Effects of Heat Treatment on Soybeans With and Without the Gene Expression for the Kunitz Trypsin Inhibitor: Chick Growth Assays

L. L. Burnham, I. H. Kim[1], J. D. Hancock* and A. J. Lewis[2]
Department of Animal Sciences and Industry, Kansas State University, USA

ABSTRACT : A total of 864 broiler chicks were used at Kansas State University and the University of Nebraska to determine the effects of heat treatment of two soybean genotypes on the growth performance. The soybeans were Williams 82 variety with (+K) and without (-K) gene expression for the Kunitz trypsin inhibitor. Heat treatment (autoclaving at 121℃ and 1.1 kg/cm^2) was applied for 0, 3, 6, 12, 18, and 24 min, resulting in a 2×6 factorial arrangement of treatments. Station and station treatment effects occurred, indicating that response in nutritional value of the soybean genotypes to heat treatment varied from year to year and location to location. However, the interactions were in magnitude of response rather than direction of response, with greater reductions in trypsin inhibitor concentrations for the soybeans heat processed at the Nebraska location. Pooled data indicated that -K supported greater ($p<0.001$) ADG, ADFI and gain/feed than the +K genotype. As the length of heat treatment increased, the ADG, ADFI, and the gain/feed ratio increased for chicks fed both soybean genotypes ($p<0.0001$). However, heating the -K soybeans resulted in a greater response in ADG, ADFI, and gain/feed than heating the +K soybeans (genotype heat treatment interaction, $p<0.001$). Pancreatic weights (mg pancreas/g of BW) of chicks fed -K soybeans were reduced compared to those from chicks fed +K ($p<0.001$). Increasing heat treatment decreased pancreas weights in chicks fed both soybean genotypes ($p<0.001$). Chicks fed heated soybeans in the Nebraska experiment had lower pancreatic weights than chicks fed heated soybeans in the Kansas experiment (station heat treatment interaction, $p<0.0001$). Chick growth performance was improved and pancreatic weights decreased by feeding raw -K soybeans versus raw +K soybeans, and by increasing heat treatment of both soybean genotypes. However, the response to heat treatment was not independent of genotype. Both +K and -K soybeans heated for 24 min supported similar ADG, ADFI, gain/feed, and pancreas weights, although chicks fed raw +K soybeans had lower growth performance than chicks fed -K soybeans. In conclusion, raw -K soybeans supported greater growth performance in broiler chicks than raw +K soybeans, although this advantage was lost when both soybean genotypes were heated for 24 min. Heat treatment of +K soybeans supported similar growth performance to heated -K soybeans, even though +K soybeans supported lower rates and efficiencies of gain than -K soybeans when fed raw. *(Asian-Aus. J. Anim. Sci. 2000. Vol. 13, No. 12 : 1750-1757)*

Key Words : Soybeans, Trypsin Inhibitor, Heat Treatment, Chicks, Growth, Pancreas

INTRODUCTION

Soybeans are used as a source of oil and protein in diets for animals and humans, but have anti-nutritional factors (i.e., lectins, goitrogens, protease inhibitors, etc.) that decrease growth in rats (Osborne and Mendel, 1917; Hancock et al., 1989), chickens (Waldroup et al., 1969; Wood et al., 1971; Veltmann et al., 1986), and pigs (Crenshaw and Danielson, 1985; Cook et al., 1988; Hancock et al., 1990). Deactivation of the anti-nutritional factors by various forms and durations of heat treatment improves growth performance of animals (Borchers et al., 1947; Vandergrift et al., 1983). However, underheating of soybeans reduces growth performance because of residual anti-nutritional factors, and over-heating reduces growth performance because of reduced bioavailability of nutrients (Borchers, 1965; Rios Iriarte and Barnes, 1966; Hancock et al., 1990), and both situations have been described in commercial soybean meals (McNaughton et al., 1981).

As an alternative to heat treatment, a soybean genotype was described by Singh et al. (1969) and later by Hymowitz (1984) with lower trypsin inhibitor activity. This soybean genotype supported greater growth performance in chicks and pigs (Yen et al., 1973; Yen et al., 1974; Bajjalieh et al., 1980; Kim et al., 1999; Kim et al., 2000). Further developments by plant geneticists have resulted in commercial genotypes without gene expression for the Kunitz trypsin inhibitor.

The experiment reported herein was conducted to determine the nutritional value of near isolines of Williams 82 soybeans, with and without gene expression for the Kunitz trypsin inhibitor. The potential for reduced heat treatment in processing low trypsin inhibitor soybeans was of primary focus.

MATERIALS AND METHOD

Experiments were conducted at Kansas State University and the University of Nebraska using the same protocol. For the experiments, near isolines of

* Corresponding Author: J. D. Hancock. Tel: +1-785-532-1230, Fax: +1-785-532-7059, E-mail: jhancock@oz.oznet.ksu.edu.
[1] Department of Animal Sciences, Dankook University, Cheonan 330-714, Korea.
[2] Department of Animal Science, University of Nebraska, USA.
Received May 15, 2000; Accepted July 21, 2000

Williams 82 soybeans, with (+K) and without (-K) gene expression for the Kunitz trypsin inhibitor, were grown in adjacent plots with identical crop management. However, the Kansas replication of the experiment was completed two years after the Nebraska experiment. The soybeans were harvested, and 3,000 g of each genotype was spread onto 6 cm ×26 cm×44 cm metal pans and autoclaved in a steam sterilizer (Model 57CR, American Sterilizer Co., Erie, PA) for 0. 3, 6, 12, 18, and 24 min at 121℃ and 1.1 kg/cm^2 steam pressure. Timing began when the autoclave chamber reached 100℃. Upon completion of the allotted time of heat treatment, the soybeans were removed promptly from the autoclave and spread onto a cool, flat, concrete floor. The soybean preparations were analyzed for DM, CP, ether extract, ash, and GE using AOAC (1990) procedures. Trypsin inhibitor content was determined with the procedure of Hammerstrand et al. (1981). For amino acid analyses, the soybean preparations were hydrolyzed in 6N HCl at 102℃ for 20 h. Amino acids from the protein hydrolysates were separated with HPLC (Waters Inc., Milford, MA), using a cation exchange resin and gradient elution with lithium buffers. The amino acids were derived post-column using o-phthaldialdehyde and detected with a fluorometer.

Before incorporation into the experimental diets (table 1), the soybeans were ground through a 1 mm screen in a Wiley Mill (Model No. 3, Arthur H. Thomas Co., Philadelphia, PA). The diets were formulated to be slightly limiting in lysine (0.96%) with all other nutrient concentrations at least 120% of National Research Council recommendations (NRC, 1984). A total of 864 Vantress×Arbor Acre broiler chicks were used in the growth assays. From Day 1 to 6 post-hatching, the chicks were fed a commercial starter diet formulated to contain 1.3% lysine, 1.1% Ca, and 0.9% total P. On Day 7, the chicks were wing-banded, sorted by sex and weight, and assigned to treatment. The chicks were housed in 38 cm×102 cm cages with 12 cages of three males and 12 cages of three females for each treatment. The cages were in heated starter batteries (Petersine Incubator Co., Gettysburg, OH), kept in constantly lighted, environmentally controlled rooms. The chicks had *ad libitum* access to feed and water during the 8-d growth assay. Upon completion of the growth assays, the intermediate weight bird in each pen was killed by cervical dislocation and the pancreas removed and weighed. Pancreatic weights were evaluated as a ratio of BW (mg of pancreas/g BW) to minimize variation from individual chick BW.

Treatments were arranged as a 26 factorial with main effects of soybean genotype (+K vs -K) and duration of heat treatment (0, 3, 6, 12, 18, and 24 min of autoclaving). Response criteria were ADG, ADFI, gain/feed and differences in pancreatic weight. Data were analyzed as a split-plot, with station as the whole plot and the weight block station interaction as the whole plot error term. Sub-plot effects were analyzed as a 2×6 factorial, with polynomial regression for unequally spaced treatments used to characterize the response trends to duration of heat treatment. All analyses were conducted using the General Linear Model Procedure of SAS (1985) with cage as the experimental unit.

RESULTS AND DISCUSSION

Laboratory analysis (table 2) of the soybean

Table 1. Basal diets used in the growth assays[a]

Ingredients, %	Kansas Exp.[b]		Nebraska Exp.[b]	
	Diets 1 to 6	Diets 7 to 12	Diets 1 to 6	Diets 7 to 12
+K Soybeans	32.90	-	36.90	-
-K Soybeans	-	32.90	-	34.05
Cornstarch	-	-	-	2.80
Corn	62.19	62.19	57.38	57.38
DL-methionine	-	-	0.20	0.20
Dicalcium phosphate	2.91	2.91	2.84	2.93
Limestone	0.80	0.80	0.83	0.79
Salt	0.35	0.35	0.35	0.35
Vitamins, minerals[c] and antibiotics[d]	0.85	0.85	1.32	1.32

[a] All diets were formulated to contain 0.96% lysine, 1.1% Ca, and 0.9% P.

[b] Diets 1 to 6 had soybeans with gene expression for the Kunitz trypsin inhibitor (+K), and diets 7 to 12 had soybeans without gene expression for the Kunitz trypsin inhibitor (-K) autoclaved (121℃ and 1.1 kg/cm2 steam pressure) for 0, 3, 6, 12, 18, and 24 minutes.

[c] Supplied (per kilogram of diet): 3,307 IU of vitamin A; 1,984 IU of vitamin D; 5.3 g of vitamin B_{12}; 17.6 mg of riboflavin; 32.5 mg of d-pantothenic acid; 52.9 mg of niacin; 153.1 mg of choline; 220 mg of Fe; 220 mg of Zn; 220 mg of Mn; 22 mg of Cu; 6.6 mg of I; 2.2 mg of Co; 0.3 mg. of Se for the Kansas exp. and; 11,000 IU of vitamin A; 4,400 IU of vitamin D; 0.011 mg of Vitamin B_{12}; 6.6 mg of riboflavin; 11.9 mg of calcium pantothenate; 77 mg of niacin; 880 mg of choline chloride; 3.3 mg menadione sodium bisulfite; 11 IU of Vitamin E;.11 mg of d-biotin; 0.66 mg of folacin; 100 mg of Fe; 100 mg of Zn; 100 mg of Mn; 10 mg of Cu; 3.0 mg of I; 1.0 mg of Co; and 0.2 mg of Se for the Nebraska Exp.

[d] Supplied (per kg of diet): 2,125 mg of amprollium and 680 mg of chopabate for the Nebraska exp. and; 125 mg of amprolliun, 40 mg chopabate, 500 mg/kg flavomycin and 2.2 mg/kg bambermycins for the Kansas Exp.

Table 2. Chemical analysis of heat treated soybeans with and without the Kunitz trypsin inhibitor[a]

Item	Heat treatment of +K soybeans, min[b]						Heat treatment of -K soybeans, min[b]					
	0	3	6	12	18	24	0	3	6	12	18	24
DM, %												
Kansas	91.1	90.7	90.8	90.3	90.1	90.0	91.7	91.2	91.1	91.0	90.8	90.7
Neraska	91.8	90.4	89.9	89.4	90.5	90.7	91.3	90.3	91.3	90.9	89.8	90.7
CP, %												
Kansas	38.1	38.1	38.1	38.1	37.4	38.1	38.4	38.1	35.6	39.2	37.3	37.5
Neraska	37.0	36.9	36.6	36.2	36.6	36.7	37.5	36.9	38.1	37.8	37.1	38.2
Ether extract, %												
Kansas	17.8	18.9	18.9	18.8	18.6	18..0	19.1	18.7	19.1	19.8	19.7	19.8
Neraska	21.5	21.8	21.9	22.3	22.3	21.0	19.0	17.4	21.0	21.4	21.8	21.4
Ash, %												
Kansas	4.8	4.9	4.7	4.7	4.9	4.8	4.9	4.9	5.0	4.9	4.9	4.9
Neraska	4.6	4.7	4.8	4.7	4.7	4.8	4.7	4.7	4.7	4.7	4.8	4.8
Gross energy, kcal/kg												
Kansas	5,009	5,030	5,116	4,947	5,063	5,159	5,048	5,092	5,091	5,082	5,034	5,037
Neraska	5,179	5,102	5,054	5,007	5,088	5,106	5,170	5,090	5,169	5,134	5,062	5,108
Trypsin inhibitor, mg/g[c]												
Kansas	21.6	19.1	18.5	14.1	10.3	9.7	12.7	11.1	10.6	8.1	5.4	3.9
Neraska	24.7	20.2	17.9	10.6	2.3	0.7	13.5	12.8	11.7	7.0	1.5	0.5

[a] AOAC (1990).
[b] Heat treatment was autoclaving at 121℃ and 1.1 kg/cm^2 steam pressure.
[c] Hammerstrand et al. (1981).

preparations indicated little effect of soybean genotype or heat treatment on percentage DM, CP, ether extract, or ash. However, increased heat treatment markedly reduced trypsin inhibitor concentration. The unheated +K soybeans had 23.2 mg/g initial trypsin inhibitor concentration, compared to 13.1 mg/g for the -K variety. As duration of heat treatment was increased, trypsin inhibitor concentration of both isolines decreased, with trypsin inhibitor concentration lower in the (-K) soybeans at each duration of heating. This corresponds to observations by Borchers et al. (1947), and Claudinin (1947) who reported reduced trypsin inhibitor content with increased duration of autoclaving. However, Claudinin (1947) and Borchers et al. (1947) gave different recommendations for optimum duration of heat treatment (4 to 15 min versus 20 min at 6.82 kg and 121℃, respectively).

Amino acid analyses of the heat-treated soybeans, with and without the Kunitz trypsin inhibitor, indicated there were only slight differences among amino acid concentration of the soybeans subjected to different durations of heat treatment (table 3). The exceptions were lysine and methionine concentrations in the -K soybeans, which were greater than the concentrations in +K soybeans with 24 min of heating. Although the Kunitz inhibitor is high in sulfur amino acids, gene expression for its activity did not result in less methionine concentration. Apparently, concentrations of other soybean proteins were increased such that no reduction in crude protein or any specific amino acids were expressed.

Evaluation in changes in trypsin inhibitor activity suggests that, although a strict protocol was followed, the soybean genotypes responded differently to duration of heating in different years at different locations. Less than 18 min was required to reduce trypsin inhibitor concentrations to less than 4 mg/g (as suggested to be a tolerable maximum by Vandergrift et al., 1983) in the Nebraska experiment, but more than 24 min was required in the Kansas experiment. This response was unexpected because the soybeans had similar moisture concentrations before autoclaving and were heated in the same model autoclave under the same conditions by the same researcher at the two test stations. However, the differences among the soybeans used in the Nebraska and Kansas experiments were in magnitude of response and not direction of response (i.e., a greater effect of heating time on trypsin inhibitor concentration in Nebraska), thus the data for growth performance of the chicks were pooled to determine the general effects of soybean genotype and heat treatment.

Improvement in performance due to heat treatment of soybean meal and raw soybeans has been described by Liener and Kakade (1969), Rackis (1972), and McNaughton et al., (1981). Wood et al., (1971)

Table 3. Amino acid concentration in heated soybeans with (+K) and (-K) the trypsin inhibitor[a]

Item	Heat treatment of +K soybeans, min[b]						Heat treatment of -K soybeans, min					
	0	3	6	12	18	24	0	3	6	12	18	24
Arginine, %												
Kansas	2.6	2.7	2.8	2.7	2.6	2.7	2.8	2.7	2.9	2.9	2.9	2.7
Neraska	2.7	2.7	2.8	2.6	2.5	2.5	2.6	2.6	2.7	2.6	2.6	2.6
Avg.	2.7	2.7	2.8	2.7	2.6	2.6	2.7	2.7	2.8	2.8	2.8	2.7
Histidine, %												
Kansas	1.0	0.9	1.0	1.0	0.9	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Neraska	1.0	1.0	1.1	1.0	0.9	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Avg.	1.0	1.0	1.1	1.0	0.9	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Isoleucine, %												
Kansas	1.6	1.7	1.7	1.6	1.6	1.7	1.6	1.7	1.7	1.7	1.7	1.7
Neraska	1.9	1.9	1.9	1.8	1.8	1.8	1.8	1.8	1.9	1.8	1.8	1.8
Avg.	1.8	1.8	1.8	1.7	1.7	1.8	1.7	1.8	1.8	1.8	1.8	1.8
Leucine, %												
Kansas	2.8	2.8	2.8	2.7	2.7	2.7	2.8	2.8	2.8	2.8	2.8	2.8
Neraska	2.9	3.0	3.0	2.8	2.8	2.8	2.9	3.0	3.0	3.0	3.0	2.9
Avg.	2.9	2.9	2.9	2.8	2.8	2.8	2.9	2.9	2.9	2.9	2.9	2.9
Lysine, %												
Kansas	2.6	2.5	2.5	2.5	2.3	2.2	2.4	2.4	2.4	2.5	2.4	2.4
Neraska	2.3	2.1	2.3	2.4	2.2	2.0	2.4	2.3	2.4	2.4	2.5	2.4
Avg.	2.5	2.5	2.4	2.5	2.3	2.1	2.4	2.4	2.4	2.5	2.5	2.4
Methionine, %												
Kansas	0.6	0.5	0.6	0.6	0.6	0.4	0.5	0.6	0.5	0.7	0.5	0.7
Neraska	0.7	0.6	0.6	0.5	0.7	0.6	0.7	0.6	0.5	0.6	0.6	0.6
Avg.	0.7	0.6	0.6	0.6	0.7	0.5	0.6	0.6	0.5	0.7	0.6	0.7
Phenylalanine, %												
Kansas	1.8	1.8	1.9	1.8	1.8	1.8	1.9	1.9	1.9	1.9	1.9	1.8
Neraska	1.9	2.0	2.0	1.9	1.8	1.8	1.9	1.9	1.9	1.9	1.9	1.9
Avg.	1.9	1.9	2.0	1.9	1.8	1.8	1.9	1.9	1.9	1.9	1.9	1.9
Threonine, %												
Kansas	1.5	1.5	1.5	1.5	1.4	1.5	1.6	1.6	1.6	1.6	1.6	1.5
Neraska	1.5	1.5	1.5	1.4	1.3	1.1	1.3	1.4	1.4	1.4	1.3	1.3
Avg.	1.5	1.5	1.5	1.5	1.4	1.3	1.5	1.5	1.5	1.5	1.5	1.4
Valine, %												
Kansas	1.6	1.7	1.7	1.8	1.7	1.7	1.8	1.8	1.8	1.8	1.9	1.8
Neraska	2.0	2.0	2.0	1.9	1.9	1.9	1.9	2.0	2.0	2.0	2.0	1.9
Avg.	1.8	1.9	1.9	1.9	1.8	1.8	1.9	1.9	1.9	1.9	2.0	1.9

[a] Heat treatment was autoclaving at 121℃ and 1.1 kg/cm^2 steam pressure.
[b] Performate oxidation prior to acid hydrolysis.
[c] Performate oxidation prior to acid hydrolysis.

determined that unheated raw soybeans fed to chicks decreased growth compared to those fed extruded soybeans. The improved performance was attributed to inactivation of trypsin inhibitor and other heat labile anti-nutritional factors, and increased digestibility with mild denaturation of storage proteins. Improved performance of animals fed raw -K soybeans compared to raw +K soybeans has been suggested by several researchers. Yen et al. (1972) and Cook et al. (1988) reported that, when unheated, low trypsin inhibitor soybeans supported greater ADG and gain/feed compared to conventional soybeans in growing and finishing swine. Improvements in ADG and gain/feed in broiler chicks was reported by Yen et al. (1973) and Bajjalieh et al. (1980) when low trypsin inhibitor soybeans were compared to raw conventional soybeans. However, in these experiments, heat-processed soybean meal was superior to the unheated conventional and low trypsin inhibitor varieties. Thus, it seems that some degree of heat treatment would be required for maximum nutritional value of all soybean varieties evaluated to date.

However, this still leaves the question of whether less heat processing is needed for soybeans genetically

altered to have reduced trypsin inhibitor activity. Thus, a firm understanding of acceptable maximum trypsin inhibitor would be beneficial. Researchers have suggested that the maximum level allowed, without impairing growth performance, varies with species (Pontif et al., 1987) and age (Crenshaw and Danielson, 1985; Saxena et al., 1963) of animal. Liener and Tomlinson (1981) demonstrated the destruction of trypsin inhibitor activity in both low trypsin inhibitor and conventional soybeans in response to autoclaving. In work with rats, Klose et al. (1946) determined that optimum performance was achieved when soybeans were heated at 33 kg steam pressure for 10 to 15 min. It has been suggested that excessive heat treatment can reduce the bioavailability of nutrients, because of the Maillard reaction (Plakas et al., 1985), thus offsetting the benefit of decreased trypsin inhibitor activity. Evans and McGinnis (1948), and Rios Iriarte and Barnes (1966) found that over-processing of soybean meal resulted in sulfur amino acids being severely limiting in rats. Thus, decreasing the need for heat treatment by decreasing trypsin inhibitor activity in raw beans could prevent the loss of availability of these limiting amino acids.

As the duration of heat treatment increased, ADG, ADFI, and gain/feed increased ($p<0.0001$) for the chicks (table 4). Also, chicks fed the -K soybeans had greater ADG ($p<0.001$), ADFI ($p<0.002$), and gain/feed ($p<0.001$) compared to chicks fed +K soybeans. It should be noted, however, that station and station × treatment interaction effects were observed for ADG and gain/feed. For ADG, chicks at Nebraska grew faster (station effect, $p<0.001$) but with less difference at heat treatments of 12 min or more (station × quadratic effect of heat treatment, $p<0.004$). The same station ($p<0.001$), station × genotype ($p<0.001$), and station × heat treatment ($p<0.002$) effects were noted for gain/feed. It is likely that these effects are related to the greater reductions of trypsin inhibitor activity with increased duration of heat treatment in the Nebraska experiment. However, the interactions were in magnitude of response rather than direction of response, thus the pooled data are presented as line graphs in figures 1 and 2.

When the data from the Nebraska and Kansas replications were pooled, ADG increased linearly (figure 1) as the duration of heat treatment increased ($p<0.001$), and chicks fed -K had greater rates of gain than chicks fed +K soybeans ($p<0.001$). Although chicks fed raw +K soybeans had lower ADG than chicks fed raw +K soybeans, rate of gain of chicks fed both soybean genotypes were similar after the soybeans had been heated for 24 min (genotype heat treatment interaction, $p<0.001$). Heat treatment of soybeans with and without gene expression for the Kunitz trypsin inhibitor decreased trypsin inhibitor intake ($p<0.001$), and chicks fed +K soybeans had greater trypsin inhibitor intake than chicks fed -K soybeans ($p<0.001$). Although chicks fed raw +K soybeans had greater trypsin inhibitor intakes, chicks

Table 4. Nutritional value of heated soybeans with (+K) and without (-K) the Kunitz trypsin inhibitor[a]

	Heat treatment of +K, min[a]						Heat treatment of -K, min[a]					
Item	0	3	6	12	18	24	0	3	6	12	18	24
ADG, g[b]												
Kansas	17.1	16.4	17.9	19.9	20.8	25.8	21.0	21.9	21.6	21.9	25.9	27.7
Nebraska	20.4	22.2	24.0	26.3	29.7	30.5	25.0	25.1	25.5	25.4	29.6	29.5
ADFI g[c]												
Kansas	35.3	34.9	36.4	37.8	38.8	42.1	38.1	39.4	37.9	38.9	41.5	42.9
Nebraska	38.1	39.3	39.3	40.1	41.1	42.2	40.4	39.5	39.6	40.2	43.0	42.4
Gain/Feed[d]												
Kansas	0.482	0.469	0.492	0.523	0.538	0.612	0.550	0.555	0.571	0.562	0.624	0.645
Nebraska	0.540	0.566	0.611	0.655	0.724	0.721	0.620	0.636	0.644	0.657	0.689	0.696
Pancreas wt, mg/g BW[e]												
Kansas	9.7	8.8	10.3	10.0	8.5	8.0	8.7	8.7	8.3	7.6	7.0	6.5
Nebraska	10.3	9.9	8.1	7.9	3.9	3.5	7.8	7.9	6.5	6.0	3.6	3.4

[a] Heat treatment was autoclaving at 121℃ at 1.1 kg/cm^2 steam pressure.

[b] Station ($p<0.001$); station by heat treatment quadratic ($p<0.004$); station × genotype ($p<0.001$); Genotype ($p<0.001$); heat treatment linear ($p<0.001$); genotype × heat treatment linear ($p<0.001$); SE=0.530.

[c] Genotype ($p<0.001$); heat treatment, linear ($p<0.05$); SE=0.669.

[d] Station ($p<0.001$); station × genotype ($p<0.001$); genotype ($p<0.001$); genotype × heat treatment linear ($p<0.001$); heat treatment linear ($p<0.001$); station × heat treatment quadratic ($p<0.002$); SE=0.010.

[e] Station ($p<0.001$); station × heat treatment, linear ($p<0.001$); genotype ($p<0.001$); heat treatment, linear ($p<0.001$); SE=0.4402.

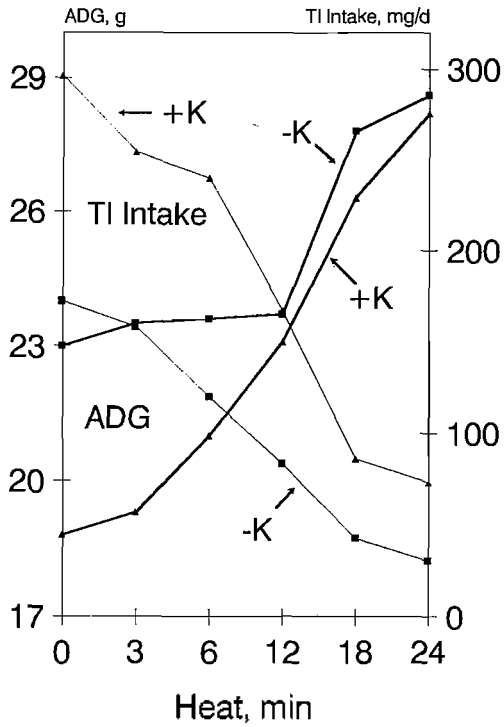

Figure 1. Average daily gain and trypsin inhibitor intake of pooled data. Heat treatment of soybeans with (+K) and without (-K) gene expression for the Kunitz trypsin inhibitor increases ADG and decreases trypsin inhibitor intake. For ADG treatment effects were: station effect ($p<0.0001$); genotype effect ($p<0.0001$); station genotype ($p<0.0002$); heat treatment linear ($p<0.0001$); genotype heat treatment linear ($p<0.0002$); and station heat treatment quadratic ($p<0.004$)

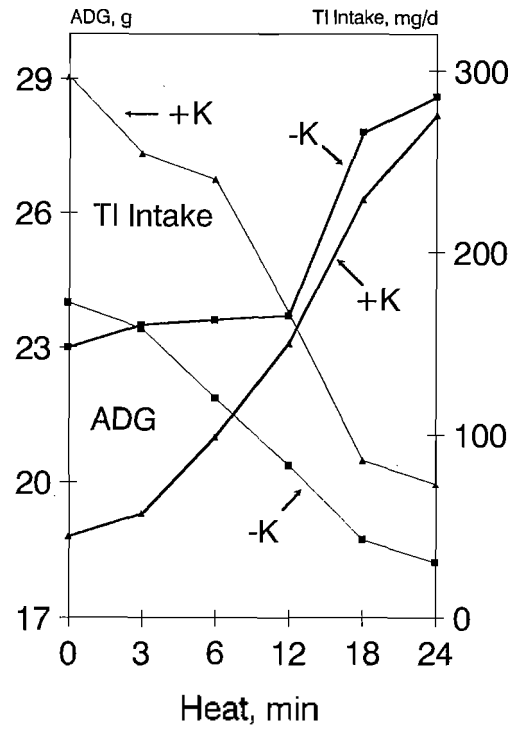

Figure 2. Gain/feed and pancreatic weights of pooled data. Heat treatment of soybeans with (+K) and without (-K) gene expression for the Kunitz trypsin inhibitor increases gain/feed and decreases pancreas weights of growing chicks. For gain/feed, treatment effects were: station effect ($p<0.0001$); genotype effect ($p<0.0001$); station genotype ($p<0.0001$); heat treatment linear ($p<0.0001$); genotype heat treatment linear ($p<0.0001$); and station heat treatment quadratic ($p<0.002$). For pancreas weights, treatment effects were: station effect ($p<0.0001$); genotype effect ($p<0.0001$); heat treatment linear ($p<0.0001$); and station heat treatment linear ($p<0.0001$).

fed both soybean genotypes heated for 24 min had similar trypsin inhibitor intakes (Genotype × heat treatment interaction, $p<0.004$).

Heat treatment of both soybean genotypes decreased chick pancreatic weights (mg pancreas/g BW) (figure 2) when data from both experiments was pooled ($p<0.001$), although pancreas from chicks fed -K soybeans were lighter in proportion to their BW ($p<0.001$). Gain/feed increased as heat treatment increased ($p<0.001$), and chicks fed -K soybeans had greater gain/feed than chicks fed +K soybeans; however, chicks fed +K soybeans had greater improvement in gain/feed over chicks fed -K soybeans, when the soybeans were heated.

The decreased trypsin inhibitor activity with heat treatment resulted in decreased pancreas weights ($p<0.001$). The pancreas respond to increased trypsin inhibitor in the diet by secreting more proteolytic enzymes (e.g. trypsin and chymotrypsin) to replace those proteases complexed by protease inhibitors. This demand for increased production of proteolytic enzymes results in hypertrophy of the organ. Kakade et al. (1973) noted that although pancreatic hypertrophy decreased in rats with decreased concentrations of trypsin inhibitor, they believed only 40% of the decrease in size was due to the decrease in trypsin inhibitor activity. Kakade et al. (1973) further suggested that the remaining pancreatic hypertrophy can be attributed to the ability of some soy proteins to complex with proteases and resist proteolysis, possibly because of their globular forms. The ineffectual complexing would trigger the same feed-back inhibition demonstrated by trypsin inhibitors (Green et al., 1973). These results are in contrast with the findings of Borchers et al. (1947), who argued that the nutritive value of the soybean product was directly related to the amount of trypsin inhibitor remaining active after various durations of heat treatment. This may be true if one considers that trypsin inhibitor activity is an indicator of the proportion of heat labile antinutritional factors that

remain, but the inhibitor itself is not solely responsible for the deleterious effects observed when raw soybeans are fed. Raw low trypsin inhibitor soybeans support greater growth performance than raw conventional soybeans, although ADG, ADFI, gain/feed, and pancreatic weights were similar when both soybean genotypes were heated to 24 min.

IMPLICATIONS

Reduced processing would translate into economic benefits for anyone processing soybeans for use in diets for animals. Decreased heat treatment could also result in less possibility for destruction of limiting amino acids. Further developments in plant breeding may produce varieties of soybeans that lack all trypsin inhibitor activity and other factors that interfere with animal growth and performance, and thus, remove the need for processing to allow maximum performance. In the current experiment, raw -K soybeans supported greater growth performance in broiler chicks than raw +K soybeans, although this advantage was lost when both soybean genotypes were heated for 24 min. Heat treatment of +K soybeans supported similar growth performance to heated -K soybeans, even though +K soybeans supported lower rates and efficiencies of gain than -K soybeans when fed raw.

REFERENCES

AOAC. 1990. Official Methods of Analysis (15th Ed.). Association of Official Analytical Chemists, Arlington, VA.

Bajjalieh, N., J. H. Of, T. Hymowitz and A. H. Jensen. 1980. Response of young chicks to raw, defatted, Kunitz trypsin inhibitor variant soybeans as sources of dietary protein. Poult. Sci. 59:328.

Borchers, R. 1965. Raw soybean growth inhibitor. Fed. Proc. 24:1494.

Borchers, R., C. W. Ackerson and R. M. Sandstedt. 1947. Trypsin inhibitor. III. Determination and heat destruction of the trypsin inhibitor of soybeans. Arch. Biochem. Biophys. 12:367.

Claudinin, D. R., W. W. Cravens, C. A. Elvehjem and J. G. Halpen. 1947. Deficiencies in over heated soybean oilmeal. Poult. Sci. 26:150.

Cook, D. A., A. H. Jensen, J. R. Fraley and T. Hymowitz. 1988. Utilization by growing and finishing pigs of raw soybeans of low Kunitz trypsin inhibitor content. J. Anim. Sci. 66:1686.

Crenshaw, M. A. and D. M. Danielson. 1985. Raw soybeans for growing-finishing pigs. J. Anim. Sci. 60:725.

Evans, R. J. and J. McGinnis. 1948. Cystine and Methionine metabolism by chicks receiving raw or autoclaved soybean oil meal. J. Nutr. 35:477.

Green, G. M., B. A. Olds, G. Matthews and R. L. Lyman. 1973. Protein as a regulator of pancreatic secretion in the rat. Proc. Soc. Exp. Biol. Med. 142:1162.

Hammerstrand, G. E., L. T. Black and J. D. Glover. 1981. Trypsin inhibitors in soy products: modification of the standard analytical procedure. Cereal Chem. 58:813.

Hancock, J. D., A. J. Lewis and E. R. Peo, Jr. 1989. Effect of methanol extraction on the utilization of soybean protein by growing rats. Nutr. Rep. Int. 39:813.

Hancock, J. D., E. R. Peo., Jr., A. J. Lewis, L. I. Chiba and J. D. Crenshaw. 1990. Effects of alcohol extraction and heat treatment on the utilization of soybean protein by growing rats and pigs. J. Sci. Food Agric. 52:193.

Hymowitz, T. 1984. Anti-nutritional factors in soybeans: genetics and breeding. World Soybean Research Conf. III Proc. 1984.

Kakade, M. L., D. E. Hoffa and I. E. Liener. 1973. Contribution of trypsin inhibitors to the deleterious effects of unheated soybeans fed to rats. J. Nutr. 103:1772.

Kim, I. H., J. D. Hancock, R. H. Hines and T. L. Gugle. 2000. Roasting and extruding affect nutrient utilization from soybeans in 5 and 10 kg nursery pigs. Asian-Aus. J. Anim. Sci. 13(2):200.

Kim, I. H., J. D. Hancock, D. B. Jones and P. G. Reddy. 1999. Extrusion processing of low-inhibitor soybeans improves growth performance of early-weaned pigs. Asian-Aus. J. Anim. Sci. 12(8):1251.

Klose, A. A., B. Hill and H. L. Fevold. 1946. Presence of growth-inhibiting substance in raw soybeans. Proc. Soc. Exp. Biol. Med. 62:10.

Liener, I. E. and M. L. Kakade. 1969. Protease inhibitors. In: Toxic Constituents of Plant Foodstuffs (Ed. I. E. Liener). Academic Press, New York.

Liener, I. E. and S. Tomlinson. 1981. Heat inactivation of protease inhibitors in a soybean line lacking the trypsin inhibitor. J. Food Sci. 46:1354.

McNaughton, J. L., F. N. Reece and J. W. Deaton. 1981. Relationship between color, trypsin inhibitor contents, and urease index of soybean meal and effects on broiler performance. Poult. Sci. 60:393.

NRC. 1984. Nutrient Requirements of Poultry (8th Ed.). 1984. National Academy Press, Washington, DC.

Osborne, T. B. and L. B. Mendel. 1917. The use of soybean as food. J. Biol. Chem. 32:369.

Pontif, J. E., L. L. Southern, D. F. Coombs, K. W. McMillin, T. D. Bidner and K. L. Watkins. 1987. Gain, feed efficiency and carcass quality of finishing swine fed raw soybeans. J. Anim. Sci. 64:177.

Plakas, S. M., T. Lee, R. E. Wolke and T. L. Meade. 1985. Effect of the browning reaction on protein utilization and plasma amino acid response by rainbow trout (*Salmo gaidneri*). J. Nutr. 115:1589.

Rackis, J. J. 1972. Biologically active components. In: Chemistry and Technology (Ed. A. K. Smith, and S. J. Circle). AVI Publishing Co., Westport, Conn. p. 158.

Rios Iriarte, B. and R. H. Barnes. 1966. The effect of overheating on certain nutritional properties of the protein of soybeans. Food Tech. 20:835.

SAS. 1985. SAS User's Guide Statistics (Version 5 Ed.). SAS Inst. Inc., Cary, NC.

Saxena, H. C., L. S. Jensen and J. McGinnis. 1963. Influence of age on utilization of raw soybean meal by chickens. J. Nutr. 80:391.

Singh, L. C. M. Wilson and H. H. Hadley. 1969. Genetic differences in soybean trypsin inhibitors separated by disc

electrophoresis. Crop Sci. 9:489.

Vandergrift, W. L., D. A. Knabe, T. D. Tanksley and S. A. Anderson. 1983. Digestibility of nutrients in raw and heated soyflakes for pigs. J. Anim. Sci. 57:1215.

Veltmann, J. R., B. C. Hansen, T. D. Tanksley, D. Knabe and S. S. Linton. 1986. Comparison of the nutritive value of different heat treated commercial soybean meals: Utilization by chicks in practical type rations. Poult. Sci. 65:1561.

Waldroup, P. W., D. R. Sloan and R. F. Davenport. 1969. The use of raw and extruded soybeans in layer diets. Poult. Sci. 48:1481.

Wood, A. S., J. D. Summers, E. T. Moran, Jr. and W. F. Pepper. 1971. The utilization of unextracted raw and extruded full-fat soybeans by the chick. Poult. Sci. 50:1392.

Yen, J. T., T. Hymowitz and A. H. Jensen. 1972. Utilization of protein from a trypsin-inhibitor variant soybean by growing gilts. J. Anim. Sci. 35:225.

Yen, J. T., A. H. Jensen, T. Hymowitz and D. H. Baker. 1973. Utilization of different varieties of raw soybeans by male and female chicks. Poult. Sci. 52:1875.

Yen, J. T., T. Hymowitz and A. J. Jensen. 1974. Effects of soybeans of different trypsin inhibitor activities on performance of growing swine. J. Anim. Sci. 38:304.