Nutritional Evaluation of Soybeans Varying in Trypsin Inhibitor Content[1]

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ABSTRACT A series of experiments was conducted to assess, by various methods, the nutritional value of a raw, Kunitz inhibitor-free, low trypsin-inhibitor soybean variant (LTS) in comparison with raw conventional soybeans (RCS) and heated dehulled soybean meal (HDS). The gross energy, protein, and amino acid concentrations of LTS were similar to those of RCS. The protein quality of the soybeans was compared in two trials in which young chicks were fed 9% or 16% CP diets containing one of the soybeans as the sole source of dietary protein. The protein quality of LTS was superior to that of RCS but inferior to that of HDS. True digestibilities of amino acids and TME_n were determined with a precision-fed cockerel assay using conventional and cecectomized birds. No significant effect of bird type on digestibility was found. The mean digestibility of 16 amino acids in RCS, LTS, and HDS was found to be approximately 68, 83, and 92%, respectively. The TME_n of LTS was greater than that of RCS. Chick growth assays indicated that amino acid bioavailability values of lysine and TSAA for LTS were greater than those for RCS but slightly lower than those for HDS.

In an additional chick assay with a corn and HDS diet (22% CP), the dietary HDS protein was replaced with LTS or RCS protein at 25, 50, 75, or 100%. Performance of chicks fed LTS was better than that of chicks fed RCS at all replacement levels, but the difference was not significant at the 25% replacement level. Feed efficiencies of chicks fed 25 or 50% of the dietary soybean protein as LTS were not significantly different from that of chicks fed the corn and HDS control diet. The results of the present study indicated that the nutritive value of LTS is substantially greater than that of RCS but somewhat lower than that of HDS.
(*Key words*: soybeans, trypsin inhibitor, protein quality, amino acid, chicken)

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INTRODUCTION

The detrimental effects on animal performance of trypsin inhibitors in raw soybeans have been extensively studied. Raw soybeans are nutritionally inferior to properly heated soybeans for rats (Osborne and Mendel, 1917; Lyman and Lepkovsky, 1957), chicks (Chernick *et al.*, 1948; Yen *et al.*, 1973) and pigs (Yen *et al.*, 1974; Cook *et al.*, 1988). The trypsin inhibitors in raw soybeans depress proteolytic activity in the small intestine, resulting in decreased release of free amino acids (AA). In addition, trypsin inhibitors cause pancreatic hypertrophy in rats and chicks due to stimulation of pancreatic secretions. The excess digestive enzymes subsequently secreted by the pancreas are largely undigested, resulting in increased AA excretion and deficiencies (Liener, 1977). Therefore, raw soybeans must be properly cooked in order to be maximally utilized by animals.

The Kunitz trypsin inhibitor is one of the major growth-inhibiting antinutritional factors present in raw soybeans (Rackis, 1965). A soybean variant low in Kunitz trypsin inhibitor activity was identified (Singh *et al.*, 1969; Clark and Hymowitz, 1972), and early studies showed that this variant was nutritionally superior to conventional commercial cultivars (Yen *et al.*, 1973). Recently, a new soybean variant has been developed at the University of Illinois that is isogenic to the commercially grown Williams 82 cultivar except that it lacks the Kunitz trypsin-inhibitor allele (Bernard and Hymowitz, 1986). Cook *et al.* (1988) reported that this variant was nutritionally superior to conventional raw soybeans for growing and finishing pigs. This new soybean variant might also be nutritionally and economically impor-

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tant to the poultry industry. Thus, the objective of the present study was to evaluate, by various methods, and compare the protein quality of the raw Kunitz inhibitor-free, low-trypsin-inhibitor Williams soybeans (LTS) with protein quality of raw conventional Williams-82 soybeans (RCS) and heated solvent-extracted dehulled soybean meal (HDS). Methods of evaluation included chick protein quality assays, chick growth assays for bioavailable AA, and an excreta collection assay with conventional and cecectomized cockerels to determine true AA digestibility and TME_n.

MATERIALS AND METHODS

Soybean Sources

The HDS was purchased from a local commercial processing plant. The RCS and LTS were obtained in two batches from the Department of Agronomy, University of Illinois and Illinois Foundation Seeds, Inc., Champaign, IL. Soybeans from the first batch were used in chick Experiments 1, 2, 4, and 5 and in the true digestibility assay. Soybeans from the second batch were used in chick Experiment 3. The RCS and the LTS were an isoline of Williams 82 (L81-4590) that was lacking Kunitz trypsin-inhibitor activity. In 1990, L81-4590 was released for commercial production under the name Kunitz. All soybeans were ground to a similar particle size and subjected to proximate analysis for DM, N, ash, and gross energy [Association of Official Analytical Chemists (AOAC, 1980)]. Trypsin-inhibitor activity was measured according to the procedure of Hamerstrand *et al.* (1981), and urease activity was determined by the AOAC (1980) method. Amino acid concentrations were analyzed (Spackman *et al.*, 1958) on each sample with at least two replicates per sample. Analyses of Met and Cys were performed separately after performic acid oxidation by the method of Moore (1963), except the excess performic acid was removed by lyophilization after dilution with water.

Chick Assays

One-week-old male chicks resulting from the cross of New Hampshire males and Columbian Plymouth Rock females were used in six chick assays. The chicks were fed a 24% CP corn and soybean meal pretest diet during the first 7 days posthatching. Following an overnight fast, chicks were weighed, wing-banded, and allotted to dietary treatment groups so that average body weights of chicks in each group were similar (Sasse and Baker, 1974). Triplicate groups of five or seven chicks were assigned to each dietary treatment, and the duration of each assay varied from 7 to 14 days. All of the chicks were housed in thermostatically controlled starter batteries with raised wire floors in an environmentally regulated room. Feed and water were supplied for *ad libitum* access, and uniform light was provided 24 h daily.

The first two chick experiments were conducted to determine and compare protein quality of soybean sources. In Experiment 1, chicks were fed a N-free basal diet (Willis and Baker, 1980) or the basal diet supplemented with one of the soybean sources to provide 9% dietary CP. The soybean sources were supplemented to the basal diet at the expense of cornstarch and dextrose. Protein efficiency ratio (PER) was calculated (in grams) as gain divided by CP intake. Net protein ratio (NPR) was calculated as [weight gain – weight gain of chicks fed N-free diet] divided by CP intake. Experiment 2 evaluated protein quality of the soybean sources

TABLE 1. *Composition of diets used in Experiment 2 for assessing protein quality of heated dehulled soybean meal (HDS), raw conventional soybeans (RCS), and raw low-trypsin-inhibitor soybeans (LTS)*[1]

Ingredient	HDS	RCS	LTS
		(%)	
Soybean source	33.76	44.56	42.33
Cornstarch	27.74	21.94	24.08
Dextrose	26.50	26.50	26.50
Corn oil	5.0	. . .	. . .
Mineral premix[2]	5.3	5.3	5.3
$NaHCO_3$	1.0	1.0	1.0
Choline·Cl	.2	.2	.2
Vitamin premix[3]	.2	.2	.2
DL-methionine	.3	.3	.3

[1]Each diet contained 16% CP.

[2]Mineral mix provided per kilogram of diet: $CaCO_3$, 30.0 g; $Ca_3(PO_4)_2$, 28.0 g; K_2HPO_4, 9.0 g; NaCl, 8.8 g; $MgSO_4·7H_2O$, 3.5 g; $MnSO_4·H_2O$, .65 g; ferric citrate, .5 g; $ZnCO_3$, .1 g; $CuSO_4·5H_2O$, 20.0 mg; H_3BO_3, 9.0 mg; $NaMoO_4·2H_2O$, 9.0 mg; KI, 40.6 mg; $CoSO_4·7H_2O$, 1.0 mg; Na_2SeO_3, .215 mg.

[3]Supplied the following amounts per kilogram of diet: vitamin A, 4,400 IU; vitamin D_3, 1,000 ICU; vitamin E, 11 IU; vitamin B_{12}, .011 mg; riboflavin, 4.4 mg; D-pantothenic acid, 10 mg; niacin, 22 mg; menadione sodium bisulfate complex, 2.33 mg.

TABLE 2. *Composition of diets used in Experiment 3*[1]

Ingredient	100 HDS[2]	75 HDS:25 RCS	75 HDS:25 LTS	50 HDS:50 RCS	50 HDS:50 LTS	25 HDS:75 RCS	25 HDS:75 LTS	100 RCS	100 LTS
	(%)								
HDS	34.58	25.94	25.94	17.29	17.29	8.64	8.64	. . .	. . .
RCS	. . .	11.36	. . .	22.73	. . .	34.10	. . .	45.46	. . .
LTS	. . .	. . .	12.05	. . .	24.10	. . .	36.15	. . .	48.20
Ground corn (8.8% CP)	43.70	43.70	43.70	43.70	43.70	43.70	43.70	43.70	43.70
Fish meal (61% CP)	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Alfalfa meal (17.5%)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Soybean oil	8.63	6.93	6.72	5.23	4.81	3.54	2.91	1.84	1.00
Cornstarch	2.08	1.67	1.56	1.26	1.04	.85	.52	.46	. . .
Dicalcium phosphate	2.20	2.20	2.20	2.20	2.20	2.20	2.20	2.20	2.20
Limestone	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
NaCl	.4	.4	.4	.4	.4	.4	.4	.4	.4
DL-methionine	.20	.20	.20	.20	.20	.20	.20	.20	.20
Choline-Cl	.10	.10	.10	.10	.10	.10	.10	.10	.10
Trace-mineral premix[3]	.05	.05	.05	.05	.05	.05	.05	.05	.05
Vitamin premix[4]	.10	.10	.10	.10	.10	.10	.10	.10	.10
Flavomycin	.05	.05	.05	.05	.05	.05	.05	.05	.05
Sand	1.96	1.65	1.47	1.35	.98	1.04	.49	.72	. . .
Wood cellulose	1.96	1.65	1.47	1.35	.98	1.04	.49	.72	. . .

[1]All diets calculated to contain 22% total CP, 16.8% soybean CP and 8.4% digestible soybean oil using analytical values for the soybeans; CP (%), 48.47 for heated dehulled soybean meal (HDS), 36.92 for raw conventional soybeans (RCS) and 34.85 for raw Kunitz-free, low trypsin-inhibitor soybean meal (LTS); ether extract (%) 2.36 for HDS, 19.71 for RCS and 20.68 for LTS. The 100 HDS diet calculated to contain 3,125 kcal ME_n/kg.

[2]Numbers indicate percentages of dietary protein supplied by HDS, RCS, or LTS.

[3]Provided the following amounts per kilogram of diet: manganese, 75 mg; iron, 75 mg; zinc, 75 mg; copper, 5 mg; iodine, .75 mg; selenium, .1 mg.

[4]Supplied the following amounts per kilogram of diet: vitamin A, 4,400 IU; vitamin D_3, 1,000 ICU; vitamin E, 11 IU; vitamin B_{12}, .011 mg; riboflavin, 4.4 mg; d-pantothenic acid, 10 mg; niacin, 22 mg; menadione sodium bisulfate complex, 2.33 mg.

TABLE 3. *Composition of heated dehulled soybean meal (HDS), raw conventional soybeans (RCS), and raw low-trypsin-inhibitor soybeans (LTS) evaluated in Experiments 1, 2, 4, and 5 and the true digestibility assay*[1]

Component	HDS	RCS	LTS
Moisture, %	11.8	8.6	9.2
Crude protein, %	47.4	35.9	37.8
Ether extract, %	2.4	20.5	18.7
Ash, %	6.3	5.0	4.6
Gross energy, kcal/g	4.219	5.244	5.181
Trypsin inhibitor activity, mg/g[2]	2.9	19.4	5.6
Urease activity, pH change	.12	2.04	2.11
Amino acid, %			
Aspartic acid	5.30 (11.2)[3]	3.87 (10.8)[3]	4.04 (10.7)[3]
Threonine	1.90 (4.0)	1.33 (3.7)	1.39 (3.7)
Serine	2.48 (5.2)	1.81 (5.0)	1.89 (5.0)
Glutamic acid	8.36 (17.6)	6.10 (17.0)	6.54 (17.3)
Proline	2.47 (5.2)	1.88 (5.2)	1.81 (4.8)
Glycine	2.40 (5.1)	2.02 (5.6)	2.13 (5.6)
Alanine	2.02 (4.3)	1.49 (4.2)	1.58 (4.2)
Cystine	.76 (1.6)	.56 (1.6)	.53 (1.4)
Valine	2.11 (4.4)	1.52 (4.2)	1.54 (4.1)
Methionine	.68 (1.4)	.49 (1.4)	.51 (1.3)
Isoleucine	2.06 (4.3)	1.46 (4.1)	1.46 (3.9)
Leucine	3.72 (7.8)	2.71 (7.5)	2.82 (7.5)
Tyrosine	1.53 (3.2)	1.12 (3.1)	1.16 (3.1)
Phenylalanine	2.34 (4.9)	1.70 (4.7)	1.76 (4.7)
Histine	1.19 (2.5)	.89 (2.5)	.94 (2.5)
Lysine	3.16 (6.7)	2.35 (6.5)	2.37 (6.3)
Arginine	3.38 (7.1)	2.45 (6.8)	2.66 (7.0)

[1]Data are on an air-dry basis except for trypsin inhibitor.

[2]Dry matter and ether-extracted basis.

[3]Values in parentheses are amino acid concentrations expressed as a percentage of the crude protein.

when included at a higher dietary CP level and with supplementation of Met. The composition of the diets is shown in Table 1. Diets were formulated to contain 16% CP solely from one of the soybean sources. All diets were supplemented with .3% synthetic DL-Met to ensure that Met and Cys were not first-limiting for growth. Corn oil was added to the diets containing HDS to reduce differences in lipid content among the diets.

Experiment 3 was a soybean replacement assay wherein the nutritional value of the soybean sources were compared when included at various levels in a practical corn and soybean meal diet. The composition of the nine experimental diets is presented in Table 2. The control diet was calculated to contain 22% CP and 3,125 kcal ME_n/kg. A portion of the soybean protein in the control diet was replaced with RCS or LTS at 25, 50, 75, and 100%. The levels of soybean oil, cornstarch, sand, and cellulose were changed accordingly to maintain all diets as isonitrogenous. The diets were similar in carbohydrate, fiber, and digestible soybean oil content. For the latter, it was assumed that the extracted, refined oil was 90% digestible and the intact-seed oil was 75% digestible based on the study by Adams and Jensen (1984).

A purified crystalline AA basal diet (Baker *et al.*, 1979) was used to determine bioavailability in the soybean sources of Lys in Experiment 4, of Met in Experiment 5, and of TSAA in Experiment 6. Graded levels of the test AA were added to a basal diet deficient in the test AA to produce a standard growth curve. Increasing percentages of the soybean sources were added to the basal diet to provide amounts of AA potency that would fall within the boundaries of the reference growth curve. In the assay for TSAA, the basal diet was deficient in both Cys (.125%) and Met (.125%). For determining bioavailability of Met per se, the basal diet was Cys-adequate (.30%) and Met-deficient (.13%). The soybean sources were added to the basal diet in place of cornstarch. Refined soybean oil was added along with HDS to approximate the digestible soybean oil levels supplied by the LTS and RCS.

TABLE 4. *Determination of protein quality of heated dehulled soybean meal (HDS), raw conventional soybeans (RCS), and raw low-trypsin-inhibitor soybeans (LTS) in Experiment 1*[1]

Treatment[2]	Weight gain	Gain:feed	PER[3]	NPR[3]
	(g)	(g:g)		
Basal (N-free)[4]	-7.2^{d}	$-.104^{d}$	. . .	. . .
Basal plus HDS	55.3^{a}	$.343^{a}$	3.81^{a}	4.31^{a}
Basal plus RCS	12.5^{c}	$.121^{c}$	1.21^{c}	2.16^{c}
Basal plus LTS	27.5^{b}	$.227^{b}$	2.52^{b}	3.18^{b}
Pooled SEM	2.3	.016	.19	.15

[a–d]Means within columns with no common superscripts differ significantly (P<.05).

[1]Means of three groups of five male chicks from 8 to 17 days posthatching.

[2]Each soybean source supplied 9% CP.

[3]PER [protein efficiency ratio (in grams)] = weight gain divided by protein intake. NPR [net protein ratio (in grams)] = [weight gain of birds fed a soybean diet minus weight gain of birds fed the N-free diet] divided by protein intake.

[4]Same as that used in protein quality assay II by Willis and Baker (1980).

True Digestibility Assay. Conventional (CONV) and cecectomized (CEC) Single Comb White Leghorn cockerels, 40 wk of age, were used to determine true digestibility of AA and TME_n in the various soybean sources. The birds were kept in individual cages with raised wire floors in an environmentally regulated room and provided with 16 h of light daily. Feed and water were supplied for *ad libitum* access before the onset of the experiment. Cecectomy was performed at 20 wk of age according to the procedure of Parsons (1985). Previous work in the authors' laboratory indicated that little or no cecal regrowth occurred in CEC cockerels within one year following the surgery (Parsons, 1985; Parsons, unpublished data). The CONV cockerels received a sham operation within a similar time period. The digestibility assay was the same as that described by Sibbald (1979), with some minor modifications. Following a 24-h fast, five CONV and five CEC cockerels were given 30 g of a test feedstuff via crop intubation. Five additional cockerels of each type were fasted throughout the experimental period to measure endogenous excretion of DM, energy, N, and AA. A plastic tray was placed under each cage, and excreta were collected for 48 h after crop intubation. Excreta samples were lyophilized, weighed, and ground to pass through a 60-mesh screen. Gross energy, N, and AA concentrations of each sample were analyzed by the procedures described previously.

Statistical Calculations and Analyses

True digestibilities were calculated for AA according to the method of Sibbald (1979) and for TME_n by the method of Parsons *et al.* (1982). Statistical significance of differences in dry matter, TME_n, and true AA digestibility values were assessed with the Student's *t* test (Steel and Torrie, 1980).

Data from chick growth assays were subjected to analysis of variance procedures for completely randomized designs (Steel and Torrie, 1980). The least significant difference test based on the pooled SE from the analysis of variance was used for comparing treatment means. Slope-ratio methodology (Finney, 1978) was used to estimate bioavailability of AA in Experiments 4 to 6. Multiple regression equations were calculated with chick weight gain (in grams) as the dependent variable and the intake of supplemental test crystalline AA and feed-

TABLE 5. *Effects of heated dehulled soybean meal (HDS), raw conventional soybeans (RCS), and raw low-trypsin-inhibitor soybeans (LTS) on performance of chicks fed a 16% protein diet supplemented with methionine in Experiment 2*[1]

Treatment	Weight gain	Gain:feed	PER[2]
	(g)	(g:g)	
HDS	98.0^{a}	$.632^{a}$	3.95^{a}
RCS	42.9^{c}	$.331^{c}$	2.07^{c}
LTS	72.3^{b}	$.489^{b}$	3.06^{b}
Pooled SEM	2.0	.016	.10

[a–c]Means within columns with no common superscripts differ significantly (P<.05).

[1]Means of three groups of seven male crossbred chicks from 8 to 15 days posthatching.

[2]PER [Protein efficiency ratio (in grams)] = weight gain divided by protein intake.

stuff AA (in milligrams) as the independent variables. In the TSAA assay, intake of sulfur (millimoles) from Met and Cys was used as the independent variable to account for molecular weight differences. The bioavailability of AA in each feedstuff was then estimated by means of the ratio of the slope of its regression line to that of the crystalline test AA, which was assumed to be 100% bioavailable. Bioavailability was computed by an additional method wherein weight gains of chicks were partitioned to reflect only gain attributable to intake of supplemental crystalline AA or supplemental AA from the test ingredient (Parsons, 1986; Hirakawa and Baker, 1986). This method attempts to minimize the effect of differences in voluntary feed intake among treatments.

RESULTS

The compositions of the soybean sources evaluated in Experiments 1, 2, 4, and 5 and the true digestibility assay are presented in Table 3. The composition of LTS was similar to that of RCS, although LTS was slightly higher in protein and lower in fat than RCS. However, these differences were reversed for the soybean sources evaluated in Experiment 3 (see Table 2, Footnote 1). The concentration of trypsin inhibitor activity in LTS was much lower than that in RCS and higher than that in HDS.

In the first protein quality assay (Experiment 1), chicks fed the diet containing LTS gained more than twice as much weight as did those fed RCS (Table 4). Feed efficiency of chicks fed the LTS diet was also superior to that of chicks fed the RCS diet. However, both weight gain and feed efficiency obtained from the LTS diet were inferior to those obtained from the HDS diet. Similar results were observed for PER and NPR values.

In Experiment 2, chicks fed a 16% protein diet containing LTS and supplemental Met gained weight more rapidly and efficiently than did chicks fed the same diet containing RCS (Table 5). As observed in Experiment 1, performance of chicks fed LTS was inferior to that of chicks fed HDS.

Replacing HDS with LTS or RCS in a 22% protein diet decreased weight gain and gain:feed ratio as the substitution level increased in Experiment 3 (Table 6). However, when 25% of the dietary soybean protein was provided by RCS or LTS, weight gain and gain:feed ratio were not significantly different ($P>.05$) from

TABLE 6. *Effects of raw conventional soybeans (RCS) and raw low-trypsin-inhibitor soybeans (LTS) on chick performance in the soybean replacement assay in Experiment 3*[1]

Amount of soybean protein replaced	Weight gain	Feed intake	Gain: feed
	(g)		(g:g)
Control[2]	223.5	323.0	.692
25% by RCS	216.9	322.9	.672
25% by LTS	219.7	324.0	.678
50% by RCS	203.6	322.5	.631
50% by LTS	211.6	319.0	.672
75% by RCS	194.7	317.0	.614
75% by LTS	208.2	320.5	.650
100% by RCS	174.9	317.4	.551
100% by LTS	198.6	319.4	.622
Pooled SEM	4.1	5.0	.008

[1]Means of three groups of seven male crossbred chicks from 8 to 19 days posthatching.

[2]Control diet was a 22% CP corn and soybean meal diet in which 100% of the dietary soybean protein was supplied by heated, dehulled soybean meal.

those of the control. In addition, feed efficiency of chicks provided 50% of the soybean protein as LTS was not significantly different ($P>.05$) from that of the control. At each replacement level, chick performance was better on LTS diets than on RCS diets, with the differences being significant ($P<.05$) at the 75 and 100% substitution rates. Regressing weight gain (in grams) on percentage replacement level of HDS protein by RCS or LTS protein yielded the regression equation: $Y = 225.6 - .4644\ (RCS) - .2592\ (LTS)$, $R^2 = .81$, indicating that each 1% replacement of HDS protein with RCS protein depressed weight gain by .46 g and each 1% replacement of HDS protein with LTS protein depressed weight gain by .26 g. The slope ratio of LTS: RCS was .558. When regressing gain:feed ratio on the same substitution levels, the equation was $Y = .697 - .001319\ (RCS) - .0007076\ (LTS)$, $R^2 = .90$, and the slope ratio of LTS:RCS was .536.

Weight gain and gain:feed ratio responded linearly to supplementation of the AA-deficient basal diets with crystalline AA or the soybean sources in the AA bioavailability assays (Tables 7 to 9). From multiple linear regression analysis and slope-ratio methodology applied to total weight gains, bioavailability of Lys was ($\bar{x} \pm SE$) 90.4 ± 5.0% for HDS, 72.0 ± 6.1% for RCS, and 86.4 ± 6.0% for LTS in Experiment 4 (Table 7). With parti-

TABLE 7. *Determination of lysine bioavailability in heated dehulled soybean meal (HDS), raw conventional soybeans (RCS), and raw low-trypsin-inhibitor soybeans (LTS) by slope-ratio chick assay in Experiment 4*[1]

Treatment	Weight gain		Gain:feed	Bioavailability[2]	
	Total	Part.[3]		Total	Part.[3]
	— (g per chick) —		(g:g)	— (%) —	
1. Basal (B)[4]	36.0	−1.6	.360		
2. B plus .15% L-Lys	66.3	19.0	.517		
3. B plus .30% L-Lys	102.4	41.5	.608	100.0	100.0
4. B plus 3% HDS	46.8	9.0	.465		
5. B plus 6% HDS	64.1	19.6	.534		
6. B plus 9% HDS	83.1	35.0	.595	90.4 ± 5.0	101.8 ± 6.5
7. B plus 3% RCS	40.5	3.6	.414		
8. B plus 6.5% RCS	56.7	15.6	.516		
9. B plus 10% RCS	63.9	18.6	.523	72.0 ± 6.1	80.4 ± 7.7
10. B plus 3% LTS	48.5	7.2	.437		
11. B plus 6.5% LTS	56.1	14.0	.496		
12. B plus 10% LTS	72.4	24.6	.559	86.4 ± 6.0	92.6 ± 7.6
Pooled SEM	2.8	1.9	.014		

[1]Values are means of triplicate groups of five male chicks from 8 to 16 days posthatching.

[2]$\bar{x}$ ± SE calculated from slope-ratio analysis for multiple regression of total or partitioned gain (grams) on supplemental crystalline lysine or soybean lysine intake (milligrams); the regression equations were Y = 36.72 + .1364(L-Lys) + .1233(HDS) + .1178(LTS) + .09825(RCS), R^2 = .96 and Y = −.48 + .08511(L-Lys) + .08667(HDS) + .06840(RCS) + .07882(LTS), R^2 = .94, respectively.

[3]Part. = Partitioned gain or bioavailability, which reflects gain or bioavailability due only to supplemental crystalline amino acid or soybean amino acid intake. The reference standard curve from regressing total gain (Y; in grams) on total lysine intake (X; in milligrams) for Treatments 1 to 3 was Y = 3.61 + .08641X, r^2 = .99.

[4]Purified crystalline amino acid diet deficient only in lysine (Lys; .4% L-Lys).

TABLE 8. *Determination of total sulfur amino acid bioavailability in heated dehulled soybean meal (HDS), raw conventional soybeans (RCS), and raw low-trypsin-inhibitor soybeans (LTS) by slope-ratio chick assay in Experiment 5*[1]

Treatment	Weight gain		Gain:feed	Bioavailability[2]	
	Total	Part.[3]		Total	Part.[3]
	— (g per chick) —		(g:g)	— (%) —	
1. Basal (B)[4]	27.5	−2.0	.277		
2. B plus .035% L-Cys + .035% L-Met	55.9	13.5	.424		
3. B plus .07% L-Cys + .07% L-Met	72.1	26.2	.511	100.0	100.0
4. B plus 4% HDS	43.1	4.9	.358		
5. B plus 8% HDS	66.3	22.6	.490		
6. B plus 12% HDS	79.9	31.7	.543	89.2 ± 6.4	92.2 ± 8.1
7. B plus 4% RCS	38.2	4.0	.344		
8. B plus 8% RCS	42.3	10.1	.400		
9. B plus 12% RCS	51.2	15.7	.448	62.3 ± 7.7	82.5 ± 9.6
10. B plus 4% LTS	40.0	5.6	.358		
11. B plus 8% LTS	52.1	13.3	.424		
12. B plus 12% LTS	61.9	19.2	.466	86.6 ± 8.0	90.0 ± 10.2
Pooled SEM	2.7	2.1	.016		

[1]Values are means of triplicate groups of five male chicks from 8 to 16 days posthatching.

[2]$\bar{x}$ ± SE calculated from slope-ratio analysis for multiple regression of total or partitioned gain (grams) on supplemental sulfur intake from crystalline TSAA or soybean TSAA (millimoles); the regression equations were Y = 30.63 + 30.11(TSAA) + 26.86(HDS) + 18.76(RCS) + 26.05(LTS), R^2 = .93 and Y = −1.31 + 19.27(TSAA) + 17.76(HDS) + 15.89(RCS) + 17.34(LTS), R^2 = .90, respectively.

[3]Part. = partitioned gain or bioavailability, which reflects gain or bioavailability due only to supplemental crystalline amino acid or soybean amino acid intake. The reference standard curve from regressing total gain (Y; in grams) on intake of sulfur from TSAA (X; in millimoles) for Treatments 1 to 3 was Y = −9.35 + 28.88X, r^2 = .99.

[4]Purified crystalline amino acid diet deficient only in TSAA (.125% L-Met, .125% L-Cys).

TABLE 9. *Determination of methionine bioavailability in heated dehulled soybean meal (HDS), raw conventional soybeans (RCS), and raw low-trypsin-inhibitor soybeans (LTS) by slope-ratio chick assay in Experiment 6*[1]

Treatment	Weight gain: Total	Weight gain: Part.[2]	Gain:feed	Bioavailability[3]: Total	Bioavailability[3]: Part.[2]
	(g per chick)		(g:g)	(%)	
1. Basal (B)[4]	18.1	−1.4	.262		
2. B plus .05% L-Met	55.1	19.0	.489		
3. B plus .10% L-Met	111.7	51.1	.633	100.0	100.0
4. B plus 4% HDS	31.6	5.5	.364		
5. B plus 9% HDS	45.7	15.9	.475		
6. B plus 14% HDS	67.0	28.4	.563	80.3 ± 4.0	87.3 ± 4.9
7. B plus 5% RCS	23.5	2.6	.322		
8. B plus 10% RCS	29.7	5.3	.362		
9. B plus 15% RCS	42.7	11.1	.424	55.4 ± 5.7	51.4 ± 7.3
10. B plus 5% LTS	26.6	3.4	.336		
11. B plus 10% LTS	33.3	6.5	.377		
12. B plus 15% LTS	48.4	17.7	.495	67.9 ± 5.7	72.1 ± 6.9
Pooled SEM	3.4	2.1	.015		

[1]Values are means of triplicate groups of five male chicks from 8 to 16 days posthatching.

[2]Part. = Partitioned gain or bioavailability, which reflects gain or bioavailability due only to supplemental crystalline amino acid or soybean amino acid intake. The reference standard curve from regressing total gain (Y; in grams) on total methionine (Met) intake (X; in milligrams) for Treatments 1 to 3 was $Y = -7.11 + .2956X$, $r^2 = .995$.

[3]$\bar{x}$ ± SE calculated from slope-ratio analysis for multiple regression of total or partitioned gain (grams) on supplemental crystalline Met or soybean Met intake (milligrams); the regression equations were Y = 19.64 + .5338(L-Met) + .4287(HDS) + .2955(RCS) + .3625(LTS), $R^2 = .98$ and Y = −.43 + .2978(L-Met) + .2600(HDS) + .1531(RCS) + .2148(LTS), $R^2 = .97$, respectively.

[4]Purified crystalline amino acid diet deficient only in Met (.13% L-Met; .30% L-Cys).

tioned weight gains, the estimated Lys bioavailabilities were slightly higher than the corresponding values obtained from total weight gains; however, the differences were not significant (P>.05).

Bioavailabilities of TSAA calculated from total weight gains were 89.2 ± 6.4, 62.3 ± 7.7, and 86.6 ± 8.0% for HDS, RCS, and LTS, respectively, in Experiment 5 (Table 8). The bioavailability estimate computed from partitioned gain was similar to that computed from total gain for HDS and LTS, but was significantly higher for RCS (82.5 ± 9.6%).

The response of chicks to supplementation of the MET-deficient basal diet with RCS was much lower than that to supplementation with HDS, with the response to LTS being intermediate in Experiment 6 (Table 9). The Met bioavailability based on total weight gain in LTS (67.9 ± 5.7%) was lower (P<.05) than that in HDS (80.3 ± 4.0%) but was greater (P<.05) than that in RCS (55.4 ± 5.7%). The estimates derived from partitioned weight gains differed among the three soybean sources in a similar manner.

True DM and AA digestibility and TME_n values are presented in Table 10. There were no significant (P>.05) differences between CONV and CEC cockerels for true digestibility of DM and AA within soybean sources except for Tyr in HDS. True digestibility values for AA in HDS were significantly (P<.05) greater than all of those in RCS and most of those in LTS. True AA digestibility values for LTS were greater (P<.05) than those for RCS. True digestibility of DM in LTS was greater than that in HDS. As expected, TME_n values of RCS and LTS were greater (P<.05) than that of HDS, and TME_n of LTS was greater (P<.05) than TME_n of RCS. The TME_n of RCS determined with CEC birds was greater than the TME_n determined with CONV birds, whereas the reverse was observed for LTS.

DISCUSSION

Proper heating of soybeans improves their nutritive value to animals by destroying trypsin inhibitors (Kunitz, 1947; Rackis *et al.*, 1962) and hemagglutinin (Liener, 1951, 1953). The chick protein quality assays (Experiments 1 and 2) clearly illustrated the benefits of

TABLE 10. *True metabolizable energy and true digestible amino acid values for heated dehulled soybean meal (HDS), raw conventional soybeans (RCS), and raw low-trypsin-inhibitor soybeans (LTS)*[1]

	HDS			RCS			LTS		
	CONV[2]	CEC[2]	SED[2]	CONV	CEC	SED	CONV	CEC	SED
True digestibility of DM, %	45.7	41.6	3.8	48.4	48.7	3.3	54.9	53.9	4.1
TME_n, kcal/g DM	2.742	2.881	.098	3.311[a]	3.623[b]	.065	3.944[a]	3.751[b]	.082
True digestibility of amino acids, %									
Aspartic acid	91.8	90.2	1.2	68.7	69.5	2.3	85.2	80.0	4.4
Threonine	90.3	88.8	1.6	61.8	63.3	2.5	82.7	76.7	4.3
Serine	92.2	92.8	1.0	66.4	67.9	2.4	85.5	79.8	4.1
Glutamic acid	94.4	95.3	1.0	76.6	81.0	2.7	88.9	86.8	4.2
Proline	94.6	94.1	1.1	72.7	76.2	2.3	86.8	82.2	3.9
Alanine	88.1	89.9	2.1	60.8	64.8	3.1	80.6	78.3	5.6
Cystine	85.5	86.2	1.4	57.0	51.9	5.1	79.1	69.3	5.0
Valine	93.1	93.5	1.8	61.7	68.3	5.8	83.1	79.6	6.4
Methionine	93.1	90.4	2.8	62.5	56.2	5.8	81.2	81.1	6.7
Isoleucine	94.2	95.0	1.2	66.4	72.2	4.7	84.9	83.7	5.5
Leucine	93.2	93.7	1.1	67.0	70.0	2.7	85.2	82.4	4.6
Tyrosine	93.2[a]	96.7[b]	1.3	64.1	63.1	2.1	84.5	77.3	4.6
Phenylalanine	94.7	96.2	1.1	67.4	70.8	3.2	86.6	82.4	5.5
Histidine	93.6	94.0	.7	73.7	70.6	5.0	86.5	87.0	5.7
Lysine	92.7	93.9	1.0	76.0	78.6	2.9	86.8	85.6	3.6
Arginine	92.0	91.9	2.2	75.5	79.9	2.7	88.7	86.9	3.9
$\bar{x}$	92.3	92.7		67.4	69.0		84.8	81.2	

[a,b]Within soybean source, means in the same row with no common superscripts are significantly different (P<.05).

[1]Values are means of five birds.

[2]CONV = Conventional birds; CEC = cecectomized birds; SED = standard error of the difference.

cooking soybeans and the deleterious effects of trypsin inhibitors and other antinutritional factors in raw soybeans. For example, the efficiency of protein utilization by chicks fed RCS was only 32% of those fed HDS. The PER from LTS was substantially superior to that from RCS but was still 34% lower than that from HDS. When diets were supplemented with Met, PER was improved for both RCS and LTS relative to HDS (Experiment 2 versus Experiment 1). The positive response to Met supplementation was probably due both to the correction of the deficiency of the first-limiting AA for growth and to the provision of precursor Cys for pancreatic enzyme synthesis, and, thus, reduction of the depletion of Cys from body tissues (Yen *et al.*, 1973; Liener, 1977).

The results of the protein quality assays indicated that LTS was nutritionally superior to RCS but inferior to HDS. These results agree with results of the early chick study of Yen *et al.* (1973) using low trypsin inhibitor soybeans of varying genetic background and with results of the pig study of Cook *et al.* (1988) using the same commercial Williams cultivars evaluated herein. The inferiority of LTS to HDS may be partially due to the fact that Kunitz trypsin inhibitor is only one of the two major classes of trypsin and chymotrypsin inhibitors present in soybeans (Stahlhut and Hymowitz, 1983). In addition, Kakade *et al.* (1973) reported that only 40% of the growth-depressing effect of raw soybeans could be accounted for by trypsin inhibitors and that heating is necessary for denaturation of the soybean proteins and subsequent digestion by pancreatic enzymes. Further, recent experiments indicate that heating of LTS increases its protein quality markedly (Friedman *et al.*, 1990; Parsons *et al.*, unpublished data).

The chick AA bioavailability assays and the true digestibility assay indicated that reduced AA availability is at least partially responsible for the lower protein quality of LTS and RCS compared with that of HDS. Bioavailability and true digestibility of AA in LTS were generally higher than those in RCS but lower than those in HDS. The lower AA digestibility probably resulted both from increased loss of endogenous protein due to increased pancreatic enzyme secretions and from decreased digestibility of dietary AA due to reduced proteolytic activity (Bielorai *et al.*, 1973; Liener, 1977). The higher TME_n of LTS compared with RCS was probably largely due to the higher availability of the AA in LTS.

True digestibility values for Lys determined by excreta collection assay with adult birds agreed very well with the bioavailability values determined by growth assay with chicks. However, digestible Met values were somewhat higher than bioavailable Met values for HDS and LTS, whereas the values were in good agreement for RCS. Calculation method (total weight gain versus partitioned weight gain) had little effect on chick growth assay bioavailability values in the present study.

It has been reported that there is a significant effect of the hindgut microflora on AA digestibility in some feedstuffs for poultry (Parsons, 1986). However, cecectomy had no significant effect on AA digestibility values for any of the soybean sources tested in the present study. In contrast, digestibility values for AA in RCS in ileal-cannulated pigs were reported to be markedly lower than digestibility values for CONV pigs (Vandergrift *et al.*, 1983). These observations suggest that digestion and metabolism of raw soybean protein by the hindgut microflora is much less in poultry than in swine.

The results of the soybean protein replacement assay showed that the growth depression caused by replacing HDS with LTS was only approximately one-half of that caused by replacing HDS with RCS. These results clearly show the nutritional superiority of HDS compared to RCS when used in a practical-type diet. Moreover, weight gain and feed efficiency of chicks fed the diets containing 25 or 50%, respectively, of the soybean protein from LTS were not significantly different from those of chicks fed a diet containing 100% of the soybean protein from HDS. It is also possible that even higher levels of LTS could be used in diets of older birds without adversely affecting performance, as the adverse response to dietary raw soybeans is, to some degree, age-dependent (Crenshaw and Danielson, 1985).

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[1]Values are means of five birds.
[2]CONV = Conventional birds; CEC = cecectomized birds; SED = standard error of the difference.

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