We conclude that surveys of mycotoxins in green coffee beans, as well as other agricultural products, should be continued.

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Autolysis of α-Galactosides of Defatted Soy Flakes: Influence on Nutritive Value for Chickens[1]

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Autolysis of the α-galactosides of defatted soy flakes (soybean meal before toasting), accomplished through in vitro incubation of soy flakes (SF), increased reducing-sugar content from 12.6 to 57.6 mg/g and decreased raffinose and stachyose from 37% of soluble carbohydrates to 5.1%. A reference diet containing 30% soybean meal (SBM) and diets in which incubated SF, unincubated SF, or soy milk (steam-infusion processed SF, 87.5% moisture) replaced all or part of the SBM were fed to broiler chicks. Chicks fed the diet containing soy milk gained more weight than chicks fed the unincubated SF-containing diets. Otherwise, no diet effects on weight gain or feed efficiency were observed. Nitrogen-corrected metabolizable energies determined with chicks and roosters showed that incubation did not significantly change the energy value of SF diets. These results indicate that the conversion of raffinose and stachyose to monosaccharides by incubation did not measurably improve the nutritional qualities of SF for chickens.

Soybean meal (SBM) is an excellent source of dietary protein, but its energy is poorly utilized by poultry. Of the gross energy contained in SBM, poultry digest and metabolize only 52%, due in part to the low digestibility of its carbohydrates (Potter and Potchanakorn, 1985). Soybean meal contains substantial amounts of stachyose, raffinose, and complex polysaccharides that are poorly digested, and these saccharides have been implicated as the causes for the poor utilization of the energy of SBM (Potter and Potchanakorn, 1985). The presence in the small intestine of unabsorbable, water-soluble sugars of low molecular weight, such as raffinose and stachyose, also may result in an osmotic effect, leading to fluid retention and an increased rate of food passage that could adversely affect the absorption of nutrients (Wiggins, 1984).

Hydrolysis of raffinose and stachyose by endogenous α-galactosidase in beans has been shown to occur in vitro under specific conditions (Crocco, 1973; Kon et al., 1973; Becker et al., 1974; Olson et al., 1975). Incubation of ground soybeans in a 0.1 M sodium acetate buffer solution (pH 5.2) for 48 h at 45 °C resulted in an almost complete hydrolysis of raffinose and stachyose and an increase in galactose and sucrose (Olson et al., 1975). Exploitation of this endogenous enzyme to reduce the raffinose and stachyose contents of defatted soy flakes (dehulled SBM before toasting) may provide a means whereby the energy content of soybean meal could be made more available to poultry.

The objectives of the research reported here were to determine a set of incubation conditions that would facilitate autolysis of the raffinose and stachyose present in defatted soy flakes without loss of their constituent monosaccharides or disaccharides and to evaluate the soy flake material obtained from in vitro incubation as a feed ingredient for chickens. In the latter instance, weight gain, feed efficiency, and metabolizable energy values were the criteria used.

EXPERIMENTAL SECTION

Soy flakes (SF) were obtained from Archer-Daniels Midland, Des Moines, IA. The SF contained, on a dry-matter basis, 53.4% protein, 6.8% ash, 0.2% ether extract, and 33.5% neutral detergent fiber.

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Extraction and Analysis of Soluble Sugars. Water-soluble sugars were extracted by the method described by Delente and Ladenburg (1972). Quantitative determination of total soluble sugars and reducing sugars was carried out according to the method described by Gaines (1973), except that the procedure was done without the assistance of automation. The glucose, fructose, galactose, raffinose, and stachyose contents of the water-soluble sugar extracts of SF were determined by gas chromatography (Delente and Ladenburg, 1972; Becker et al., 1974; Sullivan and Schewe, 1977). For this purpose, 1 mL of the water-soluble sugar extract of SF was freeze-dried for 36 h, and the weight of the dried extract was determined. Tri-Sil Z (1.5 mL) (Pierce Chemical Co., Rockford, IL) was added to the extract, and silylation of the sugars was completed by heating the mixture for 90 min at 65 °C. Glucose, fructose, galactose, raffinose, and stachyose contents of the derivatized samples were determined on a Tracor Model 540 gas chromatograph (Tracor Instruments, Austin, TX) equipped with a flame ionization detector. Nitrogen was used as the carrier gas with a flow of 60 mL/min through a stainless-steel column (183-cm length × 0.32-cm diameter) packed with 10% OV-17 on Chromosorb W-HP, 80/100 (Alltech Associates, Inc., Deerfield, IL). A mixture of hydrogen, 30 mL/min, and oxygen, 400 mL/min, was used as detector gas. Injection and detector temperatures were 320 and 350 °C, respectively. The following temperature program was used: initial temperature 140 °C, held for 5 min, increased at 5 °C/min to 165 °C, at which time the rate of temperature increase was changed to 11 °C/min until 315 °C. This final temperature was held for 6 min. The injection volume was 4 mL. Peaks were identified by comparing retention times for reagent-grade glucose, fructose, galactose, raffinose, and stachyose, all derivatized and analyzed in the same manner as the sample extracts. The area under each sugar peak was recorded as a percentage of the total area under known peaks.

Incubation of SF and Preparation of Test Ingredients. Procedures described by Kon et al. (1973), Becker et al. (1974), Olson et al. (1975), and del Campillo (1980) served as the basis for establishing conditions for inducing autolysis of the oligosaccharides in SF. Numerous in vitro tests, however, were undertaken to more precisely determine the incubation temperature, time, pH, and type of incubation solution that would result in maximal hydrolysis of oligosaccharides without loss of their constituent monosaccharides. The conditions selected on the basis of gas chromatographic analysis of products obtained as a result of incubation were as follows: incubation for 24 h at 45 °C of a mixture of SF and a 0.1 M sodium acetate buffer solution (1 g of SF/1.6 mL of buffer solution) containing 1% potassium sorbate and 0.75 mL of concentrated HCl/100 mL of buffer solution, pH 4.6.

The SF were mixed with the buffer solution for 5 min in a Hobart A-200 mixer. The mixture was placed in aluminum trays and covered with aluminum foil. Part of the mixture was incubated in a forced-draft oven for 24 h at 45 °C and then autoclaved at 110 °C (1074 mmHg) for 45 min. The resulting material constituted the test ingredient buffer-added-incubated SF. The rest of the mixture was autoclaved immediately after mixing to prevent hydrolysis of oligosaccharides and to reduce the trypsin inhibitor content of SF. The material obtained constituted the test ingredient buffer-added-unincubated SF. A third test ingredient, water-added-unincubated SF, was made by mixing SF and water (1 g of SF/1.6 mL of distilled water) and autoclaving immediately after mixing.

Table I. Composition and Calculated Analysis of the SBM Reference Diet

ingredients	compn, %
reground corn (8.6% protein)	55.04
reground SBM (47.4% protein)	30.24
corn gluten meal (61.2% protein)	5.68
limestone	1.44
dicalcium phosphate	1.76
vitamin premix[a]	0.32
salt premix[b]	0.28
DL-methionine (98%)	0.12
L-lysine hydrochloride	0.08
chromic oxide	0.24
soybean oil	4.80
total	100.00
calcd anal. (100% DM)	
dry matter, %	90.67
metabolizable energy, kcal/kg	3536.00
crude protein, %	25.30
total sulfur amino acids, %	1.03
lysine, %	1.30
calcium, %	1.10
available phosphorus, %	0.50

[a] Supplied the following per kilogram of diet: vitamin A, 5000 IU; vitamin D_3, 1500 IU; vitamin E, 12 IU; vitamin B_{12}, 11 g; vitamin K, 1.8 mg; riboflavin, 2.7 mg; pantothenic acid, 7 mg; niacin, 75 mg; choline, 509 mg; folic acid, 0.5; biotin, 75 g. [b] Supplied the following per kilogram of diet: manganese, 70 mg; zinc, 40 mg; copper, 6 mg; selenium, 0.15 mg; sodium chloride, 2.60 g.

A fourth test ingredient, soy milk was prepared by the method described by Johnson et al. (1981). This method involved forcing a SF–water mixture (12.5% dry matter) through a laboratory jet cooker by using steam infusion, during which a short-term temperature of 134.5 °C was achieved. A trypsin inhibitor assay (Hamerstrand et al., 1981) was done on SBM and on all SF test ingredients. The results showed that the trypsin inhibitor content of all test ingredients was less than 7% of that in the unautoclaved SF. Samples from all test ingredients were stored in sealed containers at −4 °C. The test ingredients were stored at 4 °C for a maximum of 4 days before being mixed into the diets.

Chick and Rooster Feeding Experiments. *Experiment 1.* The main objective of this experiment was to evaluate incubated SF on the basis of growth and feed efficiency of chicks. One-day-old (Hubbard × Hubbard) male broiler chicks were placed in electrically heated, battery brooders and were fed for 7 days a SBM reference diet (Table I) formulated to meet or exceed National Research Council (1984) nutrient level recommendations. On day 7, five chicks were placed in each of 35 brooder battery pens. Each group was of approximately the same weight. Seven diets were randomly assigned, each to five pens. Feed and water were provided ad libitum. The temperature in the brooders was maintained at 35 and 32.2 °C for the first and second weeks, respectively. Chicks were weighed by pen group, and feed consumption was recorded on days 7 and 14 of the experiment.

Seven experimental broiler starter diets were formulated, differing only in the soybean product contained. All or part of the SBM in the SBM reference diet (Table I) was replaced (on a dry-matter basis) by one of the following SF test ingredients: water-added-unincubated SF (30% of the diet), buffer-added-unincubated SF (30% of the diet), buffer-added-incubated SF (30, 20, or 10% of the diet), or soy milk (15% of the diet). The test ingredients were mixed into the diets in the "wet form" obtained from laboratory processing. The mixed diets were spread out on freezer wrap paper and air-dried for 72 h, except for

Table II. Influence of Incubating Defatted Soy Flakes on Concentration and Composition of Water-Soluble Sugar

	reducing sugar content, mg/g		relative peak area, %						rel index of oligosaccharide/ monosaccharide ratio[a]
	before HCl hydrol	after HCl hydrol (total)	fructose	galactose	glucose	sucrose	raffinose	stachyose	
raw SF	12.6	122	15.0	1.1	3.3	42.4	13.3	24.8	100
water-added-unincubated SF	12.4	119	14.4	1.3	4.7	38.9	12.9	26.1	92
buffer-added-unincubated SF	11.5	126	14.5	1.8	5.6	39.7	14.8	23.5	86
buffer-added-incubated SF	57.6	112	32.6	15.9	30.6	15.5	3.3	1.8	6
soy milk	5.6	53	17.0	nd[b]	5.9	36.7	13.2	27.2	81

[a] Relative index obtained by calculating the ratio of oligosaccharide to monosaccharide peak areas and then assigning a value of 100 to the ratio obtained for the raw SF. Ratios for other treatments were expressed relative to this value. [b] None detected.

the soy milk diet which was freeze-dried for 72 h and then air-dried for 24 h. After drying, the diets were ground to pass through a 6-mm screen.

Experiment 2. This experiment was done to determine the nitrogen-corrected metabolizable energy (ME_n) (Hill and Anderson, 1958) of the ingredients tested in experiment 1. Ten-day-old (Hubbard × Hubbard) male broiler chicks were selected on the basis of body weight from a group of chicks that had been fed the SBM reference diet (Table I) and were placed in individual cages. Five diets were randomly assigned, each to 5 of 25 cages. Test ingredients and diets were prepared as in experiment 1, and only diets containing 30% of the test ingredients were evaluated. The inclusion level of soy milk was increased to 30%. After a 5-day adjustment period the chicks were fasted for 3 h, after which individually preweighed excreta collection trays were placed under each cage and the chicks were fed the test diet ad libitum for 3 days. Feed consumption was measured and recorded daily. Excreta collection trays were replaced three times during the 3-day collection period, at 24-h intervals. At the end of day 3, feed was removed from the chicks and excreta collection terminated 4 h later. Collection trays were cleaned of feathers and spilled feed. Excreta were dried at 90 °C for 48 h, and the dry weight was recorded. Diet and excreta samples were analyzed for gross energy by using an adiabatic bomb calorimeter and for nitrogen content by the macro-Kjeldhal method (AOAC, 1980, section 2.057). Feed consumption, weight of excreta, gross energy, and nitrogen content data were used to determine ME_n (all values were on a dry-matter basis).

Experiment 3. White Leghorn roosters were used to determine nitrogen-corrected true metabolizable energy (TME_n) according to the method described by Sibbald (1986). The same diets used in experiment 2, except for the soy milk diet, were fed in experiment 3. Each diet was assigned randomly, to eight roosters.

Data obtained from all experiments were analyzed statistically by analysis of variance. Where applicable, regression analysis was done (Snedecor and Cochran, 1980). Differences between treatments were tested by Tukey's multiple-range test (Snedecor, 1956). The SAS statistical program (Barr et al., 1982) was used for this purpose.

RESULTS AND DISCUSSION

Water-Soluble Sugar in SF. The analysis of reducing sugar content of the SF samples showed that, after incubation, 5 times as much reducing sugar was present as compared with the unincubated SF samples, whereas total soluble sugar content was not changed by incubation (Table II). Qualitative analysis by gas chromatography of the soluble sugars of SF before and after incubation showed that a large shift occurred in the types of saccharides present as a consequence of incubation (Table II). The percentage peak area for raffinose and stachyose in the soluble sugars extracted from unincubated SF ingredients ranged from 38.9 to 42.4%, and total fructose, galactose, and glucose areas were 19.4–21.9%. Incubation reduced the total raffinose and stachyose area to 5.1% and increased the monosaccharide area to 79.1%. It should be emphasized that the peak areas are qualitative; i.e., the relationship between the peak area and weight of each respective sugar was not determined. However, a relative index obtained by calculating the ratio of oligosaccharide to monosaccharide peak areas, where the ratio obtained for raw SF was assigned an index value of 100 and the ratios for other ingredients were expressed relative to this value, further illustrates the effectiveness of the 24-h incubation in reducing the proportion of oligosaccharides in the soluble sugars of SF. The index value for buffer-added-unincubated SF was 86, and that for buffer-added-incubated SF was 6. These results agree with those of Olson et al. (1975), who incubated ground soybeans under similar conditions and found that only trace amounts of raffinose and stachyose remained after 48 h of incubation. The hydrolysis of raffinose and stachyose probably was the result of the activity of α-galactosidase present in soybeans (del Campillo, 1980). Activity of this enzyme in untoasted SF would be expected in a medium with pH 4.6 and kept at a temperature of 45 °C (Crocco, 1973; Olson et al., 1975). Incubation also reduced the sucrose content of the soluble sugars, probably as a result of sucrase activity, which has been found in soybeans (Kennedy et al., 1985).

Total sugar and reducing sugar contents of soy milk were approximately half of those found in raw SF (Table II). This reduction in soluble sugars brought about by steam-infusion processing of raw SF did not seem to result in a loss of specific sugars; the relative peak areas of glucose, fructose, galactose, raffinose, and stachyose, measured by gas chromatography, were similar in the raw SF and soy milk (Table II). An explanation for the reduction in total water-soluble sugar content of SF as a consequence of steam-infusion processing is not evident. The loss of soluble sugars during steam-infusion processing was unexpected and cannot be explained on the basis of research reported here. A portion of the loss (i.e., loss of reducing sugars) could have resulted from Maillard reactions. However, a more complete explanation will require further research.

Chick and Rooster Experiments. Hypothetically, the reduction in raffinose and stachyose content of SF through in vitro incubation should have improved the nutritional quality of this material. Energy availability should have been increased because much of the indigestible raffinose and stachyose was converted to digestible monosaccharides. Also, decreasing the raffinose and stachyose content of SF should have reduced the oligosaccharide × nutrient interactions, which reportedly have adverse effects on nutrient utilization by rats (Wagner et al., 1976). In general, however, the data obtained from the chick and

Table III. Weight Gain and Feed Efficiency of Chicks Fed Diets Containing Unincubated or Incubated Soy Flakes (SF), Experiment 1

test material	level of dietary inclusion	from 7 to 14 days of age[a] wt gain/chick, g	feed/gain
soybean meal (47.4% protein)	30	154^{xy}[b]	1.31
water-added-unincubated SF	30	140^{x}	1.34
buffer-added-unincubated SF	30	145^{x}	1.32
buffer-added-incubated SF	30	152^{xy}	1.37
buffer-added-incubated SF	20	146[c]	1.37
buffer-added-incubated SF	10	158[c]	1.36
soy milk	15	168^{y}	1.26
std error of means		4	.03

[a] Data represent means of five pens of five chicks each for each diet treatment. [b] Means followed by different superscript letters (x, y) are different ($P < 0.05$) on the basis of Tukey's multiple-range test. [c] There was no effect of level of inclusion of buffer-added-incubated SF on weight gain as determined by regression analysis. Therefore, the diet containing 30% buffer-added-incubated SF was the only one included in Tukey's multiple-range test.

Table IV. Metabolizable Energy (ME_n) and True Metabolizable Energy (TME_n) of Diets Containing Unincubated and Incubated Soy Flakes (SF)

test material[a]	ME_n of diets for chicks, kcal/kg, experiment 2[b]	TME_n of diets for roosters, kcal/kg, experiment 3[c]
soybean meal (47.4% protein)	3425^{xy}[d]	3685[a]
water-added-unincubated SF	3513^{y}	3729[a]
buffer-added-unincubated SF	3454^{xy}	3814[ab]
buffer-added-incubated SF	3495^{y}	3894[b]
soy milk	3383^{x}	not done
std error of means	23	45

[a] Test material constituted 30% of the diet dry matter. [b] Each mean represents five chicks. [c] Each mean represents eight roosters. [d] Means followed by different superscript letters (x, y) are different ($P < 0.05$) on the basis of Tukey's multiple-range test.

rooster bioassays did not support the hypothesis. Species differences (chickens vs rats) may have contributed to these seemingly conflicting results.

In experiment 1, weight gain responses to different levels of dietary inclusion of buffer-added-incubated SF were inconsistent and were not different ($P > 0.05$) from the weight gain of chicks fed the SBM reference diet (Table III). Also, weight gain of chicks fed the diet containing 30% buffer-added-incubated SF did not differ ($P > 0.05$) from that of chicks fed buffer-added-unincubated SF. The weight gain of chicks fed the diet containing 15% soy milk was numerically greater than that of any other treatment group, but the improved weight gain was significant only when compared with that of chicks fed the diets containing water-added-unincubated SF and buffer-added-unincubated SF. No significant differences were observed among treatment groups in kilograms of feed consumed per kilogram of weight gain (feed efficiency).

The results of experiment 2 showed that, in general, the ME_n values of diets containing SF ingredients were numerically greater than that of the SBM reference diet, irrespective of incubation treatment (Table IV). There were no significant differences between diets containing different SF ingredients. These data indicate that incubation did not have an effect on the ME_n of SF. These results, together with the growth data from experiment 1, which showed that incubation had little, if any, effect on feed efficiency, indicate that the hydrolysis of oligosaccharides to monosaccharides induced by incubation did not improve the general nutritional qualities of SF for chicks.

The lowest ME_n determined in experiment 2 was for the diet containing 30% soy milk (Table IV). Although this value was not different ($P > 0.05$) from that of the SBM reference diet, it was significantly lower than the ME_n values obtained for the diets containing water-added-unincubated SF and buffer-added-incubated SF. The low ME_n value may be due in part to the loss of half the total soluble sugars in SF during the steam-infusion process used to make soy milk. However, the lower ME_n content of soy milk in experiment 2 contrasts somewhat with the improved weight gain of chicks fed a diet containing 15% soy milk in experiment 1. These results suggest that although steam-infusion processing of soy milk reduced soluble sugar content and ME_n, other changes may have improved certain nutritional properties of this material. Additional research is needed to more adequately evaluate the nutritional value of SF processed by steam infusion.

In experiment 3, TME_n values of the diets were determined with adult roosters (Table IV). There was little difference between the TME_n of the SBM reference diet and that of the diet containing water-added-unincubated SF. However, the diet containing buffer-added-incubated SF had a significantly higher TME_n as compared with the diet containing water-added-unincubated SF. The TME_n value of the buffer-added-unincubated SF-containing diet was intermediate between the TME_n values obtained with the other two SF diets. This suggests that incubation increased the TME_n of SF, but part of this increase was due to the presence of the buffer. That is, the buffer solution seems to have increased the TME_n of SF, yet this increase became significant only when the buffer-added SF was incubated.

CONCLUSIONS

The utilization of SF by young chicks was not improved by using inherent enzymes to convert raffinose and stachyose to monosaccharides. Changes in TME_n of SF for adult chickens, as a result of raffinose and stachyose conversion, indicated only a slight favorable effect. In general, these results suggest that the poor energy utilization from soybean meal (toasted-defatted soy flakes) by poultry is not related exclusively to the presence of the oligosaccharides, raffinose, and stachyose.

ABBREVIATIONS USED

Defatted soy flakes, SF; soybean meal, SBM; nitrogen-corrected metabolizable energy, ME_n; nitrogen-corrected true metabolizable energy, TME_n.

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Registry No. Raffinose, 512-69-6; stachyose, 470-55-3; fructose, 57-48-7; galactose, 59-23-4; glucose, 50-99-7; sucrose, 57-50-1.

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Effect of Aldehyde Trapping Agents on Ethylene and Aflatoxin Biogenesis in *Aspergillus parasiticus*

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Dimedone, an aldehyde trapping agent, was found to stimulate ethylene evolution while suppressing aflatoxin biogenesis in *Aspergillus parasiticus*. Dimedone did not seem to affect the growth of the fungus. Potassium metabisulfite, another aldehyde trapping agent, was also found to be an effective inhibitor of aflatoxin biogenesis. Unlike dimedone, however, this compound inhibited the growth of the fungus. Stimulation of ethylene evolution by aldehyde trapping agents shows that ethanol is not the precursor of ethylene in fungi.

The mechanisms that trigger biosynthesis of secondary metabolites are still obscure. Ethylene has been shown to inhibit the biogenesis of aflatoxin and other secondary metabolites (Sharma et al., 1985; Sharma and Padwal-Desai, 1986). Though L-methionine is the precursor of ethylene in plants, the precursor of ethylene in microbes is yet to be established. Ethanol was earlier presumed to be the precursor of microbial ethylene (Abeles, 1973). However, recently α-ketoglutaric acid was shown to be converted to ethylene in a cell-free preparation of *Penicillium digitatum* (Fukuda et al., 1986). Aldehyde trapping agents could be employed to test the earlier hypothesis. Acetaldehyde is the known precursor of ethanol. Therefore, if ethanol were the precursor of ethylene, aldehyde trapping agents should suppress the synthesis of ethylene and hence enhance aflatoxin formation in cultures. The validity of this presumption has been tested in the present report using dimedone (5,5-dimethyl-1,3-cyclohexanedione) and metabisulfite, the two well-known aldehyde trapping agents.

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EXPERIMENTAL SECTION

Organism and Culture Conditions. Aflatoxin-producing strains of *Aspergillus parasiticus* NRRL 3145, NRRL 2999, and ATCC 15517 were used in these studies. The cultures were maintained on potato dextrose agar (Difco Laboratories, Detroit, MI), and regular transfer to fresh slants was carried out at 1-month intervals.

Preparation of Spore Suspension. The recultivation of stock cultures of the above fungi and the technique of preparation of spore suspension have been described earlier (Sharma et al., 1984). A synthetic growth medium containing 20% (w/v) of glucose and salts was used. For inoculations, 1-mL aliquots of the spore suspension containing 10^5 spores were added to the flasks containing 50 mL of growth medium.

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