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Nutrient Metabolism

Determining Optimal Heat Treatment of Soybeans by Measuring Available Lysine Chemically and Biologically with Rats to Maximize Protein Utilization by Ruminants[1,2]

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ABSTRACT Soybeans were heated in a forced air oven at 120 and 130°C for 60 and 180 min, and at 140, 150 and 160°C for 10, 30, 60, 90 and 120 min. Two types of measurements were used to determine optimal heat treatment of soybeans to maximize protein utilization by ruminants. One was to estimate the rate and extent of protein degradation in the rumen using an in vitro ruminal system. The second was to determine the nutritional availability of lysine. Methods used to determine available lysine were an indirect fluorodinitro-benzene chemical method and a rat growth assay. The product of undegraded intake protein and available lysine content was used to estimate the amount of lysine that would escape ruminal degradation and be available for intestinal absorption. As heat input increased, ruminal undegraded intake protein increased, and protein degradation rates and total and available lysine decreased. As temperature increased, the time required to maximize post-ruminal available lysine decreased. The optimal heat treatment for soybeans heated in a forced air oven was: 140°C for 120 min or greater, 150°C for 60 min or 160°C for 30 min. A loss of 15–22% of chemically determined available lysine was necessary to achieve the heat treatment that resulted in maximal post-ruminal available lysine. J. Nutr. 122: 151–160, 1992.

INDEXING KEY WORDS:

- *rat growth* • *rumen proteolysis*
- *lysine* • *Maillard reaction*

Mild heat treatment of soybeans destroys or reduces heat-labile antinutritional factors (including trypsin inhibitors, hemagglutinins, antivitamins, goitrogens and phytates), improves digestibility and availability of sulfur amino acids and increases fat digestibility by nonruminants (1–4). However, to obtain maximal supply of protein available for digestion in the small intestine of ruminants, the protein supplement may need to be heated to the point of making part of it unavailable (5, 6). The Maillard reaction is the major non-enzymatic sequence of reactions that causes protein to become nutritionally unavailable when heated. The amino acid most vulnerable to heat damage is lysine (7). Lysine also is implicated as being first limiting or co-limiting for milk production by dairy cows (8, 9). Therefore, measurement of nutritionally available lysine would be a valuable indicator of amino acid supply to the small intestine of ruminants fed heat-treated supplements.

The second measurement needed to determine the optimal heat treatment of protein supplements for ruminants is an estimate of the degree of protection against the breakdown of protein by bacteria in the rumen. Many methods are available to estimate rate and extent of microbial protein degradation. Although all methods have disadvantages, in vitro methods are relatively easy and large numbers of samples can be processed economically. An in vitro method developed by Broderick (10) provides a reliable and rapid method for estimating rate and extent of protein degradation.

Little information is available showing the relationships among protein degradation in the rumen, protein availability in the small intestine and extent

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of heat treatment of soybeans. Therefore, the objective of this research was to determine the relationship between the degree of heat treatment of soybeans and the supply of estimated available lysine to the ruminant small intestine.

MATERIALS AND METHODS

Full fat soybeans were heated in a forced air oven at 120 and 130°C for 60 and 180 min, and at 140, 150 and 160°C for 10, 30, 60, 90 and 120 min to achieve a wide range of heat exposure. Soybean samples weighing 200 g were placed on 16 × 20 × 2 cm aluminum pans and spread evenly. Soybeans heated at 140 and 160°C for 120 min were prepared in separate batches for Trials 1 and 2. For Trial 1, ~1 kg of soybeans were placed in the oven at the start. For Trial 2, ~2 kg of soybeans were placed in the oven over time for treatments with temperatures of 140, 150 and 160°C. The smaller mass of soybeans heated in Trial 1 may have resulted in a more rapid achievement of target temperature. Within a temperature treatment for Trial 2, soybean samples were placed in the oven starting with samples having the longest heat exposure, so all samples were removed at the same time. Dry matter content for soybeans before treatment was 89.8%. After heating, the soybeans were cooled and then ground sequentially through 2-mm and 1-mm Wiley mill screens. Soybean treatments were analyzed for dry matter (11), crude protein (12), trypsin inhibitor activity (13; performed by Woodson-Tenant Laboratories, Des Moines, IA) and acid detergent insoluble nitrogen (ADIN)[5] (14). Soybeans that underwent the various treatments were assayed for their fractional rate of protein degradation by an in vitro ruminal system and the proportion of protein potentially escaping the rumen was estimated assuming a ruminal passage rate of 0.06/h (10). Fractional degradation rates were computed using a single-exponential, first-order model without time lag. Rates were determined directly as the slope obtained by linear regression of the natural logarithm of the proportion of protein remaining undegraded vs. time. The undegraded portion was computed by subtracting from added nitrogen the amount of nitrogen recovered as ammonia and amino acids. Chloramphenicol and hydrazine sulfate were used in this in vitro system to inhibit microbial uptake of ammonia and amino acids, thus giving quantitative recovery of protein breakdown products. Procedures and mathematical computations are reviewed in depth by Broderick (10).

Chemical estimation of nutritionally available lysine. An indirect 1-fluoro-2,4-dinitrobenzene (FDNB) procedure was used to determine available lysine content (11, 15). Soybean samples were hydrolyzed in 20 mL of 6 mol/L hydrochloric acid at 105°C for 20 h under nitrogen in 25 × 200 mm screw-capped culture tubes sealed with Teflon tape. Total and unavailable lysine concentrations were determined using ion-exchange amino acid analysis (Model 6300, Spinco Division, Beckman Instruments, Palo Alto, CA). Total lysine was determined on a sample not treated with FDNB. Sample sizes for hydrolyzing were approximately 10 and 4 mg of nitrogen for the FDNB-treated and untreated hydrolysates, respectively. The FDNB-bound lysine (available lysine) was calculated by difference; therefore, the total lysine minus inaccessible lysine (TLMI) was considered to be nutritionally available (i.e., TLMI = total lysine − unavailable lysine). Unavailable lysine is lysine that is inaccessible to react with FDNB. The FDNB treatment of samples was performed in the same tubes used for hydrolysis. A nitrogen evaporator was used to dry the FDNB reaction mixture before hydrolyzing.

Rat growth trials measuring nutritionally available lysine. Male weanling Sprague-Dawley rats (Sprague Dawley, Teklad, Madison, WI) weighing 49 ± 3.7 g were kept in individual suspended screen-bottomed cages. The room was set for a 12-h light: dark cycle. All rats were fed ad libitum a standard diet containing wheat gluten (Teklad, Madison, WI) with

TABLE 1

Standard and test diet composition

Ingredient	Standard diet	Test diet
	g/kg	
Wheat gluten[1]	250.0	—
Soybeans	—	250.0
L-Lysine-HCl	Variable[2]	Variable[3]
L-Methionine	2.4	—
Amino acid mix[4]	—	15.9
AIN-76 Mineral mix[1]	35.0	35.0
AIN-76A Vitamin mix[1]	10.0	10.0
Choline chloride[1]	2.0	2.0
Soybean oil[1]	45.0	—
Cornstarch[1]	327.8	343.6
Cerelose[1]	327.8	343.5

[1]Teklad, Madison, WI.
[2]L-Lysine hydrochloride added to achieve L-lysine addition of 0, 1, 2, 3 and 4 g/kg diet dry matter.
[3]L-Lysine hydrochloride added to achieve L-lysine addition of 0, 1 and 2 g/kg of diet dry matter for Trial 1.
[4]See Table 2 for composition.

[5]Abbreviations used: ADIN, acid detergent insoluble nitrogen; FDNB, 1-fluoro-2,4-dinitrobenzene; PRAL, post-ruminal available lysine; TLMI, total lysine minus inaccessible lysine; UIP, undegraded intake protein.

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TABLE 2

Composition of the amino acid mixture

Amino acid (L-form)	Amount supplied: Diet dry matter[1]	Mixture[2]	Requirement[3]
		%	
Methionine	0.56	35.2	83.6
Valine	0.28	17.6	41.8
Isoleucine	0.19	12.0	33.9
Threonine	0.17	10.7	30.4
Histidine	0.08	5.0	24.2
Phenylalanine	0.20	12.6	22.5
Tryptophan	0.03	1.9	20.0
Leucine	0.08	5.0	9.6

[1]Expressed as a percentage of diet dry matter.
[2]Expressed as a percentage of amino acid mixture.
[3]Expressed as a percentage of recommended requirements (ref. 16).

no addition of L-lysine HCl (Table 1) for 2 d before receiving a standard or test diet. Five rats were assigned randomly to each diet. Rats were weighed and food intake was recorded every 2 d for 2 wk.

Two growth trials were conducted. Trial 1 was conducted to ensure a reliable standard growth curve reflecting lysine consumption and to confirm that test diets resulted in the same response as standard diets. Soybeans heated at 140 and 160°C for 2 h were tested in this study. Added to the two test diets were three levels of L-lysine HCl to obtain L-lysine additions of 0, 1 and 2 g/kg diet dry matter. The diets used as standards for determining nutritionally available lysine were prepared by adding graded levels of L-lysine HCl that corresponded to L-lysine additions of 0, 1, 2, 3 and 4 g/kg diet dry matter. Therefore, Trial 1 included five standard and six test diets to obtain two growth response curves reflecting consumption of available lysine. Trial 2 included five standard diets that were the same as those in Trial 1 and twenty test diets that included unheated soybeans and all treatments of heated soybeans with no additional lysine.

The ingredient composition of the standard and test diets is shown in Table 1. L-Methionine was included in all standard diets at 2.4 g/kg diet dry matter. The ether-extractable material of wheat gluten and soybeans was 2 and 20% on a dry basis, respectively. Soybean oil was added to the standard diets at 45 g/kg (dry matter basis). Additions to the standard and test diets were made at the expense of the dietary carbohydrate, which was a 1:1 mixture of corn starch and cerelose (glucose monohydrate) (Teklad, Madison, WI). All test diets included an amino acid mixture devoid of lysine to ensure that the other indispensable amino acids were in excess of their requirement (Table 2). Thus, the response in rat growth should have reflected the availability of lysine in the heated soybeans.

Statistical methods. Statistical procedures were those of Snedecor and Cochran (17). The hypothesis of linear time trends was tested using linear correlations for estimated ruminal undegraded intake protein (UIP), TLMI lysine and available lysine predicted from rat growth data. Linear regressions with computation of correlation coefficients, slopes with their standard errors and intercepts were conducted. Analysis of covariance was used to test similarity of slopes and intercepts of the regressions for Figures 1 and 2. Linear regressions were pooled if analysis resulted in similar slopes. The similarity of linear pattern between TLMI lysine and available lysine predicted from rat growth was evaluated with analysis of covariance within temperatures 140, 150 and 160°C.

RESULTS AND DISCUSSION

Acid detergent insoluble nitrogen content, ruminal protein degradation rates and UIP are shown in Table 3. Heat treatment of soybeans had little effect on ADIN content, except for soybeans heated at 160°C for 90 and 120 min. The difference in ADIN content between the two soybean treatments heated at 160°C for 120 min was attributed to the heating procedure. The soybeans used in Trial 1 may have had a more extensive heat treatment even though the temperature was similar, because the oven door was not opened for 120 min and the amount of soybeans being heated was less.

In general, as duration of heating increased, fractional rates of ruminal protein degradation were reduced. Degradation rates ranged from 0.152 h^{-1} for raw soybeans down to 0.019 h^{-1} for soybeans heated at 160°C for 120 min, resulting in estimated UIP of 29.7 and 75.1%, respectively. Estimated UIP increased linearly with increased heating duration within 140, 150 and 160°C ($P < 0.05$). Linear correlation coefficients were 0.99, 0.98 and 0.88 for 140, 150 and 160°C treatments, respectively.

Chemical estimation of nutritionally available lysine. The TLMI method was chosen over the direct FDNB method (18) because it circumvents the problems of nonspecificity of the FDNB reaction (because FDNB can react with other amino acids), instability of the dinitrophenyl-lysine derivatives and interference with carbohydrates and colored complexes (19, 21). The TLMI method (11, 15) involves the measurement of total lysine and lysine remaining after treatment with FDNB (before acid hydrolysis). The difference between these two values represents the lysine in the protein that has free epsilon-amino groups and is interpreted as nutritionally available in terms of the Carpenter theory (18). Although part of

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the dinitrophenyl-lysine may be lost during acid hydrolysis, it will not be detected as free lysine (unavailable lysine). The TLMI method has been used as a reference against which to compare other methods used for chemically estimating nutritionally available lysine (19, 20, 22). However, Mauron (23) and Hurrell and Carpenter (24) reported the TLMI method to be unsuitable for measuring early Maillard damage that may be prevalent when heating at low temperatures for prolonged periods.

Total, inaccessible (nutritionally unavailable) and TLMI (nutritionally available) lysine content data are shown in Table 4. Both total and TLMI lysine decreased with higher temperatures and duration of heat treatment. Generally, inaccessible lysine increased with increased heat input. Inaccessible lysine as a proportion of total lysine ranged from 4 to 57%, depending on the severity of the heat treatment. Conversely, available lysine as a proportion of original lysine available in raw soybeans ranged from 100 to 19%, depending on heat treatment. The TLMI lysine decreased linearly with increased heating duration within 140, 150 and 160°C ($P < 0.05$). Linear correlation coefficients were 0.96, 0.97 and 0.96 for 140, 150 and 160°C, respectively. The difference in total, inaccessible and TLMI lysine for treatments with a similar temperature and duration of heating indicates that a more severe heat treatment was obtained for treatments used in Trial 1 (as discussed earlier). These results and those reported by others (7, 25) support the notion that total lysine provides an indication of heat damage because blocked lysine epsilon-amino groups will not yield 100% of total lysine. However, total lysine does not give an accurate measurement of nutritional availability of lysine in heat-treated soybeans.

Rat growth trials measuring nutritionally available lysine. Results of Trial 1 on food consumed and weight gain are shown in Table 5. Linear regressions relating weight gain to available lysine consumed by rats fed standard or test diets are in Figure

TABLE 3

Effects of temperature and duration of heating of soybeans on acid detergent insoluble nitrogen (ADIN), in vitro ruminal protein degradation rate and estimated ruminal undegraded intake protein (UIP)

Temperature	Duration (min)	ADIN (C)	Rate (k_d)	Intercept (B)	UIP[2]	Time trend[3]
		% TN[1]	h^{-1}	%		r, (P)
0°C	0	3.8	0.152 ± 0.0079[4]	91.5 ± 0.03[4]	29.7 ± 0.99[4]	
120°C	60	4.3	0.108 ± 0.0037	90.6 ± 0.31	36.7 ± 0.60	
	180	4.7	0.101 ± 0.0027	91.1 ± 0.74	38.7 ± 0.31	
130°C	60	4.6	0.101 ± 0.0058	90.2 ± 0.50	38.2 ± 1.03	
	180	4.7	0.066 ± 0.0104	91.0 ± 0.52	48.0 ± 3.18	
140°C	10	4.4	0.124 ± 0.0035	90.5 ± 0.25	33.9 ± 0.50	0.99 (0.002)
	30	5.0	0.078 ± 0.0096	89.5 ± 0.23	43.9 ± 2.47	
	60	4.7	0.061 ± 0.0022	90.1 ± 0.55	49.4 ± 0.53	
	90	4.7	0.048 ± 0.0029	90.7 ± 0.70	55.0 ± 1.05	
	120	5.5	0.043 ± 0.0061	92.1 ± 0.81	59.2 ± 2.71	
150°C	10	5.1	0.108 ± 0.0023	88.2 ± 0.26	36.6 ± 0.38	0.98 (0.004)
	30	4.5	0.080 ± 0.0032	88.4 ± 0.15	42.4 ± 0.82	
	60	4.8	0.041 ± 0.0038	90.3 ± 0.32	58.4 ± 1.89	
	90	5.6	0.032 ± 0.0069	89.9 ± 0.49	64.2 ± 4.12	
	120	6.2	0.025 ± 0.0012	90.3 ± 0.03	69.9 ± 0.89	
160°C	10	5.1	0.104 ± 0.0084	88.4 ± 0.27	37.4 ± 0.156	0.88 (0.050)
	30	4.3	0.050 ± 0.0024	89.7 ± 0.49	53.2 ± 0.82	
	60	6.5	0.022 ± 0.0023	89.5 ± 0.26	72.0 ± 1.66	
	90	9.0	0.024 ± 0.0064	87.0 ± 0.55	71.1 ± 4.35	
	120	10.4	0.019 ± 0.0027	85.2 ± 0.44	75.1 ± 2.00	
140°C[5]	120	6.6	0.026 ± 0.0029	88.2 ± 0.38	68.1 ± 1.81	
160°C[5]	120	18.5	0.026 ± 0.0053	76.4 ± 0.57	71.8 ± 3.00	

[1]% TN = percent of total nitrogen.

[2]UIP (undegraded intake protein), % = $\{B [k_p/(k_p + k_d)] + C\} \times 100$, where fraction B is degradable true protein, C is protein that is considered to be indigestible, k_d is the in vitro fractional protein degradation rate and k_p is the ruminal passage rate (0.06/h) (10).

[3]Time trends were tested using linear correlation for UIP data within temperatures 140, 150 and 160°C (r = correlation coefficient, P = probability).

[4]Average for three separate in vitro analyses ± SEM.

[5]Heat treatments used for Trial 1.

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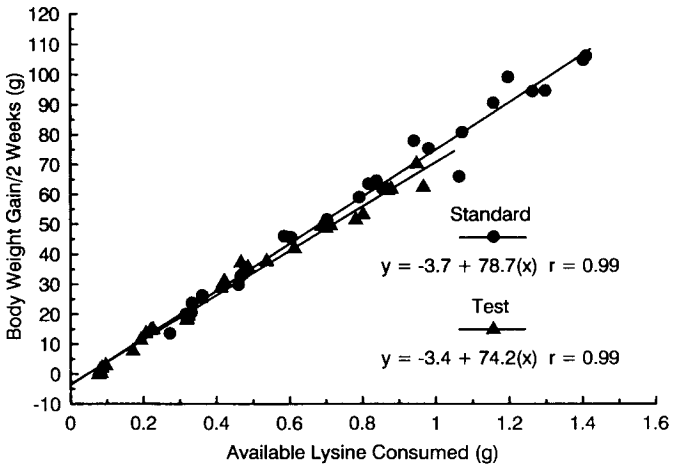

FIGURE 1 Regression lines for standard and test diets relating weight gain to available lysine consumed for Trial 1. Data points represent results from rats fed standard or test diets (Table 5). The standard and test lines are represented by 25 and 30 points, respectively. Regression lines resulted in similar equations ($P > 0.13$). The pooled regression line is: gain (y) = –4.4 + 78.4 (lysine consumed, x). The correlation coefficient and mean square error term are 0.99 and 3.61, respectively.

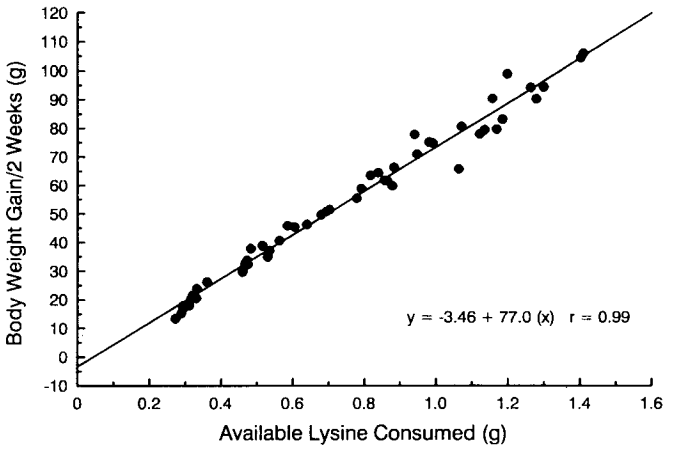

FIGURE 2 Regression line for the pooled data of rats fed standard diets for Trials 1 and 2 relating weight gain to available lysine consumed. Separate regression lines for rats fed standard diets in Trials 1 and 2 were similar ($P > 0.10$). Therefore, data from both trials were pooled (Table 5). The mean square error term for the pooled regression line is 3.8. The pooled regression line was used as a standard curve to predict available lysine content in the test diets for Trial 2.

1. Regression lines resulted in similar slopes ($P \geq 0.13$) with correlation coefficients greater than 0.98. The pooled regression equations resulted in a slope of 78.4, intercept of –4.4 g, correlation coefficient of 0.99 and a mean square error term of 3.61 g. These results indicate that lysine was the limiting amino acid in the standard and test diets. Boctor and Harper (26) observed similar results in assessing the reliability of the growth rate of rats as means of estimating the amount of available lysine in foods and feeds. The diets used as standards in their experiments for determining available lysine were prepared by adding graded levels of lysine-HCl to a 250 g/kg wheat gluten basal diet. Other researchers also found a wheat gluten diet suitable for the assessment of lysine availability by the growth method (27, 28). In the present experiment, the standard diets consisted of 250 g/kg wheat gluten and 2.4 g/kg L-methionine addition to ensure lysine as the limiting indispensable amino acid. Because satisfactory results were obtained in Trial 1, the standard diets were repeated in Trial 2, along with all heat-treated soybean test diets, with no changes in ingredient composition.

Food consumed, weight gain and the regression equation relating weight gain to available lysine consumed for rats fed standard diets in Trial 2 are also given in Table 5. Food consumed and weight gain of rats fed standard diets were lower compared with results from Trial 1. An explanation for this is not available. Average weights of rats were higher at the start of Trial 1 (53 ± 2.1 g) compared with Trial 2 (48 ± 2.9 g). To determine available lysine content in the test diets, results obtained from both growth studies relating weight gain to available lysine consumed for rats fed standard diets were used because resulting slopes for the regression equations were similar ($P > 0.10$). The pooled standard curves for Trials 1 and 2 resulted in a slope of 77.0, an intercept of –3.46 g, correlation coefficient of 0.99 and a mean square error of 3.8 g. The resulting standard curve is illustrated in Figure 2. The regression equation given in Figure 2

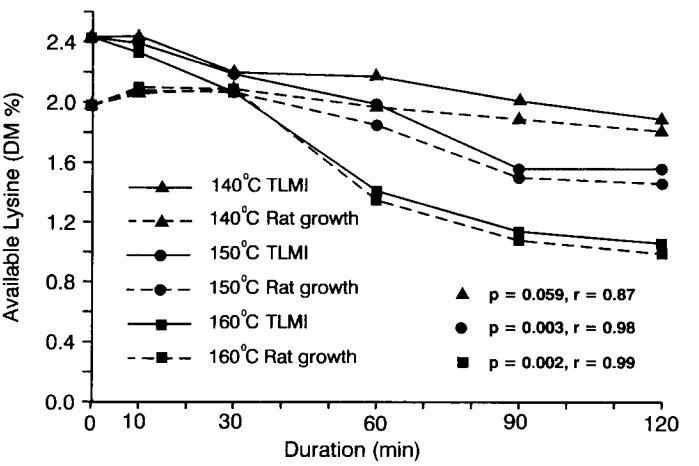

FIGURE 3 The effect of heating soybeans at 140, 150 and 160°C for 10, 30, 60, 90 and 120 min on available lysine determined by a chemical method [total lysine minus inaccessible lysine (TLMI)] or predicted by rat growth. Data points are taken from Table 4 for TLMI results and Table 6 for rat growth results. The correspondence of TLMI lysine and available lysine predicted from rat growth were evaluated with linear correlation within temperatures, starting with the 10-min heating duration. Linear correlations between TLMI lysine and available lysine were similar for 150 and 160°C ($P < 0.01$) and 140°C ($P < 0.06$).

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TABLE 4

Effect of temperature and duration of heating soybeans on total, inaccessible and total lysine minus inaccessible (TLMI) lysine

Temperature	Duration (min)	Total lysine	Inaccessible lysine		TLMI lysine		Time trend
		g/100 g dry matter[1]		%[2]	*g/100 g dry matter*[1]	%[3]	*r, (P)*[4]
0°C	0	2.57 ± 0.085[5]	0.14 ± 0.013[5]	5	2.43 ± 0.086[6]	100	—
120°C	60	2.39 ± 0.005	0.12 ± 0.016	5	2.27 ± 0.016	93	—
	180	2.34 ± 0.050	0.13 ± 0.003	6	2.21 ± 0.050	91	
130°C	60	2.47 ± 0.020	0.11 ± 0.005	5	2.36 ± 0.021	97	—
	180	2.32 ± 0.020	0.18 ± 0.002	8	2.14 ± 0.020	88	
140°C	10	2.55 ± 0.040	0.11 ± 0.004	4	2.44 ± 0.040	100	0.96 (0.008)
	30	2.36 ± 0.015	0.16 ± 0.028	7	2.20 ± 0.032	91	
	60	2.36 ± 0.035	0.19 ± 0.016	8	2.17 ± 0.038	89	
	90	2.22 ± 0.025	0.21 ± 0.009	10	2.01 ± 0.027	83	
	120	2.12 ± 0.010	0.23 ± 0.008	11	1.89 ± 0.013	78	
150°C	10	2.48 ± 0.045	0.09 ± 0.006	4	2.39 ± 0.045	98	0.97 (0.006)
	30	2.33 ± 0.030	0.14 ± 0.010	6	2.19 ± 0.032	90	
	60	2.22 ± 0.005	0.23 ± 0.006	10	1.99 ± 0.008	82	
	90	1.88 ± 0.055	0.32 ± 0.014	17	1.56 ± 0.057	64	
	120	1.88 ± 0.055	0.32 ± 0.019	17	1.56 ± 0.058	64	
160°C	10	2.46 ± 0.045	0.13 ± 0.007	5	2.33 ± 0.046	96	0.96 (0.010)
	30	2.26 ± 0.015	0.19 ± 0.004	8	2.07 ± 0.016	85	
	60	1.81 ± 0.035	0.40 ± 0.012	22	1.41 ± 0.037	58	
	90	1.57 ± 0.010	0.43 ± 0.007	27	1.14 ± 0.012	47	
	120	1.50 ± 0.005	0.44 ± 0.032	29	1.06 ± 0.032	44	
140°C[7]	120	1.85 ± 0.025	0.35 ± 0.004	19	1.50 ± 0.025	62	—
160°C[7]	120	1.07 ± 0.005	0.61 ± 0.004	57	0.46 ± 0.006	19	
Wheat gluten[8]	—	1.33 ± 0.015	0.08 ± 0.007	—	1.25 ± 0.017	—	—

[1]Total, inaccessible and TLMI lysine expressed as g/100 g of soybean dry matter.
[2]Inaccessible lysine as a percentage of total lysine.
[3]TLMI lysine as a proportion of TLMI lysine in raw soybeans.
[4]Time trends were tested using linear correlation for TLMI data within temperatures 140, 150 and 160°C (r = correlation coefficient, P = probability).
[5]Average for two analyses ± SEM.
[6]SEM difference calculation from total and inaccessible lysine.
[7]Heat treatments used for Trial 1.
[8]Wheat gluten used in standard diets for Trials 1 and 2.

was used to predict available lysine consumed from actual weight gain of rats fed test diets. The predicted available lysine consumed then was used to determine available lysine content of test diets and heat-treated soybeans by using actual food dry matter consumed.

Weight gains, food consumption and predicted available lysine content obtained with rats fed test diets are shown in Table 6. The coefficients of variation averaged across treatments for weight gain and food consumed were 11.3 and 8.6%, respectively. However, variability within test diets in predicting available lysine content was lower, with an average coefficient of variation of 4.7%. Available lysine content, predicted from rat growth, decreased linearly with increased heating duration for 140, 150 and 160°C ($P < 0.02$). Correlation coefficients were 0.97, 0.97 and 0.95 for 140, 150 and 160°C treatments, respectively. Available lysine content of raw soybeans was 1.98%, which was lower than available lysine determined by the TLMI method (2.43%). This discrepancy in available lysine can be attributed to the deleterious effects of trypsin inhibitors and other heat-labile compounds that inhibit growth. This is suggested by the apparent increase in available lysine content predicted from rat growth of soybeans heated at 140, 150 and 160°C for 10 or 30 min. The large differences (>10%) between available lysine determined by TLMI and rat growth can also be associated with trypsin inhibitor activities of >24.0 trypsin inhibitor units/mg (Table 6) in the following heat treatments: 120 and 130°C for 60 or 180 min and 140, 150 and 160°C for 10 min. It has been estimated that a reduction of ~80% of the trypsin inhibitor activity by

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TABLE 5

Effects of lysine content of diet on food consumed and weight gain by rats

Diet	Available lysine[1]	Food consumed[2]	Lysine consumed[2]	Weight gain
	%		g/2 wk	
Trial 1				
Standard diet[4,5]				
Wheat gluten	0.311	103.8 ± 4.25[3]	0.323	20.8 ± 2.16[3]
	0.411	126.0 ± 6.98	0.518	37.6 ± 3.42
	0.511	160.1 ± 6.86	0.818	63.4 ± 4.31
	0.611	172.2 ± 9.19	1.052	81.6 ± 6.38
	0.711	181.1 ± 8.08	1.288	93.1 ± 7.21
Test diet[4,5]				
160°C-120 min	0.114	74.4 ± 2.86	0.085	1.0 ± 0.53
	0.214	95.2 ± 4.65	0.204	12.3 ± 1.33
	0.314	110.7 ± 6.18	0.347	22.2 ± 2.46
140°C-120 min	0.375	124.0 ± 6.00	0.465	33.9 ± 1.76
	0.475	147.2 ± 5.69	0.700	48.1 ± 1.65
	0.575	155.3 ± 5.14	0.893	61.8 ± 2.69
Trial 2				
Standard diet[6]				
Wheat gluten	0.311	97.2 ± 1.90	0.302	17.9 ± 1.03
	0.411	125.2 ± 3.92	0.515	37.5 ± 1.40
	0.511	129.9 ± 7.92	0.664	47.6 ± 3.47
	0.611	149.4 ± 3.97	0.913	66.9 ± 2.85
	0.711	165.7 ± 3.87	1.178	82.4 ± 2.19

[1]Available lysine determined by total lysine minus inaccessible lysine (TLMI) method (Table 4) and expressed as a percentage of diet dry matter.

[2]Food and available lysine consumed are on a dry matter basis.

[3]Average for five rats ± SEM.

[4]Standard and test diets used separately for regression lines relating weight gain to available lysine consumed (Fig. 1).

[5]Pooled regression equations relating weight gain to available lysine consumed by rats fed standard and test diets for Trial 1 is: gain (y) = −4.4 + 78.4 (lysine consumed, x), r = 0.99.

[6]Regression equation relating weight gain to available lysine consumed for rats fed standard diets is: gain (y) = −2.2 + 73.7 (lysine consumed, x), r = 0.99.

heat processing is sufficient to produce optimal growth of nonruminants (3). Also, it has been estimated that 60% of the increased growth of rats fed heated soybean protein is due to improved digestibility of the protein and 40% is due to decreased trypsin inhibitor activity (29).

Another possible explanation for differences in estimates of available lysine due to method could be related to the difficulty the TLMI method has in measuring early Maillard compounds (23, 24). Results of the present study, however, favor trypsin inhibitors or other heat-labile compounds as the major factor inhibiting rat growth, because >55% of the original trypsin inhibitor activity was still present. Results from the rat growth study for the remaining heat treatments are in agreement with the TLMI method. Response curves illustrating the similarity in available lysine determined by TLMI and rat growth for soybeans heated at 140, 150 and 160°C for up to 120 min are shown in Figure 3. The correspondence of TLMI lysine and available lysine predicted from rat growth resulted in similar linear correlations for 150 and 160°C ($P < 0.01$) and 140°C ($P < 0.06$). These results suggest that lysine determined by TLMI can be used to assess nutritionally available lysine in heat-treated soybeans.

Estimated post-ruminal available lysine (PRAL). To determine the optimal heat treatment(s), the product of estimated UIP and available lysine content (TLMI or rat growth) was used to estimate the amount of lysine that potentially escapes the rumen and is available for intestinal absorption. A major assumption in calculating PRAL is that amino acid composition of the ruminal fraction of protein not degraded in the rumen and reaching the small intestine is representative of the original amino acid composition of the protein supplement. Experiments conducted in vitro (30, 31) and in vivo (32, 33) suggested that food amino acids were not degraded equally by ruminal microbes. Results from in situ

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TABLE 6

Effects of temperature and duration of heating of soybeans on trypsin inhibitor activity, food consumption, weight gain and availability of lysine

Temperature	Duration (min)	Trypsin inhibitor[1]	Weight gain	Food consumed[2]	Predicted lysine[3]	Time trend[4]
		TIU/mg	*g/2 wk*		%	*r, (P)*
0°C	0	44.0	50.1 ± 4.64[5]	140.2 ± 9.51[5]	1.98 ± 0.059[5]	—
120°C	60	29.0	50.5 ± 3.47	140.7 ± 8.10	1.99 ± 0.018	—
	180	28.0	47.3 ± 1.85	139.7 ± 4.30	1.89 ± 0.047	
130°C	60	26.0	47.4 ± 3.87	135.1 ± 6.84	1.95 ± 0.061	—
	180	24.0	38.9 ± 2.34	126.3 ± 4.02	1.74 ± 0.046	
140°C	10	38.8	51.1 ± 3.67	137.3 ± 6.45	2.06 ± 0.057	0.97 (0.007)
	30	16.1	54.9 ± 0.85	145.1 ± 2.28	2.09 ± 0.022	
	60	—	50.0 ± 1.93	141.0 ± 3.58	1.97 ± 0.024	
	90	—	45.0 ± 1.64	132.8 ± 3.02	1.89 ± 0.023	
	120	16.0	41.5 ± 2.29	129.3 ± 6.20	1.81 ± 0.038	
150°C	10	26.1	53.7 ± 3.16	142.8 ± 6.32	2.08 ± 0.035	0.97 (0.007)
	30	—	56.4 ± 2.15	150.1 ± 5.71	2.07 ± 0.049	
	60	12.0	44.6 ± 1.86	134.4 ± 3.51	1.85 ± 0.033	
	90	—	31.3 ± 1.72	120.5 ± 5.40	1.50 ± 0.019	
	120	—	27.9 ± 0.85	111.3 ± 3.53	1.46 ± 0.030	
160°C	10	25.5	52.2 ± 1.17	138.2 ± 5.59	2.10 ± 0.066	0.95 (0.013)
	30	10.0	54.6 ± 1.37	144.4 ± 1.55	2.09 ± 0.038	
	60	—	25.1 ± 1.52	109.5 ± 4.53	1.35 ± 0.031	
	90	—	18.4 ± 0.98	104.7 ± 4.93	1.08 ± 0.016	
	120	2.6	14.9 ± 0.89	95.8 ± 4.02	0.99 ± 0.028	

[1]Trypsin inhibitor activity is expressed as trypsin inhibitor units (TIU) per milligram of defatted soybeans. Not all samples were measured for inhibitor activity.

[2]Food consumed is on a dry matter basis.

[3]Predicted available lysine content of heat-treated soybeans using the standard curve shown in Figure 2.

[4]Time trends were tested using linear correlation for predicted lysine data within temperatures 140, 150 and 160°C (r = correlation coefficient, P = probability).

[5]Average for five rats ± SEM.

methods also are conflicting, with one study suggesting unequal degradation (34), and others indicating equal degradation of amino acids (35, 36). Although the majority of experiments suggest that food amino acids are not degraded equally by ruminal microbes, differences in degradation, if they exist, are small and somewhat inconsistent across studies for specific amino acids.

Results of estimated PRAL are shown in Table 7. In general, as temperature increased the time required to maximize PRAL decreased. Both estimates of available lysine (TLMI and rat growth) agreed on the point of optimal heat treatment. A loss of 15–22% of available lysine (determined by TLMI) was necessary to achieve the optimal heat treatments, i.e., the greatest increase in estimated flow of available lysine to the small intestine. The apparent optimal heat treatment for soybeans is at 140°C for 120 min or greater, 150°C for 60 min and 160°C for 30 min, when using a forced air oven. The optimal heat treatments resulted in an average increase of 56% for estimated PRAL (TLMI) relative to raw soybeans. Broderick and Craig (37) used the product of ruminal UIP (estimated by an in vitro method) and true digestibility in rats of autoclaved (120°C, 1.1 kg/cm^2) cottonseed meal to indicate the optimal heat treatment. Benefits from heat treatment increased to a maximum at 60 min of autoclaving and then declined. Ruminal escape, together with true digestibility of the protein, more accurately describes the post-ruminal value of proteins fed to ruminants. However, they also suggested that a further refinement may be to replace protein digestibility with intestinal digestibility of lysine or other specific amino acids that may be limiting ruminant performance. This was supported by further research (25) suggesting that lysine availability (TLMI method) was a more sensitive indicator than true digestibility and, therefore, of protein quality, when cottonseed was exposed to high temperatures.

Estimates of optimal heat treatment for soybeans (38), soybean meal (39, 40) and for chemically-treated soybean meal (41–43) have been reported. Abdelgadir et al. (38) reported that soybeans processed at 171°C using a roaster resulted in calf performance superior

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TABLE 7

Effect of temperature and duration of heating of soybeans on estimated post-ruminal available lysine

Temperature	Duration (min)	Post-ruminal available lysine[1] TLMI[2]	Rat[2]
		g/kg	
0°C	0	7.22 ± 0.350[3]	5.85 ± 0.262[3]
120°C	60	8.33 ± 0.148	7.30 ± 0.136
	180	8.55 ± 0.205	7.31 ± 0.191
130°C	60	9.01 ± 0.255	7.45 ± 0.307
	180	10.3 ± 0.687	8.35 ± 0.595
140°C	10	8.27 ± 0.183	6.98 ± 0.219
	30	9.66 ± 0.560	9.18 ± 0.525
	60	10.7 ± 0.221	9.73 ± 0.157
	90	11.1 ± 0.256	10.4 ± 0.234
	120	11.2 ± 0.518	10.7 ± 0.539
150°C	10	8.75 ± 0.189	7.61 ± 0.150
	30	9.28 ± 0.224	8.78 ± 0.268
	60	11.6 ± 0.379	10.8 ± 0.399
	90	10.0 ± 0.738	9.63 ± 0.630
	120	10.9 ± 0.428	10.2 ± 0.246
160°C	10	8.71 ± 0.400	7.85 ± 0.409
	30	11.0 ± 0.188	11.1 ± 0.265
	60	10.2 ± 0.354	9.72 ± 0.316
	90	8.11 ± 0.502	7.68 ± 0.483
	120	7.96 ± 0.322	7.43 ± 0.288

[1]Post-ruminal available lysine (PRAL) is a product of undegraded intake protein (UIP) (Table 3) and available lysine content expressed as g/kg soybean dry matter.

[2]PRAL estimated using total lysine minus inaccessible (TLMI) lysine (Table 4) and predicted lysine from rat growth (Table 6) as a measure of available lysine.

[3]SEM is based on Taylor series approximation (17): $\text{SEM}(\text{UIP}\cdot\text{TLMI}) = [(\text{UIP})^2\,[\text{SEM}(\text{TLMI})]^2 + (\text{TLMI})^2\,[\text{SEM}(\text{UIP})]^2]^{1/2}$.

to that obtained with soybeans processed at 138 or 191°C. The optimal heat treatment for soybean meal was identified as ~145°C (post-roasting soybean meal temperature) compared with 102, 128, 159 and 185°C (39, 40). Methods used to identify the optimal heat treatment included chick bioassay, ADIN content, in situ dacron bag (estimate of ruminal protein degradation), nitrogen balance study, duodenal flow of bacterial and food nitrogen, and total tract nitrogen digestibility. However, Abdelgadir et al. (38) emphasized that ruminant production performance remains to be determined. Cleale and co-workers (41–43) reported that soybean meal heated in a forced air oven at 150°C for 30 min with xylose (to induce nonenzymatic browning) resulted in improved utilization of the protein in soybean meal by ruminants. Their results indicated that the use of xylose decreased exposure time by half when compared with the optimal heating time for soybeans in our study.

The effect of heat treatment is a function of both temperature and time of heat exposure. Results of this study suggest that oven temperatures between 140°C and 160°C with corresponding time exposures of 120 min and 30 min are optimal for producing a protected full fat soybean supplement for ruminants. It must be remembered that it takes time for the temperature of soybeans to equilibrate with the oven temperature. Commercial drum roasters that have air temperatures of 250–400°C can bring soybeans to a temperature of 150°C much faster than a forced draft oven maintained at 150°C.

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