Chemical Treatments to Reduce Antinutritional Factors in Salseed (*Shorea robusta*) Meal: Effect on Nutrient Digestibility in Colostomized Hens and Intact Broilers

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ABSTRACT This study investigated the effects of chemical treatments of salseed meal (SSM) on nutrient digestibility and digestive enzymes in colostomized hens and intact broilers. Finely ground SSM was treated (820 mL/kg of SSM DM) with distilled water (pH 5.3), 0.67 *M* acetic acid (pH 2.4), or 0.67 *M* sodium hydrogen carbonate (pH 8.2), and incubated for 12 h at 37°C. Five isonitrogenous and isocaloric diets each for colostomized hens (25.6 g of N/kg of DM and 12.5 MJ/kg of DM) and for broilers (33.6 g of N/kg of DM and 12.3 MJ/kg of DM) were formulated. For each group, 1 of these diets was wheat-based (control) whereas the other 4 were SSM-based diets (untreated SSM or SSM treated with water, acetic acid, and sodium bicarbonate). Inclusion of SSM in diets markedly reduced apparent protein and starch digestibility in hens and broilers. Chemical treatments of SSM improved the protein and starch digestibilities in colostomized hens and broilers. Treatment of SSM with alkali reduced the pancreatic hypertrophy in broilers that was observed when SSM was included in the diet. Inclusion of SSM in broiler diets did not affect pancreatic trypsinogen activity; however, chymotrypsinogen and α-amylase activities were depressed with its inclusion. Activities of these enzymes were improved by all chemical treatments of SSM. Dietary inclusion of SSM in broiler diets depressed the activities of trypsin, chymotrypsin, and α-amylase in the jejunal digesta. Alkali treatment proved to be the most effective in reducing the adverse effects of SSM upon trypsin and chymotrypsin activities. The hens receiving SSM in their diets produced eggs with discolored yolks (dirty greenish-yellow). In conclusion, nutrient digestibility, and pancreatic and intestinal enzymes were markedly depressed with the inclusion of SSM in the diets of the fowl. Bicarbonate was the most effective treatment to improve nutrient digestibility and mitigate, to some extent, the poor digestion of SSM.

Key words: tannin, protein, starch, digestive enzyme, colostomized chicken

2006 Poultry Science 85:2207–2215

INTRODUCTION

Tannins are naturally occurring plant polyphenols with molecular weights between 500 and 3,000. These polyphenols have the ability to form stable complexes with proteins and other polymers such as cellulose, hemicellulose, and pectin (Mangan, 1988; Choct and Hughes, 1999). Tannins are widely distributed in plant barks, roots, fruits, leaves, and seeds (Mangan, 1988). However, dicotyledonous plants, particularly of the *Leguminosae* family, contain a substantial amount of tannins (Salunkhe et al., 1990; Evers et al., 1999).

Tannins are potentially toxic substances for simple-stomached animals (Santos-Buelga and Scalbert, 2000) and their inclusion in the diets of fowl reduces feed intake, weight gain, and efficiency of feed utilization (Ahmed et al., 1991; Mahmood and Smithard, 1993). Tannin from faba bean (Longstaff and McNab, 1991) and sorghum grains (Mitaru et al., 1984) has reduced the digestibilities of amino acids, starch, and lipids when fed to young chicks and adult cockerels.

Salseed meal (**SSM**) is produced from the nuts (seed) of the Sal (*Shorea robusta*), which is a tall (35 m) tree widely grown for timber along the Himalayan foothill belt in India and South Asia. The botanical family of Sal is *Dipterocarpaceae*. The kernel of its nut contains 15 to 18% oil, which is removed and used locally in food or sold to the confectionery industry worldwide. The SSM is either used by local feed industry or exported to European markets to be used for animal feeding. The presence of SSM tannins in the diets of fowl results in lowered digestibility of protein, reduced activities of digestive enzymes (trypsin, chymotrypsin, α-amylase, dipeptidase, and disaccharidases) in the gut lumen, and an increase in the relative weight of the pancreas (Mahmood et al., 1997).

Received April 25, 2006.
Accepted July 29, 2006.
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Table 1. Proximate chemical composition (mean ± standard deviation) of salseed meal (n = 10) produced through solvent oil extraction from the kernel (nut) of the Sal (*Shorea robusta*)

Parameter	g/kg of DM
Moisture	50.62 ± 8.01
CP	350.71 ± 12.30
Lipids	29.0 ± 5.04
Crude fiber	19.51 ± 2.11
Nitrogen-free extract	513.21 ± 19.4

The results of our in vitro study (Mahmood, 1993) revealed that the tannin content and trypsin inhibitor (**TI**) activity of SSM were markedly reduced by the addition of water or aqueous solutions of sodium bicarbonate or acetic acid, followed by anaerobic storage for 12 h at 37°C. Results obtained from in vitro chemical testing may not provide results equivalent to in vivo studies because of the more complex biochemical and biophysical environment that prevails inside the body of the chicken. Therefore, this study was planned to test the hypothesis that the chemical treatments of SSM might improve the nutrient digestibility by mitigating the negative effects of its tannin and TI contents on pancreatic and intestinal enzymes in chicken.

Because excreta are composed of both feces and urine, in vivo digestibility studies in intact poultry require the determination of total nitrogen, ammonia nitrogen, chromium III oxide, and uric acid to determine the digestibility of protein. These determinations require many chemicals, most of which are used in the separation of uric acid. The chemicals used in such studies are scarce and expensive in underdeveloped countries and the procedures are time consuming. However, the use of colostomized hens eliminates the contamination of excreta samples with uric acid and determination of uric acid and ammonia nitrogen may thus be avoided. Therefore, use of colostomized hens for in vivo digestibility trials might be a less expensive and time-consuming method for research workers in developing countries.

The aim of this investigation was to evaluate the nutritional value of SSM treated with water, acetic acid, and sodium bicarbonate in colostomized hens and intact broilers. Enzyme activities in pancreatic tissue and jejunal digesta were also determined to evaluate the relationship with the improvement in nutritional quality of SSM, if any, from the experiment with broilers.

MATERIALS AND METHODS

Analysis and Treatment of Salseed Meal

Salseed meal was analyzed for moisture, CP, ether extract, and nitrogen-free extracts by the methods of AOAC (1990). The proximate composition of SSM is presented in Table 1.

Three batches of finely ground SSM (25 kg each) were thoroughly mixed with distilled water (pH 5.3), 0.67 *M* acetic acid (pH 2.4), or 0.67 *M* sodium hydrogen carbonate (pH 8.2) solutions at 820 mL/kg of SSM DM and incubated (in polyethylene bags) for 12 h at 37°C. The treated material was dried after incubation at the same temperature in a forced-draft oven.

Five isonitrogenous (25.6 g of N/kg of DM) and isocaloric (12.5 MJ/kg of DM) diets (Table 1) containing chromium III oxide (5 g of Cr_2O_3/kg) as an indigestible marker, with or without SSM, were formulated. One of these diets was wheat-based (control, with no SSM) whereas the other 4 were SSM-based. The SSM-based diets had the same composition of ingredients except that they contained untreated SSM (**USSM**), or SSM treated with water, acetic acid, or sodium bicarbonate (alkali).

Analysis of Tannins

A modified vanillin-HCl method (Price et al., 1978) was used for the estimation of condensed tannins. Four representative samples of SSM (USSM, plus SSM treated with water, acid, and alkali) were separately extracted for 1 h with acidified methanol. The extracts were centrifuged at 1,000 × *g* for 15 min and the resultant supernatants were immediately analyzed. Vanillin dissolved in acidified methanol was used as reagent. Twenty minutes after the addition of the reagent to the sample aliquots, the absorbance of the color developed was measured spectrophotometrically at 500 nm. A portion of supernatant from each sample was mixed with 4% HCl-methanol solution to serve as a blank. Catechin was used as standard to compare the values of the samples.

The dye-labeled protein precipitation method (Asquith and Butler, 1985) was used to estimate the hydrolysable tannin contents of treated SSM or USSM. Bovine serum albumin covalently bound to the dye Remazol Brilliant Blue was used as dye-labeled protein. The 4 representative samples of the meal were extracted with aqueous methanol (50% vol/vol) for 1 h. The extracts were centrifuged at 1,000 × *g* for 15 min and the resultant supernatant was immediately analyzed for the hydrolyzable tannin. Aliquots of the supernatants were mixed with dye-labeled BSA and allowed to stand for 15 min. During this time a tannin–protein complex was formed, the precipitate separated by centrifugation at 1,000 × *g* for 20 min, and the supernatant discarded. The precipitate was dissolved in SDS solution and the absorbance of the colored solution was read at 590 nm. A tube containing the extracting solution for each sample was run through the method to serve as a blank. A series of standard solutions containing 0.2 to 1.0 mg of tannic acid per mL of aqueous methanol was run through the method to compare the values of the samples.

TI Activity

Treated or untreated samples (n = 5 per treatment) of SSM were extracted with water for determination of TI activity. The extracts were centrifuged at 1,000 × *g* for 20 min and the supernatant retained. The supernatants of the extracts were diluted with Tris buffer and centrifuged

again to remove the precipitated material from the extracts. Supernatant from the diluted samples was used for TI determination according to the method of Liu and Markakis (1989). Porcine trypsin was used as standard and the antitryptic activity of the sample was calculated in terms of trypsin units inhibited (**TUI**)/g of sample.

Experiment with Colostomized Birds

Adult White Leghorn pullets (18 wk age) were transferred to the experimental farm 1 wk before surgery to acclimate to the environment. The hens were housed in individual cages (1 pullet/cage) in an environmentally controlled room. The cages were constructed according to the guidelines of University Federation for Animal Welfare (United Kingdom) to provide maximum comfort (to avoid feather and foot damage and overgrowth of claws, better physical condition, and more freedom of movement) to the birds. Each cage was 80 cm high, 60 cm wide, and 60 cm long. A mesh size of 5 × 5 cm and 2.5 × 2.5 cm was used for the sidewalls and floor of the cages, respectively. Each cage was supplied with a plastic feeder and a drinker inside the cage. An aluminum tray underneath the wire-netting floor of the cage was used for collection of the excreta. The temperature of the house was maintained at 24 to 26°C by using 2 electric fan heaters.

The colostomy procedure on the pullets was performed in a surgical theater. The surgical instruments were thoroughly sterilized before use. The birds were anesthetized using halothane. As a bird became unconscious it was restrained on a board with strings where feathers were carefully plucked from the right side, between the keel bone and pubis, and the area was disinfected. A longitudinal incision (3 to 4 cm) was made in the skin about 3 cm to the right of the midline at a point halfway between the point of the keel bone and ischium, 3 to 4 cm anterior to the ventral lip of the cloaca. The muscles under the incision were penetrated by blunt dissection and the peritoneum was cut. Muscle and skin were excised from the center of each side of the incision to create an opening of about the same size as the diameter of the cloaca.

The colon was pushed out through the incision using the small finger of the right hand inserted into the colon through the cloaca. The caudal end of the colon was tied with suture string and cut. A polythene tube about 1 cm in diameter was clamped into the lumen of the exposed colon. The colon was everted around itself onto the tube. The colon was supported by the tube during the suturing. The everted edges of the colon were sutured to its own serosal wall at 3 places (equally spaced). Four triangular sutures (muscle–colonic serosa–muscle) were applied to promote the adhesion of muscles to the colon. The skin incision was closed by using 3 triangular sutures (skin–colonic serosa–skin) to fix the skin to the colonic serosa. The polythene tube was then taken out and the wound was cleaned with an antiseptic solution. The bird was untied and then put in a thermostatically controlled incubator to recover from the anesthesia.

It took about 5 min for the bird to recover from the anesthesia after the surgical procedure and the bird was kept in the incubator for another 15 min. The bird was then transferred to an individual cage in a controlled-environment room. The cage had a small piece of vetbed serving as bedding material. Each bird was provided with commercial layer ration and water to provide energy to the bird to overcome stress due to the surgical procedure. The colostomy was carefully washed twice a day and antiseptic cream was applied to keep the tissue soft and free from infection. The bird was closely watched for any pecking at the colostomy. If pecking was noticed, a thin cardboard collar was applied around the bird's neck and the collar was removed after the site was healed.

Any bird suspected of being infected was given antibiotic treatment (Ceporex, 50 mg of Cephalexin/kg of BW per d, Schering-Plough Ltd., Welwyn Garden, UK) intramuscularly. If the colostomy became blocked, it was flushed with lukewarm water. Usually the hens started eating 3 to 4 h after the operation. However, those that did not eat on the second day after colostomization were fed by hand to initiate their appetite. Scab formation on the mucosa started during the first day and became a scale on d 3; the scale fell off eventually, leaving a fresh and healthy mucosa. It took 1 to 4 d for the hens to reach normal feeding levels.

After complete healing, the colostomized hens were housed in the same individual cages in an environmentally controlled room as described earlier. Old newspapers were used underneath the cages for the collection of droppings. The paper was replaced daily to avoid any mess under the birds. Clean and fresh water was available to the hens at all times.

Five colostomized hens were used in a Latin square design for digestibility trial. The allocation of the hens to each diet during the periods of feeding was made by following the schedule (selected randomly) for Latin square design. During each period, the hens were fed experimental diets ad libitum for 7 d as an adaptation period followed by a 7-d collection period when each bird was fed 120 g/d of the diet. Half of the allocated diet was fed in the morning (0900 h) and the rest in the evening (1700 h). A plastic flange, designed to hold a polythene collection bag, was sutured to the skin around the colostomy 2 d before beginning collection of excreta. A polythene sample collection bag (7 × 10 cm) was attached to the flange. Five consecutive collections at 2-h intervals were made daily from 0900 to 1700 h)]. After each collection the excreta were transferred to aluminum foil trays, covered with polythene bags, and immediately frozen.

At the end of the experiment the frozen excreta were dried in a freeze drier. Freeze-dried excreta were finely ground and stored in airtight plastic containers at –20°C pending analysis. Feed and excreta samples were analyzed for total nitrogen, ether extract, starch, and chromium III oxide contents (AOAC, 1990). Digestibilities of starch, protein, and ether extract were calculated using the equations described by Mahmood et al. (1997).

Table 2. Composition of the experimental salseed meal (SSM) diets for colostomized hens and broilers

	Hens		Broilers	
Ingredients (g/kg)	Control diet	SSM diet	Control diet	SSM diet
Wheat	620.2	246.1	400.0	100.0
Salseed meal[1]	—	500.0	—	300.0
Wheat bran	120.0	—	—	—
Flaked corn	—	—	236.5	176.7
Soybean meal (48%)	76.3	49.8	258.8	279.2
Fish meal	50.0	50.0	50.0	50.0
Vegetable oil	34.5	55.1	11.7	51.1
Dicalcium phosphate	14	14	10	10
Limestone	60	60	8.0	8.0
Vitamin-mineral premix[2,3]	25.0	25.0	25.0	25.0
Chemical composition (calculated)				
CP (N × 6.25; g/kg)	160.0	160.0	210.0	210.0
ME (MJ/kg)	12.50	12.50	12.30	12.30
Oil content (g/kg)	53.46	66.91	33.51	68.80

[1]Untreated SSM (USSM) or SSM treated with distilled water (pH 5.3), 0.67 *M* acetic acid (pH 2.4), or 0.67 *M* sodium hydrogen carbonate (pH 8.2) and incubated at 37°C for 12 h was used to formulate the 4 treatment diets (USSM, water, acid, and alkali, respectively) for hens and broilers. The calculated amounts of tannins were 40.3, 22.7, 25.0, and 14.3 g/kg in the USSM, water, acid, and alkali diets of colostomized hens, respectively; respective values of tannins for broilers diets were 24.2, 13.7, 15.0, and 8.6 g/kg.

[2]Supplied per kilogram of diet of hens: vitamin A, 3,500 IU; vitamin D_3, 300 IU; vitamin E, 10 IU; vitamin K, 1 mg; thiamine, 1 mg; riboflavin, 4 mg; pyridoxine, 3 mg; vitamin B_{12}, 0.005 mg; folic acid, 0.35 mg; niacin, 12 mg; Ca pantothenate, 2.5 mg; biotin, 0.13 mg; choline, 1,050 mg; Mn, 30 mg; Zn, 50 mg; Fe, 50 mg; Co, 0.40 mg; I, 0.04 mg; Cu, 2 mg; Se, 0.07 mg; Banox, 75 mg.

[3]Supplied per kilogram of diet of broilers: vitamin A, 1,600 IU; vitamin D_3, 200 IU; vitamin E, 11 IU; vitamin K, 0.5 mg; thiamine, 1.7 mg; riboflavin, 3.6 mg; pyridoxine, 3.6 mg; vitamin B_{12}, 0.01 mg; pantothenic acid, 10 mg; niacin, 38 mg (30 mg in finisher); choline, 1,300 mg (1,000 mg in finisher); biotin, 0.15 mg; folic acid, 0.55 mg; Mn, 60 mg; Zn, 40 mg; Co, 8 mg; Fe, 80 mg; antioxidant (Santoquin), 125 mg.

Experiment with Broiler Cockerels

Five isonitrogenous (33.6 g of N/kg of DM) and isocaloric (12.3 MJ/kg of DM) diets (Table 2) containing 5 g of Cr_2O_3/kg as an indigestible marker, with or without SSM, were formulated as described above for colostomized hens.

Sixty 1-d-old male broiler (Cobb) chicks were purchased from a commercial hatchery and were reared in a group on deep litter system for 17 d. On d 18 the broilers were weighed individually and 40 birds with BW between 450 and 500 g were selected and put into separate cages (1 chick/cage) to be used as experimental birds. The caged broilers were allocated to 5 groups (control, USSM, SSM treated with water, acid, or alkali; n = 8/group) and were fed the experimental diets. For the first 7 d of the experimental period (d 18 to 24), no excreta collection was made while chromium III oxide passage was becoming stabilized. During the excreta collection period (d 25 to 31) the amount of feed offered to all groups was the same. Each bird was offered 70 g of feed/d at the start of the collection period; feed was gradually increased, reaching 90 g/d at the end of the trial. Collection of feed and excreta and their chemical analyses were made as described earlier for colostomized hens. Starch and ether extract digestibilities were also calculated by the methods mentioned above. Nitrogen retention (NR) in broiler was calculated using following equation:

$$NR = 100 - \left(100 \times \frac{\%\ Feed\ Marker}{\%\ Excreta\ Marker} \times \frac{Total\ Excreta\ N}{Total\ Feed\ N}\right).$$

At the end of the collection period (d 31), each bird was killed by cervical dislocation, and the digestive tract and pancreas were excised. Each pancreas was weighed, immediately frozen in liquid N, and stored at –70°C. Each pancreas was thawed, sliced into smaller pieces, and homogenized in 30 mL of buffer solution (50 m*M* Tris, 0.154 *M* KC1, pH 7.9). The homogenate was centrifuged at 35,000 × *g* for 20 min at 4°C and the supernatant was collected for enzymatic analysis. The aliquots of supernatants were analyzed for trypsinogen, chymotrypsinogen, and amylolytic activity. The activities of the enzymes were expressed as units per gram of pancreatic tissue or units per kilogram of live weight.

Sections (150 mm) of jejunum were taken. Digesta were collected from the jejunal sections and stored at –20°C until analyzed. Jejunal digesta (0.8 to 1.0 g) were homogenized in 15 mL of buffer solution (0.04 *M* Tris, 0.01 *M* CaCl, pH 8.1) and the homogenates were centrifuged at 35,000 × *g* for 20 min at 4°C. The resulting supernatants were analyzed for trypsin, chymotrypsin, and α-amylase activities. The activities of the enzymes in the supernatant were expressed as units per gram of dry chyme.

Supernatants of jejunal digesta homogenates were assayed for trypsin activity (Liu and Markakis, 1989) using benzoyl-DL-arginine-4-nitroanilide as substrate. The enzyme activity was based on its ability to hydrolyze the substrate when incubated at 37°C. The resultant colored compound (4-nitroaniline) was measured spectrophotometrically at 410 nm and the activity was calculated from the absorbance values.

Trypsinogen in the supernatant aliquots of pancreatic homogenates was activated to trypsin by incubating with

Table 3. Digestibility of protein, starch and ether extract in colostomized hens

		SSM treatment[2]				
Digestibility (%)	Control diet[1]	USSM	Water	Acid	Alkali	SEM
CP	81.43[a]	41.83[d]	49.69[c]	45.86[cd]	58.10[b]	1.73
Starch	98.80[a]	92.44[c]	95.09[b]	96.94[ab]	93.21[c]	0.59
Ether extract	91.37	89.18	90.36	89.40	90.75	1.20

[a–d]Means in the same row followed by the different superscript letters are significantly different ($P < 0.05$). SEM is among means.

[1]The control diet contained no salseed meal (SSM).

[2]Untreated salseed meal (USSM) or SSM treated with distilled water (pH 5.3), 0.67 *M* acetic acid (pH 2.4), or 0.67 *M* sodium hydrogen carbonate (pH 8.2) and incubated at 37°C for 12 h was used to formulate the 4 treatment diets (USSM, water, acid, and alkali, respectively). The calculated amounts of the tannins were 40.3, 22.7, 25.0, and 14.3 g/kg in the USSM, water, acid, and alkali diets, respectively.

enterokinase (EC 3.4.21.9) at 37°C for 1 h (Gertler and Nitsan, 1970). In a modified procedure, tryptic activity (EC 3.4.21.4) was estimated by rate measurements at 385 nm, and the enzyme activity was calculated using the procedure of Collington (1990). The enzyme activity was expressed in international units (**IU**), the amount of the enzyme that hydrolyzes 1 micromole of substrate per minute.

Chymotrypsin (EC 3.4.21.1) activity was determined with the modified method described by Collington (1990) in which rate measurements were made rather than taking single final absorbance value after a fixed period of time. The substrate used in the assay was N^2-succinyl-l-alanyl-L-alanyl-L-proly-L-phenylalanyl-4-nitroanilide. The amount of 4-nitroaniline produced during the hydrolysis of the substrate was determined by rate measurement. Chymotryptic activity was proportional to the rate of absorbance increase at 385 nm. Chymotrypsinogen in pancreatic homogenates present in the form of chymotrypsinogen was activated by incubating the homogenate with trypsin (EC 3.4.21.4) solution for 10 min at 37°C. The activated cymotrypsinogen was assayed by the method described above. Chymotryptic activity was expressed in IU. One international unit was defined as the amount of enzyme that hydrolyzed 1 micromole of the substrate per minute.

Activity of α-amylase (EC 3.1.1.1) was determined using MA-KIT (Roche Diagnostics GmbH, Mannheim, Germany). The enzyme activity was based on its ability to hydrolyze the substrate maltotetraose. The catalytic α-amylase activity was expressed in IU, defined as the amount of enzyme that hydrolyzes 1 micromole of substrate per minute assuming that hydrolysis of 1 molecule of maltotetraose yielded 2 molecules of NADH.

Statistical Analysis

Data on hydrolyzable tannins, condensed tannins, and TI contents in treated and untreated SSM were analyzed as a completely randomized design. The nutrient digestibility data from colostomized hens were subjected to ANOVA as a 5 × 5 Latin square design. The nutrient digestibility and enzyme activity data from broilers were analyzed using ANOVA as a completely randomized design. Duncan's multiple range test was applied to separate means. Analyses were performed using SAS (SAS Institute, 1994).

RESULTS

Hydrolyzable tannin, condensed tannin, and TI activity of untreated and chemically treated SSM are presented in Figure 1. Hydrolyzable and condensed tannins in SSM were reduced ($P < 0.05$) by all chemical treatments. Alkali treatment of SSM was more effective ($P < 0.05$) in reducing its hydrolyzable and condensed tannins compared with acid and water treatments. Hydrolyzable tannins were similar in SSM treated with acid and water. All treatments

Table 4. Nitrogen retention and digestibility of starch and ether extract in broilers

		SSM treatment[2]				
Parameter (%)	Control diet[1]	USSM	Water	Acid	Alkali	SEM[3]
N retention	68.57[a]	41.45[c]	49.39[b]	41.51[c]	53.94[b]	0.41
Starch	98.24[a]	88.20[c]	92.61[b]	91.81[b]	89.57[c]	0.80
Ether extract	88.37	86.10	87.53	87.20	88.00	0.00

[a–c]Means in the same row followed by the different superscript letters are significantly different ($P < 0.05$).

[1]The control diet contained no salseed meal (SSM).

[2]Untreated salseed meal (USSM) or SSM treated with distilled water (pH 5.3), 0.67 *M* acetic acid (pH 2.4), or 0.67 *M* sodium hydrogen carbonate (pH 8.2) and incubated at 37°C for 12 h was used to formulate the 4 treatment diets (USSM, water, acid, and alkali, respectively). The calculated amount of tannin in the USSM, water, acid and alkali diets was 24.2, 13.7, 15.0, and 8.6 g/kg, respectively.

[3]SEM is among means.

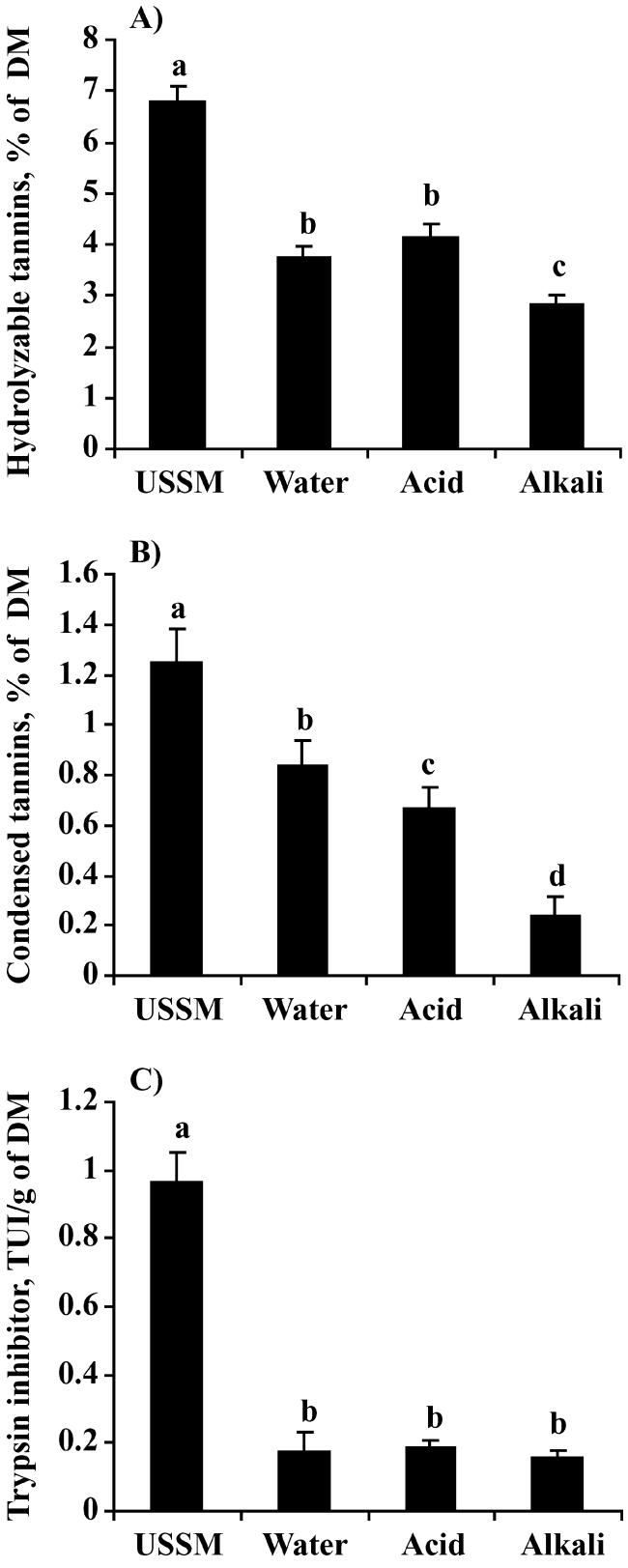

Figure 1. Mean (± standard error) of a) hydrolyzable tannins, b) condensed tannins, and c) trypsin inhibitor contents in untreated salseed meal (USSM) or in salseed meal treated with distilled water (pH 5.3), acid (0.67 *M* acetic acid, pH 2.4), or alkali (0.67 *M* sodium hydrogen carbonate, pH 8.2), and incubated at 37°C for 12 h. [a–d]Means with different superscript letters are significantly different ($P < 0.05$). For evaluation of trypsin inhibitor content, porcine trypsin was used as standard and the antitryptic activity in salseed meal was expressed in terms of trypsin units inhibited (TUI)/g of DM.

exerted a similar reduction (80 to 85%) in the TI activity of the SSM.

The apparent digestibility of protein in colostomized hens and NR in broilers are shown in Tables 3 and 4, respectively. Apparent digestibility of protein in hens and NR in broilers were markedly depressed ($P < 0.05$) with the inclusion of SSM in their diets compared with the control diet. Treatment of SSM with water, acid, or alkali significantly improved ($P < 0.05$) the protein digestibility in colostomized hens and NR in the broilers. Alkali treatment of SSM was the most effective ($P < 0.05$) to improve protein digestibility in hens and NR in broilers compared with water and acid treatments.

The hens and the broilers fed the control diet digested the starch content of the diet more ($P < 0.05$) effectively than those fed USSM or treated SSM-based diets. Treatment of SSM with water or acid was most effective ($P < 0.05$) in improving the digestion of starch in colostomized hens. The trial with broilers showed the same trend in improvement of starch digestibility when birds were fed SSM treated with acid, water, or alkali. Inclusion of SSM (whether treated or untreated) in the diets of the hens or broilers did not show any significant effect on the digestion of ether extract. However, the presence of SSM in the diets slightly lowered ether extract digestibility.

Relative pancreatic weight and enzymatic activities in the pancreatic tissue of broilers are shown in Table 5. Inclusion of SSM in the diets significantly ($P < 0.05$) increased the pancreatic weight of broilers compared with those fed the control diet. Alkali and water treatments of SSM minimized ($P < 0.05$) this effect in broilers. However, pancreatic weight of broilers fed SSM treated with acid was similar to those fed untreated SSM. Pancreatic trypsinogen activity was similar in broilers fed different experimental diets. The broilers fed the control diet showed higher ($P < 0.05$) chymotrypsinogen and α-amylase activities than those fed USSM or treated SSM diets. Chymotrypsinogen activity (IU/g of pancreatic tissue) in broilers fed diets containing SSM treated with water, acid, or alkali was higher than in those fed the USSM diet. Alkali and water treatments were more effective in improving the activity (IU/kg of live weight) of pancreatic chymotrypsinogen in broilers compared with acid treatment of SSM. Pancreatic α-amylase activity was higher in broilers fed diets containing SSM treated with water, acid, or alkali compared with those fed the USSM diet. Similar activities of pancreatic α-amylase were noticed in broilers fed diets treated with water, acid, or alkali.

Activities of trypsin, chymotrypsin, and α-amylase in the jejunal digesta of the broilers are shown in Table 6. Dietary inclusion of SSM in broilers diet markedly ($P < 0.05$) depressed the activities of these enzymes. The depression in trypsin activity in jejunal digesta of broilers with dietary inclusion of SSM was significantly ($P < 0.05$) alleviated with alkali treatment. However, acid and water treatments of SSM brought a numerical improvement in the activity of trypsin in jejunal digesta of broilers. Depressed jejunal chymotrypsin activity with the inclusion of SSM in the diets of broilers was significantly ($P <$

Table 5. Relative pancreas weight and enzyme activities in the pancreatic tissue of broilers

Parameter	Control diet[1]	SSM treatment[2]				SEM[3]
		USSM	Water	Acid	Alkali	
Pancreas (g/kg of live weight)	2.24[a]	3.34[d]	2.76[b]	3.02[c]	2.77[b]	0.09
Trypsinogen						
IU/g of pancreatic tissue	5.94	5.12	5.83	5.65	5.83	0.52
IU/kg of live weight	15.17	15.09	16.66	16.01	17.07	1.39
Chymotrypsinogen						
IU/g of pancreatic tissue	852[a]	440[c]	548[b]	530[b]	575[b]	58
IU/kg of live weight	1,905[a]	1,295[d]	1,604[b]	1,490[c]	1,605[b]	51
α-Amylase						
IU $\times 10^{-3}$/g of pancreatic tissue	26.81[a]	13.02[c]	15.78[b]	17.59[b]	15.59[b]	0.8
IU $\times 10^{-3}$/kg of live weight	60.47[a]	37.54[c]	47.72[b]	49.76[b]	43.51[b]	3.88

[a–d]Means in the same row followed by the different superscript letters are significantly different ($P < 0.05$).

[1]The control diet contained no salseed meal (SSM).

[2]Untreated salseed meal (USSM) or SSM treated with distilled water (pH 5.3), 0.67 *M* acetic acid (pH 2.4), or 0.67 *M* sodium hydrogen carbonate (pH 8.2) and incubated at 37°C for 12 h was used to formulate the 4 treatment diets (USSM, water, acid, and alkali, respectively). The calculated amount of tannin in the USSM, water, acid, and alkali diets was 24.2, 13.7, 15.0, and 8.6 g/kg, respectively.

[3]SEM is among means.

0.05) improved with water and alkali treatments of SSM; however, acid treatment was unable to bring any significant improvement in chymotrypsin activity. Acid treatment of SSM significantly improved ($P < 0.05$) the depressed α-amylase activity in jejunal digesta of broilers fed USSM; however, no significant improvement in α-amylase activity was noticed with water and alkali treatments of SSM.

DISCUSSION

Reduction in protein digestibility or NR with SSM-based diets may be the relative contribution of tannin–enzyme (Mahmood and Smithard, 1993) and tannin–dietary protein interaction (Mahmood et al., 1997). In present study the jejunal trypsin and chymotrypsin activities were significantly depressed with inclusion of SSM in the broiler diet. However, the treatment of SSM with alkali and water significantly improved the protein digestibility (32 and 20%) and NR (30 and 19%) in colostomized hens and broiler cockerels, respectively. The improvement in chymotrypsin activity in the digesta of the broilers fed diets containing alkali-treated or water-treated diets was also comparable to the improvement in the nitrogen digestibility or retention. This improvement was probably due to reduction in tannin content of the treated SSM, which may have spared more nutrients such as protein, starch, and enzymes in the digestive tract of the birds. The degree of tannin–protein complex formation depends upon the ratio of tannin to protein concentration (Schaffert et al., 1974). Feeny and Bostock (1968) reported that tannin completely bound protein when present at a ratio of 1:1. In this investigation the tannin:protein ratios in USSM, water-, acid-, and alkali-treated diets were 1:4, 1:7, 1:6, and 1:11 in colostomized hens, and 1:8, 1:15, 1:13, and 1:24 in broiler cockerels, respectively. In support of the discussion above, the digestibility of protein and NR in this investigation followed almost the same trend as indicated by tannin:protein ratios in the diets.

Lower digestibility values of nutrients with higher levels of tannin compared with low tannin have been reported in chicks (Ahmed et al., 1991), pigs (Buraczewska et al., 1989; Jansman et al., 1989), and rats (Horigome et al., 1988). It is possible that, in the presence of moisture, the tannins are polymerized to larger molecules that are insoluble and lose their ability to precipitate proteins. Evers et al. (1999) reported that tannin molecules comprising more than 10 flavan monomers are either highly insol-

Table 6. Enzyme activities (IU/g of dry chyme) in the jejunal digesta of broilers

Parameters	Control diet[1]	SSM treatment[2]				SEM[3]
		USSM	Water	Acid	Alkali	
Trypsin	1.28[a]	0.19[c]	0.32[bc]	0.25[bc]	0.46[b]	0.10
Chymotrypsin	27.32[a]	8.99[c]	15.23[b]	9.86[c]	18.31[b]	1.27
α-Amylase	251.8[a]	71.7[c]	95.9[bc]	133.3[b]	85.7[bc]	18.8

[a–c]Means in the same row followed by different superscript letters are significantly different ($P < 0.05$).

[1]The control diet contained no salseed meal (SSM).

[2]Untreated salseed meal (USSM) or SSM treated with distilled water (pH 5.3), 0.67 *M* acetic acid (pH 2.4), or 0.67 *M* sodium hydrogen carbonate (pH 8.2) and incubated at 37°C for 12 h was used to formulate the 4 treatment diets (USSM, water, acid, and alkali, respectively). The calculated amount of tannin in the USSM, water, acid, and alkali diets was 24.2, 13.7, 15.0, and 8.6 g/kg, respectively.

[3]SEM is among means.

uble, have few reactive sites, or are too large to fit the protein orientation required for cross linking. Intake of sorghum grains with moisture has been shown to increase the digestibility of sorghum protein in broiler chicks (Mitaru et al., 1983). Perhaps a similar phenomenon occurred in the treated SSM in this sudy, resulting in reduced tannin content and causing a beneficial effect on the digestibility of protein.

Treatment with alkali was more effective than acid or water treatments, which coincides with the degree of improvement in the activities of trypsin and chymotrypsin in the digesta of the broiler cockerels. The reduction in tannin content of the treated meal also paralleled the improvement in the protein digestibility and NR in the birds fed alkali-treated SSM. Sodium bicarbonate may improve the electrolyte balance in the diet, creating favorable conditions for an improvement in apparent protein digestibility (Banda-Nyirenda and Vohra, 1990). Soaking of winged beans (Sathe and Salunkhe, 1981) and seeds of cow peas (Laurena et al., 1986) in alkaline solution has been shown to increase their in vitro protein digestibility. The results of this study are compatible with the findings of Mohammed and Ali (1988), who reported increased digestibility of protein and NR in broiler chicks fed sorghum grains treated with alkaline solution.

Tannins are also known to form complexes with carbohydrates, particularly with starch. Deshpande and Salunkhe (1982) studied the interaction of tannic acid and catechin with starches of different legumes and reported a decrease of 9 to 17% in digestibility of starches (in vitro) due to the formation of tannin–starch complexes. The birds fed diets containing SSM in this experiment showed significantly lower digestibility of starch. The activity of α-amylase was also markedly reduced in the digesta of the tannin-fed birds. Therefore, lower digestibility of starch may be due to the formation of tannin–enzyme or tannin–carbohydrate complexes or both, rendering starch indigestible. These findings are in agreement with the results of Longstaff and McNab (1991), who observed a significant decrease in the starch digestibility in chicks fed tannin from faba beans hulls. However, the decrease in that study was more severe than observed in this study.

Treatment of SSM with acid and water improved starch digestibility in broilers compared with the USSM. A similar trend for the digestibility of starch was observed in colostomized hens. The improvement in the digestibility of starch was proportional to the improvement in the α-amylase activity of broilers consuming acid-treated SSM compared with those fed the USSM diet. The improvement in the digestibility of starch may also be attributed to the decrease in the tannin contents of the treated material resulting in decreased tannin–starch and tannin–enzyme (α-amylase) associations in the digesta of the chicks fed diets containing water- or acid-treated SSM.

In contrast to the reduction in overall digestibility of nitrogen and starch observed in both colostomized and intact broiler cockerels consuming diets containing SSM, there was no significant adverse effect on the digestibility of ether extract. Lipase plays an important role in the digestibility of fat contents of an ingredient. Increased activity of lipase in the intestinal contents of rats due to the dietary inclusion of tannin has been reported by Griffiths (1979) and Horigome et al. (1988). These results indicate that tannins have less affinity for lipase. It is possible that the presence of SSM tannins in the gut did not have any adverse effect on the activity of lipase and subsequently no adverse effect on digestibility of ether extract. A slight inhibition in the activity of lipase in the intestinal contents of chicks due to tannins from field beans and consequently, slightly lower digestibility of lipid, has been reported by Longstaff and McNab (1991).

Although the quality of eggs produced from the colostomized hens was not an objective of the experiment, it was noted that the hens receiving SSM in their diets produced eggs with discolored yolks (dirty greenish-yellow). However, no such effect was observed in the eggs laid by hens fed the SSM-free diet. The reason for the discoloration of the yolks was not investigated. A possible explanation for this may be the absorption of SSM pigment from the diet. Discoloration of the yolk due to the presence of SSM would be an important economic factor to be considered if SSM were to be used in layer rations.

In conclusion, protein and starch digestibility, nitrogen retention, and pancreatic and intestinal enzyme activities were markedly depressed with the inclusion of SSM in the diets of hens and broilers but the effect on digestibility of ether extracts was moderate. Chemical treatments of SSM improved the digestibility of protein and starch as well as nitrogen retention, with the alkali treatment being the most effective. Although the quality of eggs produced from the colostomized hens was not an objective of the experiment, it was noted that the hens receiving SSM in their diets produced eggs with discolored yolks (dirty greenish-yellow). Discoloration of the yolk due to the presence of SSM would be an important economic factor to be considered if SSM were to be used in layer rations.

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