Effects of variability in trypsin inhibitor content of soya bean meals on true and apparent ileal digestibility of amino acids and pancreas size in broiler chicks.

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Abstract

Variability in the nutritional value of processed soya bean meal (SBM) and full fat soya bean (FFSB) currently restrict its use in diets for non-ruminant livestock. Two metabolism trials determined the amino acid digestibility and effect on pancreas size in young broilers of 8 commercially obtained products. Trypsin inhibitor (TI) values varied from 1.1 to 3.6mg/g but were all within the tolerated range of trypsin inhibitor activity (TIA). The results of the trials reported demonstrated that the coefficients of digestibility for individual amino acids varied widely between samples (e.g. coefficient of ileal apparent digestibility, CIAD, for lysine was from 0.755 to 0.884 for SBM and 0.778 to 0.848 for FFSB) but did not correlate with TIA levels, indicating other factors also affect amino acid digestibility of FFSB and SBM. Pancreas to body weight ratio (PBWR) increased with rate of soya inclusion in both the SBM (from 2.25 to 2.44g pancreas/kg body weight) and the FFSB (from 2.19 to 2.40g pancreas/kg body weight). The linear increase in pancreas size with increasing TIA intake suggested a dose dependent response that should be considered in feed formulation.

1. Introduction

As soya beans are recognised for their high nutritive value (high oil and protein contents and superior amino acid profile compared to other plant proteins employed as diet raw materials), increased amounts of both full fat soya bean (FFSB) and hexane-extracted soya bean meal (SBM) are being used in the feed compounding industry. However, the nutritional potential of SBM and FFSB for non-ruminant and immature ruminant animals is limited by the presence of anti-nutritional factors (ANFs) which interfere with the intake, digestion, absorption and metabolism of nutrients as well as the health status of the animal (Liener and Kakade, 1980; Nitsan and Nir, 1986).

The main proteinaceous ANFs present in FFSB are trypsin inhibitors (TIs). Trypsin inhibitor activity (TIA) varies with source of bean and processing conditions as TIs are heat labile. The wide variation in processing conditions of soya bean meal means that the actual amount of TI ingested by poultry will vary considerably between batches. Previously when only a small proportion of the diet consisted of soya bean products, these fluctuations were largely undetectable in terms of altered bird performance. However, the importance of plant proteins continues to increase as use of animal and fish proteins in diets becomes more controlled. As the proportion of soya bean in the diet continues to increase, the effect of variation in both ANF activity and protein quality will continue to be reflected in bird performance.

Osborne and Mendel (1917) observed that only soya beans that had been cooked could support growth in rats. Following this observation, research was extended to many other animal species (Liener, 1958). Initially it was assumed that this growth reduction was due to limited proteolysis in the gut due to trypsin inhibition. However, it was reported that there was still a growth reduction in rats when predigested proteins or free amino acids were fed together with a high antitryptic fraction prepared from soya beans (Liener and Kakade, 1980). This result indicated that the anti-nutritional effect of TI cannot only be explained by the inhibition of trypsin activity in the gut. In other studies it was shown that TI also influenced the secretion of other pancreatic enzymes (Schneeman *et al.*, 1977). When trypsin is inhibited by TI, cholecystokinin production is enhanced resulting in an increased production of pancreatic digestive enzymes leading to pancreatic hypertrophy (Hasdai *et al.*, 1989; Grant *et al.*, 1995) Hence the growth depression observed is a combined effect of endogenous loss of essential amino acids and decreased intestinal proteolysis.

The literature contains contrasting views on whether variability in processing conditions (and, therefore, TIA values) produces detectable changes in bird performance. Lee and Garlich (1992) found that the performance in terms of weight gain and feed to gain ratio of chicks fed diets containing 310g soya bean meal (SBM) /kg was unaffected by increasing the toasting time or temperature by up to 50%. Chang *et al.* (1987) also found that a range of TIA values from 1.6 to 5.3 mg/g gave no significant difference in nutrient digestibilities for pigs. Veltman *et al.* (1986) reported similar findings in broiler chick assays despite having found highly significant ($P<0.001$) differences between soya samples using *in vitro* tests. More recently, many other authors have reported negative effects of feeding FFSB and

SBM high in TIA levels to broiler chicks, for example Leeson and Atteh (1996), Zhu *et al.* (1996) and Zollitsh *et al.* (1996). The physiological demands now placed on young broilers to attain target weights may have rendered the modern broiler even more sensitive to variability in nutritional value of their diets.

It would appear from previous reports that the nutritional consequences of including FFSB and SBM with low but variable concentrations of trypsin inhibitor activity have not been examined. Accordingly, the current trial was designed to assess the ileal digestibility of amino acids (which are the components of major relevance) within both full fat and oil-extracted soya bean samples selected on the basis of TIA content, with the highest still being considered as acceptable for the young broiler chicken.

2. Materials and methods

2.1 In vivo trial

Male broiler chickens (Ross 308) were obtained at one day of age and held in wire cages (four per cage) in an environmentally-controlled metabolism room maintained at 35°C on day 1 and reduced by 1°C per day until 22°C was reached. Birds had free access to water and feed; lighting was maintained at 23h on : 1 h off. Birds were recaged into groups of 3 on day 6 and sorted into pairs matched within 10 g of live weight on day 13. A cage of two birds was a replicate. Birds were fed a standard mash broiler starter diet until 19 days old after which they were fed one of the test diets for six days.

2.2 Soya bean samples

Four different samples of FFSB and of SBM were obtained in the UK and a chemical analysis of each sample was conducted, including nitrogen content, TIA (Smith *et al*., 1981), dry matter (DM) and ether extract performed by Soxhlet extraction according to the method outlined by the Association of Official Analytical Chemists (1990) method no 920.39B. The FFSB and SBM samples were ground using a hammer mill fitted with a 3mm screen.

2.3 Diets

Twelve experimental diets were formulated for each study, using the four FFSB or SBM samples with three diets per sample. Diets were designed so that the FFSB / SBM under investigation provided the sole source of amino acids. Diets were based on 600, 400 or 200 g soya/kg, 50 g oil/kg (to reduce dust) and 50g mineral mixture /kg (designed to met the macro and micro mineral and vitamin requirements of the young broiler), 5 g titanium dioxide/kg as an inert internal marker and were made up to one kg with a 50: 50 maize starch : glucose mixture.

Experimental diets were fed to 72 pairs of birds for 6 days from 19 to 25 or 26 days of age, with each pair of birds being allocated one of the 12 test diets, giving 6 replicates per diet. To spread the work load, the trial was carried out in two halves with one half (three replicates) being slaughtered at 25 days of age and the second half at 26 days of age. Birds were slaughtered using carbon dioxide asphyxiation and death was confirmed by cervical dislocation. They were then quickly dissected to reveal the lower gastrointestinal tract between Meckel's diverticulum and the ileo-caecal-colonic junction. After rapid removal of this section, digesta were squeezed, using gentle digital pressure, into a collection vessel. Samples from both birds in a cage were pooled in order to provide enough material for analysis. The weight of each bird was then recorded prior to excision of the pancreas. All connective tissue was removed from the pancreas and its weight recorded. The protocol of the trial was within official guidelines for experimentation on birds.

2.4 Digesta treatment

Samples of ileal digesta obtained from the birds were deep-frozen (-20ºC) immediately after removal and later freeze dried before being ground using a pestle and mortar. The lower ileum was used as it was thought that all possible dietary protein digestion and absorption would be complete before digesta reached this portion of the intestine and thus only indigestible and endogenous proteins and amino acids would be present. The samples were then analysed for amino acid and titanium concentrations.

2.5 Amino acid analysis

Amino acid concentrations in FFSB / SBM samples and ileal digesta were determined using samples (0.2g) which had been oxidised using performic acid to allow measurement of cysteine and methionine prior to hydrolysation in 6 M HCl for 18 hours at 110°C. Samples were neutralised using sodium hydroxide and 2ml of L-norleucine (0.025M) added as an internal standard. 50ml of sodium citrate buffer were added and the pH was adjusted to 2.2 before making up the volume to 100ml. 40μl aliquots of these samples were assayed on a cation exchange column (Pharmacia Biochrom Amino Acid Analyser). The samples were eluted using sodium citrate buffers and eluted amino acids were detected by a ninhydrin colour reaction at 570 nm.

2.6 Titanium dioxide analysis

Titanium dioxide was analysed using a modified version of the AOAC method (Short *et al*., 1996). Samples (0.1 g) were ashed at 580°C for 13 h prior to dissolving in 7.4M H_2SO_4. The solutions were diluted with water and H_2O_2 (30%v/v). The absorbance was measured at 410 nm on a spectrophotometer.

2.7 Trypsin inhibitor acitivity

The trypsin inhibitor activities of the soya bean samples were measured using the method of Smith et al. (1980). Trypsin inhibitor was extracted overnight from the soya bean using 0.01M sodium hydroxide before measuring the degree of inhibition of trypsin acting on a chromogenic amino acid substrate using a UV spectrophotometer.

2.8 Protein dispersibility index

The protein dispersibility index of the soya bean samples was measured using the AOCS official method Ba 10-65. 20 g of ground sample was placed in a variable speed blender with 300 ml of distilled water and mixed for 10 minutes at 7800 rpm. An aliquot of the resultant slurry was centrifuged and the nitrogen content of supernatant measured using a nitrogen elemental analyser (Fison, UK). The nitrogen content of a dry sample of the soya bean was compared to the nitrogen content of the slurry sample and the proportion of total nitrogen content dispersible in water was calculated.

2.9 Calculations

The titanium dioxide and amino acid concentrations were used to calculate the apparent amino acid digestibility content using the following equation:

$$1-(aa_{dig} * marker_{feed}) / (aa_{feed} * marker_{dig})$$

where:
aa_{dig} = amino acid concentration in the digesta
$marker_{feed}$ = titanium concentration in the diet
aa_{feed} = amino acid concentration in the diet
$marker_{dig}$ = titanium concentration in the digesta.

The determined apparent digestible amino acid (ADAA) content of the diets was regressed against the rate of inclusion of soya for each amino acid studied. The linear regression thus derived was extrapolated to a rate of inclusion of 1000g soya /kg (i.e. a diet based only on soya bean). This gave the ADAA content (for a specific amino acid) within the soya bean sample under investigation. Dividing this figure by the total content of the specific amino acid in the soya sample under investigation gave the coefficient of ileal apparent digestibility (CIAD) of the amino acid.

The y intercept of the linear regression described above should in principle be negative. Soya bean intake at this point is zero and therefore the ADAA value at the y intercept represents endogenous losses. When this value is deducted from the ADAA content determined at 1000g soya /kg the true digestible amino acid content (TDAA) of the soya is determined.

The method developed by Short *et al*. (1996) does not allow for alterations in endogenous losses due to changes in ileal amino acid flow but the three inclusion levels used allow a greater degree of accuracy in estimating contents of digestible amino acids than methods where a single inclusion level is used. However, it must be remembered that small errors in the slope of the line plotted from the digestible amino acid content of the three inclusion levels of diets will result in some degree of error at both the y-intercept (endogenous losses) and at the extrapolated value for digestible amino acid content of 1000g soya/kg diet.

2.10 Statistical analysis

Data were analysed as a 3 * 4 factorial design using Genstat version 5 for windows, release 4.1 to examine the effects of TIA and rate of inclusion (ROI) on digestibility of amino acids and establishing linear and non-linear contrasts for ROI and TIA level in FFSB via linear (L) and quadratic (Q) partitioning of the treatment sum of squares.

3. Results

3.1 Chemical analysis

The results of the chemical analyses performed on the FFSB samples are given in table 1 and for SBM samples in table 2. The TIA values for the FFSB samples divide the samples into two groups. Samples A and C both had higher TIA values and correspondingly higher PDI values than samples B and D. Raw beans were available from samples A and D and TIA analysis gave values of 21.7 and 17.7 mg/g DM respectively.

The higher nitrogen content of the SBM samples compared to the FFSB is a result of removing the oil from the former samples and therefore increasing the relative concentration of nitrogen. The three SBM samples processed in the UK (sample W, Y and Z) were considerably higher in TIA content than sample X, processed in South America.

3.2 Total amino acid content

The total amino acid content of the SBM samples showed samples Y and Z to have similar content of the amino acids reported. Sample W had the lowest content of all amino acids apart from methionine, where sample X showed slightly lower levels.

The lowest concentrations of amino acids present in the FFSB samples were seen in methionine and cysteine with levels of 4.48 to 5.24g/kg FFSB DM for methionine and 4.82 to 5.36g/kg for cysteine; lysine was the amino acid present in the highest concentration (20.66 to 21.86 g Lys/kg FFSB). For some amino acids, particularly glycine, there was a wide range of total amino acid content.

Table 1 near here

Table 2 near here

3.3 Concentration of apparently digestible amino acids

The apparently digestible amino acid contents of each diet (g/kg DM diet) for the five representative amino acids selected (4 were nutritionally essential: lysine, methionine, threonine and cysteine and 1 was not: glycine) are shown in tables 3 and 4 for FFSB and SBM respectively. The amino acids present in lowest digestible concentrations were methionine and cysteine, in both the FFSB and SBM samples. Lysine was the amino acid found in highest digestible concentrations. Concentration of apparently digestible lysine varied considerably between both FFSB and SBM samples and was lower in the FFSB than the SBM.

Table 3 near here

Table 4 near here

Both the SBM and FFSB samples showed a significant linear response to increasing ROI. Analysis of variance on the effect of ROI of the FFSB samples (table 5) and the SBM samples (table 6) on digestible amino acid concentration gave $P<0.001$ for all amino acids. This allowed extrapolation to a theoretical figure for the concentration of digestible amino acid in pure SBM or FFSB.

Table 5 near here

Table 6 near here

The contents of apparently digestible amino acids in each FFSB or SBM sample were calculated by extrapolation of the diet values to 1000g soya /kg diet and are presented in tables 7 and 8. Sample X showed a consistently lower digestible amino acid content than the other SBM samples. Data for each of the amino acids reported ranked samples W, Y and Z in a consistent order where Z contained the highest concentration of digestible amino acids, followed by Y.

Apparently digestible amino acid content was more variable within the FFSB samples. Sample A had the highest digestible lysine, threonine and methionine contents but sample D showed higher cysteine and glycine digestibility. No significant differences between true and apparently digestible amino acid content were found for either the FFSB or SBM samples.

3.4 Coefficient of ileal apparent amino acid digestibility (CIAD)

The CIAD for all amino acids studied in FFSB was highest in sample A whilst sample C consistently showed the lowest digestibility coefficients.

Table 7 near here

Table 8 near here

Sample A had a consistently higher CIAD for all amino acids considered. Conversely, sample C consistently showed a consistently values. CIAD for lysine ranged from 0.778 (sample C) to 0.848 (sample A). Sample X consistently showed the lowest coefficient of amino acid digestibility among the SBM samples.

3.5 Pancreatic enlargement

Pancreas to body weight ratio (table 9) was affected by FFSB (P = 0.028) and also by increasing ROI (P = 0.014); the response to ROI was linear (P=0.004) but no non-linear responses or ROI * FFSB interactions were recorded. Chicks fed diets containing samples A and C (the higher TIA samples) showed greater pancreatic enlargement than chicks fed diets containing samples B and D.

Conversely, variation in SBM fed to chicks did not affect pancreas to body weight ratio (table 10). However, once again, increasing ROI of SBM significantly (P = 0.018) linearly increased pancreas to body weight ratio linearly; there was no non-linear response recorded. There was a significant ROI * SBM meal interaction (P=0.052, linear) The grand mean of both FFSB and SBM was 2.32 g pancreas /kg BW but the variation between samples was greater in FFSB than SBM.

Table 9 near here

Table 10 near here

4. Discussion

The data reported demonstrate that young broilers are sensitive to variation in quality of both FFSB and SBM even when samples are processed to below the recommended TIA threshold of 4 mg/g. However, whilst a highly significant difference was observed between digestible amino acid contents for diets based on the four FFSB samples, the content did not correlate with TIA level. Samples A and C had very similar TIA values (3.6 and 3.4 mg/g DM respectively) but widely differing digestible amino acid content. Sample A consistently showed the highest digestible amino acid content and sample C showed the lowest. Data expressed as the CIAD allow comparison of amino acid digestibility without influence of the total volume of amino acid present in the sample. The content of apparently ileal digestible amino acids in FFSB and SBM is a reflection of both the total amount and the CIAD; the latter term is a more valid base on which to draw comparisons between samples in terms of relationships with TIA concentration. However, the same trends are seen in the coefficient data; sample C shows the lowest values for all amino acids and sample A the highest. This suggests that a factor other than TIA content of the FFSB sample is affecting amino acid digestibility.

The data for the SBM samples also showed wide variation in apparent digestible amino acid content and CIAD. The low nitrogen content of sample W was reflected in its low total amino acid content. The differences between ranking the samples by total amino acid content or apparent digestible amino acid content demonstrates the importance of digestibility in determining nutritional value of a product.

Leeson and Atteh (1996) studied the response of broiler chicks to dietary FFSB and found that the reduction in TIA caused a trend toward an increase in N retention and little or no effect on fat, calcium or phosphorus retention and diet metabolisable energy (ME); it was therefore concluded that, while TIA was reduced with extrusion, factors other than TIA may still play important roles in the observed results. Kakade *et al.* (1972) reported that trypsin inhibitor accounted for only 40% of the growth depression and pancreatic hypertrophy observed with raw soya beans.

In contrast to the current experiment, Lee and Garlich (1992) compared four samples of soya bean meal and found that amino acid content and amino acid "availability" differed little or not at all between the four samples in the study. However, they collected excreta samples for amino acid analysis rather than ileal samples which may account for differences in findings. The influence of hindgut microflora on protein digestibility / modifications of amino acids in chickens is not as clearly established as for the pig (Sibbald, 1987). Although it is assumed by some that the influence of the avian hindgut is insignificant (McNab, 1995), a comparison of ileal and excreta digestibilities by Ravindran *et al.* (1999) demonstrated that amino acid metabolism by hindgut microflora in chickens may be substantial and that determination of amino acid digestibility by excreta analysis may not be a valid procedure for all diet ingredients.

In contrast to samples A-C, FFSB sample D was produced by jet sploding rather than toasting so it is possible that this method is able to reduce TIA levels to as low as 1.1mg/g DM without over-processing whereas the conditions during toasting may be more favourable for the Maillard browning reaction. However, Lee and Garlich

(1992) studied the effects of increasing toasting temperature and found no differences in lysine availability and no change in the glucose or soluble oligosaccharide content which suggests that no progressive Maillard reaction occurred with increasing heat treatment used in the study.

A measurement specifically directed towards estimating the sensitivity of young broiler chicks to TI content of FFSB is pancreas to body weight ratio (Chernick *et al*., 1948; Singh *et al*., 1964). A significantly larger pancreas to body weight ratio was observed in birds fed diets containing either of the FFSB samples with the higher TIA values. This indicated that broiler chicks were affected by TIA content of FFSB even when it was below the previously suggested threshold level of 4 mg/g (Smith *et al*., 1980; Chang *et al.,* 1987). The SBM diets gave less variation in pancreas size between samples but the highly significant, linear increase in pancreatic enlargement with increasing rate of inclusion of FFSB or SBM in the diet indicates a dose dependent response to increasing TIA levels.

The dose dependent response of pancreatic enlargement to TI intake observed suggests that intake of SBM or FFSB (and therefore TI intake) should be an important consideration in diet formulation. The results of the current study suggest further work is required to determine that exact TIA intake above which degree of pancreatic enlargement becomes unacceptable. This information, combined with knowing the TIA value of the FFSB or SBM would allow the feed compounder to adjust the amount of soya included in the diet to avoid pancreatic enlargement. For example, a FFSB sample with a very low TIA level could be used at a higher inclusion rate without causing as much pancreatic enlargement as small amounts of FFSB with a high TIA level.

The overall conclusions to be drawn from the current study are that both FFSB and SBM samples with variable levels of TIA below the currently accepted threshold of 4mg/g will have variable nutritional values in terms of apparent ileal digestibility. However, there was no correlation between TIA value and apparent ileal amino acid digestibility, suggesting factors other than TIA were also affecting amino acid digestibility. Conversely, pancreatic enlargement showed a dose dependent effect suggesting nutritional consequences should be viewed in terms of overall TI intake rather than TI concentrations

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Table 1 Chemical analysis of FFSB samples

Sample	Dry Matter (g/kg)	Ether extract (g/kg DM)	Nitrogen content (g/kg DM)	PDI (%)	TIA (mg/g DM)
A	864	218	69.1	29.5	3.4±0.02
B	876	224	64.9	15.3	1.7±0.01
C	891	219	66.2	29.9	3.6±0.05
D	883	236	64.3	13.6	1.1±0.02

Table 2 Chemical analysis of SBM samples

Sample	Dry Matter (g/kg)	Ether extract (g/kg DM)	Nitrogen content (g/kg DM)	PDI (%)	TIA (mg/g DM)
W	884	26	80.0	24.3	2.3
X	879	26	84.3	14.5	1.7
Y	893	24	88.8	23.1	2.8
Z	883	26	87.4	26.4	2.9

Table 3 Concentration of apparently digestible amino acids (AA) in diets using four FFSB samples with varying trypsin inhibitor activities (TIA, mg/g DM) at three inclusion levels (g/kg).

	200				Inclusion	400				
FFSB	A	B	C	D		A	B	C	D	
TIA	3.4	1.7	3.6	1.1	Mean	3.4	1.7	3.6	1.1	Mean
AA										
Lys	3.56	3.52	3.68	3.91	3.67	7.10	6.66	7.69	7.63	7.27
Met	0.88	0.82	0.78	0.96	0.86	1.77	1.49	1.60	1.89	1.69
Cys	0.61	0.70	0.64	0.81	0.69	1.36	1.21	1.55	1.66	1.45
Thr	1.88	1.88	1.79	2.10	1.91	4.07	3.45	4.22	4.36	4.02
Gly	1.83	1.89	1.82	2.24	1.95	3.77	3.48	4.06	4.45	3.94

	600				Inclusion	Mean				
FFSB	A	B	C	D		A	B	C	D	
TIA	3.4	1.7	3.6	1.1	Mean	3.4	1.7	3.6	1.1	Mean
AA										
Lys	10.71	10.11	10.19	10.86	10.46	7.12	6.76	7.19	7.47	7.13
Met	2.70	2.28	2.14	2.70	2.46	1.79	1.53	1.51	1.85	1.67
Cys	2.02	2.22	1.91	2.24	2.10	1.33	1.38	1.37	1.57	1.33
Thr	6.02	5.71	5.42	5.89	5.76	3.99	3.68	3.81	4.12	3.90
Gly	5.76	5.53	5.07	6.12	5.62	3.79	3.63	3.65	4.27	3.83

Table 4 Concentration of apparently digestible amino acids (AA) in diets using four SBM with varying trypsin inhibitor activities (TIA, mg/g DM) at three inclusion levels (g/kg).

	200				Inclusion	400				
SBM	W	X	Y	Z		W	X	Y	Z	
TIA	2.3	1.7	2.8	2.9	Mean	2.3	1.7	2.8	2.9	Mean
AA										
Lys	3.20	3.74	4.28	4.31	3.88	7.46	7.10	8.48	8.27	7.83
Met	1.01	0.98	1.11	1.09	1.05	2.02	1.83	2.22	2.14	2.05
Cys	0.74	0.69	0.94	0.80	0.79	1.77	1.41	1.85	1.61	1.66
Thr	2.45	2.52	2.75	2.73	2.61	4.76	4.77	5.58	5.34	5.11
Gly	1.73	1.81	2.12	2.10	1.94	3.32	3.25	4.27	4.06	3.73

	600				Inclusion	Mean				
SBM	W	X	Y	Z		W	X	Y	Z	
TIA	2.3	1.7	2.8	2.9	Mean	2.3	1.7	2.8	2.9	Mean
AA										
Lys	11.24	10.18	11.82	12.23	11.37	7.30	7.00	8.19	8.27	7.69
Met	2.98	2.77	3.14	3.21	3.02	2.00	1.86	2.15	2.15	2.04
Cys	2.40	1.88	2.92	2.78	2.49	1.64	1.33	1.90	1.73	1.65
Thr	7.53	7.22	8.04	8.24	7.76	4.91	4.83	5.46	5.44	5.16
Gly	5.16	4.88	5.91	6.09	5.51	3.40	3.31	4.10	4.08	3.72

Table 5 Analysis of variance of content of apparent ileal digestible amino acids of 4 FFSB samples

Amino acid	Analysis of Variance		
	Factor	SEd	P
LYS	FFSB	0.130	<0.001
	ROI	0.113	<0.001
			<0.001(L)
			0.043 (Q)
	FFSB*ROI	0.226	0.014
			0.167 (L)
			0.010 (Q)
MET	FFSB	0.027	<0.001
	ROI	0.023	<0.001
			<0.001(L)
			0.142(Q)
	FFSB*ROI	0.046	<0.001
			<0.001 (L)
			0.005 (Q)
CYS	FFSB	0.057	<0.001
	ROI	0.050	<0.001
			<0.001(L)
			0.225(Q)
	FFSB*ROI	0.100	0.004
			0.372(L)
			<0.001(Q)
THR	FFSB	0.144	0.020
	ROI	0.125	<0.001
			<0.001(L)
			0.089(Q)
	FFSB*ROI	0.249	0.060
			0.535 (L)
			0.019 (Q)
GLY	FFSB	0.117	<0.001
	ROI	0.101	<0.001
			<0.001(L)
			0.081(Q)
	FFSB*ROI	0.202	0.008
			0.086 (L)
			0.008 (Q)

Table 6 Analysis of variance of content of apparent ileal digestibile amino acids of 4 SBM samples

Amino acid	Analysis of Variance		
	Factor	SEd	P
LYS	SBM	0.192	<0.001
	ROI	0.166	<0.001
			<0.001(L)
			0.165 (Q)
	SBM*ROI	0.332	0.027
			0.005 (L)
			0.758 (Q)
MET	SBM	0.027	<0.001
	ROI	0.024	<0.001
			<0.001(L)
			0.370(Q)
	SBM*ROI	0.047	<0.001
			<0.001 (L)
			0.118 (Q)
CYS	SBM	0.102	<0.001
	ROI	0.088	<0.001
			<0.001(L)
			0.840(Q)
	SBM*ROI	0.177	0.017
			0.008(L)
			0.271(Q)
THR	SBM	0.102	<0.001
	ROI	0.088	<0.001
			<0.001(L)
			0.343(Q)
	SBM*ROI	0.176	0.028
			0.015 (L)
			0.249 (Q)
GLY	SBM	0.097	<0.001
	ROI	0.084	<0.001
			<0.001(L)
			0.978(Q)
	SBM*ROI	0.169	0.005
			0.002 (L)
			0.241 (Q)

Table 7 Calculated apparently digestible amino acid (AA) concentration, total AA contents by chemical analysis and calculation of coefficient of ileal apparent digestibility (CIAD) for five amino acids in four FFSB samples.

AA	FFSB	TIA (mg/gDM)	Apparently Digestible AA (g/kg soya)	Total AA (g/kg)	CIAD	S.E.
LYS	A	3.4	17.84	21.05	0.848	(±0.021)
	B	1.7	16.60	20.66	0.804	(±0.017)
	C	3.6	17.00	21.86	0.778	(±0.022)
	D	1.1	17.32	21.10	0.847	(±0.019)
THR	A	3.4	10.20	13.84	0.737	(±0.024)
	B	1.7	9.33	13.25	0.704	(±0.037)
	C	3.6	9.29	13.59	0.684	(±0.033)
	D	1.1	9.80	13.96	0.702	(±0.041)
MET	A	3.4	4.52	5.09	0.887	(±0.016)
	B	1.7	3.71	4.58	0.809	(±0.024)
	C	3.6	3.55	4.48	0.793	(±0.021)
	D	1.1	4.47	5.24	0.853	(±0.014)
CYS	A	3.4	3.44	4.82	0.714	(±0.030)
	B	1.7	3.58	5.08	0.706	(±0.054)
	C	3.6	3.29	5.14	0.639	(±0.040)
	D	1.1	3.72	5.36	0.693	(±0.031)
GLY	A	3.4	9.67	12.88	0.751	(±0.025)
	B	1.7	9.03	12.88	0.701	(±0.032)
	C	3.6	8.56	13.27	0.645	(±0.033)
	D	1.1	10.09	14.12	0.715	(±0.025)

Comment [DJW1]: Do you want to include true as well as apparent, and then comment on differences? You do refer to this in the paper.

Table 8 Calculated apparently digestible amino acid (AA) concentration, total AA contents by chemical analysis and calculation of coefficient of ileal apparent digestibility (CIAD) for five amino acids in four SBM samples.

AA	SBM	TIA (mg/gDM)	Apparently Digestible AA (g/kg soya)	Total AA (g/kg)	CIAD	S.E.
LYS	W	2.3	19.38	21.92	0.884	(±0.041)
	X	1.7	16.65	22.04	0.755	(±0.014)
	Y	2.8	19.54	24.70	0.791	(±0.014)
	Z	2.9	20.15	24.57	0.820	(±0.015)
THR	W	2.3	12.53	15.96	0.785	(±0.016)
	X	1.7	11.90	16.51	0.721	(±0.016)
	Y	2.8	13.41	18.16	0.738	(±0.014)
	Z	2.9	13.69	17.95	0.763	(±0.021)
MET	W	2.3	4.96	5.56	0.893	(±0.018)
	X	1.7	4.56	5.41	0.842	(±0.010)
	Y	2.8	5.22	6.21	0.840	(±0.015)
	Z	2.9	5.31	6.10	0.871	(±0.019)
CYS	W	2.3	4.17	5.25	0.749	(±0.077)
	X	1.7	3.08	5.32	0.580	(±0.041)
	Y	2.8	4.86	6.48	0.750	(±0.045)
	Z	2.9	4.65	5.96	0.781	(±0.048)
GLY	W	2.3	8.54	12.05	0.710	(±0.022)
	X	1.7	7.93	13.08	0.595	(±0.020)
	Y	2.8	9.80	14.54	0.661	(±0.018)
	Z	2.9	10.07	14.37	0.696	(±0.024)

Table 9 Effect of FFSB and ROI on pancreas to body weight ratio (PBWR)(g/kg) in broiler chicks

PBWR (g/kg)		Rate Of Inclusion (g/kg)				Analysis of Variance		
		200	400	600	Mean	Factor	SEd	P
FFSB	A	2.34	2.39	2.62	2.45	FFSB	0.095	0.028
	B	2.14	2.26	2.29	2.23	ROI	0.083	0.014 0.004 (L) 0.897(Q)
	C	2.17	2.45	2.56	2.39			
	D	2.09	2.20	2.30	2.19	FFSB*ROI	0.165	0.910 0.752(L) 0.838 (Q)
Mean		2.19	2.32	2.44	2.32			

Table 10 Effect of SBM and ROI on pancreas to body weight ratio (PBWR)(g/kg) in broiler chicks

PBWR (g/kg)		Rate Of Inclusion (g/kg)				Analysis of Variance		
		200	400	600	Mean	Factor	SEd	P
SBM	W	2.23	2.25	2.40	2.30	SBM	0.072	0.713
	X	2.10	2.36	2.54	2.34	ROI	0.062	0.052 0.018 (L) 0.554(Q)
	Y	2.32	2.21	2.27	2.28			
	Z	2.32	2.35	2.40	2.36	SBM*ROI	0.124	0.185 0.052(L) 0.808 (Q)
Mean		2.25	2.30	2.40	2.32			