The effect of soybean meal replacement with raw full-fat soybean in diets for broiler chickens

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ABSTRACT
The aim of this study was to examine the effects of soybean meal replacement by multiple levels of raw full-fat soybean (RFFS) in broiler diets on the growth performance, apparent ileal amino acids digestibility (AIAAD), intestinal histometric characteristic, pancreas weight and trypsin activity in digesta. In the experiment, in total 208 × ROSS 308 male chickens were used to examine the effect of RFFS on growth performance, AIAAD, intestinal morphology and trypsin activity in the intestinal content. Four dietary treatments were used: control group (without RFFS) and groups containing 4%, 8% and 12% of RFFS. The experiment lasted from 10th to 38th day of age. The presence of RFFS in broiler diets markedly decreased body weight and feed conversion ratio merely in group fed by 12% of RFFS in its diet. The AIAAD was lower when diets contained RFFS in all observed amino acids expect methionine. Higher AIAAD was obtained in RFFS12 in comparison with RFFS8 and RFFS4 due to a higher weight of pancreas and higher trypsin activity. Pancreas weight and trypsin activity increased with increasing RFFS in the diets. The morphology parameters villus height and crypt depth were negatively by the presence of RFFS.

1. Introduction
Soybean (Glycine max) is one of the most important components in diets for broilers, and for other animals too. It is widely used mainly because of its high nutrient value: a high content of crude protein and energy (Waldroup 1982). Soybean proteins have a high biological value and have a high content of fat and unsaturated fatty acids (with about 50% linoleic acid) (Leeson & Summers 2005). Currently, most soybeans in poultry diets are used in the form of soybean meal (SBM) and extruded soybeans (Karr-Lienthal et al. 2005). Both of these soybean forms are treated by heat and pressure to reduce the effect of antinutritional substances (trypsin inhibitors, antinutritional proteins, oestrogens, lectins, saponins and non-starch polysaccharides) present in soybeans (Liener & Kakade 1980), which limit their use. But the thermal treatment could also destroy other essential nutrients (Chunmei et al. 2010). Trypsin inhibitors (TI) are considered as the most problematic antinutritional compounds in soybean and can be classified into two types: Bowman–Birk and Kunitz-TI. TI reduces the proteolytic action of the pancreatic enzyme trypsin and chymotrypsin (Rackis et al. 1986) in the digestive tract of animals and thereby reduces the digestibility of feed protein and it is associated with growth reduction. It also causes hypertrophy and hyperplasia of the pancreas (Leeson & Summers 2001). In the earlier studies focused on the use of raw full-fat soybean (RFFS), negative results of broilers were found to be linked to TI impact, especially Kunitz TI (Han et al. 1991; Zhang et al. 1991; Chohan et al. 1993). There are not so many research works available on the less-required treatment of the lower TI soybean hybrids for optimal inclusion of full-fat soybeans into the broilers’ diets. In recent years, there has been progress in soybean breeding with lower TI content in soybean hybrids as outcome (Bernard & Hymowitz 1986; Hymowitz 1986; Han et al. 2005). These hybrids can be used to avoid the energy- and cost-intensive operation needed for soybean treatment and to get better use of soy nutrients.

The aim of this study was to examine the effects of SBM replacement by multiple levels of RFFS in broiler diets on the growth performance (body weight – BW; feed conversion ratio – FCR), apparent ileal amino acids digestibility (AIAAD), intestinal histometric characteristic, pancreas weight and trypsin activity in digesta.

2. Material and methods
2.1. Birds management and diets
Four treatments that differed in the percentage of RFFS included in the diet were used in this experiment. The broilers were fed diets formulated to resemble industry ingredient and nutrient specifications. Diets were offered in two feeding phases: starter from 0 to 10th day; grower from 10th to 38th day. Starter diet had the same composition (21.5% CP; 12.2 MJ ME/kg) for all treatments. Grower diets were the experimental and based primarily on wheat, SBM and corn, and contained 0, 4, 8 or 12% RFFS (20.5% CP; 12.7 MJ ME/kg). Diets were formulated to be isocaloric and similar in limiting and
**Table 1.** Composition of the experimental diets (g/kg) fed to broilers in days 10–38 of the growing period.

| Ingredient                  | C    | RFFS4 | RFFS8 | RFFS12 |
|-----------------------------|------|-------|-------|--------|
| Wheat                       | 390.8| 390.8 | 390.8 | 390.8  |
| Maize                       | 250.0| 250.0 | 250.0 | 250.0  |
| Soybean meal               | 273.0| 240.2 | 207.4 | 174.6  |
| Raw full-fat soybean       | 0    | 40    | 80    | 120    |
| Soybean oil                | 45   | 37.8  | 30.6  | 23.4   |
| L-tyrosine HCl            | 3.0  | 3.0   | 3.0   | 3.0    |
| L-Threonine                | 1.0  | 1.0   | 1.0   | 1.0    |
| dl-Methionine              | 2.7  | 2.7   | 2.7   | 2.7    |
| L-Lysine HCl               | 3.0  | 3.0   | 3.0   | 3.0    |
| Sodium phosphate           | 12.5 | 12.5  | 12.5  | 12.5   |
| Sodium carbonate           | 2.0  | 2.0   | 2.0   | 2.0    |
| Complex of minerals and vitamins | 3.0  | 3.0   | 3.0   | 3.0    |

Calculated composition:

- Dry matter: 881.7 g/kg
- Crude protein: 204.7 g/kg
- Metabolizable energy (MJ/kg): 26.8
- Fibre: 26.8 g/kg
- Fat: 64.2 g/kg
- Lysine: 12.2 g/kg
- Methionine: 5.73 g/kg
- Methionine + cysteine: 9.26 g/kg
- Threonine: 8.16 g/kg
- Tryptophan: 2.41 g/kg
- Arginine: 12.7 g/kg
- Ca: 8.31 g/kg
- P: 6.47 g/kg
- Available P: 4.76 g/kg
- Na: 1.66 g/kg

Other nutrients (Table 1). Broilers consumed feed and water on an ad libitum basis.

Experiment was executed at the Mendel University Brno, Czech Republic. The cage technology with collecting belt for excreta was used. In total, 208 × ROSS 308 male broiler chicks were obtained from a commercial hatchery and were kept according to the principles referred to breeding instructions for this hybrid. At the beginning of the experiment, broilers were individually labelled by wing marks and distributed equally across 16 cages so that each treatment was replicated four times with 13 broilers each. Chicks were vaccinated at the hatchery for Marek’s disease and Infectious Bronchitis. Each cage was equipped with a lying feeder and a nipple drinker line.

Broilers were individually weighed on the digital scale with 0.1 g accuracy always in the morning on the 10th, 17th, 24th, 31st and 38th day during the experimental period. At those same days, the feeders were emptied and the residual feed was weighed in order to estimate the feed consumption. Feed and broilers’ weights were recorded for determination of growth intensity and FCR. Feed conversion was determined by individually weighing feed mixture for each cage (replication). All dead cocks were weighed and the FCR was corrected for the dead cocks. The broilers were killed by decapitation. After exsanguinations, the broilers were scalced at 85°C water for 15 s and picked manually. The pancreases were removed manually at an average of three individual cocks from each replication (that means 12 samples per experimental group) and were weighed on the digital scale with 0.1 g accuracy.

### 2.2. Amino acids and fat digestibility

This experiment was conducted to determine the ileal amino acids digestibility of diets with different content of RFFS. All birds at day 38 were killed by decapitation after stunning according to Czech law 246/1992 to protect animals against cruelty. Then the birds were dissected in order to obtain the digesta content of ileum (last one-third section between Meckel’s diverticulum and 4 cm from ileocecal junction). Digesta collected from each bird was stored at −30°C (one sample – five chickens, six replicates per treatment). Samples were lyophilized, ground and analysed for amino acids, dry matter and insoluble ash in 4 mol l⁻¹ HCl, which was used as an indicator. The 0.5 g samples of the feed and ileal digesta were treated by HCl oxidative acid hydrolysis (c = 6 mol l⁻¹). The chromatographic analysis of the hydrolysed samples was performed in the analyzer AAA 400 (Ingos, Prague, Czech Republic) using Na-citrate buffers and ninhydrin detection to find the amounts of certain amino acids.

The samples of excreta were collected on the end of the experiment under each cage (replication). Three samples were taken from each replication. The content of fat in the diets and excreta was determined according to Soxhlet. AIAAD (fat digestibility) was calculated using the following formula:

\[
\text{AIAAD} = \frac{1 - (100 - \text{I}_{dl} \times \text{AA}_{dc}/\text{I}_{dc} \times \text{AA}_{dd})}{100}
\]

where AIAAD denotes apparent ileal amino acid digestibility, I₉d – content of indicator in the diet, AA₉dc – content of amino acid in the digesta, I₉dc – content of indicator in the digesta and AA₉dd – content of amino acid in the diet.

The effect of feeding RFFS on AIAAD for each amino acid was expressed by polynomial function:

\[
y = ax^2 + bx + c
\]

where y represents coefficient of digestibility; a, b, c – parameters of the polynomial function; x – the level of RFFS.

### 2.3. Trypsin activity in the digesta

Digesta from small intestine, which was gently pushed from part of first one-third between duodenum and the Meckel’s diverticulum, was used for determination of trypsin activity. Samples of digesta were diluted 10x, based on the sample weight, with ice-cold PBS (pH 7.0) and homogenized in refrigerator for 10 min. Then the samples were centrifuged at 1500 g at 4°C for 10 min. The supernatant was transferred to the Ependorf tubes and stored at −30°C for enzyme assays.

Trypsin activity was analysed using method of constant time. N-α-Benzoyl-dl-arginine-p-nitroaniline (BAPNA) diluted with 50 mM Tris–HCl was used as substrate. One millilitre of substrate was incubated at 37°C for 5 min. Then 0.1 ml of supernatant (digesta samples) was added and the solution was incubated for 10 min. The reaction was stopped by adding 1 ml of acetic
Table 2. Dietary treatment effects on Live body weight (BW).

| Groups | 10 days | 17 days | 24 days | 31 days | 38 days |
|--------|---------|---------|---------|---------|---------|
| C      | 291 ± 3.8a | 665 ± 9.5a | 1146 ± 22.9a | 1823 ± 36.0a | 2443 ± 56.3a |
| RFFS4  | 287 ± 4.2a | 639 ± 8.9a | 1078 ± 25.1a,b | 1722 ± 30.2a | 2306 ± 41.2a,b |
| RFFS8  | 286 ± 4.1a | 625 ± 8.7a,b | 1090 ± 19.2a,b | 1677 ± 33.2a,b | 2296 ± 38.3a,b |
| RFFS12 | 289 ± 3.6a | 585 ± 7.2a,b | 1039 ± 13.9a | 1603 ± 26.0a,b | 2158 ± 37.9a,b |

Note: Different superscripts (a, b) indicate statistical significant difference between groups (p < .05).

Table 3. Dietary treatment effects on Feed conversion ratio (FCR).

| Groups | FCR (kg/kg) |
|--------|-------------|
| C      | 1.69 ± 0.04a |
| RFFS4  | 1.73 ± 0.07a,b |
| RFFS8  | 1.76 ± 0.08a,b |
| RFFS12 | 1.90 ± 0.05b |

Note: Different superscripts (a, b) indicate statistical significant difference between groups (p < .05).

2.4. Morphometric light microscopy

For morphometric analysis, the small intestine was quickly removed after killing the birds. A 4-cm segment from Meckel’s diverticulum was removed and rinsed with saline. Sections were fixed in 10% neutral buffered formalin solution. The cross-sections of each small intestinal sample were processed in low-melt paraffin, sectioned at 9 μm thickness, mounted on glass slides and stained with haematoxylin–eosin.

Villus height (VH) and crypt depth (CD) were measured using the ANALYSYS program, and VH:CD ratios were calculated. For each segment, the best-situated villi and associated crypts were measured.

2.5. Statistical analysis

Data obtained from these experiments were expressed as mean and standard error of the mean. They were analysed using the single-factor analysis of variation. Data of live weight, trypsin activity and intestinal morphology were followed by Scheffe’s test. For ileal amino acid and fat digestibility, the Kruskal–Wallis analyses followed by LSD (least significant difference) test were performed. Software package UNISTAT 5.1 (UNISTAT Ltd., England) was used.

3. Results

3.1. Growth performance

The effect of dietary treatments on growth performance – live BW and FCR – are shown in Tables 2 and 3. BW and FCR were depressed in RFFS groups (RFFS4, RFFS8 and RFFS12) when compared to group C with feeding of SMB. Broilers performance decreased with increasing dietary levels of RFFS. Significantly (p < .05) lower BW had the group RFFS12 from the 17th day of age till the end of the experiment (38th day). Groups RFFS4 and RFFS8 had lower BW during the whole experiment compared to group C, but without significant difference. The BW and FCR of group RFFS12 were significantly impaired (p < .05) in comparison to those of the control group (C) (BW 2157.8 g vs 2442.5; FCR 1.90 vs 1.69 kg/kg). Groups RFFS4 and RFFS8 achieved lower BW and deteriorated FCR compared to control group, but without significant difference (p < .05).

3.2. Amino acids digestibility and fat digestibility

The coefficients of AIAAD are shown in Table 4. The effect of RFFS levels on AIAAD and fat digestibility was expressed by polynomial functions, and the parameters of the polynomial functions are shown in Table 5. The dietary AIAAD coefficient of Thr, Ile, Leu, Phe, His, Arg and Val decreased with increasing levels of RFFS and lowest levels were seen in RFFS8. The presence of RFFS in the broiler diets negatively (p < .05) influenced the AIAAD coefficients of Ileu and His in all experimental treatments. Compared to those in the C group significantly (p < .05) lower AIAAD coefficients in groups RFFS4 and RFFS8 have been observed for Thr, Leu, Arg and Val. However, significantly increased AIAAD coefficients of RFFS12 were estimated for Thr, Ileu, Leu, Phe, His, Arg and Val in comparison to RFFS8. For Lysine, a significantly (p < .05) reduced AIAAD coefficient was seen in RFFS4 and RFFS8 compared to that in C. For AIAAD of Methionine, no significant differences were detected.

The presence of RFFS in the broiler diets significantly (p < .05) decreased fat digestibility in all experimental groups.

3.3. Pancreas weight and trypsin activity

The results of pancreas weight and trypsin activity are given in Table 6. Pancreatic weights increased significantly (p < .05) as a response to the presence of RFFS in the broiler diets. The pancreas weight of RFFS4, RFFS8 and RFFS12 was increased by 38%,
The presence of RFFS in broiler diets negatively affected VH and CD, as shown in Table 7. All experimental groups, with RFFS in diets, showed lower villi, but the animals fed with basal diet merely in groups RFFS4 and RFFS8 showed significant (p < .05) differences compared to C. In terms of CD, significantly (p < .05) lower crypts had groups RFFS4 and RFFS8. VH and CD ratio markedly (> .05) decreased by 20–40% in animals fed diets containing raw soybean or high at groups RFFS12 were similar to control group without significant difference.

4. Discussion

When raw full-fat soya is included in broiler diets, the broiler performance will be impaired because of the presence of anti-nutritional compounds (primarily TI) (Rackis et al. 1986). Nearly 40% of growth reduction of animals fed RFFS, according Kakade et al. (1973), is due to the TI. In the present experiment, feeding RFFS resulted in submaximal rate FCR and this was associated with high trypsin inhibitor activity TIA. These findings are in close agreement with the report of Perez-Maldonado et al. (2003), who observed significantly (p < .05) better FCR and BW in broilers fed a diet with SBM than broilers fed a diet with RFFS. Many studies have confirmed that daily gain in BW and feed utilization efficiency are lower in animals fed with diets containing TI. One of the possible reasons for this result is that TI leads to the loss of endogenous nitrogen (Chunmei et al. 2010). The effect of soybean TI on animal growth is related to their dietary levels. As Palacios et al. (2004) found that chicks fed diets containing no raw soybean varieties gained less weight (p < .05) than did chicks fed SBM. They also considered that the poorest weight gain was achieved by the group fed the raw commercial soybean (p < .05). In our chick experiment, TIA was almost twice higher in RFFS than in SBM (Table 1) and that affected final BW in groups fed diets containing RFFS. However, a significant (p < .05) reduction was only seen in when the diet contained 12% of RFFS. On the other hand, some soybeans lacking the Kunitz TI (Kunitz trypsin inhibitor-free; KF) were developed (Bernard & Hymowitz 1986) and several experiments have been conducted regarding the dietary value of these soybeans for chicken. For instance, feeding diet was beneficial in terms of better growth performance compared with raw soybeans (which contained Kunitz TI), but again inferior to the growth performance obtained by SBM (where Kunitz trypsin inhibitor was reduced by heat treatment) (Han et al. 1991; Zhang et al. 1991, 1993). In our experiment, we used RFFS in grower diets for broilers older than 10days. Up to a dietary level of 8%, the presence of RFFS did not affect growth performance much. According to Baker (2000), the effect of Kunitz TI is age-dependent, and might be more harmful for young animals than for adults. Pancreatic hypertrophy is an attempt of the body to offset the effect of ingested TI, as it will increase the enzyme production and secretion of trypsin and chymotrypsin (Liener 1981; Waldroup et al. 1985). In the present experiment, the highest dietary inclusion of RFFS resulted in the highest pancreas weight and highest trypsin activity while the group fed the basal diet (without RFFS) resulted in the lowest pancreas weight and Trypsin activity. Although the trypsin activity increased with increasing dietary inclusion of RFFS, this did lead to improved animal performance. TI activity, according to Ruiz et al. (2004), is significantly correlated with BW, and FCR and pancreas weight increase with increasing dietary inclusion of raw soybean (Perez-Maldonado et al. 2003). Similar results were achieved in our study. Many studies have found that protein digestibility was decreased by 20–40% in animals fed diets containing raw soybean or high...
levels of TI compared with those fed diets containing heated soybeans or SBM (Qin 1996; Caine et al. 1998; Li et al. 1998) compared with conventional soybeans when fed to growing pigs. Likewise, Herkelman et al. (1992) showed that low-trypsin-inhibitor soybean had significantly greater amino acid and nitrogen digestibility compared with conventional soybean when fed to growing pigs. Contrary to these findings, Batal and Parsons (2003) showed that the amino acids digestibility values of the Williams 82 soybeans (Kunitz-free and lectin-free soybeans) diets were much lower than those for the SBM diet. In the present study, RFFS was used as a source of crude protein, and the results showed that amino acids digestibilities were decreased by its presence. On the other hand, many authors reported that thermal treatment of soybeans improved the digestibility and nutritional value of soybean (Wuersch et al. 1986; Bengala Freire et al. 1991). Our result showed that RFFS in diets depressed broiler fat digestibility. In contrast with Chunmei et al. (2010), who discovered that fat digestibility in rats fed by RFFSs was significantly higher than that of the control group fed a common diet. Structural changes in intestinal function can decrease the absorptive function and efficiency in both chickens and turkeys (Croom et al. 1999). Shortening of the villi causes villus atrophy and a decrease in surface area for nutrient absorption. As Ma and Gou (2008) discovered, the crypt can be regarded as the villus factory, and a large crypt indicates fast tissue turnover and a high demand for new tissue. In the present study, RFFS negatively influenced villus height and CD, which could have a negative impact on lower BW and FCR in groups fed diets containing RFFS.

5. Conclusion
The present study has shown that the addition of RFFS to the broiler diets up to 8% is possible without having a significantly negative effect on the growth rate and FCR. Both of these aspects were shown to be negatively influenced when 12% of RFFS were included in the diet.

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