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Treated Extruded Soybean Meal as a Source of Fat and Protein for Dairy Cows

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Abstract: The influence of treated, extruded, partially expelled soybean meals as undegradable protein and bypass fat sources was studied on lactation performance and ruminal fermentation of dairy cows. Experiment 1: nine cows were used in a replicated 3 x 3 Latin square design with each period being 3 weeks in duration. Cows were fed 440 g/kg forage and 560 g/kg grain diet with one of three extruded soybean meals fed at 110 g/kg of the diet. The 3 soybean meals were 1) twice-extruded soybean meal (ESM; as a control); 2) lignosulfonate-treated, twice-extruded soybean meal (LSM); and 3) calcium oxide plus lignosulfonate-treated, twice extruded soybean meal (CLSM). Experiment 2: 3 ruminally cannulated cows were used in a 3 x 3 Latin square to study the treatment influence on ruminal fermentation. Feeding treated soybean meal to cows in LSM and CLSM treatments did not improve feed intake, milk yield, or milk composition except that cows fed the LSM and CLSM treatments produced less milk protein compared with the ESM treatment. The proportion of $C_{18:2}$ was greater in milk fat of cows fed CLSM compared with that of cows fed the ESM or LSM treatments. Ruminal pH, ammonia, and total volatile fatty acidsVFA were not affected by treatment. An increased proportion of $C_{18:2}$ in milk fat suggest that there is a potential use of calcium salts of fatty acids in protecting the lipid portion of extruded soybean meal and further research is needed to explore this potential with full-fat extruded soybeans.

Key words: Extruded, Soybean meal, Source of fat, Protein, Dairy cows

Introduction

Increasing the ruminally undegraded protein (RUP) content of protein feeds with inherently high degradation rates can have a positive influence on milk production and milk composition in high-producing dairy cows by allowing a greater flow of essential amino acids available for absorption in the small intestine (NRC 2001). For this reason, many methods have been explored to increase the RUP content of soybean meal (SBM) for ruminants. These methods include various forms of heat such as roasting, expelling, and extrusion (Broderick 1986; Faldet and Satter 1991; Schwab 1995). There are also several chemical methods that have been studied in detail such as treatment with aldehydes, acid, alkali, and ethanol (Schwab 1995). The use of chemical methods alone has not proven to be very effective at increasing the RUP content of SBM. A study conducted by Bowman *et al*. (1988) found that NaOH-treated SBM reduced protein degradation in situ, but had no effect on milk production of dairy cows. Researchers have also tried combinations of chemical and heat treatments in an attempt to enhance the RUP of SBM. An example of this combination is the addition of lignosulfonate, a byproduct of the wood pulp industry that contains xylose sugar, to SBM before heat treatment. Limited animal studies suggest that feeding lignosulfonate-treated SBM did not result in a significant improvement in animal performance (Nakamura *et al*. 1992; Mansfield and Stern, 1994).

The feeding of fat sources has become common in the dairy industry as improved genetics have increased the cow's need for energy to maintain health and production. Besides being high in protein, partially expelled SBM is also a source of dietary fat for dairy cows. However, the feeding of supplemental fats high in polyunsaturated fatty acids such as those found in SBM, can lead to problems such as milk fat depression (Griinari *et al*. 1998). The mechanism whereby this occurs is not completely clear, but one theory is that milk fat depression is due to the incomplete biohydrogenation of unsaturated fatty acids, resulting in *trans*-fatty acids that may be toxic to fiber-digesting microbes or causing a lack of available lipid precursors in the mammary gland. For this reason, methods for protecting fat sources from ruminal metabolism have been developed, the major one being protection by making calcium salts of long-chain fatty acids.

PAs mentioned earlier, partially expelled SBM is an excellent source of protein and fat; therefore it would be beneficial to devise a way to protect both of these nutrients from ruminal degradation and metabolism. Very little research has been conducted that compares combinations of methods for increasing RUP content or protecting fat in SBM from ruminal metabolism. Our hypothesis is that protecting protein and fat in partially expelled SBM from ruminal metabolism will enhance the milk, fat, and protein yields of lactating dairy cows by providing more protein and energy for absorption in the lower digestive tract.

The objectives of the present research were: 1) to enhance the availability to dairy cows of fat and RUP in extruded, partially expelled SBM, 2) to quantify the milk yield and milk composition responses of dairy cows fed treated SBM,

and 3) to study ruminal fermentation characteristics of cows fed treated SBM.

Materials and Methods

Experiment 1: Animals and Experimental Procedures: Nine primaparous and multiparous Holstein dairy cows in mid-lactation were used to measure milk production and milk composition responses to feeding treated SBM. At the start of the experiment, cows averaged 184 ± 81 days in milk and were producing 40.0 ± 4.1 kg milk/day. Cows were blocked into three groups based on their milk yield during the 7 days prior to beginning the experiment. Cows within each group were randomly assigned to one of three treatments.

The study was conducted from August through October 2002 at the George B. Caine Dairy Teaching and Research Center, Utah State University, Logan, UT, USA. The experimental design was a replicated 3 x 3 Latin square. The duration of the experiment was 9 weeks, with periods of 3 weeks each. The first 2 weeks in each period served as an adaptation period, and measurements were made during the last week. Cows in the three treatments were fed diets containing either untreated, twice-extruded, partially expelled SBM (ESM) as a control, lignosulfonate-treated ESM (LSM), or calcium oxide plus lignosulfonate-treated ESM (CLSM) at 110 g/kg of the dietary DM. Animal care and procedures were approved and conducted under established standards of the Utah State University Institutional Animal Care and Use Committee.

Preparation of Extruded, Partially Expelled SBM: Raw cracked soybeans were extruded at 149°C using an Insta-Pro® Extruder Model 9600 (Des Moines, IA, USA) and oil was expelled using a Continuous Horizontal Press Model 1500 (Insta-Pro® International, Des Moines, IA, USA). The temperature for extrusion was based on previous research on optimum temperature for heat treatment of soybeans (Faldet and Satter 1991). One batch of extruded SBM containing 90 g/kg of oil on a dry matter (DM) basis was used to prepare the three experimental SBM. To prepare the ESM, extruded SBM was added to a ribbon-style mixer (Model HR-30-0198, Hayes Stolz, Fort Worth, TX, USA). Mixing was continued for 15-20 min while water was added to bring the product up to a moisture content of 180 g/kg. To prepare the LSM, extruded SBM was mixed with a lignosulfonate premix (Pharmtech, Des Moines, IA; 79 kg/ t of extruded SBM on a DM basis). Manufacturer listed ingredients of the lignosulfonate premix were sodium bentonite, lignin sulfonate, and hemicellulose extract. Lignosulfonate is a byproduct of the wood pulp industry that contains the the reducing sugar xylose (200 g/kg). Addition of water and mixing were conducted as described previously. To prepare the CLSM, extruded SBM was mixed with the lignosulfonate mix at the same level used to make the LSM while calcium oxide was also added at 27 kg/ t of extruded SBM on a DM basis. Water was added while mixing for 15-20 min to maintain the same conditions as in the other two treatments. Each mixture was re-extruded at a temperature maintained between 148-157°C. The temperature used in the second extrusion was higher than with the first extrusion in order to achieve a reaction between the lipid in the extruded SBM and the calcium oxide. Additional water was added if needed at a rate of 18.9 l/h to maintain the flow of the product through the extruder. The extrusion rate was 1,363.6 kg/h. The first extrusion of the SBM was to expel the oil while the second extrusion was used to facilitate a reaction between calcium oxide and the lipid portion of the SBM, using high temperature and pressure caused by extrusion.

Estimation of Protein Degradability of Treated SBM: The RUP fraction of each treated SBM was estimated using three different techniques: 1) an *in vitro* enzyme system using the *Streptomyces griseus* protease method (Roe and Sniffen 1990), 2) standardized *in situ* techniques for concentrates (Vanzant *et al.* 1998), and 3) *in situ* techniques combined with an intestinal digestibility procedure described by Calsamiglia and Stern (1995). In method 3, RUP and intestinal digestibility were determined separately. Intestinal digestibility was determined and used as an objective measure to detect heat-damaged protein. Results are presented in Table 1.

Each treated SBM was also tested for heat damaged protein using acid detergent insoluble crude protein (ADICP) as a criterion for evaluation. The ADICP was determined by performing the macro Kjeldahl nitrogen test using AOAC Official method 954.01 (AOAC 2000) with Kjeltec digestor 20 and Kjeltec System 1026 distilling unit; (Tecator AB, Hoganas, Sweden) on feed acid detergent fiber residue. Acid detergent insoluble crude protein is considered to be both undegradable in the rumen and indigestible in the small intestine. Values for ADICP are presented in Table 1.

Feeding and Management of Cows: Cows were housed in a tie-stall barn and fed individually. Diets were fed as a total mixed ration (TMR) and contained 440 g/kg of forage and 560 g/kg of grain mix. Soybean meal treatments were fed at 110 g/kg dietary DM. Diets were formulated to meet the nutrient requirements of cows producing 50 kg of 3.5% fat-corrected milk yield/day according to NRC (2001) recommendations. Diets were offered immediately after the morning milking at 07:00 h. and were fed once daily with push-up in the evening. All diets had similar ingredient composition, except for the three extruded SBM (Table 2). Amounts of feed offered were adjusted daily to ensure feeding an excess of 50-100 g/kg of ad libitum fresh feed intake. Cows were weighed after the morning milking on two consecutive days at the beginning of the experiment. Average body weight of the cows at the

beginning of the experiment was 657 ± 58 kg.

Sample Collection, Analyses, and Calculations: Daily amounts of feed offered and refused for individual cows were recorded during the entire experiment. Samples of the TMR and feed refusals from individual cows were collected daily during week 3 of each period. The TMR samples were stored at -20°C. Feed refusal samples were collected from individual cows, mixed within each treatment, and a representative sample was frozen daily. Weekly composite samples of TMR and feed refusals were analyzed for DM. Once per week, samples of all feed ingredients were collected for determination of DM. The DM content of the feed ingredients was determined by drying in a forced-air oven at 60°C for 48 h. Dietary formulations were adjusted weekly (if necessary) to account for small changes in ingredient DM content.

Samples of dried feed and refusals from each period were composited for the 3 weeks and then ground through a Wiley mill (Arthur H. Thomas, Philadelphia, PA, USA) using a 1 mm screen. Composite samples of feed ingredients and refusals from each treatment during each period were analyzed for chemical composition. Crude protein (CP) was determined using the macro Kjeldahl procedure as described previously. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined with the ANKOM200 Fiber Analyzer (ANKOM Technology Corporation, Fairport, NY, USA), using the basic procedure of Van Soest *et al.* (1991). Sodium sulfite was not used in the procedure for NDF determination, but pre-treatment with heat stable amylase (Type XI-A from *Bacillus subtilis*; Sigma-Aldrich Corporation, St. Louis, MO, USA) was included. During analysis, the samples were further dried at 105°C for 8 h to determine the absolute DM. Chemical analyses were expressed on the basis of this final absolute DM. with residual ash.

The chemical composition of the TMR was calculated from the chemical composition of individual ingredients of the diet. The DM content of the diets was 635 ± 5 g/kg of fresh feed during the entire experiment (Table 2). The alfalfa hay used in the diets contained 923 g/kg of fresh feed as DM and 174, 412, and 312 g/kg DM as CP, NDF, and ADF, respectively. Corn silage contained 380 g/kg of fresh feed as DM and 81, 427, and 261 g/kg DM as CP, NDF, and ADF, respectively.

Daily DM intake was calculated by subtracting the weekly mean of feed DM refused from the amount of feed offered on an individual cow basis. The CP and NDF intakes were calculated by subtracting CP and NDF amounts in feed refused from feed offered. The amount of CP and NDF in feed refused was calculated by multiplying weekly mean feed DM refused for individual cows with treatment average CP and NDF contents in feed refused during that week. The NE_L content of the diet was calculated by multiplying ingredient parts by the NE_L values found in NRC (2001) for individual dietary ingredients. The RUP content of each diet was calculated using NRC (2001) values for individual dietary ingredients, except that the RUP values used for treated SBM were those estimated using the *in situ* procedure combined with intestinal digestibility (Calsamiglia and Stern 1995; Table 1). The NE_L values used for alfalfa hay, corn silage, steam rolled corn, steam rolled barley, distillers dried grain, whole linted cottonseed, dried beet pulp, and molasses were 5.44, 5.77, 8.74, 7.78, 8.24, 8.12, 6.15, and 7.36 MJ/kg feed DM, respectively.

Daily a.m. and p.m. milk weights were recorded. During the last week of each period, milk samples were collected from individual cows on three consecutive days (three morning and three evening milkings over a period of 72 h). Milk samples from individual cows were analyzed at the Rocky Mountain Dairy Herd Improvement Association Laboratory (Logan, UT, USA) for fat, protein, and lactose contents with near mid-infrared procedures using a Bentley 2000 (Bentley Instruments, Chaska, MN, USA). An enzymatic procedure was used to determine milk urea nitrogen using a Chemspec 150 instrument (Bentley Instruments). Final milk composition for each week was expressed on weighted milk yield of a.m. and p.m. samples. Average fat and protein yields were calculated by multiplying the milk yield by the fat and protein content for the respective week on an individual cow basis.

Weighted composite milk samples (6 samples) from each cow were analyzed during each period for fatty acid composition including conjugated linoleic acid (CLA) as described by Dhiman *et al.* (2002). Heptadecanoic acid was used as an internal standard. Fat samples were analyzed in a gas chromatograph (Model 6890 Series II, Hewlett-Packard Co., Wilmington, DE, USA) fitted with a flame ionization detector. Gas chromatography conditions were the same as described by Dhiman *et al.* (1999). Fatty acids were identified by comparing the retention times with methylated fatty acid standards including CLA. The CLA reported is *cis*-9, *trans*-11 $C_{18:2}$. The percentage of each fatty acid was calculated by dividing the area under the fatty acid peak by the sum of the areas under the total reported fatty acid peaks.

Energy output in milk was calculated using Equation 1 of Tyrrell and Reid (1965) as follows: (41.63 x fat content) + (24.13 x protein content) + (21.6 x lactose content) – 11.72. Gross feed efficiency was calculated as daily energy output in milk per kg of feed DM intake on an individual cow basis.

Feed DM and NDF digestibility coefficients were measured during the last week in each period using acid-insoluble ash as an internal marker (Van Keulen and Young 1977). Fecal grab samples (200 to 300 g fresh basis) were collected from individual cows at 05:00, 10:00, 16:00, and 22:00 h on day 20 and at 03:00, 08:00, 13:00, and 19:00 h on day 21 of each period.

Fecal samples were dried in a forced-air oven at 60°C for 72 h and ground through a 1 mm screen using a Wiley

mill. Composite fecal samples for each cow in each period were analyzed for NDF as described previously, and for acid insoluble ash using the procedure described in Van Keulen and Young (1977). Samples of TMR and feed refusals for each period and each treatment were also analyzed for acid insoluble ash. The apparent total tract DM digestibility for individual cows was calculated as described by Dhiman *et al.* (1995).
Apparent digestibility of NDF, expressed as a coefficient, was computed as the difference between NDF intake and the amount of NDF excreted in feces divided by NDF intake. The fecal output was calculated by multiplying feed DM intake by 1 minus fractional feed DM digestibility on an individual cow basis. The amount of NDF excreted in the feces was calculated by multiplying fecal output by NDF content of feces on an individual cow basis.
During the last 2 days of each period, blood samples (15 ml) from individual cows were collected from the coccygeal vein or artery at 5 h post-feeding. The blood samples were collected in serum separation tubes (Vacutainer brand SST Gel and clot activator; Becton Dickinson and Co., Franklin Lakes, NJ, USA). Blood samples were allowed to clot for a minimum of 30 minutes at room temperature and stored in the refrigerator overnight. The samples were centrifuged at 2200 x g for 15 min at 4°C to separate the serum. The serum samples were stored at -20°C until further analysis. Serum samples were ~~then~~ analyzed for glucose concentration colorimetrically using the Beckman glucose kit #442640 (The Beckman Synchron CX Systems, Brea, CA, USA).

Experiment 2: Animal and Experimental Procedures: Three lactating Holstein dairy cows fitted with ruminal cannulae were used in a 3 x 3 Latin square arrangement of treatments to study the influence of feeding treated extruded SBM on ruminal fermentation characteristics. Cows were fed ESM, LSM, or CLSM treatment diets from experiment 1. The total duration of the experiment was 9 weeks with three periods of 3 weeks each. Two weeks were allowed in each period for diet adaptation and measurements were made during the last week in each period. Experiments 1 and 2 were conducted simultaneously. Feeding, animal management, and procedures for feed and milk sample collections, analyses, and calculations were the same as in experiment 1. Milk samples were analyzed for composition as described in experiment 1; however, fatty acid analysis of milk was not conducted in experiment 2. At the start of the experiment, cows averaged 202 ± 86 days in milk and were producing 29 ± 6 kg of milk/day. Average body weight of the cows at the beginning of the experiment was 787 ± 39 kg.

Rumen Fluid Sampling and Analysis: Rumen fluid samples were collected from the ventral sac of the rumen during the last 2 days in each period at 0, 1, 2, 3, 6, 9, 12, 18, and 24 h after the morning feeding. Ruminal liquor samples were strained through two layers of cheesecloth. The pH was determined in strained rumen fluid samples immediately after collection using a pH meter (model #310, Orion Research Inc., MA, USA). Samples of strained rumen fluid (16 ml) were preserved in plastic vials containing 0.3 ml of 50% sulfuric acid for ammonia analysis and were stored at -20°C until analysis. Rumen fluid samples (8 ml) were acidified with 88% formic acid (1:1; vol/vol) and stored at −20°C before preparation and analysis of volatile fatty acids (VFA). The rumen fluid samples collected for ammonia analysis were thawed and centrifuged at 30,000 x g for 20 min at 4°C. Supernatants were analyzed for ammoniausing an alkaline phenolhypochloride colorimetric procedure (Chaney and Marbach 1962) on a Beckman DU® 640 Spectrophotometer (Beckman Instruments, Inc., Fullerton, CA, USA). Acidified ruminal fluid samples were analyzed by gas chromatograph (Erwin *et al.* 1961; Technical bulletin #856A; Supelco, Inc., Bellfonte, PA).
for VFA analysis were centrifuged at 10,000 x g at 4°C for 20 min. For sample extraction, samples were blended with deionized water for 2 min, filtered through cheesecloth, and then filtered through a disposable syringe filter. This extract was then mixed in a 1:1 ratio with 0.06 M oxalic acid containing 100 ppm trimethylacetic acid, which served as an internal standard. Samples were injected into a Perkin Elmer Autosystem XL gas chromatograph containing a packed column with the following specifications: 2 m x 2 mm; tightspec ID; 4% carbowax 20 M on 80/120 B-DA (Supelco, 1990).

Statistical Analyses: Data were analyzed as a replicated 3 x 3 Latin square arrangement of treatments using the mixed models procedures of SAS (1999-2000). The mixed model used for analyzing production variables in experiment 1 included block, cow within block, period, treatment, period x block interaction, block x treatment interaction, and residual error. The mixed model used for analyzing production variables in experiment 2 included cow, period, treatment, and residual error and was not analyzed as a replicated design. The mixed model used for analyzing rumen fermentation characteristics included cow, hour, treatment, period, hour x treatment interaction, and residual error. Hour was specified in the program as a repeated measure. Different variance-covariance matrices were used, depending upon which one was deemed the best fit for the model. Significance was declared at $P < 0.05$ unless otherwise noted. Significance at $P < 0.01$ was mentioned as $P = 0.01$ to simplify the tables. Block x treatment and block x period interaction effects were non-significant ($P > 0.05$) for production variables and therefore are not reported. Hour x treatment interaction effects were also non-significant ($P > 0.05$) for rumen fermentation measurements and, therefore, only overall means over time are reported.

Diet Composition: Diets fed to cows in experiments 1 and 2 were formulated to be similar in nutrient composition

(Table 2). The ESM treatment diet contained higher CP because of the slightly higher CP of the SBM used in ESM over the LSM or CLSM treatment diets. The RUP values in the three treatment diets ranged from 74 to 79 g/kg feed DM. As discussed earlier, this difference in dietary RUP content was due to the lower RUP in treated SBM used in LSM compared with the ESM and CLSM treatments. According to NRC (2001), RUP and ruminally degradable protein requirements were met for cows consuming each diet.

The NDF content was higher in the CLSM treatment diet because of the higher NDF content in CLSM compared with ESM or LSM (Table 2). Samples were sent to another laboratory, which confirmed these results (data not shown). It is unclear why CLSM was higher in NDF content because no fiber was added that was not also added to the LSM treatment diet. It is possible that the method used for determining NDF is biased toward feedstuffs containing calcium salts of fatty acids. Another possible explanation is that the addition of calcium oxide is causing some reaction that is artificially increasing the NDF content of the SBM. However, the NDF and ADF contents of all the diets were within an acceptable range for dairy cattle (NRC 2001). An attempt was made to formulate diets so that Ca and P contents were similar. However, actual analysis showed that the CLSM had slightly higher Ca content owing to the addition of calcium oxide to the SBM used in this treatment.

The fatty acid profiles of the experimental diets were very similar (Table 3). Total fat content in the CLSM diet was slightly lower than that of the ESM or LSM diets. Addition of calcium oxide lowered the concentration of fat in SBM used in CLSM.

Results

RUP Content of Treated SBM: The determination of RUP content using the three techniques produced mixed results (Table 1). Overall, the *in situ* procedure used when determining intestinal digestibility (Calsamiglia and Stern 1995) gave the lowest values for RUP followed by the *in vitro* procedure of Roe and Sniffen (1990). The standard *in situ* procedure (Vanzant *et al.* 1998) showed the highest values of RUP for the three treated SBM. However, there was a trend for LSM to be lower in RUP than ESM and CLSM, regardless of the technique used.

Lactation and Ruminal Fermentation Characteristics: In experiment 1, cows fed untreated SBM (ESM) or treated SBM (LSM and CLSM) treatments had similar ($P > 0.05$) feed DM intake, CP intake, NDF intake, apparent total tract digestibility of DM and NDF, milk yield, fat yield, milk energy output, milk energy/kg feed DM intake, milk composition, milk urea content, and blood serum glucose concentrations (Table 4). Cows fed ESM and LSM treatments tended to have higher protein content in milk compared with cows fed the CLSM treatment. The slight increase in protein content and numerically higher milk production observed in milk from cows in the ESM treatment resulted in a higher ($P = 0.01$) protein yield for that group. Milk fat from cows fed the CLSM treatment contained a higher ($P = 0.01$) proportion of $C_{18:2}$ fatty acid than that from cows in either of the other two treatments (Table 5). Proportions of other fatty acids did not differ among treatments.

In experiment 2, daily feed DM intakes ranged from 20.9 to 21.5 kg/d across all three treatments and did not differ ($P > 0.05$) among them. Intakes and apparent total tract digestibility of DM and NDF were also not different ($P > 0.05$) among treatments (data not shown). Milk yields ranged from 23.5 to 27.2 kg/d across the three treatments. As observed in experiment 1, cows in experiment 2 fed untreated (ESM) or treated SBM (LSM and CSLM) treatments also had similar ($P > 0.05$) milk yield, milk energy output, fat yield, milk composition, and milk urea content (data not shown).

Mean values for rumen fluid measurements are presented in Table 6. Feeding treated SBM had no influence on ruminal pH, ammonia, and total VFA concentrations. Molar proportions of acetate, propionate, and isobutyrate, and the acetate to propionate ratio in rumen fluid were similar ($P > 0.05$) across the three treatments. However, butyrate (mol/100 mol) was higher ($P = 0.03$) in rumen fluid of cows in the ESM treatment compared with that of cows fed the CLSM treatment.

Discussion

RUP Content and Intestinal Digestibility: The RUP estimations for the treated SBM were performed in three different laboratories. As discussed in detail by Stern *et al.* (1994), the method used and sample particle size can greatly influence the RUP estimations. Coefficients for intestinal digestibility of RUP estimated by the Calsamiglia and Stern (1995) procedure were 0.79, 0.77, and 0.79 for ESM, LSM, and CSLM, respectively. According to the NRC (2001), the intestinal digestibility coefficient for the RUP of all types of SBM is 0.93. It should be noted that very little testing has been done that compares the intestinal digestibility of differently treated SBM. If this current NRC (2001) value is used as the standard, it seems that there could have been some overprotection of the protein in the three treatments, but not more overprotection in any one over another. However, ADICP contents for all three SBM (Table 1) were close to the reported ADICP (16 g/kg DM) for lignosulfonate treated SBM (NRC 2001), suggesting that there was very little heat damage to protein in any of the SBM. The values for ADICP were not compared to that of regular untreated solvent extracted SBM because all three treated SBM in the present study had undergone the heating process. An interesting observation was that the protein in LSM did not seem to be well-protected using

any of the three different methods of RUP analysis. This is contrary to what was expected, based on other studies using lignosulfonate treatment to protect protein (Mansfield and Stern 1994). A possible explanation is that the second extrusion of the SBM had already protected protein from ruminal degradation, thereby causing no further increase in RUP by adding lignosulfonate.

Nutrient Intake and Production Response: Intakes of DM, CP, and NDF were numerically higher for cows in experiment 1 than in experiment 2. This is attributed to the lower milk production of the cows in experiment 2. Despite small differences in CP, NDF, and ADF contents of the diets, intakes of DM, CP, and NDF were not different across treatments within experiments. Others researchers have reported similar results in nutrient intakes when feeding treated SBM. Intakes of feed DM or CP were not affected by feeding chemically treated SBM (lignosulfonate or alkali-treated) to dairy cows in place of solvent-extracted SBM (Bowman *et al.*, 1988 and Santos *et al.*, 1998). Consistent with similar nutrient intakes, the apparent values for total tract digestibility of DM and NDF in the present study were also not influenced by the method of SBM treatment in experiments 1 and 2. These results along with intestinal digestibility and ADICP contents (Table 1) of treated SBM indicate that there was no overheating or overprotection of protein in one treatment compared to another. Mansfield and Stern (1994) observed no difference in apparent total tract DM digestibility when feeding SBM or lignosulfonate-treated SBM. However, Windschitl and Stern (1988b) reported a reduction in digestibility of DM when cows were fed diets containing lignosulfonate-treated SBM in place of solvent-extracted SBM. In another study by Windschitl and Stern (1988a), lignosulfonate-treated SBM also inhibited ruminal digestion of organic matter and cellulose in continuous culture. As mentioned previously, milk production was lower for cows in experiment 2 than for cows in experiment 1, but there was no change in milk yield, fat yield, or energy output in milk within experiments (Table 4). Feeding treated SBM to cows in the LSM or CLSM treatments did not change milk fat, lactose, or urea contents. However, feeding treated SBM to cows in CSLM resulted in a tendency for lower milk protein content as compared to the other two treatments. This tendency coupled with a numerical decrease in milk yield resulted in significantly lower protein yields for LSM and CLSM treatments compared to feeding untreated SBM.

Other researchers also reported no increases in milk yield when lignosulfonate-treated SBM was compared with untreated solvent extracted SBM (Nakamura *et al.* 1992; Mansfield and Stern 1994). Bowman *et al.* (1988) reported no difference in milk yield when NaOH-treated SBM was compared to solvent-extracted SBM.

One of our objectives was to enhance the supply of protein to dairy cows through lignosulfonate-treated SBM using extrusion technology and thereby increase the yields of milk and protein. However, cows fed treated SBM produced less milk protein than cows fed untreated SBM (Table 4), suggesting that we failed to increase the supply of protein by treating SBM. One possible explanation as to why no increases were observed in milk and protein yields is that the second extrusion of the three SBM had already protected the protein to the extent that no further increase in RUP was observed by adding lignosulfonate to the SBM. As discussed earlier, the first extrusion was done to expel the oil and the second extrusion was necessary to facilitate a reaction between the calcium oxide and lipid portion of the SBM at high temperature and pressure caused by extrusion. In future experiments, we recommend the use of full-fat extruded soybeans that are extruded only once in order to obtain maximum benefits from ruminal protection of protein and fat.

Depression in milk protein is often observed when dairy cows are fed fats or feeds containing fat (Broderick 1986; Grummer 1988; Mohamed *et al.* 1988; Schingoethe *et al.* 1988; DePeters *et al.* 1989; Drackley and Elliott 1993). We hypothesized that if protein depression as a result of feeding supplemental fat is due to the negative effects of fat in the rumen, then protecting fat from ruminal biohydrogenation by adding calcium oxide may alleviate the depression in milk protein content and enhance protein yield. However, results from the present study suggest that protecting fat in the CSLM treatment did not improve protein yield, but rather decreased it (Table 4). A possible explanation for these results is that the negative effect of feeding fat on milk protein could be a post-ruminal rather than a ruminal effect as suggested by Wu and Huber (1994). Limited observations in this study indicate that treatment had no effect on blood serum glucose concentrations (Table 4).

The existing literature concerning the influence of feeding treated SBM on milk fat content presents mixed results. Bowman *et al.* (1988) observed increases in milk fat content when cows were fed NaOH-treated SBM compared with untreated SBM. Mansfield and Stern (1994) reported no increase in milk fat content when cows were fed lignosulfonate-treated SBM compared to solvent-extracted SBM. However, Abel-Caines *et al.* (1998) observed an increase in milk fat percentage when increasing levels of lignosulfonate-treated SBM were added to the diet. In the present study, treating SBM with lignosulfonate or calcium oxide plus lignosulfonate did not increase milk fat content.

Table 1. Protein characteristics of treated soybean meals (SBM)

Item	Treated soybean meal[a] ESM	LSM	CLSM
CP (g/kg DM)	460	431	423
RUP (g/kg DM) In vitro enzymeb	225	220	241
In situc	280	235	287
In situ + intestinal digestibilityd	213	170	215
Soluble proteind (g/kg DM)	44	38	52
Intestinally digestible RUPd (g/kg DM)	167	130	170
ADICPe, (g/kg DM)	9.0	18.0	11.0

a ESM = extruded, partially expelled SBM; LSM = lignosulfonate-treated SBM, CLSM = calcium-oxide and lignosulfonate-treated SBM.
b Determined using the *Streptomyces griseus* protease method (Roe & Sniffen 1990).
c Determined using the in situ procedure of Vanzant *et al.* (1998)
d Determined using the procedure of Calsamiglia & Stern (1995).
e ADICP = Acid-detergent insoluble crude protein.

Table 2. Ingredient and chemical composition of diets fed to cows (experiments 1 and 2)

Composition	Treatment ESM	LSM	CLSM
Ingredient (g/kg DM)a			
Alfalfa hay	330	330	330
Corn silage	110	110	110
Grain mixb	421	421	421
Untreated SBMc	110	---	---
Lignosulfonate-treated SBMc	---	110	---
Calcium-oxide and lignosulfonate-treated SBMc	---	---	110
Calcium salts of long chain fatty acids	10	11	11
Monosodium phosphated	2	2	4
Calcium supplemente	3	2	---
Trace minerals, vitamins, othersf	14	14	14
Chemical (g/kg DM)			
CP	177	173	172
RUPg	79	74	79
NDF	325	332	354
ADF	210	216	211
Ca	8.1	7.9	8.8
P	4.1	4.0	4.2

a Average DM and NEL contents in all three diets were 635 g/kg fresh feed and 6.90 MJ/kg feed DM, respectively.
b Contained 170 g steam rolled corn, 80 g steam rolled barley, 40 g distiller dried grain, 59 g whole linted cottonseed, 60 g dehydrated sugar beet pulp and 12 g of sugar beet molasses.
c Untreated SBM (ESM) contained 942 g/kg of fresh feed as DM and 465, 183, and 123 g/kg DM as CP, NDF, and ADF, respectively. Lignosulfonate-treated SBM (LSM) contained 940 g/kg of fresh feed as DM and 432, 251, and 180 g/kg DM as CP, NDF, and ADF, respectively. Calcium-oxide plus lignosulfonate-treated SBM (CLSM) contained 938 g/kg of fresh feed as DM and 425, 447, and 139 g/kg DM as CP, NDF, and ADF, respectively.
d Contained: minimum 260, 193, and maximum 0.03 g/kg P, Na, and Fl, respectively.
e Contained: 360 to 380 g/kg Ca.
f Contained 2, 7, 3, and 2 g/kg of yeast, sodium bicarbonate, trace-mineralized salt and vitamin mixture, respectively. Trace-mineralized salt contained 950 to 970 g/kg NaCl, 5.5 g/kg Zn, 5.5 g/kg Mn, 3.5 g/kg Fe, 1.4 g/kg Cu, 0.08 g/kg I, 0.06 g/kg Se, and 0.02 g/kg Co. Vitamin mix contained 1,102,500 IU of vitamin A, 330,750 IU vitamin D, and 6,615 IU of vitamin E/kg of DM.
g Calculated using NRC (2001) RUP values for feedstuffs; the RUP values used for treated SBM were those obtained when determining intestinal digestibility using the procedure described in Calsamiglia & Stern (1995).

Table 3: Fatty acid composition and total fatty acids of experimental diets

	Fatty acids, g/100 g of total fatty acids									
Treatment	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:4	C22:0	Total fatty acids (g/kg diet DM)
ESM	6.86	18.1	0.52	4.62	21.3	33.4	7.77	5.32	2.00	53.5
LSM	6.83	18.2	0.52	4.67	21.3	33.4	7.73	5.26	2.00	53.8
CLSM	6.67	18.2	0.52	4.70	21.3	33.5	7.75	5.26	2.01	51.9

Table 4: Nutrient intake, production and blood glucose response of cows fed treated SBM (experiment 1)

	Treatment				
Item	ESM	LSM	CLSM	SEM	P > F
Intake (kg per day)					
DM	24.9	24.1	24.1	1.03	0.50
CP	4.05	3.83	3.81	0.17	0.17
NDF	7.03	6.87	7.39	0.35	0.35
Apparent total tract digestibility coefficient					
DM	0.83	0.78	0.79	0.01	0.13
NDF	0.66	0.54	0.62	0.10	0.15
Production (kg per day)					
Milk	36.4	34.4	34.5	1.20	0.28
Fat	1.16	1.05	1.12	0.09	0.22
Protein	1.11a	1.04b	1.01b	0.04	0.01
Milk energy (MJ per day)c	100.6	95.3	95.0	4.33	0.11
Milk energy (MJ /kg DM intake)	4.06	3.95	4.03	0.13	0.84
Milk composition (g per kg)					
Fat	30.8	29.6	31.6	0.22	0.47
Protein	30.4	30.6	29.1	0.10	0.08
Lactose	49.0	48.5	48.0	0.08	0.72
Milk urea N (mg/dl)	14.9	14.2	14.6	0.64	0.27
Blood serum glucose (mg/dl)	37.7	38.3	39.6	1.91	0.78

a,b Means in the same row with different superscripts differ significantly for treatment effect with *P* value as mentioned in column for significance.
c Calculated using Equation 1 of Tyrell & Reid (1965).

Milk Fatty Acid Composition: The feeding of treated SBM in the CLSM treatment significantly increased the proportion of the $C_{18:2}$ fatty acid in milk fat compared with either of the other two treatments in experiment 1 (Table 5). The predominant polyunsaturated fatty acids in dairy cattle diets are $C_{18:2}$ and $C_{18:3}$ from plant lipids. However, under normal ruminal conditions, these fatty acids are usually biohydrogenated to $C_{18:0}$. Because milk from cows in the CLSM treatment contained a higher proportion of $C_{18:2}$, this is indicative that the lipid portion of SBM in CLSM was somewhat protected from ruminal metabolism. It is also interesting to note that *trans* $C_{18:1}$ content was also numerically lower, but not significantly, in milk from cows fed the CLSM diet. The *trans* $C_{18:1}$ (*trans*-11 $C_{18:1}$ in particular) is an intermediary product in the biohydrogenation of $C_{18:2}$ to $C_{18:0}$ (NRC 2001). A lower level of *trans* $C_{18:1}$ in milk fat as a result of less ruminal fatty acid biohydrogenation is also an indication of protected lipid in the CLSM treatment. No differences in other milk fatty acids suggest that feeding treated SBM in LSM or CLSM treatments did not have a negative influence on the absorption of fatty acids from the lower tract or on the synthesis of fatty acids in the mammary gland.

Ruminal Fluid Characteristics: The pH values observed for all three treatments were within an acceptable range to maintain a healthy rumen environment (NRC 2001). Supplying unsaturated fatty acids at amounts greater than 20 g/kg of diet DM can negatively influence rumen microbial activity (Jenkins 1993) and ruminal fermentation characteristics. No significant differences in ruminal pH, ammonia concentration, or total VFA (Table 6) suggest that treating SBM with lignosulfonate or calcium oxide plus lignosulfonate did not have any negative effects on the rumen microbial environment. Ruminal butyrate concentration in cows fed the CLSM treatment was significantly lower than that in the ESM treatment. As the other ruminal parameters were not affected by treatment, the small difference in butyrate between ESM and CLSM is probably of little biological significance. There was a tendency for CLSM cows to have a higher ratio of acetate to propionate, suggesting that unsaturated fatty acids in this treatment were more protected and thus had less negative effect on fiber digestion than either of the other two treatments.

Table 5: Fatty acid composition of milk from cows fed treated SBM (experiment 1)

Fatty acid	Treatment				
(g/100 g fat)	ESM	LSM	CLSM	SEM	P > F
C8:0	0.89	0.86	0.84	0.11	0.87
C10:0	0.31	0.30	0.30	0.02	0.62
C12:0	2.43	2.47	2.45	0.17	0.94
C14:0	11.7	11.9	11.7	0.26	0.72
C14:1	0.54	0.55	0.53	0.14	0.55
C15:0	1.68	1.88	1.76	0.08	0.26
C16:0	33.8	34.0	33.9	0.51	0.88
C16:1	1.96	2.10	1.78	0.24	0.41
C17:1	0.23	0.25	0.22	0.02	0.26
C18:0	11.2	10.5	11.3	0.72	0.41
C18:1-*trans*	5.30	5.09	4.58	0.56	0.44
C18:1-*cis*	24.0	24.1	24.6	0.71	0.54
C18:2	4.66b	4.67b	4.89a	0.10	0.01
C18:3	0.46	0.48	0.50	0.02	0.42
CLAc	0.55	0.52	0.50	0.04	0.50
C20:4	0.12	0.11	0.12	0.01	0.87
C22:0	0.11	0.10	0.10	<0.01	0.37

a,b Means in the same row with different superscripts differ significantly for treatment effect with *P* value as mentioned in column for significance.
c Conjugated linoleic acid, *cis*-9, *trans*-11 C18:2 isomer.

Table 6: Ruminal fluid measurements in cows fed treated SBM (experiment 2)

	Treatment				
Item	ESM	LSM	CLSM	SEM	P > F
pH	6.11	6.06	6.29	0.09	0.17
NH3 (m*M*)	7.12	7.13	6.15	0.56	0.39
Total VFA (m*M*)	164.3	163.2	150.1	5.82	0.20
VFA (mol/100 mol)					
Acetate	66.3	66.8	67.2	0.82	0.74
Propionate	18.3	18.0	18.5	0.93	0.93
Isobutyrate	1.0	1.0	1.0	0.05	0.97
Butyrate	14.4a	14.3a,b	13.4b	0.45	0.03
Acetate:Propionate	3.7	3.8	4.0	0.09	0.09

a,b Means in the same row with different superscripts differ significantly for treatment effect with the *P* value as mentioned in column for significance.

Conclusions

Treating extruded, partially expelled SBM with lignosulfonate or calcium oxide plus lignosulfonate using extrusion technology did not result in a significant improvement in RUP availability. Feeding either of the treated SBM to lactating dairy cows had no influence on nutrient intake, milk production, milk composition, or rumen fermentation characteristics, except in that cows fed treated SBM that yielded less protein in milk. Cows fed calcium oxide plus lignosulfonate-treated SBM had higher proportions of $C_{18:2}$ fatty acid in milk fat, suggesting that the lipid portion of the SBM was somewhat protected from ruminal biohydrogenation as compared to SBM used in the ESM and LSM treatments.

Feeding lignosulfonate or calcium oxide plus lignosulfonate-treated, extruded, partially expelled SBM to dairy cows did not improve animal performance in the present study. We reject the hypothesis that feeding treated, twice-extruded SBM will enhance the protein yield or content of milk. However, based on the findings of the current study, there appears to be a potential for further research in the area of protecting the unsaturated fatty acid portion of full-fat extruded soybeans in order to enhance milk production and avoid milk fat depression that is commonly seen when feeding high levels of unsaturated fatty acids in the diet.

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