intake, FI, 791 versus 679 g DM/day, \( P < 0.001 \)) and had a significantly better feed conversion ratio (FCR, 1.211 versus 1.404, \( P < 0.001 \)). There were no effects of temperature or steam × temperature interactions. Performance characteristics were highly correlated with reduced intake of TIA (dietary intake of TIA × feed intake; \( P < 0.001 \) for DLWG and \( P = 0.002 \) for FI and FCR).

In Trial 2, there was a significant linear improvement in DLWG (\( P = 0.004 \), but not quadratic) a significant change in FI (\( P = 0.026 \) quadratic, but not linear) and a significant linear improvement in FCR (\( P < 0.001 \), but not quadratic) with increasing extrusion temperature. DLWG and FCR were negatively correlated to increasing daily TIA intakes (\( P < 0.05 \)).

Both trials allowed an assessment of the response to TIA intake; performance was optimum at low daily intakes of TIA (between 0.5 and 1.5 g/day) irrespective of processing method. Decisions...
on optimum processing conditions should be based on TIA levels in FFSB, the rate of inclusion and subsequent feed intake (giving, collectively, daily TIA intake) and not simply on named processes.

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Keywords: Micronization; Extrusion; Full fat soya bean; Piglets; Performance

1. Introduction

Full fat soya beans (FFSBs) contain many heat-labile biological compounds, of which the most important are the heat-labile trypsin inhibitors (TIs). The degree of improvement in nutritive value following their reduction depends upon heating temperature, duration of heating and moisture content. Thus defining optimum conditions during processing assumes considerable importance. Although heating destroys most of the anti-nutritional factors (ANFs), care must be taken to avoid either under- or over-heating of FFSB (Liener and Kakade, 1980; Van der Poel, 1989) as excessive heat treatment markedly impairs protein digestibility.

The beneficial effect of heat treatment on the nutritive value of FFSB has been attributed, in part, to the destruction of protease inhibitors (Liener, 1989). However it is also a consequence of the denaturation of the soya protein which leads to improved digestibility by increasing susceptibility to enzymatic attack (Kakade et al., 1973). Additional processing variables include the presence of moisture. Although soaking had no effect on TI content, soaking followed by cooking or steaming and cooking eliminated TI levels in immature soya beans, but only soaking plus cooking removed TI from mature samples (Liu and Markakis, 1987).

A considerable volume of literature describes the effects of processing on the nutritive value of FFSB for piglets by referring to named processes. However, this is an uninformative approach as reference to conditions operating during processing and data on TI levels are rarely given. There is little information available on the effects of controlled processing on TI reduction and subsequent nutritional value. The objective of the current programme was to subject FFSB to a precisely defined sequence of processes generating material of measured trypsin inhibitor activity (TIA) which would be used subsequently in performance studies.

2. Materials and methods

2.1. Processing conditions for FFSB

In Trial 1, FFSB were micronized with low steam (LS) or full steam (FS) combined with high (dwell time = 40 s approximately, HC) or low (dwell time = 10 s, LC) cooking conditions by using a Micro Red 20 cereal micronizer with a burner temperature of approximately 200 °C (Dale Country Foods Ltd., North Yorkshire, UK). Thus there were four treatment combinations (LSHC, LSLC, FSHC and FSLC).

In Trial 2, FFSB, from the same batch, were finely ground, pre-conditioned with water (moisture content of 250–260 g/kg) and processed with a twin screw co-rotating extruder
Table 1
Diet specification of Trials 1 and 2

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Inclusion (g/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full fat soya bean</td>
<td>300</td>
</tr>
<tr>
<td>Maize</td>
<td>75</td>
</tr>
<tr>
<td>Wheat</td>
<td>400</td>
</tr>
<tr>
<td>Barley</td>
<td>83</td>
</tr>
<tr>
<td>Fish meal</td>
<td>75</td>
</tr>
<tr>
<td>Whey powder</td>
<td>25</td>
</tr>
<tr>
<td>Vegetable oil</td>
<td>15</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>5</td>
</tr>
<tr>
<td>Limestone</td>
<td>3</td>
</tr>
<tr>
<td>Salt</td>
<td>1</td>
</tr>
<tr>
<td>Lysine</td>
<td>2.8</td>
</tr>
<tr>
<td>Methionine</td>
<td>1.2</td>
</tr>
<tr>
<td>Threonine</td>
<td>1.5</td>
</tr>
<tr>
<td>Vitamin mineral premix</td>
<td>12.5</td>
</tr>
</tbody>
</table>

Provided by Trouw Ltd., Wincham, Northwich, Cheshire, UK.

*Premix supplied, per kg diet: Vitamin A, 12,000 IU; Vitamin D₃, 2000 IU; Vitamin E, 50 IU; Vitamin B₁, 1 mg; Vitamin B₂, 3 mg; Vitamin B₆, 1 mg; Vitamin B₁₂, 10 μg; Vitamin K, 2 mg; copper (cupric sulphate), 175 mg; nicotinic acid, 12 mg; pantothenic acid, 10 mg; iron, 200 mg; cobalt, 0.5 mg; manganese, 40 mg; zinc, 90 mg; iodine, 1 mg; selenium, 0.2 mg; calcium, 31.25 g; salt, 25 g; sodium, 10 g.

(MPF50 manufactured by APV Baker) at the Campden and Chorleywood Food Research Association, UK. The following conditions were employed: die plate with 2 mm × 25 mm holes, feed rate of 800 g/min, screw speed of 250 rpm, dwell time of approximately 30 s and exit temperatures of 70, 90, 110 and 150 °C. After overnight drying moisture content was generally less than 150 g/kg.

2.2. Experimental diets

Processed FFSB were ground through a 5-mm screen and transported to the University of Nottingham animal feed mill where the diets were produced. For each trial there were four experimental diets containing FFSB at a rate of 300 g/kg; other ingredients were maize, wheat barley, fish meal, whey powder and vegetable oil; micronutrients were also added (Table 1).

2.3. Experimental animals

Thirty-two post-weaned pigs were employed for each trial (16 of each sex) of approximately 8.84 ± 0.18 kg (Trial 1) and 10.40 ± 0.24 kg (Trial 2) initial live weight, obtained from the University herd (Landrace × (Landrace × Large White)) 1 week after weaning at 25 days of age. The criteria for selection were that animals were of similar weight, healthy (no evident pathologies) and appropriate sex. The trial was conducted under official welfare guidelines and protocols covering animal experimentation at the University.
2.4. Chemical analysis of FFSB

All analyses were carried out in duplicate; any variation between samples above 5% was regarded as unacceptable and the analysis was repeated. FFSB were dried for approximately 48 h in an oven at 105 °C for dry matter (DM), analysed for nitrogen/crude protein (CP) by using a NA 2000 Nitrogen and protein analyser (Fisons, UK) and extracted with a 40–60 °C petroleum ether for ether extract (EE) with a preliminary step of acid hydrolysis treatment with hydrochloric acid prior to the extraction (Sanderson, 1986).

Trypsin inhibitor activity (TIA) was measured according to the method established by Kakade et al. (1974), as modified by Smith et al. (1980) (allowing large numbers of widely differing samples to be assayed on a routine basis) and described below. TIA is usually measured by the loss of activity of added trypsin under standard conditions.

“Tris Buffer”: 6.05 g Tris-(hydroxymethyl)-methylamine and 2.94 g calcium chloride dihydrate (Fisher Scientific U.K. Ltd., Loughborough, UK) were completely dissolved in 900 ml of distilled water and the pH was adjusted to 8.2. The buffer was diluted to 1 l and the pH confirmed at 8.2.

BAPA solution: 0.04 g of N-α-benzoyl-DL-arginine-p-nitroanilide hydrochloride (Sigma Chemical Co., St Louis, MO, USA) was completely dissolved in 1 ml of dimethyl sulphoxide and diluted to 100 ml with Tris buffer previously warmed to 37 °C.

Standard Trypsin solution: 0.04 of crystalline bovine trypsin (Sigma Chemical Co., St Louis, MO, USA) was dissolved in 0.001 M of hydrochloric acid and diluted to 2 l with 0.001 M HCl.

Approximately 1 g of sample, following ether extraction, was mixed with 50 ml of 0.01 M sodium hydroxide at pH 9.4–9.6 for extraction of the inhibitors and soaked overnight. After filtering, a diluted suspension of the sample (dilution factors ranged from 5× to 50×) was mixed with 2.0 ml of a standard trypsin and incubated in a water bath at 37 °C. After exactly 10 min, 5.0 ml of BAPA solution previously warmed to 37 °C was added and followed by a second 10-min incubation phase.

To stop the tryptic hydrolysis reaction, 1 ml of 30% acetic acid was added and the sample was mixed. The absorbance of the solutions (activity of the remaining trypsin) was measured by using a SP6-500 UV spectrophotometer (Pye Unicam Ltd., Cambridge) at 410 nm. One trypsin unit is defined as an increase of 0.01 absorbance units at 410 nm per 10 ml of reaction mixture under the strict conditions of the Kakade test (Kakade et al., 1974). TIA may be expressed as TIA per gram nitrogen, trypsin units per gram protein or most commonly as milligram pure trypsin inhibited per gram sample; the latter was employed in the current study.

2.5. Animals, housing and management

Full details of procedures during the performance trial are given in Zarkadas and Wiseman (2001).

Average daily live weight gain (DLWG) was calculated by linear regression of the weekly live weight on days, with the slope of the linear response being DLWG. Total feed intake (on dry matter basis so that small differences in dry matter content of FFSB would not confound results) was divided by the number of days that the animal took to grow over the
live weight range of the experiment (giving average daily feed intake, FI). Feed conversion ratio (FCR) was the ratio of total feed intake (kg dry matter basis) per total weight gain (kg) over the experimental period.

2.6. Statistical analysis

Pig performance data for Trial 1 were analysed as a $2 \times 2$ factorial design with the factors in the model being steam levels (2) and heat levels (2) using the Genstat V program (Lawes Agricultural Trust, 1984). Trial 2 was analysed with either extrusion exit temperature or concentration of trypsin inhibitor activity as numerical values; linear and non-linear contrasts were established through a POLYANOVA routine within GENSTAT 6 where the effects of factor are partitioned into polynomial contrasts (linear, quadratic, etc.) up to order $s$, where $s$ is a scalar containing an integer between 1 and 4 (in the current programme, 2—quadratic—was the upper limit). Variate $v$ defines a numerical value for each level of the factor. In a TREATMENTSTRUCTURE formula, the contrasts are orthogonalized. Significant differences were when $P < 0.05$; actual $P$ values were however presented (except when $P < 0.001$).

3. Results

Chemical analyses for FFSB are presented in Tables 2 and 3, respectively, for Trials 1 and 2. The principal differences were for TIA which, for both trials, showed a progressive decrease with increasing degree of cooking being degree of steam application and length of cooking during micronization in Trial 1; temperature of extrusion cooking in Trial 2, a
<table>
<thead>
<tr>
<th></th>
<th>Full S</th>
<th>Low S</th>
<th>Steam</th>
<th>Temperature</th>
<th>SED</th>
<th>S</th>
<th>T</th>
<th>S × T</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>High T</td>
<td>Low T</td>
<td>High T</td>
<td>Low T</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(FSHC)</td>
<td>(FSLC)</td>
<td>(LSHC)</td>
<td>(LSLC)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DLWG&lt;sup&gt;a&lt;/sup&gt; (g/day)</td>
<td>678</td>
<td>627</td>
<td>492</td>
<td>481</td>
<td>652</td>
<td>487</td>
<td>585</td>
<td>554</td>
</tr>
<tr>
<td>Feed intake (g DM/day)</td>
<td>812</td>
<td>771</td>
<td>678</td>
<td>680</td>
<td>791</td>
<td>679</td>
<td>745</td>
<td>725</td>
</tr>
<tr>
<td>FCR&lt;sup&gt;b&lt;/sup&gt; (g DM/g gain)</td>
<td>1.19</td>
<td>1.23</td>
<td>1.40</td>
<td>1.41</td>
<td>1.21</td>
<td>1.40</td>
<td>1.30</td>
<td>1.32</td>
</tr>
</tbody>
</table>

<sup>a</sup> DLWG, daily live weight gain; FCR, feed conversion ratio.

<sup>b</sup> Standard error of difference.
### Table 5
Effect of extrusion temperature on performance of pigs fed diets based on full fat soya beans (Trial 2)

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>DLWGa (g)</th>
<th>Feed intake (g DM/day)</th>
<th>FCRa (g DM/g gain)</th>
</tr>
</thead>
<tbody>
<tr>
<td>70</td>
<td>413</td>
<td>776</td>
<td>1.82</td>
</tr>
<tr>
<td>90</td>
<td>362</td>
<td>642</td>
<td>1.71</td>
</tr>
<tr>
<td>110</td>
<td>428</td>
<td>639</td>
<td>1.61</td>
</tr>
<tr>
<td>150</td>
<td>539</td>
<td></td>
<td>1.48</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Analysis of variance</th>
<th>(A) Using temperature as factor</th>
<th>(B) Using trypsin inhibitor level in FFSB as factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>SEDb</td>
<td>P</td>
<td>SED</td>
</tr>
<tr>
<td>48.4</td>
<td>0.012</td>
<td>94.1</td>
</tr>
<tr>
<td>0.004 (L)</td>
<td>0.280 (L)</td>
<td>&lt;0.001 (L)</td>
</tr>
<tr>
<td>0.109 (Q)</td>
<td>0.026 (Q)</td>
<td>0.560 (Q)</td>
</tr>
<tr>
<td>0.306 (Dev)</td>
<td>0.797 (Dev)</td>
<td>0.924 (Dev)</td>
</tr>
<tr>
<td>48.4</td>
<td>0.012</td>
<td>94.1</td>
</tr>
<tr>
<td>0.015 (L)</td>
<td>0.740 (L)</td>
<td>&lt;0.001 (L)</td>
</tr>
<tr>
<td>0.017 (Q)</td>
<td>0.026 (Q)</td>
<td>0.655 (Q)</td>
</tr>
<tr>
<td>0.498 (Dev)</td>
<td>0.288 (Dev)</td>
<td>0.376 (Dev)</td>
</tr>
</tbody>
</table>

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*DLWG, daily live weight gain; FCR, feed conversion ratio.

**b Standard error of difference.

A response which was non-linear (*P* < 0.001) over the whole range of temperatures employed although linear (*P* < 0.001) for 70, 90 and 110 °C.

#### 3.1. Performance—Trial 1

Growth performance data are presented in Table 4, together with the main effects and interactions. Piglets offered the diets containing the low steam treated FFSB (LSHC and LSLC) had similar growth rates, consumed similar amounts of feed offered and, therefore, had similar FCR. The higher temperatures associated with full steam (FSHC and FSLC) led to better DLWG, higher FI and improved FCR; the differences between LSHC and FSLC were significant for all measurements (*P* < 0.001). However there were no significant effects of temperature associated with cooking time (HC versus LC) nor were there any steam × cooking time interactions.

#### 3.2. Performance—Trial 2

Mean daily live weight gain (DLWG), feed intake (FI) and feed conversion ratio (FCR) of the pigs fed diets containing FFSB are presented in Table 5. Establishing linear and non-linear contrasts during the analysis of variance revealed that the effect of temperature during extrusion was significant for DLWG (*P* = 0.004, linear) and FCR (*P* < 0.001, linear), with responses improving the higher the temperature. There was a non-linear effect of treatment on FI (*P* = 0.026, quadratic) indicating that there was an initial reduction in FI followed by a subsequent increase as temperature increased.
4. Discussion

The most desirable consequence of heat treating FFSB is to inactivate anti-nutritional factors without affecting any of the heat labile amino acids, mainly expressed by the degree of available lysine. The degree of protein denaturation is affected by moisture content, temperature and time of heating. Steam or dry heat can inactivate anti-nutritional factors in full fat soya beans but control over protein solubility in either method is limited (Bastiaens, 1976). Little is known on the effect of micronization on FFSB for piglets thus making it difficult for comparison with the results reported in the current work. Hutton and Foxcroft (1975) examined in vitro micronized flaked soya bean samples under different conditions. With a dwell time varying from 25 to 95 s and temperatures ranging from 180 to 225 °C, TIA was reduced to a minimum level without loss of lysine availability, with values for TIA close to those measured in Trial 1.

Although no actual comparisons between the different techniques can be made, it is clear that FFSB with TIA levels below 5 mg/g of sample in both experiments supported higher growth rates, thus leading to the conclusion that, for piglets of this age, this level of TIA can be considered as safe, at the rate of inclusion of FFSB employed.

Although no safe limits have been yet established for pigs, FFSB that do not exceed a maximum level of 5.0 mg pure trypsin inhibited/g of substrate, have been shown to have little or no effect on digestibility (Chang et al., 1984). This conclusion was confirmed to an extent in the current programme although it should be noted that levels below this supported improved performance. Thus there does appear to be a critical stage at approximately 5 mg TIA/g when adverse responses to TIA are found as indicated by performance data in Trials 1 and 2.

Feed intake for the FFSB with the low TIA levels (Trial 1) were similar. This is in agreement with the observation made by Hansen et al. (1987), who stated that slight differences in heat treatment of soya bean meal, but with adequate degree of inactivation of trypsin inhibitor, did not greatly affect diet preference of pigs weaned at 4 weeks of age. However, although FI was reduced when TIA increased (Trial 1), there was no difference in intake between levels of 5.2 and 16.0 mg/g FFSB.

When four different solvent-extracted soya bean meals (characterized as under, normal, over heated and rumen escape) were included (273–285 g/kg) in weaner pig diets (approximately 7.0 to 19–22 kg live weight), Hansen et al. (1987) did not observe any significant difference for daily gain and feed/gain ratio; however, when heat treatment was increased, growth rate and FCR values were poorer which may have been the result of lower lysine content.

When under (5 min), intermediate (20 min) or over-processed (60 min) autoclaved soya flakes at 121 °C without, before or after ether extraction were fed to weaned pigs (Hancock et al., 1988), it was observed that utilization of nutrients was affected; growth rate was increased quadratically in relation to duration of heat treatment. In a more recent study, improved (P < 0.01) weight gains and feed gain were associated with TIA levels falling from 6.8 to 1.4 mg/g (Herkelman et al., 1992).

When considering extrusion, Seerley et al. (1974) observed that undercooking was responsible for lowering growing pig performance when FFSB were extruded at temperatures of 115 °C for 15 s, 132 °C for 18 s and 143 °C for 22 s. However, Ferrier and Lopez (1979)
stated that FFSB flour with low TIA activity, but with a good protein solubility, can be produced by partially rehydrating soya beans (moisture content up to 200–230 g/kg) to increase heat transfer and then heating them at a temperature approximately 100 °C. The extrusion process rapidly inactivates TIs but only slowly reduces the protein solubility because the proteins are heat denatured sufficiently to inactivate the active site of the trypsin inhibitor but not enough to cause extensive protein insolubilization (Ferrier and Lopez, 1979). Therefore, it seems likely that exposure time of FFSB to a temperature of approximately 100 °C is more important in TIA inactivation than exposure temperature.

Moreover, when Friesen et al. (1993) compared the effect of moist (120 °C) versus dry extrusion (160 °C) processing on soya bean meal (485 g protein/kg) fed to weaned piglets (5.4–5.8 kg), average daily gain was increased for the moist extruded soya bean meal (354 versus 313 g/day, \( P < 0.05 \)), however feed:gain and DM and nitrogen digestibilities were similar for both treatments for the period 0–28 days post-weaning. Friesen et al. (1993) also studied the effect of extrusion processing (temperatures of 120, 116, 103 and 96 °C, steam and water was also added to the extruder) or not for different soya products (soya flakes, toasted soya flour, commercial soy protein concentrate, respectively) when fed to nursery piglets (5.4–5.8 kg). Moist extrusion and soya bean product origin influenced average daily gain (433, 429, 431 versus 346 g/day, respectively) and feed:gain ratio \(( P < 0.05 \)) from day 0 to day 14 post-weaning, which compared well with the results observed in Trial 2. The same authors (Friesen et al., 1993) observed greater \(( P < 0.05 \)) DM and nitrogen digestibilities for moist extruded soya flakes, results that indicated a heat processing × protein source interaction \(( P < 0.06 \)) post-weaning.

In order to be useful, in vitro variables used to predict the nutritive value of the FFSB must correlate well with performance. Daily live weight gain (DLWG) and feed conversion ratio (FCR) values were regressed against the levels of TIA present in the FFSB for both Trials 1 and 2. There was a positive relationship for all parameters for micronized FFSB. Both GR \(( P < 0.001 \)) and FCR \(( P = 0.001 \)) were highly correlated to FFSB TIA levels. However, of much greater importance is the TIA intake rather than concentration in the diet. Thus there were significant non-linear relationships between daily TIA intake and performance characteristics (Fig. 1). It should be noted that, when considering DLWG, both extrusion and micronization generated similar responses. However, when examining FCR, extrusion tended to result in inferior performance at the same level of daily TIA intake. Thus the level of inclusion of FFSB is another factor that should always be taken into account when comparisons are made between the current results presented and previous observations. If a correlation between TIA intake and performance is established (as was the case in the current programme) then it is important to consider whether this effect was attributable to TIA intake itself or overall feed intake (as a reduction in intake would result in a general reduction in energy and nutrients). The diets were all of the same nutritional value, with the only difference being level of TIA which reduced overall feed intake. It is therefore considered that, although higher dietary levels of TIA would indirectly cause a reduction in energy and nutrient intake, it is TIA intake itself which is the more important factor. This conclusion is confirmed by the digestibility and histological assessments reported in Zarkadas and Wiseman (2005).

In conclusion, from the performance results presented, it is more accurate to assess processed FFSB in terms of daily TIA intake rather than dietary concentrations; lower daily
TIA intake resulted in high GR with daily TIA intakes of 0.55, 0.77 and 1.11 g/day. Similar observations could also be made for FCR. Therefore, it may be concluded that daily TIA intakes of less than 1.5 g/day will result in high performance. Knowledge of TIA levels and rate of inclusion of FFSB together with predicted feed intake is necessary to achieve diets of optimum quality, all other things being equal.

Acknowledgements

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References


