Changes in Nitrogenous Compounds and Rates of in Situ N Loss of Soybean Meal Treated with Sodium Hydroxide or Heat

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Rates of in situ N loss of soybean meal (SBM) were altered by various combinations of sodium hydroxide and heat treatment; some nitrogenous compounds involving the modified proteolysis were investigated. The amount of lysinoalanine (LAL), neutral detergent insoluble nitrogen, 1-fluoro-2,4-dinitrobenzene reactive lysine, and reducing power of SBM were linearly related ($P < 0.01$) to rate of in situ N loss with correlation coefficients of −0.847, −0.835, 0.793, and −0.846, respectively. The results indicate that the LAL formation and the Maillard reaction are related to change in susceptibility of SBM protein to microbial degradation in the rumen.

Keywords: *Lysinoalanine; in situ N loss; nonenzymatic browning; feed processing*

INTRODUCTION

Degradation of dietary protein by ruminal microorganisms decreases the amount of protein digested and absorbed in the intestine when proteolysis exceeds the synthesis of protein by microorganisms. Rumen proteolysis could be altered by feed processing, feed additives, and type of diet fed to animals; its control and prediction have been investigated (Broderick et al., 1991). A well-defined factor which is inherent to the feedstuff and influences the extent of degradation is the degree of cross-linking present in or introduced to proteins. Intrinsic disulfide bonds are involved in the susceptibility of protein to microbial degradation (Mahadevan et al., 1980; Nugent et al., 1983; McNabb et al., 1994). Moreover, heat and formaldehyde treatments, which produce melanoidin polymers and methylol and methylene bridges, respectively, reduce the rate of proteolysis in the rumen (Ferguson, 1975; Broderick and Craig, 1980). Nishino et al. (1995) reported that alkaline treatment could protect soybean meal (SBM) protein from degradation in the rumen and that protection was correlated with formation of the cross-linked amino acid, lysinoalanine (LAL). However, other factors were not investigated and the SBM was artificially dried at 80 °C after being alkalized. The drying process may have reduced proteolysis because the Maillard reaction is enhanced under alkaline conditions (Ashoor and Zent, 1984). For establishing precise control and prediction, factors affecting rumen proteolysis should be fully understood and their relationships need to be quantified. This experiment was designed to study the relevance of LAL formation and nonenzymatic browning to the rate of rumen proteolysis of SBM which had been artificially modified by sodium hydroxide (NaOH) and heat treatment.

EXPERIMENTAL PROCEDURES

Treatment of Soybean Meal. A commercial solvent-extracted SBM was used. It was sprayed with 0 (water), 1, 2, 4, or 8% NaOH (DM basis) and dried in a forced-draft oven at 60 or 100 °C for 7 h. The concentrations of NaOH solution were varied from 0 to 50% (w/v) so that the same amount of water was added to the SBM. The treatments were made in duplicate. Samples for chemical analyses were ground to pass a 1-mm screen; for in situ incubation, unground samples were used.

In Situ Incubation Trial. Four mature castrated male goats were used. They were fitted with permanent ruminal cannulae and maintained on alfalfa hay cubes during the experiment. Daily feed was provided at 50 g of DM per kilogram of body weight $(BW)^{0.75}$ in two equal meals at 08:00 and 20:00 h. Water and a mineral block were accessible at all times.

Six bags, each containing 4 g of SBM, were inserted into the rumen just before the morning meal. The dimensions of the nylon bag were 100 × 70 mm with a pore size of 42 μm; stitches were closed with waterproof glue. Incubations were stopped after 3, 6, 9, 12, 24, and 48 h; bags were removed from the rumen, washed with a domestic washing machine until running tap water was cleared and dried at 60 °C for 24 h. Two of four goats were randomly used to replicate the incubation; the other two were allocated for the corresponding duplicate sample. Coefficient of variation of protein degradation was less than 8% at each incubation time when nonalkaline SBM was incubated in the four goats rumen. Efforts to correct N influx to the bags and microbial attachments to incubation residues were not made in this study.

Analyses of Nitrogenous Compounds. Total N of SBM and residues of the in situ incubation trial was determined by the Kjeldahl procedure. Neutral and acid detergent insoluble nitrogen (NDIN and ADIN, respectively) were determined after the preparation of neutral and acid detergent fiber (Goering and Van Soest, 1970). Amino acid analysis was performed on an acid hydrolysate using an automated amino acid analyzer (JEOL JLC-300). Hydrolysis was conducted in a sealed tube with 6 N HCl at 110 °C for 24 h. A lysinoalanine dihydrochloride standard was purchased from Sigma Chemical Co. (St. Louis, MO). 1-Fluoro-2,4-dinitrobenzene (FDNB) reactive lysine was determined according to Booth (1971) with a correction factor of 1.18 (= 100/0.85).

Analysis of Reducing Power. One gram of SBM dissolved in 0.05 M potassium phosphate buffer (pH 6.60) was homogenized using a Polytron disperser (Kinematica, PT-3000). After centrifugation at 12000*g* for 10 min, the reducing power of the supernatant fluid was determined with potassium ferricyanide, and absorbance at 700 nm was measured (Adachi, 1958).

Data and Statistical Analysis. Results of protein degradation were fitted to an exponential equation including a discrete lag time (McDonald, 1981) $p = a + b(1 - e^{-c(t-t_0)})$, where p is degradation at time t, a is soluble fraction, b is degradable fraction, c is rate of degradation, and t_0 is degrada-

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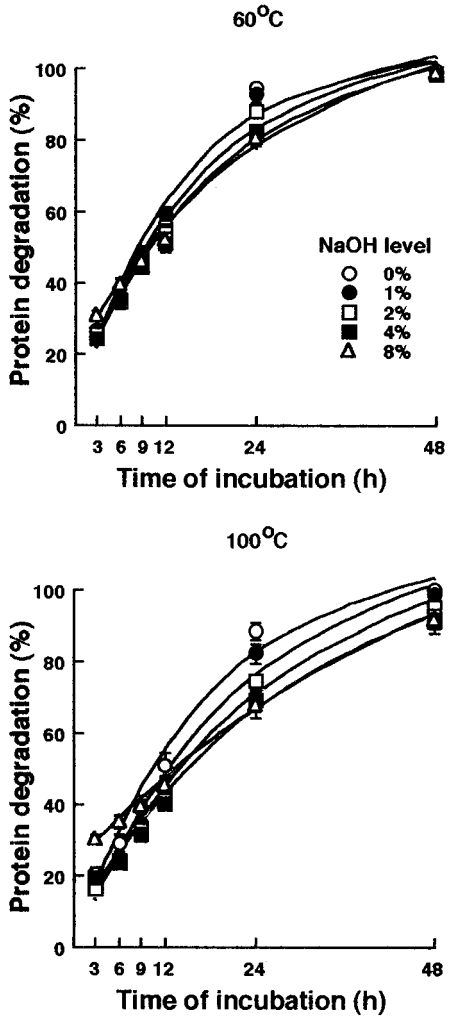

Figure 1. Protein degradation of soybean meal treated with various levels of sodium hydroxide followed by drying at 60 or 100 °C for 7 h. Plots indicate means of four observations.

tion lag time. The data fitting was carried out by nonlinear regression analysis procedure of the Statistical Analysis Systems Institute Inc. (SAS, 1985). Parameters obtained from replicated incubations were averaged, and the mean values were used for subsequent statistical analysis. The soluble fraction of SBM protein was determined as the loss of N during the incubation in McDougall's artificial saliva (pH 6.80) at 39 °C for 1 h. Potential degradation was computed by the equation at 48 h; degradable fraction was calculated by subtracting soluble fraction from potential degradation. Orthogonal polynomials were used to investigate linear and quadratic responses to the level of NaOH treatment.

RESULTS

Protein degradation of SBM heated at 60 °C appeared to be nearly maximum at 24 h incubation in nonalkaline material (Figure 1). Addition of NaOH gradually decreased the degradation; the highest level of treatment increased ($P < 0.05$) the initial (3 h) degradation. Disappearance of SBM protein reached nearly 100% after 48 h; this value was not influenced ($P > 0.05$) by NaOH treatment.

When heating temperature was increased from 60 to 100 °C, the degradation was low at any incubation times regardless of the level of NaOH. The higher temperature enhanced the effect of alkaline treatment and differences of degradation due to NaOH became more obvious compared with those found in SBM heated at 60 °C. Addition of 8% NaOH gave the highest degradation at 3 and 6 h incubation, but the degradation determined at 48 h was reduced ($P < 0.05$) to around 90% as the level of NaOH increased.

The soluble fraction of SBM protein was lowered by adding up to 4% NaOH. But SBM treated with 8% NaOH showed a similar proportion to nonalkaline SBM (Figure 2). This response was quadratic at each heating temperature although the effect of NaOH was more pronounced for SBM heated at 100 °C. The effects of NaOH less appeared on degradable fraction but both linear and quadratic response were significant irrespective of heating temperature. Rates of in situ N loss decreased linearly with increasing level of NaOH; a high drying temperature enhanced the effect of alkaline treatment. When SBM was treated with 8% NaOH and heated at 100 °C, rate of N loss was only one-third that of untreated material.

LAL was not detected in nonalkaline SBM, but NaOH treatment linearly increased concentration of the cross-linked amino acid (Figure 3). Heating at a higher temperature enhanced the LAL production; a quadratic effect was significant only when the SBM heated at 100 °C was included in the regression. The LAL content in SBM treated with 8% NaOH was 0.43 g/16 g N when heated at 60 °C; LAL was more than doubled (1.00 g/16 g N) by heating SBM to 100 °C.

The nonalkaline SBM heated at 60 °C contained only 4.1% of N as NDIN. Addition of 4% NaOH increased this proportion to 13.0%. Heat at 100 °C greatly increased NDIN at any NaOH levels; 26.7% was the maximum for SBM treated with 4% NaOH. The highest level of NaOH lowered NDIN compared with the 4% treatment and both linear and quadratic effects were significant regardless of heating temperature.

The NaOH treatment slightly decreased ADIN when SBM was heated at 60 °C; the linear response to NaOH was significant. Heating SBM to 100 °C increased ADIN, but the effect of NaOH was not consistent.

The amount of FDNB-reactive lysine decreased with NaOH addition. Heating at a high temperature had little impact on FDNB-reactive lysine except for SBM treated with 8% NaOH. The highest level of NaOH followed by heating at 100 °C greatly reduced its content to 4.5 g/16 g N; the effect of NaOH was shown to be quadratic.

Alkaline treatment increased the reducing power of SBM although the effect was not large when NaOH was less than 4%. Heat at 100 °C consistently increased the reducing power compared with SBM heated at 60 °C, but the extent was small. Both linear and quadratic effects were significant regardless of heating temperature.

The rate of in situ N loss was significantly correlated with LAL, NDIN, FDNB-reactive lysine, and reducing power of SBM and linearly related to all four relations (Figure 4). The three measurements of LAL, FDNB-reactive lysine, and reducing power had close relationships in every combination; none was closely related to NDIN content (Table 1). ADIN was not significantly related to rate of in situ N loss or to concentrations of the other nitrogenous components.

DISCUSSION

Nishino et al. (1995) reported that when SBM was treated with NaOH and heated at 80 °C, 5% NaOH was excessive for the efficient protection from rumen proteolysis. In this experiment, we used from 0 to 8%

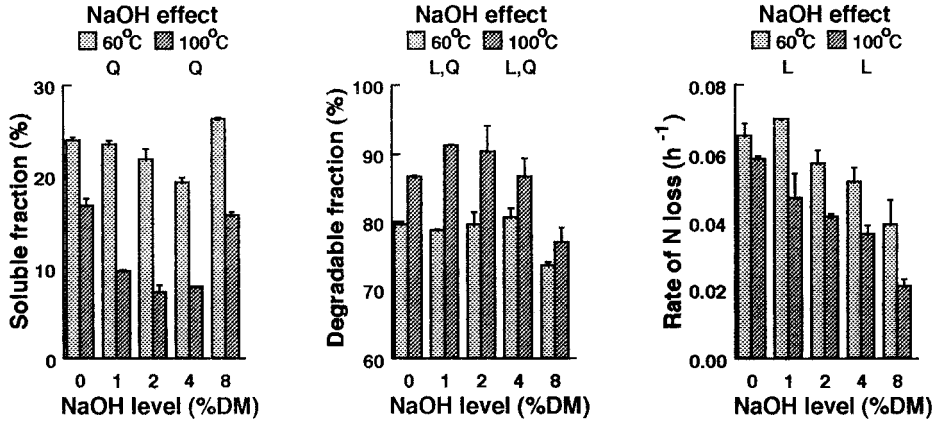

Figure 2. Changes in the characteristics of in situ N loss of soybean meal due to various levels of sodium hydroxide and heat treatments. Columns and vertical bars indicate means and standard errors of duplicate treatments, respectively. L and Q represent linear and quadratic responses to the level of sodium hydroxide treatment ($P < 0.05$), respectively.

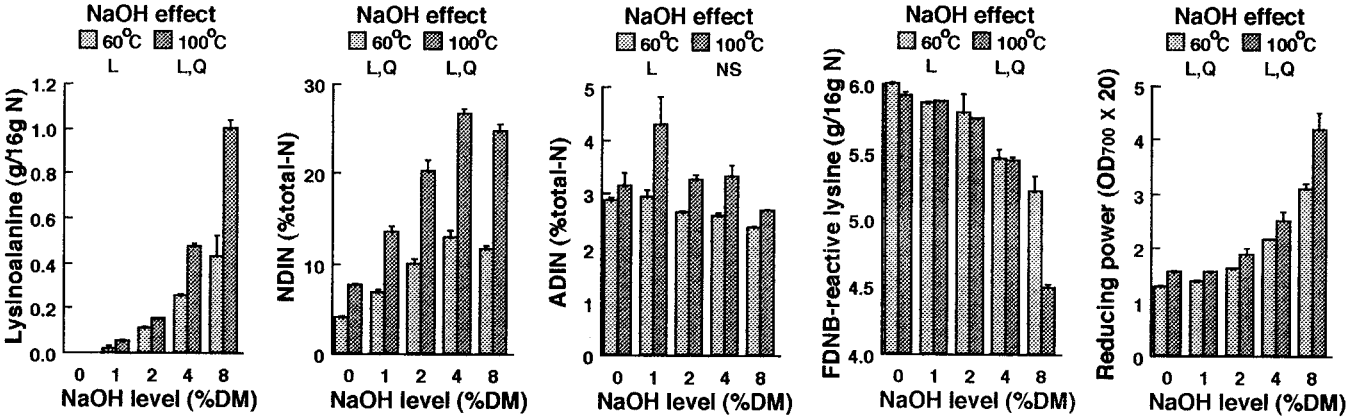

Figure 3. Changes in lysinoalanine, neutral detergent-insoluble nitrogen (NDIN), acid detergent-insoluble nitrogen (ADIN), 1-fluoro-2,4-dinitrobenzene (FDNB) reactive lysine, and reducing power of soybean meal due to various levels of sodium hydroxide and heat treatments. Columns and vertical bars indicate means and standard errors of duplicate treatments, respectively. L and Q represent linear and quadratic responses to the level of sodium hydroxide treatment ($P < 0.05$), respectively.

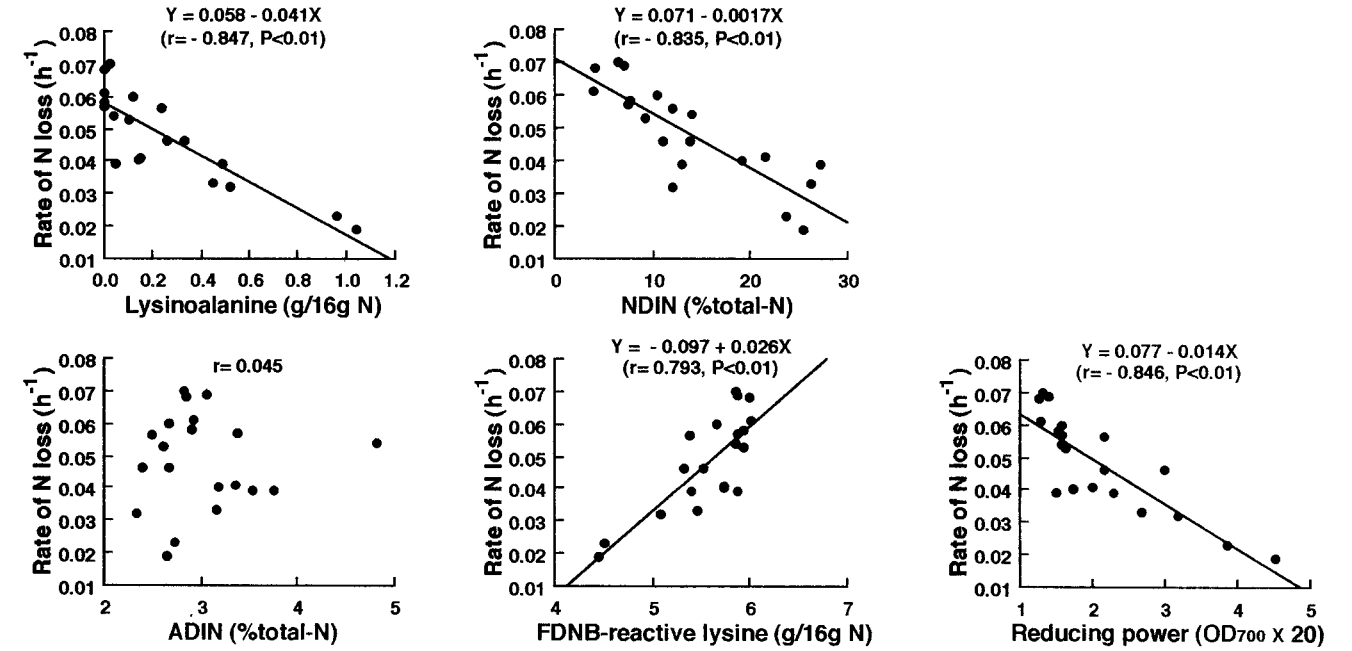

Figure 4. Relationships between in situ N loss with lysinoalanine, neutral detergent-insoluble nitrogen (NDIN), acid detergent-insoluble nitrogen (ADIN), 1-fluoro-2,4-dinitrobenzene (FDNB) reactive lysine, and reducing power of soybean meal.

because mild heating was expected to diminish the effect of alkaline treatment. The temperature of 60 °C has been considered a critical for the nonenzymatic browning of feedstuffs (Van Soest, 1982). Use of a higher temperature may shorten the time of heating required for an equal effect on protein degradation. Disappearance of SBM protein (Figure 1) indicated that the effect of NaOH was virtually limited to SBM heated at 100

Table 1. Correlation Coefficients of Lysinoalanine (LAL), 1-Fluoro-2,4-dinitrobenzene Reactive Lysine (FDNB-L), Reducing Power (RP), Neutral Detergent Insoluble Nitrogen (NDIN), and Acid Detergent Insoluble Nitrogen (ADIN) of Soybean Meal[a]

	LAL	FDNB-L	RP	NDIN	ADIN
LAL		−0.975**	−0.966**	0.737**	−0.300
FDNB-L			−0.969**	−0.647**	0.365
RP				0.664**	−0.348
NDIN					0.170
ADIN					

[a] Asterisks (**) indicate $P < 0.01$.

°C while the response of in situ N loss was evaluated the same between two heating conditions.

A quadratic effect of NaOH on the soluble fraction was observed in the previous study and might be explained by partial hydrolysis of SBM protein due to the severe alkalinity. Protein solubility in the rumen is known to be a factor limiting N utilization of ruminants (Aitchison et al., 1976), while some soluble proteins like ovalbumin and bovine serum albumin resist ruminal degradation due to their high content of disulfide linkages (Mahadevan et al., 1980; Nugent et al., 1983; McNabb et al., 1994).

The extent of protein degradation is markedly influenced by the retention time in the rumen and a fast outflow rate increases the amount of undegradable protein. When outflow rate was assumed to be equal among treatments and range from 0.02 to 0.08 h^{-1}, the lowest degradation could be obtained by either 2 or 4% NaOH at any heating conditions. The addition of 2% NaOH followed by heat at 100 °C could most efficiently protect SBM protein because formation of LAL inevitably causes destruction of some essential amino acids (Friedman et al., 1984). High levels of NaOH and heat might decrease the digestibility of undegradable protein because protein degradation at 48 h suggested a production of undegradable and indigestible protein. Although NaOH had little impact on ADIN which is related to the digestibility of undegradable protein (AFRC, 1993), protein digestibility could be deteriorated according to the increase in LAL cross-linking (Friedman et al., 1981).

The changes in LAL in this experiment demonstrate that LAL formation depends on pH, temperature, and duration of treatment (Friedman et al., 1984). LAL can be detected in heated materials (Sternberg et al., 1975), but the amino acid was not found in nonalkaline SBM at any heating temperatures. Hydroxide ion-catalyzed β-elimination reactions of threonine, serine, and cystine residues produce dehydroalanine intermediate which reacts with lysine residue to form a LAL cross-linking (Friedman et al., 1984); the amino acids were reduced when the level of NaOH increased (data not shown). The maximum LAL (1.00 g/16 g N) was less than that (1.26 g/16 g N) previously found in SBM treated with 10% NaOH and heated at 80 °C. This treated SBM had no detrimental effect on N retention when fed to goats as a mixed diet (1:9 w/w) with sudangrass hay (Nishino et al., 1995).

Although the chemical and nutritional properties are not well understood, NDIN has been regarded as fiber-bound protein (Van Soest, 1982). NDIN increases with NaOH and heat treatment of alfalfa (Nishino et al., 1994) and may reflect the Maillard reaction. NaOH treatment cleaves ester bonds of lignin−carbohydrate complex (Morrison, 1974) and may have increased the substrates for the Maillard reaction. In addition, alkaline condition accelerates the advances of nonenzymatic browning (Ashoor and Zent, 1984). Defatted soybean contains sucrose and stachyose as its principal soluble sugars (Celga and Bell, 1977). Although these are not reducing ones, sucrose is hydrolyzed by heat into glucose and fructose which can be involved in the Maillard reaction. The slight decrease of ADIN in SBM heated at 60 °C may indicate that NaOH addition can release some amino acids integrated in the cell wall matrix. This effect, however, may have been masked when nonenzymatic browning was enhanced by heating at 100 °C. ADIN contains amino acids and increases in heated forages; its digestibility varies according to the degree of heat damage (Amos et al., 1984; Weiss et al., 1986).

The reduction of FDNB-reactive lysine parallels the increases of LAL and Maillard reaction products; the ϵ-amino group of lysine is essential for LAL formation and likely is involved in the Maillard reaction. The Maillard reaction also will produce various compounds including furfurals, dicarbonyls, and reductones (Swaisgood et al., 1991). Therefore, the reducing power of an extract may reflect the extent of the Maillard reaction.

Although production of LAL does not relate directly to increases in reducing power, the close relationship between these two values suggests that the combination of NaOH and heat may have similarly promoted both protein cross-linking and nonenzymatic browning. The more distant relationship between NDIN and LAL, FDNB-reactive lysine, or reducing power could be due to the characteristics of NDIN which is insoluble and may involve various amino acids in addition to lysine.

The changes in LAL, NDIN, FDNB-reactive lysine and reducing power demonstrate that SBM protein was being cross-linked. Therefore, significant correlations of rates of in situ N loss with the above four measurements could confirm the findings that cross-linking renders feed protein less susceptible to microbial degradation. However, residual alkali in SBM might have mediated the effects of treatments by delaying the attack of microorganisms to protein because the treated SBM was incubated intact without removing the added NaOH. The fate of LAL and Maillard reaction products during ruminal digestion should also be considered although it has not been elucidated in this study. The high correlation coefficients suggests that these parameters might be used to predict the in situ ruminal N loss of protein supplements modified by combined NaOH and heat treatments.

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