

# Chemical, In Vitro, and In Situ Evaluation of Heat-Treated Soybean Proteins<sup>1</sup>

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## ABSTRACT

The effect on protein degradability of roasting soybeans and soybean meal and holding them at elevated temperatures for 0, .5, 1, 1.5, 2, 2.5, and 3 h was assessed using in vitro, in situ, and chemical techniques. Ruminant in vitro degradation rates decreased with initial roasting for both soybeans and soybean meal from .165 to .065 h<sup>-1</sup> and .155 to .092 h<sup>-1</sup>, respectively. Roasting and holding for 3 h resulted in the lowest degradation rates, .037 and .029 h<sup>-1</sup> for soybeans and soybean meal, respectively. In situ degradation rates decreased from .174 to .090 h<sup>-1</sup> and from .069 to .012 h<sup>-1</sup>, respectively, for soybeans and soybean meal roasted and held 3 h. There were no increases in ADIN with heat processing of soybeans. However, ADIN increased from 2.3 to 4.1% when soybean meal was roasted and held 3 h. In contrast, nutritionally available lysine decreased dramatically during holding with a loss after 3 h of 22% for soybeans and 17% for soybean meal. Roasting and holding significantly increased estimated postruminally available lysine (g/kg DM) of soybeans from 6.5 to 10.5 (0 h) and 11.2 (mean, .5 to 3 h) and of soybean meal from 7.6 to 10.5 (0 h) and 14.2 (mean, .5 to 3 h). Holding soybeans and soybean meal at an elevated temperature resulted in a more thorough and exten-

sive heat treatment than typically is obtained in commercial processing.

(Key words: rumen degradation, roasted soybeans, available lysine)

Abbreviation key: FDNB = fluorodinitrobenzene, PRNAL = postruminal nutritionally available lysine, RSB = roasted soybeans, RSBM = roasted soybean meal, SB = soybeans, SBM = soybean meal, TLMI = total lysine minus inaccessible lysine, UIP = undegradable intake protein.

## INTRODUCTION

Soybean products are the most common source of protein supplements fed to ruminants in the US. Unfortunately, the protein in full fat soybeans (10) and soybean meal (2) is degraded readily by rumen microbes, leading to surplus ammonia production in the rumen. Several procedures have been used to reduce microbial protein degradation; however, these methods have met with variable success. Heat treatment may have the greatest potential for safe and economical treatment of protein. Heat has been used to decrease protein degradation in the rumen and to increase the supply of dietary protein to the duodenum (4, 22, 23). However, there have been conflicting results regarding animal response to feeding heat-treated proteins (14, 17, 24). The diversity of results reported in the literature in part may be due to the method of heat treatment (oven-heating, roasting, extruding, and autoclaving) and the variation in heat actually applied to the protein supplements. These different methods provided varying amounts of protection from microbial protein degradation in the rumen. The objective of our experiments was to determine the optimal holding time after roasting of soybeans and soybean meal. Effectiveness of heat treatment was assessed using in vitro and

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in situ ruminal protein degradation, in situ mobile bag digestion, plus changes in ADIN and nutritionally available lysine.

#### MATERIALS AND METHODS

Full fat soybeans (SB) and solvent-extracted soybean meal (SBM) were roasted in a Gem Roaster (Gem Roaster, Winona, MN) and held at elevated temperatures for up to 3 h in 208-L barrels covered with canvas. Mean air temperatures of the roaster for SB and SBM were 420 and 205°C, respectively (the difference possibly reflecting lower efficiency of heat transfer during roasting of SB). Initial and final (3-h) temperatures at midpoint locations in the barrels were 120 and 110°C for SB and 116 and 110°C for SBM. Samples were removed from the barrels at 0, .5, 1.5, 2, 2.5, and 3 h postroasting and spread out to allow rapid cooling. All samples were ground through a 2-, then 1-mm, Wiley mill screen (Arthur H. Thomas, Philadelphia, PA). Soybeans, roasted SB (RSB), SBM, and roasted SBM (RSBM) were analyzed for DM (1), N (6), and proportion of total N found in ADIN (12).

Because lysine is the amino acid most vulnerable to heat damage, its nutritional availability should be a sensitive indicator of the effects of the heating process. Therefore, nutritionally available lysine was determined using an indirect 1-fluoro-2,4-dinitrobenzene (FDNB) procedure (1). Untreated and FDNB-treated samples were hydrolyzed in 20 ml of 6N HCl at 105°C for 20 h in 25- × 200-mm screw-capped culture tubes that were flushed with N<sub>2</sub> and sealed with Teflon tape; lysine concentrations were determined using an amino acid analyzer (Model 6300, Beckman Instruments, Spinco Division, Palo Alto, CA). Total lysine was determined on the untreated FDNB sample, and inaccessible (unavailable) lysine was determined on the FDNB-treated samples. Nutritionally available lysine was calculated by difference and will be referred to as total lysine minus inaccessible lysine (TLMI). This procedure was validated by Faldet et al. (10) using a rat growth assay.

Relative rates of ruminal protein degradation were determined with an inhibitor in vitro system on the unheated and heat-treated protein sources as described by Broderick (3), except for a scaled down procedure utilizing 5

ml of McDougall's buffer and 10 ml of inoculum. Net release of NH<sub>3</sub> and total amino acids between 0 and 4 h was used to estimate fractional degradation rates, assuming protein degradation can be described as a single exponential. Ruminal undegradable intake protein (UIP) was estimated using inhibitor in vitro degradation rates, assuming ruminal passage rate of .06 h<sup>-1</sup>. The product of UIP and TLMI was used to estimate the amount of lysine that escaped the rumen and was available for intestinal absorption [postruminal nutritionally available lysine (PRNAL)].

Statistical analyses of ADIN, TLMI, inhibitor in vitro degradation rates, and PRNAL were conducted using SAS (19). The total 15 df were partitioned between replication (1 df, two separate roasting dates: October 1984 and September 1985) and treatment (7 df) effects, and the replication × treatment interaction (7 df) was used as the error term. Where significant ( $P < .05$ ) *F* values were detected due to treatment, separation of means was by least significant differences.

#### In Situ Trial

Two lactating Holstein cows with ruminal and proximal duodenal cannulas were used in a switchback trial to determine the rate and extent of DM and N disappearance from Dacron bags in the rumen and lower gastrointestinal tract. Cows were fed, for ad libitum intake, total mixed rations containing a 55:45 ratio of forage to concentrate DM (Table 1) at 0400, 1000, 1600, and 2200 h. The RSB diet was fed (DM and CP intakes, 19.3 and 3.0 kg/d) when SB and RSB samples were tested, and the solvent SBM diet was fed (DM and CP intakes, 16.9 and 2.7 kg/d) when SBM and RSBM samples were tested. Cows were fed their respective diets for 21 d with the last 6 d of each period used for in situ incubations.

Unroasted and roasted SB and SBM, excluding the 1.5- and 2.5-h holding times, were tested. One-gram samples were placed in 6- × 10-cm Dacron polyester bags with mean pore size of 52 μm prepared as described by Weakley et al. (26). Duplicate samples were incubated on 2 different d for 1, 4, 8, 16, 24, and 36 h. Times when bags were suspended in the rumen were staggered such that all bags were removed from the rumen at the same

TABLE 1. Diet composition for cannulated cows in the in situ trial.

	Diets	
	Soybean meal	Roasted soybeans
	— (% of DM) —	
Alfalfa silage	27.5	27.5
Corn silage	27.5	27.5
High moisture ear corn	32.8	29.6
Soybean meal	10.2	...
Roasted soybeans <sup>1</sup>	...	13.4
Dicalcium phosphate	.82	.82
Calcium carbonate	.64	.64
Trace-mineral salt <sup>2</sup>	.43	.43
Vitamin A-D-E premix <sup>3</sup>	.11	.11

<sup>1</sup>Roasted soybeans held at elevated temperature for 3 h.

<sup>2</sup>Composition (g/100 g): NaCl (95 to 99); Mn (>.2); ferrous iron (>.16); ferric iron (>.04); Cu (>.033); Zn (>.01); I (>.007); and Co (>.003).

<sup>3</sup>Vitamin A-D-E (IU/kg): 3,525,000 of vitamin A; 661,000 of vitamin D; and 661 of vitamin E.

time and frozen. Zero-hour bags were soaked in water for .5 h and then washed to estimate disappearance of DM and CP due to both solubility and the washing procedure. Bags that had been incubated in the rumen were thawed and washed (26). All bags then were dried at 60°C for 24 h followed by 24 h at 105°C, and the remaining DM was determined. Nitrogen content (1) was determined on the entire bag, and the fractional degradation rate was computed from residual N by the procedure of Ørskov and McDonald (15).

The mobile bag technique (9) was modified as follows: quadruplicate samples were placed in the rumen for 16 h, then removed, washed, and incubated 3 h in pepsin (Sigma Chemical Co., St. Louis, MO) in dilute HCl (2 g pepsin/L .12N HCl) at 40°C with constant stirring. Bags (n = 28) were incubated in 3 L of pepsin-HCl solution. Following incubation, bags were placed in ice and then inserted via cannulas into the proximal duodenum at a rate of two bags/2 h. Bags were prepared and analyzed for DM and N as described previously.

Degradation rates for bags incubated in the rumen were analyzed statistically using one-way ANOVA (21). Separation of means was by least significant differences if treatment effects were significant ( $P < .05$ ).

## RESULTS AND DISCUSSION

Dry matter content for both RSB and RSBM averaged 97%. Results for ADIN, inhibitor in vitro rates of protein degradation, TLMI, and estimated PRNAL are in Table 2. Roasting had no effect on ADIN content for SB. However, ADIN content increased from 2.3% for SBM to 3.3% for 0-h RSBM, and up to a maximum of 4.2% for RSBM held more than 1.5 h.

Degradation rates of SB and RSB were reduced at each heating time from .165 h<sup>-1</sup> for unheated SB to .065 h<sup>-1</sup> for 0-h RSB and .051 h<sup>-1</sup> for RSB held for .5 h. There were no significant differences ( $P > .05$ ) among RSB held for .5 h (.051 h<sup>-1</sup>) to 2 h (.045 h<sup>-1</sup>); however, rates at 2.5 h (.040 h<sup>-1</sup>) and 3 h (.037 h<sup>-1</sup>) were lower. This agrees with the results of Pena (16), who observed differences in in vitro degradation rates between unheated SB and SB roasted at air temperatures of 300 and 370°C and held for 3 h at 120 and 151°C, respectively.

Unheated solvent SBM was degraded at a rate of .155 h<sup>-1</sup>; RSBM, allowed to cool immediately (0 h), was degraded at .092 h<sup>-1</sup> and was different from both SBM and all other RSBM. Roasted soybean meals held for .5 (.057 h<sup>-1</sup>) or 1 h (.057 h<sup>-1</sup>) were degraded more rapidly than RSBM held for 3 h (.029 h<sup>-1</sup>). The standard protein sources (casein, solvent SBM, and expeller SBM) used for the inhibitor in vitro procedure were the same as those used by Broderick (3) and yielded similar rates of degradation, indicating consistency among inhibitor in vitro experiments.

Generally, as holding time increased, TLMI content decreased for both SB and SBM. However, within the SB group there was greater divergence among treatments. Raw soybeans contained (DM basis) 2.29% TLMI and were greater than all RSB treatments. The TLMI content was similar for 0-h RSB (2.15%) and RSB held for .5 h (2.05%). The TLMI content was lowest in RSB held for 3 h (1.78%), which was lower than all remaining treatments except 2.5-h RSB. The TLMI content of 3-h RSB was 22% lower than for raw SB.

The TLMI content of SBM was (DM basis) 2.65% and 0-h RSBM was 2.55%, and TLMI decreased to 2.24 and 2.21% for RSBM held for 2.5 and 3 h, respectively. The RSBM were

TABLE 2. Effect of roasting and duration of holding on ADIN, in vitro protein degradation rate, nutritionally available lysine, and estimated postruminal nutritionally available lysine.<sup>1</sup>

Protein	Holding time	ADIN <sup>2</sup>	Rate <sup>2</sup>	Intercept antilog <sup>2</sup>		TLMI <sup>3</sup>	PRNAL <sup>4</sup>
				(% N)	(% N)		
Raw soybeans	. . .	3.1	.165 <sup>a</sup>	94.9	28.4	2.29 <sup>a</sup>	6.5 <sup>c</sup>
Roasted soybeans	0	2.5	.065 <sup>b</sup>	96.3	48.7	2.15 <sup>b</sup>	10.5 <sup>b</sup>
	.5	2.6	.051 <sup>c</sup>	96.4	54.7	2.05 <sup>b,c</sup>	11.2 <sup>a</sup>
	1	2.3	.044 <sup>c,d</sup>	96.0	57.7	1.92 <sup>c,d</sup>	11.1 <sup>a</sup>
	1.5	2.7	.042 <sup>c,d</sup>	95.7	59.0	1.92 <sup>c,d</sup>	11.3 <sup>a</sup>
	2	2.5	.045 <sup>c,d</sup>	96.3	57.5	1.94 <sup>c,d</sup>	11.2 <sup>a</sup>
	2.5	3.0	.040 <sup>d</sup>	95.8	60.5	1.84 <sup>d,e</sup>	11.1 <sup>a</sup>
SEM	. . .	.2	.003	. . .	. . .	.05	.2
Solvent SBM	. . .	2.3 <sup>c</sup>	.155 <sup>a</sup>	94.2	28.6	2.65 <sup>a</sup>	7.6 <sup>c</sup>
Roasted SBM	0	3.3 <sup>b</sup>	.092 <sup>b</sup>	96.0	41.2	2.55 <sup>a</sup>	10.5 <sup>b,c</sup>
	.5	3.6 <sup>a,b</sup>	.057 <sup>c</sup>	98.9	54.3	2.47 <sup>a,b</sup>	13.4 <sup>a,b</sup>
	1	3.8 <sup>a,b</sup>	.057 <sup>c</sup>	96.9	53.5	2.41 <sup>a,b</sup>	12.9 <sup>a,b</sup>
	1.5	4.2 <sup>a</sup>	.050 <sup>c,d</sup>	98.5	57.9	2.39 <sup>a,b</sup>	13.8 <sup>a,b</sup>
	2	4.0 <sup>a</sup>	.035 <sup>c,d</sup>	94.7	63.8	2.38 <sup>a,b</sup>	15.2 <sup>a</sup>
	2.5	4.2 <sup>a</sup>	.038 <sup>c,d</sup>	96.0	63.0	2.24 <sup>b</sup>	14.1 <sup>a,b</sup>
SEM	. . .	.2	.009	. . .	. . .	.10	1.2
Casein	. . .	. . .	.431	99.6	12.2	7.19	8.8
Solvent SBM	. . .	1.9	.162	93.1	27.1	2.75	7.5
Expeller SBM	. . .	2.8	.039	93.8	59.6	2.37	14.1

<sup>a,b,c,d,e</sup>Means in the same column with different superscripts differ ( $P < .05$ ). Statistical analyses were performed on the soybean and soybean meal (SBM) groups separately.

<sup>1</sup>Means are from two roasting dates (October 1984 and September 1985).

<sup>2</sup>Estimated undegradable intake protein (UIP, %) =  $(B[k_p/(k_p + k_d)] + C)$ , where C is ADIN as a proportion of total N, B is the intercept antilog,  $k_p$  is the rumen passage rate (.06/h), and  $k_d$  is the in vitro fractional degradation rate.

<sup>3</sup>TLMI = Total lysine minus inaccessible lysine on a DM basis.

<sup>4</sup>PRNAL = Postruminal nutritionally available lysine, a product of estimated UIP and TLMI (g/kg) on a DM basis.

similar in TLMI content across holding times. The lack of statistical difference and large SEM were attributed to the differences between roasting dates; on one roasting date, there was little effect of holding time on TLMI content. Holding RSBM for 3 h resulted in an average loss of 17% of the TLMI relative to unheated SBM.

Estimated PRNAL for SB were different among unheated SB, 0-h RSB, and RSB held for .5 to 3.0 h (6.5, 10.5, and an average of 11.2 g/kg DM, respectively). Faldet et al. (10) observed the optimal temperature and time for heating SB in a forced-air oven was 150°C for 1 h, and 160°C for .5 h, and averaged 11.3 g of PRNAL/kg DM for both. There appears to be a substantial benefit from the heating process. Our results showed 62 and 72% increases over unheated SB for 0-h RSB and RSB held for .5

to .3 h. Voss et al. (25) reported an advantage of 4.6 kg/d of 4% FCM with feeding cows RSB held for 3 h versus SBM when alfalfa silage was fed as the only forage. Some reports (14, 17, 24) have shown little or no benefit from feeding commercially roasted or extruded SB compared with unheated SB or SBM. Recently, Faldet and Satter (11) compared RSB held for .5 h to SBM and raw SB protein supplements. Results indicated a 4.5 kg/d increase in milk production for the RSB held for .5 h. The results of Voss et al. (25) and Faldet and Satter (11) may not be directly comparable with those from commercially roasted SB because these workers obtained more thorough and extensive heat treatment by using the holding process.

Estimated PRNAL for the SBM was 7.6 g/kg DM and differed from the RSBM held .5 to

TABLE 3. Nitrogen extracted from Dacron bags during soaking and washing and in situ rumen N degradation rates from Dacron bags for soybeans (SB) and soybean meal (SBM).

Protein <sup>1</sup>	Hours held postroasting	N Extracted <sup>2</sup>		Degradation rate	
		—— (%) ——		—— (h <sup>-1</sup> ) ——	
		$\bar{X}$	SE	$\bar{X}$	SE
Raw soybeans	. . .	42.0 <sup>a</sup>	.8	.174	.038
Roasted soybeans	.0	12.4 <sup>b</sup>	2.1	.118	.017
	.5	12.4 <sup>b</sup>	2.9	.112	.011
	1.0	14.9 <sup>b</sup>	4.3	.114	.001
	2.0	15.0 <sup>b</sup>	1.9	.110	.017
	3.0	13.8 <sup>b</sup>	2.2	.090	.021
Solvent SBM	. . .	18.5	1.7	.069 <sup>a</sup>	.004
Roasted SBM	.0	13.1	2.4	.036 <sup>b</sup>	.005
	.5	10.2	1.6	.018 <sup>c</sup>	.002
	1.0	10.5	1.2	.019 <sup>c</sup>	.003
	2.0	9.2	3.5	.015 <sup>c</sup>	.003
	3.0	8.9	1.6	.012 <sup>c</sup>	.002

<sup>a,b,c</sup>Means with different superscripts differ ( $P < .05$ ). The soybeans and SBM were tested as separate groups for mean separation. Significant treatment effects were not detected ( $P > .05$ ) for N extracted, for SBM, and for degradation rate for SB.

<sup>1</sup>Soybeans and soybean meal were from a March 1985 roasting date.

<sup>2</sup>Nitrogen loss after .5 h soaking in water.

3 h, which averaged 14.0 g/kg DM across all holding times. The 0-h RSBM yielded 10.5 g PRNAL/kg DM and was intermediate between SBM and RSBM held for .5, 1, 1.5, and 2.5 h. The highest PRNAL values were for RSBM held for 2 and 3 h (15.2 and 14.8 g/kg DM, respectively), an average of about twice as great as unheated SBM. If lysine were first-limiting (20), then the increased PRNAL for RSBM compared with SBM should result in increased milk production. Voss et al. (25) did not observe significant benefit with cows fed RSBM held for 3 h, compared with unheated SBM when the forage consisted of a mixture of alfalfa and corn silage (50:50, DM basis). The PRNAL value for expeller SBM (14.1 g/kg DM) was similar to all RSBM held .5 to 3 h. Results with cows fed expeller SBM have shown a benefit in milk production when alfalfa silage was the sole forage (5, 7) compared with a mixture of alfalfa and corn silage (2). Sahlu et al. (18) observed increased milk production by cows when heat-treated SBM replaced solvent SBM in cows fed corn silage plus alfalfa hay as forage.

Degradation rates computed from CP disappearance from in situ bags are in Table 3. Although heating did not influence rate for SB,

as holding time increased, the rate of CP disappearance decreased for SBM. Results showed trends similar to inhibitor in vitro degradation rates (Table 2) in which roasting and roasting and holding had significant effects on decreasing protein degradation rates. Pena (16) reported in situ degradation rates for CP of .171 and .061 h<sup>-1</sup> for SB and SB roasted at 300°C (air temperature of roaster) and held for 3 h. Grummer and Clark (13) found no difference in rate of CP disappearance between 4 and 16 h of in situ incubation for solvent-extracted SBM (.14 h<sup>-1</sup>) versus SBM flakes heated at 250°C for 30 min (.12 h<sup>-1</sup>).

Although roasting had no effect on SBM, roasting SB decreased "instantaneously" soluble N, as indicated by N losses from 0-h bags determined after soaking in warm water for .5 h (Table 3). Soluble N loss in RSB (12.4 to 15.0%) differed dramatically from unheated SB (42%). This agrees with the findings of Stern et al. (22), who reported N disappearances of 51.3 and 21.9% after 1-h incubations with unheated SB and SB extruded at 132°C, respectively.

Nitrogen disappearance determined with the mobile bag technique was not influenced by heating and averaged 99.7% (SE = .18) for SB

and 99.0% (SE = .38) for SBM. However, we do not think these data reflect true intestinal digestibilities of protein. Undigested N-containing compounds may have diffused out of the bags, making the mobile bag technique insensitive to differences in intestinal digestibility. The pH of the pepsin-HCl solution was 1.6 at the start of the incubation, which was slightly lower than the expected pH of 2.0 in the abomasum. Average residence times of the bags in the intestinal tract were 12.1 h with SBM and 12.7 h for SB. These times may have been overestimated somewhat because feces were checked only every 2 h. Deacon et al. (8) reported disappearances of 96.5 and 94.9% for DM and 99.0 and 98.8% for N, with SBM and extruded SBM, respectively, when incubated for 12 h in the rumen and then placed into the duodenal cannulas of Holstein cows. Similarly, de Boer et al. (9) reported overall N disappearance for SBM of 99.3%.

#### CONCLUSIONS

In vitro and in situ methods both indicated decreased rates of ruminal protein degradation from roasting SB and SBM and holding at elevated temperatures; degradation decreased with increasing holding time. Although both methods provided estimates of ruminal protein degradation rates, the rates were not similar.

Levels of ADIN did not change with roasting and holding for SB but were slightly higher for SBM, suggesting the RSB and RSBM had low extents of heat damage. In contrast, TLMI content decreased dramatically from the heating process with a loss of 22% for RSB and 17% for RSBM when held for 3 h. This indicates that ADIN levels did not reflect extent of heat damage. The roasting and holding process significantly increased PRNAL.

If data from only ADIN and in situ and in vitro degradation rates are considered, the best heat treatment would be roasting and holding for 3 h. However, because PRNAL values were similar across holding times, there was no additional benefit from holding SB or SBM longer than .5 h after roasting. Overall, the added benefit from the holding process resulted in more thorough and extensive heat treatment than is typical for commercial RSB and RSBM.

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