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ARTICLE

Soy Protein Isolate Extruded with High Moisture Retains High Nutritional Quality

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High-moisture extrusion of soy protein isolate generates a highly palatable meat substitute. No systematic evaluation of the nutritional quality of soy processed in this manner has been performed. This study compared the growth rate of male and female mice fed diets containing soy protein isolate without extrusion or with high-moisture extrusion. Other measures of overall growth and animal health were examined. Minor differences in the parameters were observed. Overall, the extruded soy protein was equally nutritious as the unextruded soy protein for the animals. Hence, high-moisture extrusion may be considered a useful method to generate high-quality protein foods. A longer term feeding trial may be recommended to further define the nutritional adequacy of this protein.

KEYWORDS: Soy protein isolate; high-moisture extrusion; animal growth; bone mineral content; serum hormones

INTRODUCTION

Proteins are an essential component of the human diet, and high-quality protein is required for adequate growth and health (*1*). Meat in the form of animal flesh or fish is the most common source of high-protein food for many people. However, the high cost of meat may prohibit its affordability in some parts of the world (*2*). In addition, there are people who, for health or religious reasons, choose to not eat meat. Although soy protein is an excellent source of high-quality protein and is readily supplied in a nutritional form, such as powders in health foods stores, it is often unacceptable because of taste and texture. Advances have been made in improving the taste characteristics of soy protein through plant breeding (*3*) and processing technology (*4*). Soy protein has characteristics that allow it to be processed through extrusion to alter the texture. A highly palatable soy protein-based food, such as a fibrous meat analogue from a high-moisture twin-screw extrusion process, would be much more acceptable to most consumers (*5*).

In the manufacture of protein meat analogues, most proteins must be made insoluble during extrusion cooking and given structural integrity and viscoelastic properties similar to those of meat. It has long been assumed that the protein is insoluble and aggregated into a macroscopic structure due to molecular changes in the protein fraction (*6*). These changes are complex, involving alteration of both covalent and non-covalent interactions (*5*, *7*). Liu and Hsieh (*8*) concluded recently that disulfide bonding plays a more important role than non-covalent interactions in forming not only the rigid structure but also the fibrous texture of soy protein meat analogue.

Alteration of disulfide and non-covalent interactions in the meat analogue formation during extrusion cooking may lead to changes in the nutritional value of final products (*9*). There have been many studies conducted to monitor such changes in extruded foods. Yet, almost all studies regarding the effects of the extrusion process on nutritional values of soy protein were carried out under low- to moderate-moisture extrusion conditions that cause significant reduction in protein digestibility due to decreased bioavailability of lysine resulting from the Maillard reaction (*10*, *11*). Under high-moisture extrusion conditions, these changes would be expected to be different from traditional extrusion methods because extrusion at higher moisture would reduce viscous dissipation, which lowers the extrusion temperature. The texture and chemical and sensory characteristics, but not nutritional value, of a soy meat protein analogue extruded at high moisture have previously been reported (*5*, *7*). The objectives of this study were to evaluate the nutritional value of a meat analogue derived from high-moisture extrusion. The study involved comparison of overall growth, circulating indicators of muscle and bone growth, organ weights, intestinal tract measures, and bone strength and composition in both male and female mice fed the experimental diets for 90 days.

MATERIALS AND METHODS

Soy Protein Extrusion. The soy protein isolate (Profam 974) was obtained from Archer Daniels Midland Co. (Decatur, IL), and unmodified wheat starch (Midsol 50) was from MGP Ingredients (Atchison, KS). The ingredients were blended in a 9:1 ratio using an 18.9 L Hobart mixer (model A-200-F, Hobart Corp., Troy, OH) for 10 min. This blend was extruded using an APV Baker MPF 50/25 twin-screw extruder (APV Baker Inc.,

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Grand Rapids, MI) with a cooling die attached at the end of the extruder. The dimensions of the cooling die were 30 × 10 × 300 mm (W × H × L), and refrigerated cold water (4.4 °C) was used as the cooling medium for the die. The screw profile (from feeder to die) used was as follows: 250 mm twin-lead screws, 25 mm 90° paddles, 75 mm single-lead screws, 50 mm 30° reverse kneading pitch, 100 mm single-lead screws, 50 mm 30° forward kneading pitch, 100 mm single-lead screws, 50 mm 30° forward kneading pitch, and 75 mm single-lead screws. The feed rate was fixed at 6.8 kg/h, and the screw speed was 150 rpm. Water was injected 0.108 m downstream from the center of the feed port to the extruder and adjusted to give a feed moisture content of 60% (wet basis). The temperature profile in the extruder from the first zone to the fourth zone preceding the cooking temperature was 26.6, 60.0, 107.2, and 129.4 °C. The last two zones were set at 160 °C. These extrusion conditions were selected because the resulting meat analogue was more fibrous and had a more orderly directional structure than that extruded at higher moisture (65 or 70%) or lower temperature (137.8 and 148.9 °C) reported previously (*5*, *7*). Furthermore, 60% feed moisture and 160 °C represent severe high-moisture extrusion conditions, and therefore if changes in nutritional quality were not observed under these conditions, it would be expected that less severe treatments would also not have a negative effect. Extruded products were collected and stored in airtight plastic containers without dehydration in a walk-in freezer at −18 °C. Once frozen, the products were freeze-dried using a laboratory freeze-dryer (LyphLock 12, Labconco Freeze-Dry System, Kansas City, MO). The resulting powdered protein was used for the animal feeding trial.

Isoflavone Extraction and Analysis. The extraction and determination of isoflavones in the protein samples were made according to the procedure of Klump et al. (*12*). In brief, test samples were extracted first at 65 °C for 2 h with methanol/water (80:20). Extracts were then saponified for 15 min at room temperature (23 °C) with dilute (0.13 M) sodium hydroxide solution, filtered, centrifuged at 7000*g*, and analyzed in triplicate by reversed-phase liquid chromatography with UV detection at 260 nm. All chemical standards including diadzin, diadzein, genistein, and genistin were ordered from Fisher Scientific (Pittsburgh, PA) and were of HPLC grade. Isoflavone glucosides and aglycones were separated on a Supelco LC-18 column (5 μm, 25 cm × 4.6 mm i.d.; Supelco, Bellefonte, PA) with a methanol/water mobile phase delivered at 1.5 mL/min by a Perkin-Elmer series 410 pump and determined at 260 nm using a Perkin-Elmer LC-90 UV (Waltham, MA).

Animals. All animal protocols were approved and supervised by the Iowa State University Animal Care and Use Committee and were performed in compliance with all regulations. Twenty male and 20 female C57Black/J mice (14 ± 1.3 g) were purchased from Charles River (Wilmington, MA) and randomly divided into groups of 10. Mice were housed in groups of three (females) or singly (males) in standard shoe-box cages with pine shavings and free access to tap water. The mice were given free access to either a diet prepared with the unextruded dry mix (CSP; soy protein isolate/unmodified starch = 9:1) or the extruded soy protein isolate (ESP; **Table 1**). The two diets were formulated using the AIN-93G standard (*13*) with the only difference being the source of soy protein. After 90 days, mice were injected intraperitoneally with Nembutal, and blood was collected from the left ventricle using a 1 cm^3 tuberculin syringe previously rinsed with 0.01% heparin in saline. Blood was placed on ice and total glucose determined using a glucose meter (One-Touch, Lifescan, Johnson & Johnson, Langhorne, PA) and hematocrit analyzed by capillary centrifugation. The blood was then centrifuged for 5−7 min at 15000*g* at 4 °C and the plasma aliquoted into three separate tubes and stored frozen.

Plasma Biomarkers. The plasma samples were analyzed for the following biomarkers: insulin, IGF-I, cortisol, and growth hormone, using commercial ELISA kits [Alpco (Salem NH), R&D Systems (Minneapolis, MN), IBL America (Minneapolis, MN), and Linco Research Inc. (St. Charles, MO), respectively]. Samples were analyzed in duplicate on a single 96-well plate containing internal standards and controls according to the manufacturer's instruction.

Table 1. Diet Composition

	g/kg	
ingredient	CSP	ESP
choline bitartrate[a]	2.5	2.5
DL-methionine[a]	4.0	4.0
vitamin mix AIN 93[a]	10.0	10.0
mineral mix AIN 93 M[a]	35.0	35.0
corn starch[b]	396.5	396.5
cellulose BW200[a]	50.0	50.0
Dyetrose[c]	132.0	132.0
sucrose[b]	100.0	100.0
soy protein[d]	200.0	200.0
safflower oil[a]	20.0	20.0
corn oil[b]	50.0	50.0

[a] MP Biomedicals, Solon OH. [b] General Stores, Iowa State University. [c] Dyets Inc., Bethlehem, PA. [d] Protein used was Profam 974, Archer Daniels Midland, either without extrusion (CSP) or with extrusion (ESP) as described under Materials and Methods.

Intestine and Organ Preparation. Colon and small intestine were cleaned of adhering fat and flushed with cold phosphate-buffered saline. The tissues were weighed, then flushed with 10% neutral-buffered formalin, and placed in a glass beaker containing formalin for fixation. After 30 min, a 0.7 cm long midsection of the colon and small intestine were cut with a scalpel and placed into a tissue cassette, which was incubated in formalin for 2 h, and then transferred into 95% ethanol until they were embedded in paraffin on end to obtain ring-shaped slices. Paraffin embedding and section preparation were done by the ISU Veterinary Medical Clinical Pathology Department. The following organs were dissected and weighed: heart, lung, liver, kidney, spleen, retroperitoneal fat pads, abdominal fat and reproductive fat pads, gastrocnemius muscle, triceps muscle, reproductive tract, and pancreas.

Bone Preparation. At termination, the hind quarters of each mouse were removed and frozen. The femurs were collected after removal of adhering skin and muscle. Bones were prepared and subjected to breaking strength analysis as defined by Yannicelli and Medieros (*14*). Muscle and soft tissue were cleaned from the right femur, and the width and the length (including trochantor) were measured. Breaking strength was determined at room temperature using a TA-XT2i texture analyzer (Texture Technologies Corp., Algonquin, IL) with a Warner-Bratzler shear. Each femur was placed on two lower supports (10 mm apart), and a 0.5 N force was applied at the mid-diaphysis on the anterior surface (to measure compression force) at a test speed of 0.4 mm/s over a distance of 1.2 mm. The point of rapid decrease in force (measured in newtons, N) was used as the point of fracture force from force−strain curves using instrument software. After testing for breaking, bones were dried in an oven and then placed in a high-temperature oven to remove organic materials. The remaining ash was weighed and used to estimate the total mineral content per bone (*15*). Mineral content is expressed as the ratio of weight of the residual ash to total bone weight.

Statistical Analysis. The data were analyzed with SAS using two-way ANOVA to determine effects of gender, diet, and the interaction of gender × diet. One-way ANOVA was used to evaluate effects of diet within each gender.

RESULTS

As expected, male mice grew at a faster rate than female mice (**Figure 1**; $P < 0.006$). Within gender, the ESP diet provided

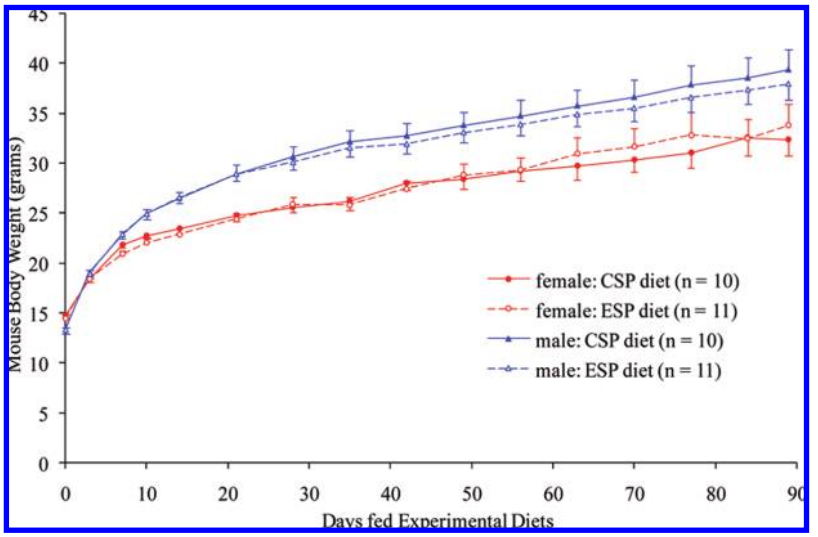

Figure 1. Average growth rate of male and female mice fed the ESP or CSP diet. Data are expressed as mean with standard error shown in error bars. The data were analyzed using ANOVA to determine statistically significant difference at each time point. By two-way ANOVA it was determined that males had significantly higher body weight compared to females beginning on day 3, and there was no diet × gender interaction. Using one-way ANOVA to compare within gender, no differences between the ESP and CSP diets were observed.

similar rates of growth as the CSP diet. In males, mice fed the ESP diet began to show a slight decrease in rate of gain starting at 35 days compared to the CSP-fed mice; however, this did not reach statistical significance. When combined across gender (data not shown), the growth rate of mice fed the diet containing the ESP was not statistically different from that of mice fed the CSP. Food consumption was also similar within gender for the ESP and CSP diets (**Figure 2**). Overall, females had a tendency to consume less of the ESP than the CSP diet, but this reached statistical significance only on days 7 and 10. For the males, the food consumption was similar for both diets throughout the study.

Twelve organs were dissected and weighed and expressed as a percent of body weight (**Figure 3**). For 11 of these, no effect of diet was observed in either gender. In females only, liver weight was significantly less in mice fed the ESP compared to the CSP diet.

The histological analysis of the colon and small intestine revealed no differences due to dietary protein source (data not shown). The number and height of colon crypts were similar in the mice fed the two proteins. All sections examined appeared normal with no obvious pathology.

Femur length and width were found to be unaffected by the dietary treatments in either gender (**Table 2**). In females, breaking strength was significantly reduced in mice fed the ESP compared to the CSP diet. Males did not show this effect. No differences in mineral concentration were observed in either gender.

Diet had no affect on growth hormone, IGF-I, insulin, or glucose/insulin ratio in either the male or female mice (**Table 3**). Growth hormone levels were significantly higher in female than male mice. In male mice, cortisol levels were significantly higher when fed the ESP compared to the CSP diet. This response was not observed in female mice, although there was a nonsignificant trend for a higher mean in females fed the ESP compared to the CSP diet. Hematocrit concentration was slightly lower in males fed ESP compared to CSP, but this effect was not present in females.

DISCUSSION AND CONCLUSIONS

To test our hypothesis that a high-moisture extruded soy protein was nutritionally equivalent to an unextruded soy protein, we used the rapidly growing mouse model. The parameters were selected to provide an overall view of the nutritional quality of the soy protein and to determine if physiological regulators of growth and metabolism were affected. The experimental diets were well tolerated by both male and female mice and supported growth and development similarly. No pathological effects due to diet were observed during the 90 day feeding trial. The protein quality of soy has been found to be similar to that of cow's milk and egg white, which are the standards for protein quality (*16*). The extruded soy protein in our study provided growth rates in the male and female mice similar to those provided by the nonextruded protein, which suggests no loss of protein quality occurred during the processing procedure. In some cases, the digestibility of extruded soy protein has been found to be higher than for nonextruded protein (*17*). In our study, we did not directly determine digestibility; however, the similar rate of growth suggests digestibility was not reduced by the extrusion process. Using a corn−soy flour mixture, Baskaran and Bhattacharaya (*18*) reported an increase in food intake and body weight gain in rats fed diets containing an extruded compared to a nonextruded mixture. No difference in protein efficiency ratio was found for the diets; therefore, the enhanced growth was likely a consequence of increased food intake due to greater palatability of the diet.

Bone breaking strength in females fed the ESP diet was lower compared to females fed the CSP diet. None of the other bone parameters were different, including bone length, width, and mineral content; hence, this difference in breaking strength may not be of physiological significance. Comparison of the data showed that breaking strength of males on both diets was similar to that of females fed the ESP; hence, the results may be due to a slightly higher breaking strength in females fed the CSP diet. Given that the breaking strength of females fed the ESP was similar to that of the males, this suggests the ESP diet did not negatively affect bone strength in the females. However, it may be of value to determine effects of the ESP diet on bone strength over a longer time. In other studies, soy protein consumption has been reported to improve bone strength and mineralization, when compared to other dietary protein sources. Female rats fed a diet

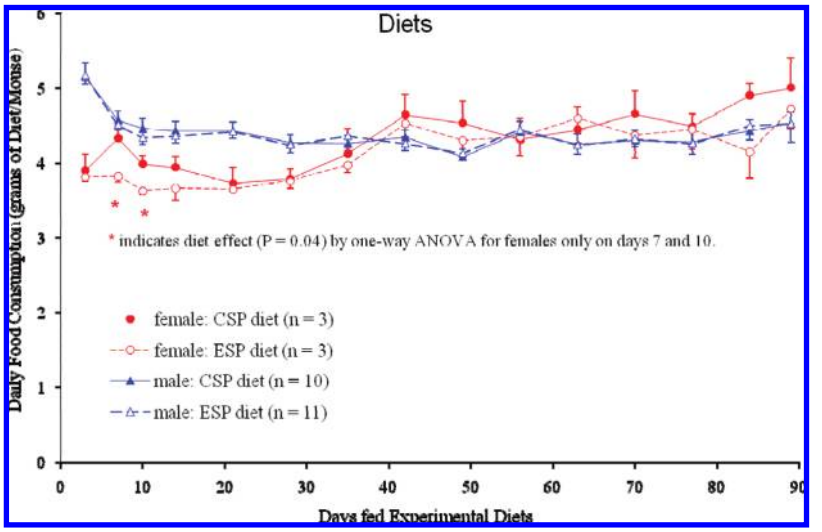

Figure 2. Average daily food consumption of male and female mice fed the ESP or CSP diet. Males were housed individually; therefore, food intake data were collected from 10 or 11 cages. Females were housed in groups of three or four; therefore, food intake data were collected from three cages. Data are expressed as mean with standard error shown in error bars. By two-way ANOVA it was determined that food consumption was not different due to diet treatment when all of the mice were compared. By one-way ANOVA, there was an effect of diet in females on days 7 and 10.

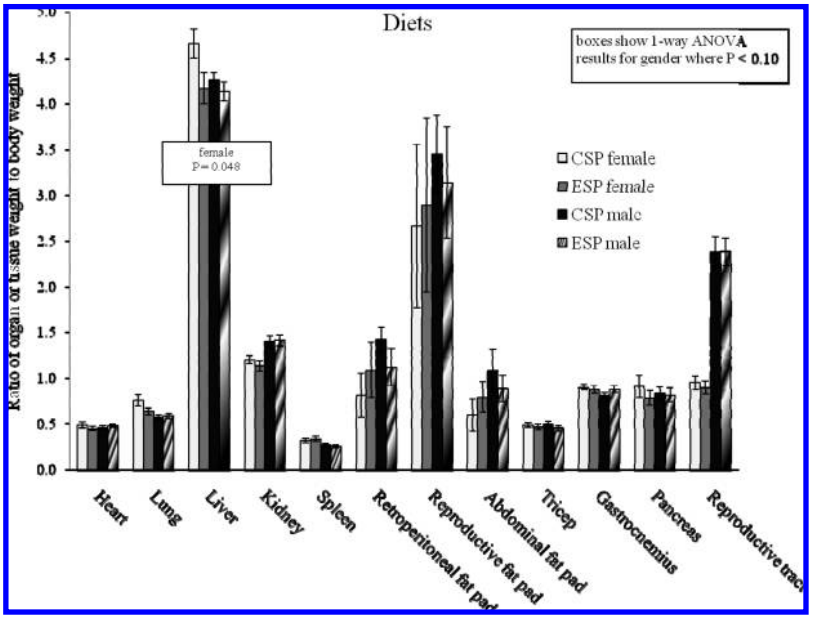

Figure 3. Average weights of selected organs from male and female mice fed the ESP or CSP diet. Data are expressed as mean with standard error shown in error bars. Box inserts show one-way ANOVA differences for gender; $P < 0.10$.

Table 2. Bone Parameters from Mice Fed Experimental Diets[a]

	femur length (cm)	femur width (cm)	breaking strength (units)	mineral concn, ash/bone ratio
Males				
CSP	1.67 ± 0.03	2.5 ± 0	23.9 ± 3.7	0.62 ± 0.01
ESP	1.63 ± 0.03	1.8 ± 0.5	27.5 ± 2.9	0.64 ± 0.01
Females				
CSP	1.73 ± 0.03	2.5 ± 0	36.1 ± 0.1 a	0.65 + 0.01
ESP	1.70 ± 0.00	2.5 ± 0	29.7 ± 1.2 b	0.68 ± 0.02

[a] Values are mean ± standard error, $n = 3$ per group. By gender, values within a column with different letters are significantly different as determined by one-way ANOVA, $P < 0.005$.

containing soy protein isolate had higher bone mineral density (BMD) than mice fed a casein-based diet (*19*). Because estrogens play a role in bone maintenance, isoflavones in soy may affect bone response to soy diets. Chen et al. (*19*) observed the response to SPI and estrogens to be bone specific in that ovariectomized rats fed SPI and treated with estrogen had higher tibial bone BMD, but trabecular BMD was lower, compared to casein-fed mice with estrogen treatment. In our study, the isoflavone concentrations of the two diets were similar upon chemical analysis. Daidzin, genistin, daidzein,

Table 3. Serum Hormone Concentrations in Mice Fed Experimental Diets[a]

	cortisol (ng/mL)	growth hormone (ng/mL)	IGF-1 (ng/mL)	insulin (ng/mL)	glucose/insulin ratio	hematocrit
Males						
CSP	22.5 ± 3.1 a	5.0 ± 2.0	454.8 ± 47.1	0.28 ± 0.06	7.7 ± 1.0	43.6 ± 0.5 a
ESP	43.3 ± 6.0 b	8.8 ± 3.1	489.3 ± 34.4	0.36 ± 0.07	6.4 ± 0.7	41.9 ± 0.6 b
Females						
CSP	68.6 ± 9.9	32.5 ± 8.1	421.1 ± 41.1	0.29 ± 0.04	4.2 ± 0.9 a	41.4 ± 1.4
ESP	79.3 ± 17.9	27.0 ± 7.7	441.7 ± 58.2	0.28 ± 0.04	6.2 ± 0.5 b	43.7 ± 0.8

[a] Values are mean ± standard error, $n = 10$ per group. By gender, values within a column with different letters are significantly different as determined by ANOVA $P < 0.01$.

and genistein levels in CSP were 181 ± 10.2, 466 ± 21.3, 49 ± 0.6, and 66 ± 2.0 μg/g and in ESP were 184 ± 12.1, 471 ± 15.5, 50 ± 0.8, and 68 ± 1.6 μg/g, respectively. However, it is possible that the bioavailability of the isoflavones may have differed in the two diets.

Several of the circulating markers measured showed dietary differences in response to diet in both males and females. In males, the ESP diet induced higher cortisol and lower hematocrit levels. In females, the ESP diet induced higher glucose/insulin ratios. Cortisol is released in an episodic manner and, when present in high concentrations, can inhibit growth. Hematocrit is a measure of hemoglobin production in red blood cells, and hemoglobin is essential for oxygen delivery. Insulin is released from the pancreas in response to a rise in blood glucose and facilitates the utilization of glucose by muscle and adipose tissue. Soy protein has been found to not alter carbohydrate metabolism compared to casein (*20*). Male, cynomolgus monkeys fed diets containing soy isolate with or without isoflavones or casein as the protein source for 25 months had similar circulating glucose and insulin concentrations. However, a beneficial effect of soy with isoflavones on insulin resistance was reported in old, obese monkeys compared to casein or the low-isoflavone soy protein diet. Whereas we have observed statistical differences in some of these parameters due to diet, the values are all within the normal physiological range for these parameters and therefore do not suggest an abnormal effect due to diet. However, given these trends it might be of value to undertake a longer feeding study to determine if the ESP diet has an impact on these markers.

In our study, IGF-I was not different in either males or females in response to the dietary protein source. IGF-I mediates the effect of growth hormone within muscle and bone to facilitate growth. Others have found that soy protein, when substituted equally for other dietary proteins over a 2 year trial, had no effect on serum IGF-I in premenopausal women (*21*). Because the total dietary protein intake was the same across the treatment groups and because of the demonstrated adequacy of the ESP to support growth, a lack of effect on IGF-I in our study would be expected.

When expressed as a percent of body weight, the liver weighed less in female mice fed the ESP diet compared to the CSP diet. A physiological basis for this observation is not readily apparent as there is no overall trend for lower organ weights in these mice. Although there were no statistical differences, the three fat pads tended to weigh less in males and more in females when fed the ESP compared to the CSP diet. This suggests that the dietary protein source may have more positive effects on body composition in males and more negative effects in females. Because we had no difference in isoflavone concentration in the two diets, the effect on body fat was not likely due to these estrogenic compounds. However, it is possible that the extrusion process altered the bioavailability of the isoflavones or another compounds in the soy which affected body fat. A longer term feeding study would be necessary to determine if these changes were important. We did not measure lean body mass directly, but did evaluate selected muscles. No differences in heart, triceps, or gastrocnemius muscle weights were observed in either gender in response to the diets. Hence, muscle mass does not seem to be affected by the dietary protein source, as would be expected from the absence of an effect on growth or body weight.

Overall, this study demonstrates that ESP is of similar nutritional value as CSP. There is no evidence of reduced growth or intolerance of the diet in male or female mice. Minor differences in some parameters were observed; however, these tended to be small and within the expected normal range. It may be concluded that ESP is a high-quality protein that provides adequate nutrition in this animal model.

ABBREVIATIONS USED

CSP, commercial soy protein; ESP, extruded soy protein; BMD, bone mineral density.

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