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Impact of different processing of full-fat soybeans on broiler performance

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ABSTRACT: The response of broilers to various conditions of expander processing of full-fat soybeans (FFSB) was studied in an experiment involving 5040 birds allocated to 10 dietary treatments with six replicates. In treatments 1–8, the proportions of FFSB in starter, grower, and finisher diets were 40, 39–43, and 34–37%, respectively. Diets for treatments 9 and 10 contained commercial soybean meal (SBM) as a main protein source. All diets were isocaloric and were formulated to contain standardized ileally digestible amino acids at levels corresponding to 95% (treatments 1–7 and 10) or 100% (treatments 8 and 9) of requirement. In treatment 1, raw FFSB was used while diets for treatments 2–8 contained FFSB processed by various combinations of times and temperatures used during conditioning and expanding. Urease activity, protein dispersibility index, and trypsin inhibitor activity of FFSB decreased as processing time and/or temperature increased while protein solubility remained relatively unchanged. Concurrently, growth performance of chickens improved ($P < 0.05$) and their relative pancreas weight decreased ($P < 0.05$). The best results in terms of feed intake, growth rate, feed conversion ratio, and carcass quality of FFSB-fed birds were obtained using expanding for 15 s at 125°C preceded by short-term (1 min, 100°C) and long-term (5 min, 100°C) conditioning. These results were not significantly different from those achieved with SBM-based diets ($P > 0.05$). Numerically better performance was found in 100% amino acid diets as compared with 95% diets ($P > 0.05$). Switching the raw FFSB diet to SBM diet caused relative pancreas weight decrease by 31% during 3 days. An opposite switch resulted in a 65% increase in relative pancreas weight during 4 days.

Keywords: soybean treatment; expanding; pancreatic hypertrophy; heat treatment

INTRODUCTION

In the last years, the feed industry showed a growing interest in the use of full-fat soybeans (FFSB) and, as a result, its large-scale processing became a common practice. Due to their high oil content, FFSB are particularly suitable for manufacturing high-energy poultry diets, as post-pellet application of fat may be reduced or eliminated (Waldroup 1982). However, the nutritional potential of FFSB is limited by the presence of antinutritional factors, mainly trypsin inhibitors, which interfere with digestion, absorption, and metabolism of nutrients (Liener and Kakade 1980). Trypsin inhibitors form complexes with pancreatic proteases, thus reducing their acitivity in the upper small intestine. In an attempt to offset the lack of enzymes, hypersecretion by the exocrine pancreas is evoked followed by pancreatic hypertrophy and hyperplasia (Grant et al. 1995). As a result of the combined effect of endogenous loss of essential amino acids (particularly sulphur amino acids) and decreased intestinal proteolysis, growth performance of chickens is reduced.

Thus, sufficient inactivation of trypsin inhibitors is crucial for optimal broiler performance. However, over-processing must be avoided to prevent reduction of the nutritional value of the product. Excessive heat treatment may result in decreased amino acid availability due to the early Maillard reaction. Lysine is particularly susceptible because of its exposed ε-amino group which readily reacts with reducing sugars. During the advanced phase of Maillard reaction, cross-linking may occur be-

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tween most amino acids and polypeptide chains, thus reducing the efficiency of intestinal proteases. Part of amino acids may be totally destroyed as a result of the advanced Maillard reaction. Thus, an optimal balance between under- and over-processing must be found to ensure an efficient utilization of soyabean protein. In this experiment, an attempt was made to find such optimum and to investigate which amount of trypsin inhibitors was tolerated by growing broilers without affecting their performance. The aim of the present experiment was to investigate the effect of different heat treatments imposed on full-fat soybeans on the performance and organ weights in broilers.

MATERIAL AND METHODS

Processing of soybeans. One batch of raw soybeans was purchased from Rieder Asamhof GmbH & Co. KG (Kissing, Germany). The raw soybeans were further processed at the facilities of Amandus Kahl GmbH & Co. KG (Reinbeck, Germany). The following equipment was used for the processing of the soybeans: a short-term (60 s) conditioner DLM I, a long-term conditioner LK 1605-2, and an expander OEE 15.2 (all Amandus Kahl GmbH & Co. KG). Various combinations of short-term conditioning at 80 or 100°C, long term conditioning at 100°C for 5 or 15 min, and expanding at 115 or 125°C were applied to manufacture six FFSB products used in experimental diets (Table 1). The expander had a throughput of 1.5–1.8 t/h. After short-term conditioning at 80°C, a specific energy input of 30.0 and 39.2 kWh/t was needed to reach expansion temperatures of 115 and 125°C, respectively. After short-term conditioning at 100°C and long-term conditioning at 100°C for 5 and 15 min, a specific energy input of 13.5 and 20.0 kWh/t was needed to reach expansion temperatures of 115 and 125°C, respectively.

All FFSB products were dried in a belt dryer with 85°C air and left the dryer after 5 min with a temperature of 43°C. Then they entered the belt cooler and left it after 5 min with a temperature of 30°C.

Animals and procedures. The experiment was conducted at the International Poultry Testing Station Ústrašice, Czech Republic. The animal procedures were reviewed and approved by the Animal Care Committee of the Mendel University in Brno. A total of 5040 one-day-old male Ross 308 broiler chicks were allocated randomly to 10 dietary treatments using a randomized complete block design with six replicates per treatment. There were 70 chicks per replicate pen except for treatments 1 and 10, in which 140 chicks per pen were used. Stocking density was 17 broilers per square meter. The chickens were kept in a windowless house with full climatic control, on deep litter from wood shavings. Each pen was equipped with manually filled tube feeders and nipple drinkers. Heating and lighting programmes were in accordance with Ross Broiler Management Manual (2009). On day 1, the birds in each pen were weighed together while on days 10, 24, and 35, all birds were weighed individually. At the same time, feed consumption per pen was recorded. On day 35, when the first part of the experiment terminated, five birds of each pen having body weights closest to the pen mean were selected, tagged, and after 12 h of fasting they were weighed, sacrificed and carcass, breast meat (boneless and without skin), and pancreas weights were recorded. The experiment continued with the chickens remaining on treatments 1 and 10 for another 6 days. From day 36 onwards, the chickens on treatment 1 received the diet previously fed to birds on treatment 10 while the birds on treatment 10 were switched to treatment 1. During this period, the recovery from raw soybeans (treatment 1) and the adaptation to raw soybeans (treatment 10) was studied. Five birds from each pen were sacrificed each day and slaughter analysis was performed as described above.

Diets. Starter (days 1–10), grower (days 11–24), and finisher (days 25–35) diets were formulated to contain FFSB or soybean meal (SBM) as main protein sources. Except for amino acids, the diets, isocaloric for each period, were formulated to be in line with the Ross Nutrition Supplement (2009). To enable a sensitive detection of changes in soybean protein quality, the diets for treatments 1–7 and 10 were calculated to contain standardized ileally digestible amino acids at levels corresponding to 95% of the recommended requirement. The diet containing raw FFSB served as a negative control (treatment 1). Various combinations of processing conditions were applied to manufacture six further FFSB products used in diets of the same composition as the control diet (treatments 2–7). Treatment 8 was the same as treatment 7 except that the amino acid level was increased to 100% of the requirement. Diets for treatments 9 and 10 were positive controls using commercial SBM as the main protein source and containing ileally

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Table 1. Processing conditions and treatment characteristics

Treatment	Soybean source	Conditioning			Expanding (15 s) (°C)	Amino acid level[1] (%)
		short-term (60 s) (°C)	long-term			
			min	(°C)		
T1	FFSB	–	–	–	–	95
T2	FFSB	80	–	–	115	95
T3	FFSB	100	5	100	115	95
T4	FFSB	100	15	100	115	95
T5	FFSB	80	–	–	125	95
T6	FFSB	100	5	100	125	95
T7	FFSB	100	15	100	125	95
T8	FFSB	100	15	100	125	100
T9	SBM	–	–	–	–	100
T10	SBM	–	–	–	–	95

FFSB = full-fat soybeans, SBM = soybean meal

[1] as compared with ileally digestible amino acid requirements given by Ross Nutrition Supplement (2009)

digestible amino acids at levels of 100 and 95%, respectively. Processing conditions and diet characteristics are summarized in Table 1. Ingredient composition and nutrient contents of diets are given in Tables 2 and 3. The diets in crumbled form (starter phase) or pellets (grower and finisher phase) were supplied *ad libitum*.

Chemical analyses. The raw and processed FFSB as well as commercial SBM were analyzed for urease activity (UA) according to ISO 5506:1988 (Soya bean products – Determination of urease activity), protein solubility in a 0.2% potassium-hydroxide solution (PS) as described by Araba and Dale (1990a), protein dispersibility index (PDI) using the AOCS Official Method Ba 10-65 proposed by the American Oil Chemists' Society (AOCS 1996), and trypsin inhibitor activity (TIA) according to ISO 14902:2001 (Animal feeding stuffs – Determination of trypsin inhibitor activity of soya products). Reactive lysine was determined in raw and FFSB samples using homoarginine reaction as described by Fontaine et al. (2007). The diets were analyzed for nitrogen using Dumas procedure and for amino acids by ion-exchange chromatography (Llames and Fontaine 1994).

Statistical analysis. Experimental data were analyzed as a completely randomized block design using ANOVA procedure of Statgraphics Plus package (Version 3.1., 1994). When a significant value for treatment effect ($P < 0.05$) was observed, the differences between means were assessed by Tukey's HSD test. The experimental unit was a replicate pen. Linear, quadratic or rectilinear (Robbins et al. 2006) models were fitted to experimental data to describe the response of chickens to TIA values found in FFSB and SBM samples. Only treatments with 95% amino acid levels were included into this analysis. Pearson's correlation coefficients between *in vitro* indicators of soybean treatment as well as between *in vitro* indicators and *in vivo* performance characteristics were calculated. In the change-over part of the experiment, relative pancreas weight was related to time after the diet change.

RESULTS AND DISCUSSION

Growth experiment. Except for PS, all other *in vitro* indicators responded strongly to changes in FFSB treatment (Table 4). Urease activity fell almost to zero in treatment 7 while PDI and TIA were reduced to 20% of their initial values (Figure 1). The lowest TIA was slightly above the recommended threshold level of about 4.0 mg/g (Leeson et al. 1987; Loeffler et al. 2012). The increase of the expanding temperature from 115 to 125°C (T2 vs T5) resulted in a reduction of PDI and TIA values by 57 and 70%, respectively. The PS gradually decreased from 94 to 89%. Although it has been suggested that this assay may be useful in assessing protein quality in under-processed samples (Araba and Dale 1990b), it is commonly used as an indicator of soybean over-processing (Parsons et al. 1991; Anderson-Haferman et al.

Table 2. Composition of diets (%, as-fed basis)

Diet	Starter (days 0–10)				Grower (days 11–24)				Finisher (days 25–35)			
Treatment	T1–7	T8	T9	T10	T1–7	T8	T9	T10	T1–7	T8	T9	T10
Amino acid level (%)	95	100	100	95	95	100	100	95	95	100	100	95
FFSB	40.00	40.00	–	–	38.92	42.57	–	–	33.66	36.8	–	–
SBM	5.61	8.07	37.18	34.55	–	–	31.12	28.45	–	–	26.90	24.61
Maize	20.00	20.00	20.00	20.00	30.00	30.00	30.00	30.00	35.00	35.00	35.00	35.00
Wheat	29.30	27.36	33.50	36.45	27.09	23.62	29.32	32.31	26.80	23.80	28.74	31.29
Soybean oil	–	–	5.07	4.77	0.37	0.19	5.83	5.53	1.19	1.04	5.91	5.65
Dicalcium phosphate	1.92	1.92	1.93	1.93	1.70	1.69	1.71	1.71	1.56	1.56	1.58	1.58
Calcium carbonate	1.91	1.35	0.97	0.99	0.75	0.73	0.78	0.80	0.75	0.74	0.78	0.79
Sodium chloride	0.31	0.31	0.37	0.37	0.31	0.31	0.37	0.37	0.32	0.32	0.37	0.37
DL-Methionine	0.27	0.29	0.28	0.25	0.23	0.25	0.24	0.21	0.19	0.21	0.20	0.18
Biolys®[1]	0.27	0.27	0.29	0.29	0.24	0.24	0.25	0.25	0.19	0.18	0.19	0.20
L-Threonine	0.06	0.07	0.06	0.06	0.05	0.06	0.05	0.05	0.04	0.04	0.03	0.03
L-Valine	0.05	0.06	0.05	0.04	0.04	0.04	0.03	0.02	–	0.01	–	–
Supplementary premix[2]	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30

FFSB = full-fat soybeans, SBM = soybean meal

[1] lysine content of 50.7%

[2] premix for Starter diet supplied (mg/kg diet): retinyl acetate 4.64, cholecalciferol 0.128, DL-α-tocopherol acetate 83.0, menadione 3.99, thiamine 3.99, riboflavin 8.1, pyridoxine 4.5, hydroxycobalamine 0.02, niacin amide 60.0, pantothenic acid 15.6, biotin 0.2, folic acid 2.0, choline chloride 240.0, betaine 138.0, Maxiban 95.0, Cu 17.4, Fe 60.0, Zn 89.7, Mn 120.0, I 1.0, Co 0.3, Mo 0.5, Se 0.3; premix for Grower diet supplied (mg/kg diet): retinyl acetate 4.13, cholecalciferol 0.128, DL-α-tocopherol acetate 56.0, menadione 3.0, thiamine 3.0, riboflavin 6.0, pyridoxine 4.1, hydroxycobalamine 0.015, niacin amide 50.0, pantothenic acid 18.0, biotin 0.2, folic acid 1.7, choline chloride 240.0, betaine 100.0, Narasin 70.0, Cu 17.0, Fe 50.0, Zn 80.0, Mn 100.0, I 1.0, Co 0.4, Mo 0.5, Se 0.3; premix for Finisher diet supplied (mg/kg diet): retinyl acetate 3.43, cholecalciferol 0.125, DL-α-tocopherol acetate 50.0, menadione 3.0, thiamine 2.0, riboflavin 5.0, pyridoxine 4.0, hydroxycobalamine 0.013, niacin amide 40.0, pantothenic acid 14.0, biotin 0.2, folic acid 1.5, choline chloride 198.0, betaine 77.0, Cu 17.0, Fe 50.0, Zn 80.0, Mn 100.0, I 1.0, Co 0.4, Mo 0.5, Se 0.3

Table 3. Nutrient content of diets (as-fed basis)

Diet	Starter (days 0–10)				Grower (days 11–24)				Finisher (days 25–35)			
Treatment	T1–7	T8	T9	T10	T1–7	T8	T9	T10	T1–7	T8	T9	T10
Amino acid level (%)	95	100	100	95	95	100	100	95	95	100	100	95
Calculated composition (g/kg)												
AME_n (MJ/kg)	12.65	12.65	12.65	12.65	13.20	13.20	13.20	13.20	13.40	13.40	13.40	13.40
Crude protein	225.0	234.4	233.8	224.8	199.3	208.4	207.4	198.4	182.9	190.8	189.9	182.2
Crude fat	87.0	87.2	70.4	67.3	91.0	95.4	79.9	76.8	91.3	95.1	81.7	79.0
SID Lysine	12.1	12.7	12.7	12.1	10.4	11.0	11.0	10.4	9.2	9.7	9.7	9.2
SID Met+Cys	8.7	9.1	9.1	8.7	7.7	8.1	8.1	7.7	7.0	7.4	7.4	7.0
SID Threonine	7.6	8.0	8.0	7.6	6.7	7.0	7.0	6.7	6.0	6.3	6.3	6.0
SID Valine	9.6	10.0	10.0	9.6	8.3	8.8	8.8	8.3	7.4	7.8	7.8	7.4
SID Isoleucine	8.2	8.6	8.6	8.2	7.2	7.6	7.6	7.2	6.5	6.9	6.9	6.5
Analyzed composition (g/kg)												
Crude protein	230.2	240.3	238.9	230.7	199.3	206.1	212.6	203.5	182.9	194.1	197.8	183.8
Lysine	13.6	14.3	13.6	13.2	11.8	12.5	12.2	11.6	10.4	11.2	11.2	9.9
Methionine	5.8	6.2	5.9	5.6	5.1	5.2	5.1	4.7	4.6	4.9	4.8	4.2
Cysteine	3.8	4.0	3.7	3.5	3.7	3.8	3.6	3.7	3.3	3.4	3.3	3.2
Threonine	8.9	9.6	9.0	8.9	7.7	8.1	8.1	7.8	7.2	7.6	7.5	6.9
Arginine	15.3	16.2	15.9	15.4	12.6	13.3	13.7	13.1	11.8	12.6	13.0	11.6
Valine	10.8	11.5	11.4	10.9	9.4	9.8	10.0	9.6	8.5	9.2	9.4	8.4
Isoleucine	9.3	9.8	9.8	9.5	8.2	8.6	8.8	8.5	7.6	8.1	8.3	7.4

AME_n = apparent metabolizable energy, SID = standardized ileal digestible

1992). The critical level of PS associated with maintaining optimal performance is a matter of debate. Lee et al. (1991) reported that a 10% drop in PS resulted in a significant decrease in growth rate of turkeys while Araba and Dale (1990a) and Parsons et al. (1991) identified the critical PS levels at 70 and 59%, respectively. The PS values found in the present study thus suggested that none of the FFSB treatment led to soybean over-processing. As indicated by correlation coefficients (Table 4), there was a close correlation between UA, PDI, and TIA, the *R* values ranging between 0.97 and 0.99. A moderately strong relationship was found between PS and other FFSB processing indicators. Reactive

Table 4. *In vitro* indicators of soybean processing

Treatment	Urease activity (mg N/g)	Protein dispersibility index (%)	KOH protein solubility (%)	Trypsin inhibitor activity (mg/g)
T1	4.15	72.2	94.2	27.3
T2	3.88	50.7	99.0	24.0
T3	1.53	34.6	93.3	14.4
T4	1.19	27.1	91.9	8.7
T5	0.42	22.0	90.0	7.2
T6	0.36	24.6	89.5	5.9
T7	0.05	14.7	88.7	5.3
T10	0.01	12.5	80.2	2.6
Pearson's correlation coefficients				
UA	–	0.96	0.78	0.99
PDI	–	–	0.72	0.97
PS	–	–	–	0.80

UA = urease activity, PDI = protein dispersibility index, PS = potassium-hydroxide solution lysine content in raw FFSB expressed as percentage of total lysine was 92.4. The values found in other FFSB samples ranged between 91.4 and 92.9, thus indicating no deleterious effect of processing on lysine availability.

As expected, growth performance of chickens fed raw or under-processed FFSB was markedly reduced in comparison with the SBM-fed birds (Table 5). At 95% amino acid level, both weight gain (WG) and feed conversion ratio (FCR) were maximized in treatment 6 and were not significantly different from those obtained with SBM-based diets. Similar results were reported by other authors comparing SBM and properly heat-treated FFSB included into broiler diets at moderate levels (Herkelman et al. 1991; Perilla et al. 1997; Mirghelenj et al. 2013). Increasing the amino acid concentration from 95 to 100% of the requirement resulted in an insignificant improvement of growth rate and FCR in both types of diet. Feed intake followed a similar trend as growth performance. In contrast, Perilla et al. (1997) reported a negative relationship between extrusion temperature and feed intake in chickens fed FFSB-based diets. Carcass and breast meat yields responses to processing time and temperature were similar to those of growth rate and FCR (Table 6). Relative pancreas weight was reduced from 4.47 g/kg in raw FFSB to 1.85 g/kg in treatment 3. In other treatments, the values ranged between 1.73 and 2.26 g/kg including birds fed SBM-based diets. The only exception was treatment 5 in which unexpectedly higher pancreas weights were found.

It is generally accepted that the reduction of chicken performance and pancreatic hypertrophy is largely due to the presence of trypsin inhibitors in raw soybeans (Applegarth et al. 1964; Han and Parsons 1991; Leeson and Atteh 1996; Foltyn et al. 2013). In the present study, a close correlation was found between *in vivo* chicken response and *in vitro* indicators of FFSB treatment (Table 7). TIA and PDI were the best predictors of chicken growth performance. The relationships between TIA and WG or FCR in chickens fed FFSB-based diets are shown in Figure 2. Growth rate and FCR did not change appreciably at TIA levels below 15 mg/kg which suggests that the TIA threshold might be higher than the commonly accepted values of 4–5 mg/kg (Leeson et al. 1987; Leeson and Atteh 1996). PDI is considered to be a sensitive indicator of minimum adequate heat processing of SBM (Batal et al. 2000). The results of the present study suggest that, to attain sufficient heat treatment, the PDI value should not exceed 30–35%. A slightly higher threshold (45%) was suggested by Batal et al. (2000) while the American Soybean Association recommended optimum PDI between 15 and 30% (Balloun 1980). In accordance with the results of other studies (Anderson-Haferman et al. 1992; Ruiz et al. 2004), a relatively weak correlation was found between KOH protein solubility and *in vivo* characteristics which indicated

Table 5. Effects of soybean treatment on growth performance of chickens

	Feed intake (g/day)				Weight gain (g/chick)				Feed conversion ratio			
Days	1–10	11–24	25–35	1–35	1–10	11–24	25–35	1–35	1–10	11–24	25–35	1–35
Treatment												
T1	28.2^{a}	72.3^{c}	133.8^{d}	79.0^{d}	137^{d}	602^{c}	560^{d}	1299^{d}	2.07^{a}	1.68^{a}	2.66^{a}	2.13^{a}
T2	28.1^{a}	76.1^{c}	142.6^{cd}	83.2^{cd}	157^{c}	737^{b}	664^{cd}	1558^{c}	1.79^{b}	1.45^{b}	2.37^{ab}	1.87^{b}
T3	29.0^{a}	88.3^{ab}	146.0^{bcd}	89.4^{bc}	211^{ab}	875^{a}	756^{bc}	1842^{b}	1.38^{c}	1.42^{b}	2.15^{bc}	1.70^{cd}
T4	29.3^{a}	91.0^{ab}	162.0^{ab}	95.6^{ab}	220^{ab}	930^{a}	791^{abc}	1940^{ab}	1.34^{c}	1.37^{b}	2.26^{bc}	1.73^{bcd}
T5	27.9^{a}	85.4^{b}	153.5^{abc}	90.4^{b}	204^{b}	837^{ab}	782^{abc}	1823^{b}	1.38^{c}	1.43^{b}	2.17^{bc}	1.74^{bc}
T6	29.5^{a}	93.5^{a}	166.5^{a}	98.0^{a}	228^{ab}	953^{a}	880^{ab}	2060^{a}	1.30^{c}	1.38^{b}	2.08^{bc}	1.66^{cd}
T7	29.8^{a}	91.6^{ab}	158.1^{abc}	94.8^{ab}	221^{ab}	930^{a}	791^{abc}	1942^{ab}	1.35^{c}	1.39^{b}	2.21^{bc}	1.71^{cd}
T8	29.0^{a}	91.8^{ab}	161.1^{ab}	95.5^{ab}	223^{ab}	945^{a}	933^{a}	2101^{a}	1.30^{c}	1.36^{b}	1.91^{c}	1.59^{cd}
T9	31.2^{a}	88.8^{ab}	159.0^{ab}	94.3^{ab}	229^{ab}	939^{a}	917^{a}	2086^{a}	1.36^{c}	1.32^{b}	1.92^{c}	1.58^{d}
T10	30.9^{a}	90.0^{ab}	147.8^{bcd}	91.3^{ab}	232^{a}	882^{a}	800^{abc}	1914^{ab}	1.33^{c}	1.44^{b}	2.06^{bc}	1.68^{cd}
Pooled SEM	0.79	1.71	3.48	1.50	5.5	26.6	32.6	41.2	0.051	0.033	0.084	0.032

$^{a–d}$means within a column not sharing a common superscript were significantly different (Tukey's HSD test, $P < 0.05$)

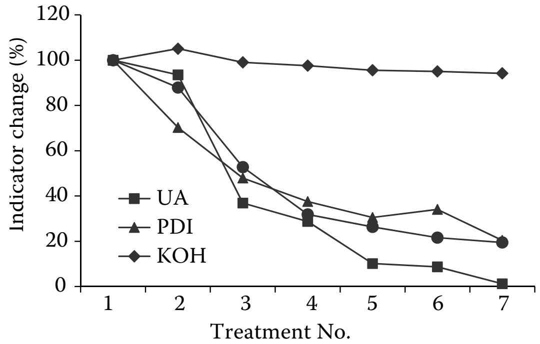

Figure 1. Effect of various treatments of full-fat soybeans on various *in vitro* indicators (% of initial value)

UA = urease activity, PDI = protein dispersibility index, KOH = potassium hydroxide

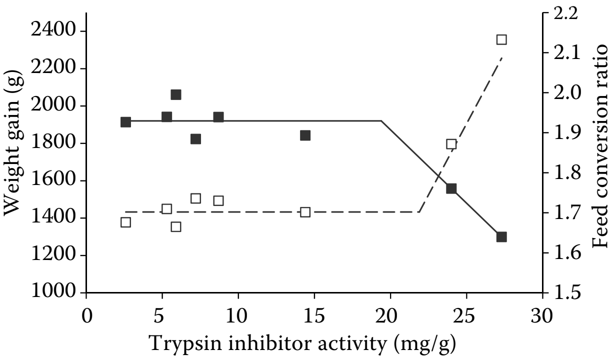

Figure 2. Weight gains (WG) (■) and feed converson ratio (FCR) (□) of chickens (1–35 days of age) in relation to trypsin inhibitor activity. The points are treatment means. Plotted from the equations y_1 = 1920.0, y_2 = 3441.8 – 78.49x, R^2 = 0.78 (WG) and y_1 = 21.89, y_2 = 0.148 + 0.071x, R^2 = 0.78 (FCR)

that this index was not sufficiently sensitive for evaluating protein quality of underprocessed FFSB. Correlation coefficients for UA were comparable with those for TIA and PDI thus suggesting that, under conditions of the present study (i.e. using FFSB not impaired by over-processing), this assay may be a good predictor of chicken performance. However, as shown by Dale et al. (1986) and Parsons et al. (1991), UA is unsuitable for detecting over-processed FFSB or SBM samples. There was a negative correlation between TIA and relative pancreas weight but, in contrast to other reports (Herkelman et al. 1991; Perilla et al. 1997; Clarke and Wiseman 2007), only a moderately strong relationship between the variables was observed (Figure 3). Even though significant differences were found between most of the treatments, pancreas weights in treatments 1–7 and 10 ranged within a relative narrow limit, thus suggesting that the pancreas to BW ratio might not be a sensitive indicator of TIA at lower trypsin inhibitor levels. Nevertheless, linear regression model relating the present growth performance data to relative pancreas weight showed a good relationship between the variables. The regression equations were y = 2532 – 286.4x (R^2 = 0.78) and y = 1.301 + 0.1861x (R^2 = 0.77) for WG and FCR, respectively.

Changeover experiment. Switching the dietary treatments from T1 to T10 and *vice versa* resulted in rapid changes in pancreas weights. During the first 3 days after the switch from raw FFSB to the SBM diet, relative pancreas weight decreased by 31%. When the SBM diet was replaced by the raw FFSB diet, relative pancreas weight on day 4

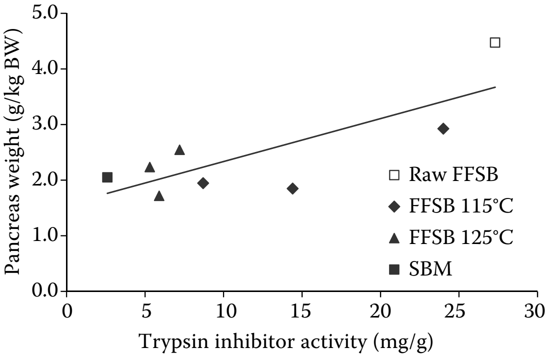

Figure 3. Relative pancreas weights of chickens at 35 days of age in relation to trypsin inhibitor activity. The points are treatment means. Plotted from the equation y = 1.563 + 0.0772x, R^2 = 0.62

FFSB = full-fat soybeans, SBM = soybean meal, BW = body weight

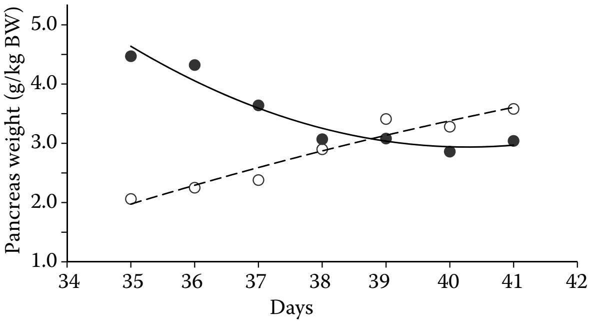

Figure 4. Relative pancreas weights of chickens after the changeover from treatments T1 to T10 (●) and T10 to T1 (○), respectively, during six following days. The points are treatment means. Plotted from the equations y = 101.98 – 4.918x + 0.0610x^2, R^2 = 0.80 (T1 to T10) and y = –7.501 + 0.272x, R^2 = 0.71 (T10 to T1)

BW = body weight

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Table 6. Effects of soybean treatment on carcass characteristics of chickens

Treatment	BW (g)	Carcass (% BW)	Breast meat (% BW)	Pancreas (g/kg BW)
T1	1447^{e}	62.46^{d}	16.44^{c}	4.470^{a}
T2	1677^{d}	64.92^{c}	18.43^{b}	2.920^{b}
T3	1972^{bc}	66.54^{abc}	19.87^{ab}	1.850^{f}
T4	2115^{ab}	67.08^{ab}	19.33^{ab}	2.000^{e}
T5	1892^{c}	66.23^{bc}	19.00^{ab}	2.567^{c}
T6	2183^{a}	67.94^{a}	20.25^{a}	1.734^{g}
T7	2046^{abc}	66.94^{ab}	19.31^{ab}	2.258^{d}
T8	2197^{a}	67.00^{ab}	20.17^{a}	1.848^{f}
T9	2159^{a}	67.31^{ab}	19.79^{ab}	1.856^{f}
T10	2061^{ab}	67.69^{ab}	19.45^{ab}	2.062^{e}
Pooled SEM	15.1	0.292	0.292	0.0222

BW = body weight

$^{a-g}$means within a column not sharing a common superscript were significantly different (Tukey's HSD test, $P < 0.05$)

increased by 65%. No significant changes were observed on days 5 and 6 (Table 8) even though there was a further numerical increase in pancreas weight. As shown in Figure 4, the response of T10 to T1 chickens was linear ($P < 0.001$), thus suggesting that the final pancreas weight might reach a similar level as that found in T1 chickens on day 35. On the other hand, the recovery of T1 to T10 chickens does not seem to follow a linear pattern. Very little information is available on the time-course of the pancreatic response to trypsin inhibitors in poultry. Madar (1979) found that pancreatic hypertrophy in chickens fed Bowman-Birk soybean trypsin inhibitor was evident after a minimum of 7 days of feeding. In contrast, Duarte et al. (2014) reported that the pancreas weight and pancreatic enzyme activities of feed-restricted chickens returned to normal after 3 days of realimentation. In experiments with rats, Rackis (1965) observed maximum pancreatic hypertrophy within 9 days after feeding raw SBM while Oates and Morgan (1984) found that a significant increase in pancreatic weight was apparent in rats already after 2 days of feeding raw soybean flour. It has been shown that the trypsin inhibitor-induced pancreatic enlargement is mediated through a negative feedback mechanism regulating enzyme secretion in response to the concentration of trypsin or chymotrypsin in the upper part of the small intestine (Schneeman and Lyman 1975). The process is under hormonal control by cholecystokinin (CCK), the release of which is inhibited by intraduodenal proteases (Slaff et al. 1984). When the activity of intestinal proteases is reduced by soybean trypsin inhibitor, CCK production is stimulated which results in an increased secretion of pancreatic enzymes and consecutive pancreatic hypertrophy (Grant et al. 1995; Clarke and Wiseman 2005). In experiments with chickens, Furuse et al. (1990) observed a considerable increase in plasma CCK within 90 min of feeding a diet supplemented with soybean trypsin inhibitors. The pancreatic response to CCK was also quick; continuous infusion of CCK in rats caused pancreatic hyperplasia already after 36 h of infusion. Conversely, blockage of endogenous CCK by

Table 7. Pearson's correlation coefficients describing the relationships between *in vivo* chicken parameters and *in vitro* indicators of full-fat soybeans treatment (calculated for treatments 1–7)

Item	Feed intake (g/day)	Weight gain (g)	Feed conversion ratio	Pancreas weight (g/kg BW)
KOH protein solubility (%)	-0.67^{***}	-0.65^{***}	0.49^{***}	0.44^{*}
Urease activity (mg N/g)	-0.81^{***}	-0.87^{***}	0.78^{***}	0.78^{***}
Protein dispersibility index (%)	-0.81^{***}	-0.88^{***}	0.84^{***}	0.85^{***}
Trypsin inhibitor activity (mg/g)	-0.85^{***}	-0.89^{***}	0.79^{***}	0.80^{***}

BW = body weight

$^{*}P < 0.05$; $^{***}P < 0.001$

Table 8. Mean relative pancreas weights during the change-over experiment (g/kg BW; n = 30)

Treatment change	Day							Pooled SEM
	35	36	37	38	39	40	41	
T1 to T10	4.47^{a}	4.32^{a}	3.64^{b}	3.07^{c}	3.08^{c}	2.86^{c}	3.04^{c}	0.115
T10 to T1	2.06^{d}	2.25^{d}	2.38^{cd}	2.90^{bc}	3.41^{ab}	3.28^{ab}	3.58^{a}	0.141

BW = body weight

$^{a–d}$means within a row not sharing a common superscript are significantly different (Tukey's HSD test, $P < 0.05$)

a receptor antagonist led to decreased pancreas weight after 3 days of infusion (Ohlsson et al. 1995). Similarly, Brannon (1990) in her review on pancreas adaptations concluded that diet-induced changes in pancreatic enzyme synthesis occur within several hours. Whether or not the pancreatic enlargement may be fully reversed after switching to a TIA-free diet, is not clear. In the present experiment, the relative pancreas weight significantly decreased during the first 3 days of T1 to T10 changeover, but did not reach the values found in SBM-fed chickens at day 35 (Figure 4). In contrast, Booth et al. (1964) found that pancreatic hypertrophy in rats fed raw SBM-based diet was reversible when a casein-based diet was fed for 71 days. However, the time-course of the recovery phase was not studied in detail in this experiment.

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