Chemical, In Vitro, and In Vivo Evaluation of Soybeans Heat-Treated by Various Processing Methods[1]

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ABSTRACT

In trial 1, eight Holstein heifers weighing 410 kg were used in an 8 × 8 Latin square and fed TMR containing 79.3% alfalfa silage and 20% soybeans. The first four treatments were raw soybeans, soybeans roasted and held for 3 h at the roasting temperature, extruded soybeans, and soybeans roasted in a California Pellet Mill Jet-Sploder®. The remaining four treatments were obtained by altering the residence time of soybeans in the Jet Sploder®. The temperatures of soybeans exiting the roaster were 117, 126, 138, and 154°C for the last four treatments. The soybeans held 3 h postroasting and the extruded soybeans resulted in the highest estimate of postruminal available lysine. Blood plasma concentrations of essential and branched-chain AA were highest in heifers fed soybeans held 3 h postroasting. In trial 2, 44 Holstein heifers weighing 150 to 250 kg were assigned randomly to one of four TMR. Diets consisted of 91.8% alfalfa silage and 7.5% of one of four soybean treatments. Treatments were raw soybeans, soybeans roasted in a drum roaster with an exit temperature of 146°C, and those roasted with exit temperatures of 141 or 146°C and held for .5 h. Estimated postruminal available lysine was higher for soybeans roasted and held versus roasted or raw soybeans. However, BW gain for heifers was similar across diets, averaging .90 kg/d for 12 wk. Concentrations of AA in plasma were not affected by diet. Overall, results support the recommendation of holding soybeans for at least .5 h following roasting.

(Key words: ruminant, protein, soybean, lysine)

Abbreviation key: ADG = average daily gain, BCAA = branched-chain amino acids, EAA = essential amino acids, ESB = extruded soybeans, JS = soybeans roasted in a California Pellet Mill Jet-Sploder®, PRAL = postruminal available lysine, SB = raw soybeans, SBHT = soybeans held at high temperature following roasting, SBM = soybean meal.

INTRODUCTION

Faldet et al. (12) reported on the relationship among protein degradation in the rumen, protein availability in the small intestine, and heat input for full fat soybeans in order to determine the optimal amount of heat for treating soybeans to maximize postruminal available lysine (PRAL). Methods used to identify optimally heated soybeans were an inhibitor in vitro procedure for estimating fractional rate and extent of protein degradation by rumen microbes and nutritionally available lysine content (by chemical analysis and rat growth). However, laboratory estimates of the optimal amount of heat for treating soybeans must be confirmed in vivo.

The concentration of plasma AA can reflect changes in AA absorption from the small intestine (2). More specifically, plasma concen-

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trations of branched-chain AA (BCAA) reflect more closely their absorption, because they are metabolized to a lesser extent by the liver than are other essential AA (EAA) (15). Responses in plasma AA, especially BCAA, have been observed when heated soybean meal (SBM) was fed to lambs (21), steers (22), and lactating cows (17) and when extruded (ESB) (3) or roasted (18, 23) soybeans were fed to lactating cows.

Responses in average daily gain (ADG) and feed conversion in ruminants also have been used to determine the effectiveness of heat treatment of proteins. Researchers have reported increased ADG or improved feed conversion, or both, by feeding heat-treated SBM to young lambs (13, 16) and cattle (9, 27). Dysli et al. (11) reported a linear increase in ADG in sheep by feeding soybeans that had progressively greater exposure to autoclaving. In contrast, calves fed ESB showed no benefit in ADG compared with those fed SBM (10).

The purpose of our research was to test in vivo, using heifer plasma AA concentrations and growth, what the laboratory methods (in vitro and chemical assays) were suggesting as the optimal heat treatment for soybeans that maximizes PRAL.

MATERIALS AND METHODS

Trial 1

Eight Holstein heifers weighing an average of 410 kg were assigned randomly to one of eight experimental diets in an 8 × 8 Latin square design (8) with 5-d periods. Period 1 was preceded by a 10-d adaptation period. Diets were fed as TMR for ad libitum intake once a day. Composition of the TMR is given in Table 1.

Eight soybean treatments were used. The first four treatments were 1) raw soybeans (SB), 2) soybeans roasted (Gem Roaster, Winona, MN) and immediately placed and held (SBHT) for 3 h in 208-L barrels covered with canvas before being allowed to cool, 3) ESB (Insta Pro, Des Moines, IA), and 4) soybeans roasted in a California Pellet Mill Jet-Sploder® (JS) (California Pellet Mill Co., San Francisco, CA). The remaining four treatments were obtained by changing the transit setting of the JS. Temperature of soybeans exiting the roaster were 117, 126, 138, and 154°C. Temperature of soybeans exiting the roasters for SBHT and JS treatments were not obtained. The JS treatment was a roasted soybean product marketed by a local feed manufacturer. Mean air temperature inside the Gem Roaster for the SBHT treatment was 430°C. Initial and final temperatures at a depth of 30 cm in the barrels for the SBHT treatment were 120 and 110°C. The SB and SBHT soybeans were cracked into halves and quarters, and the JS, 117, 126, 138, and 154°C soybeans were crimped prior to storage. A sample of each soybean treatment was obtained for each period and composited into one sample for analysis of DM (1), CP (7), total lysine (1), as well as an indirect 1-fluoro-2,4-dinitrobenzene procedure for determining available lysine (1, 24). All samples were ground through a 2-mm and then a 1-mm Wiley mill (Arthur H. Thomas, Philadelphia, PA) screen. Soybean treatments also were assayed for fractional rate of ruminal protein degradation by an inhibitor in vitro system, and the proportion potentially escaping the rumen was estimated (5). Results of these assays are in Table 2.

Samples of silage, TMR, and orts were collected daily, stored frozen, and composited by period. Silage DM was determined by toluene distillation (1) and DM content of other ration ingredients by oven drying at 60°C for 48 h. Crude protein content (7) of all feed

TABLE 1. Composition of diets (trials 1 and 2).

Composition	Trial 1	Trial 2
	— (% dry basis) —	
Alfalfa silage[1]	79.3	91.8
Soybean	20.0	7.5
Trace-mineralized salt[2]	.5	.5
Monophosphate	.1	.1
Vitamin ADE premix[3]	.1	.1
CP[4]	25.4	20.3

[1]Averaged 30% DM, 23% CP, 32% ADF, and 39% NDF for trial 1. Averaged 53% DM, 19% CP, 34% ADF, and 43% NDF for trial 2.

[2]Composition (grams per 100 g): NaCl (96 to 98.5); Zn (>.350); Mn (>.200); Fe (>.200); Cu (>.030); I (>.007); Se (>.007); Co (>.005).

[3]Composition (international units per kilogram): vitamin A, 2,664,600; vitamin D, 900,000; vitamin E, 900.

[4]An average of all diets within a trial.

TABLE 2. Protein composition and degradability measurements of soybean treatments (trial 1).

Component	Dietary treatment[1]							
	SB	SBHT	ESB	JS	117°C	126°C	138°C	154°C
DM, %	91.3	97.3	98.0	94.4	95.4	96.4	97.4	98.3
CP, % of DM	37.2	36.2	33.3	36.4	35.9	36.1	36.3	37.2
Total lysine, % of DM	2.38	1.93	2.11	2.38	2.30	2.24	2.08	1.71
Available lysine, % of DM	2.30	1.79	2.06	2.28	2.24	2.18	1.96	1.54
In vitro degradation rate,[2] (k_d)/h	.175	.029	.040	.085	.068	.060	.048	.033
Intercept (B), %	94	95	95	94	94	94	95	95
Estimated UIP,[3] %	24	64	57	39	44	47	53	61
PRAL,[4] g/kg DM	5.5	11.5	11.7	8.9	9.9	10.2	10.4	9.4

[1]SB = Raw soybeans; SBHT = soybeans held for 3 h postroasting without cooling; ESB = extruded blend of SB (92.5%), sodium bentonite (5%), lignin sulfonate (1.25%), and hemicellulose extract (1.25%); JS = Jet-Sploded® soybeans; 117, 126, 138, and 154°C are the temperatures of soybeans immediately exiting a California Pellet Mill Jet-Sploder®.

[2]Ruminal degradation rate determined with an inhibitor in vitro system (5).

[3]Estimated rumen undegraded intake protein (UIP), % = $\{B \times [k_p/(k_p + k_d)]\} \times 100$, assuming that k_p = .06/h (5).

[4]PRAL = Estimated postruminal available lysine; a product of available lysine and estimated UIP.

ingredients was determined. Alfalfa silage also was analyzed for NDF (25) and ADF (14). Samples of TMR and orts were analyzed for DM (60°C, 48 h) and CP (7). Dry matter and CP fed were calculated from analysis of individual feed ingredients, and DM and CP intakes were calculated from the TMR formulation and DM and CP content of orts.

Five hours after feeding on d 4 and 5 of each period, 10-ml blood samples were taken by venipuncture from the jugular vein of each heifer, heparinized, and placed immediately on ice. Plasma was prepared within 1 h of sampling and deproteinized with sulfosalicylic acid (6). Deproteinized plasma samples from d 4 and 5 were composited for each heifer and stored (–20°C) until analyzed for plasma free AA using a Beckman 6300 AA analyzer (Beckman Instruments, Palo Alto, CA) with S-2-aminoethyl cysteine as internal standard.

Data were analyzed statistically as an 8 × 8 Latin square design. When significant *F* values were detected because of soybean treatment ($P < .05$), mean separation was by least significant differences (26).

Trial 2

Forty-four Holstein heifers weighing from 150 to 250 kg were randomly assigned to one of four experimental diets. Diets were fed as TMR for ad libitum intake once a day. A 10-d adaptation period preceded the experimental period, which lasted 12 wk. Heifers were grouped by treatment and placed in freestall housing.

Composition of diets is given in Table 1. The four soybean treatments were SB, soybeans roasted (Gem Roaster) with an exit temperature of 146°C, and soybeans roasted with exit temperatures of 141 and 146°C and immediately placed and held for .5 h in 208-L barrels covered with canvas before being allowed to cool. Mean air temperatures inside the roaster were 430 and 490°C for the soybeans exiting at 141 and 146°C, respectively. Both initial and final temperatures at a depth of 30 cm in the barrels were 117°C for the 141°C soybean treatment and 124 and 122°C for the 146°C treatment. All soybeans for treatments were coarsely cracked, giving mostly halves and quarters, prior to storage. Two subsamples of each soybean treatment were obtained by compositing six weekly samples. Subsamples were analyzed for DM, CP, total lysine, available lysine, fractional rate of ruminal protein degradation, and proportion of protein escaping the rumen by procedures described in trial 1. Results of these assays are in Table 3.

A weekly composite of silage, TMR, and orts were collected from daily samples and stored frozen. Analyses of composited samples for DM and CP for TMR and orts and for silage DM, CP, ADF, and NDF were as

TABLE 3. Protein composition and degradability measurements of soybean treatments (trial 2).[1]

Component	SB	146°C	Held for .5 h at high temperature	
			141°C	146°C
DM, %	89.1	96.1	95.7	97.3
CP, % of DM	37.3	38.3	38.6	35.9
Total lysine, % of DM	2.41	2.30	2.22	2.18
Available lysine, % of DM	2.28	2.16	2.08	2.02
In vitro degradation rate,[2] (k_d)/h	.167	.056	.029	.034
Intercept (B), %	97	98	96	96
Estimated UIP,[3] %	26	51	65	61
PRAL,[4] g/kg DM	5.9	11.0	13.5	12.3

[1]SB = Raw soybeans; 141 and 146°C are the temperatures of soybeans immediately exiting a drum roaster; holding = soybeans held in barrels .5 h postroasting without cooling.

[2]Ruminal degradation rate determined with an inhibitor in vitro system (5).

[3]Estimated rumen undegraded intake protein (UIP), % = $\{B \times [k_p/(k_p + k_d)]\} \times 100$, assuming that k_p = .06/h (5).

[4]PRAL = Estimated postruminal available lysine; a product of available lysine and estimated UIP.

detailed in trial 1. Dry matter and CP intakes were determined as in trial 1. Heifer BW were recorded on 3 consecutive d at the beginning and end of the experiment and weekly during the experiment. Samples of blood from jugular venipuncture were obtained 3 h after feeding on the last 2 d of wk 10. Plasma samples were deproteinized, composited for each heifer, and analyzed for free AA as described in trial 1. Means for ADG and plasma AA profiles were analyzed as a completely randomized design. When significant F values were detected because of soybean treatment ($P < .05$), means were separated by least significant difference (26).

RESULTS AND DISCUSSION

Trial 1

Intakes of DM and CP were similar across diets, averaging 8.5 and 2.2 kg/d, respectively. Plasma free AA concentrations, which were significantly affected by diet, are in Table 4. The SBHT treatment gave rise to greater plasma concentrations of individual and total BCAA and total EAA than other soybean treatments. Increases in blood plasma concentrations of BCAA and EAA reflect a greater supply of protein to the intestine (2). More specifically, concentrations of BCAA in extrahepatic blood plasma reflect their absorption from the intestine because BCAA are metabolized to a lesser extent by the liver than are other EAA (15). Therefore, concentrations of plasma BCAA can be an indicator of protein uptake from the small intestine.

It was expected that the SBHT treatment would lead to relatively higher concentrations of BCAA, as it did, because SBHT had one of the highest estimates of PRAL (Table 2). The 138°C treatment resulted in the next highest plasma concentrations of individual and total BCAA and total EAA. Treatments 154°C, JS, ESB, and 126°C were intermediate in individual and total BCAA concentrations, whereas treatments 117°C and SB were lowest. It was surprising that ESB did not result in higher BCAA concentrations because calculated PRAL values for ESB and SBHT were very similar. Total EAA were similar for treatments 154°C, JS, ESB, 126 and 117°C, and SB. Total BCAA:glycine ratio, also an indication of protein supply to the intestine (2), was highest for treatments SBHT and 138°C, intermediate for treatments 154 and 126°C, and lowest for the remaining treatments. Increases in BCAA concentrations have been reported in blood plasma of lambs fed heated (149°C for 4 h) compared with unheated SBM (21) and in blood serum of cows fed ESB compared with SB (3). Mohamed et al. (18) also reported increases in plasma concentrations of BCAA in cows fed SBHT (held for 3 h postroasting) compared with SB, but BCAA concentrations were similar to those of cows fed SBM. However, in

TABLE 4. Concentrations of free amino acids in blood plasma (trial 1).[1]

Component	Dietary treatment[2]								SEM
	SB	SBHT	ESB	JS	117°C	126°C	138°C	154°C	
	(nmol/ml plasma)								
Asparagine	110^{c}	130^{a}	113^{bc}	123^{ab}	112^{bc}	111^{bc}	122^{abc}	113^{bc}	4
Citrulline	89^{c}	102^{a}	94^{bc}	96^{ab}	92^{bc}	91^{bc}	94^{bc}	93^{bc}	2
Valine	283^{d}	338^{a}	293^{cd}	301^{bcd}	285^{d}	298^{bcd}	315^{b}	311^{bc}	7
Isoleucine	115^{c}	140^{a}	119^{c}	124^{bc}	115^{c}	121^{bc}	130^{b}	124^{bc}	3
Leucine	144^{cd}	181^{a}	148^{cd}	159^{bc}	141^{cd}	150^{bcd}	162^{b}	159^{bc}	5
Phenylalanine	47^{cd}	54^{a}	47^{cd}	49^{bc}	45^{d}	47^{cd}	51^{ab}	50^{bc}	1
Ornithine	101^{ab}	106^{a}	96^{bc}	95^{c}	98^{bc}	99^{bc}	100^{abc}	98^{bc}	2
Lysine	110	111	103	108	109	103	110	97	4
BCAA[3]	542^{de}	659^{a}	560^{bcde}	584^{bcd}	541^{e}	569^{bcde}	607^{b}	594^{bc}	15
BCAA:Glycine	3.18^{c}	3.80^{a}	3.26^{bc}	3.36^{bc}	3.25^{bc}	3.51^{ab}	3.71^{a}	3.51^{ab}	.10
EAA[3]	1076^{bc}	1208^{a}	1082^{bc}	1108^{bc}	1058^{c}	1079^{bc}	1145^{ab}	1116^{bc}	26
NEAA[3]	1036	1095	1040	1064	1040	1020	1027	1023	24

^{a,b,c,d,e}Means in the same row with different superscripts differ ($P < .05$).

[1]Includes all plasma free AA that were significantly affected by diet (except lysine and total NEAA).

[2]SB = Raw soybeans; SBHT = soybeans held for 3 h postroasting; ESB = extruded soybeans; JS = Jet-Sploded® soybeans; 117, 126, 138, and 154°C are the temperatures of soybeans immediately exiting a California Pellet Mill Jet-Sploder®.

[3]BCAA = Branched-chain AA (sum of valine, isoleucine, and leucine); EAA = essential AA (threonine, valine, methionine, isoleucine, leucine, phenylalanine, tryptophan, lysine, histidine, and arginine); NEAA = nonessential AA (aspartic acid, serine, asparagine, glutamic acid, glutamine, proline, glycine, alanine, and tyrosine).

their study, DM and CP intakes were lower for cows fed SB or SBHT compared with those fed SBM, which may account for the similarity in plasma AA concentrations between cows fed SBM and SBHT. Pena-Castellanos (23) also reported significant increases in plasma EAA concentrations (specifically BCAA) in cows fed moderately or well-roasted SBHT (both held for 3 h postroasting; air temperature of drum roaster was 300 and 370°C, respectively) compared with SB.

Estimates of PRAL for soybean treatments followed a similar trend as the results for plasma concentrations of BCAA and BCAA:glycine ratio, except for ESB. Estimated PRAL was highest for the SBHT treatment (11.5 g/kg, excluding ESB), followed by treatment 138°C (10.4 g/kg), and the remaining heat treatments ranged from 8.9 to 10.2 g/kg. Plasma concentrations of BCAA and EAA followed the same trend.

Trial 2

Intakes of DM and CP were similar across diets, averaging 7.0 and 1.43 kg/d, respectively. Heifer weights averaged 197 kg at the beginning of the study. Body weight gains for heifers were similar ($P > .05$) across diets, averaging .91 kg/d (Table 5). It appears that the protein requirement of the heifers may have been met with these diets and that the soybean treatments, therefore, could not influence growth rate. Another possible explanation for the lack of growth response is that soybeans accounted for only 14% of dietary CP, and this may have reduced sensitivity of the experiment. However, there is evidence that the protein in legume forages, particularly alfalfa, is utilized poorly by ruminants. Several studies have suggested that protein supplements with a higher proportion of undegraded intake protein would be more effective with alfalfa than with corn silage-based diets (4, 28, 29). Also, the proportion of total N as NPN ranged from 50 to 89% for alfalfa ensiled at 35 to 85% moisture (19). Undegraded protein as a proportion of total CP is considered to be 23% for alfalfa silage (20), and we estimated undegraded protein for treatments SB, 146°C, and 141 and 146°C with .5 h of holding to be 26, 51, 65, and 61%, respectively (Table 3). The proportion of undegraded intake protein from soybeans ranged from 15 to 31% of total undegraded intake protein, depending on treatment. Therefore, ADG and feed efficiency for heifers were expected to reflect treatment ef-

TABLE 5. Average daily gain and blood plasma AA concentrations (trial 2).[1]

Component	SB	146°C	Held for .5 h at high temperature		SEM
			141°C	146°C	
Gain, kg/d	.91	.90	.89	.92	.04
BCAA,[2] nmol/ml	684	650	731	700	34
Essential, nmol/ml	1273	1197	1354	1291	54
Nonessential, nmol/ml	1166	1072	1153	1131	38

[1]SB = Raw soybeans; 141 and 146°C are the temperatures of soybeans immediately exiting a drum roaster; held = soybeans held in barrels .5 h postroasting without cooling.

[2]BCAA = Branched-chain AA.

fects. Daniels et al. (10) reported similar ADG (1.02 kg/d) for heifers (157 kg of BW) fed SBM, SB, or heat-treated soybeans (ESB or SBHT). However, the diet containing SB was lower in apparent digestible DM, CP, and energy than were diets containing either SBM or heat-treated soybeans. In contrast, Dysli et al. (11) reported a significant linear increase in ADG of sheep as autoclaving time for soybeans increased from 0 to 30 min. The CP content of diets fed to heifers (10) averaged 15.5%, whereas the diets fed to sheep (11) averaged 12%. It is possible that the protein requirement of the heifers was met by the diet, whereas a response in weight gain for sheep could be attributed to the improvement in quality and amount of protein because of heat treatment.

Total BCAA, EAA, and nonessential AA are in Table 5. In the present trial, blood plasma concentrations of AA in heifers were not affected by treatment diets. The standard error of the means was at least twice as high for BCAA and EAA in this trial as in trial 1.

CONCLUSIONS

In both trials, the percentage of estimated rumen undegraded intake protein in soybeans increased because of heat treatment; however, the increase was variable (range 39 to 65%) and was influenced by the method of treatment. Loss of available lysine in heat-treated soybeans depended on the severity of treatment; the 154°C treatment resulted in the largest loss of available lysine (33% relative to SB). In trial 1, plasma AA concentration appeared to be a useful indicator of AA absorption from the intestine and generally showed a positive relationship with estimated PRAL content of soybean treatments. However, in trial 2, plasma AA concentrations did not differ among treatments. Body weight gain in heifers also was similar across treatments.

When dietary intake of CP is similar between treatments and animal variability is controlled (e.g., in a Latin square design), then the concentration of BCAA and EAA in blood plasma can be useful indicators of whether a heat treatment is effective or not. However, it is difficult to quantitate how much better a treatment may be using BCAA and EAA concentrations alone. If data from both the laboratory analyses and plasma AA concentrations are considered, holding soybeans for at least .5 h following roasting (soybeans at 146°C when leaving the roaster) appears to result in a more thorough and extensive heat treatment than is typical for commercially roasted soybeans. Holding soybeans at an elevated temperature for approximately 30 min probably is beneficial for two reasons. First, the residence time of soybeans in many commercial roasters is not more than 1 to 2 min, and this does not give adequate time for heat transfer to the middle part of the soybean. Second, the Maillard reaction, which is caused by heating, is time-dependent, and longer heating times result in more complete protection of protein from degradation in the rumen.

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