




Soy Protein is an Efficacious Alternative to Whey Protein in Sorghum–Soy Fortified Blended Foods in Rats

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ABSTRACT

Background: Previously we found that extruded corn–soy blend (CSB) and sorghum–soy blend (SSB) fortified blended foods (FBFs) containing whey protein concentrate (WPC) were equally nutritious food aid products. WPC provides high-quality protein; however, it is the most expensive ingredient in these FBFs.

Objectives: The primary objective of this study was to determine if soy protein can serve as an alternative to WPC and the secondary objective was to evaluate different sucrose amounts in the FBFs.

Methods: Nine extruded FBFs were formulated: 1 CSB and 1 SSB, both containing 9.5% WPC and 15% sucrose, served as comparison FBFs. Three additional CSB and 4 SSB FBFs were formulated containing no WPC, but with increased soy flour to meet protein requirements and varying sucrose concentrations. The sucrose content ranged from 0% to 10% for the CSBs and 0% to 15% for the SSBs. Male weanling Sprague Dawley rats were individually housed and divided into 10 diet groups ($n = 9–10$) which consumed either AIN-93G or a dry FBF for 28 d. At study conclusion, blood, livers, and body composition data were collected. Results were analyzed using 1-factor ANOVA with Tukey's test.

Results: Outcomes were not significantly different between the SSB groups, with the exception of significantly higher protein efficiency for the WPC-containing group. Among the CSB groups, caloric and protein efficiencies were significantly higher for the WPC-containing CSB group. There were no significant differences in hemoglobin or hepatic iron concentrations between FBF groups, but hepatic iron concentrations were significantly higher in all FBF groups than in the AIN-93G group. Groups consuming diets with $\leq 10\%$ sucrose had significantly higher bone mineral density than groups consuming diets with 15% sucrose.

Conclusions: These results suggest that extruded SSB, but not necessarily CSB, FBFs with soy protein and 5%–10% added sucrose are efficacious and cost-effective alternatives to WPC-containing FBFs. *Curr Dev Nutr* 2020;4:nzaa115.

Keywords: fortified blended foods, soy, sorghum, corn, sucrose, iron, rats, growth, protein quality, bone mineral density

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Abbreviations used: BMD, bone mineral density; CSB, corn–soy blend; CSB-15WPC, corn–soy blend with whey protein concentrate and 15% sucrose; CSB-0, corn–soy blend with 0% sucrose; CSB-5, corn–soy blend with 5% sucrose; CSB-10, corn–soy blend with 10% sucrose; FAQR, Food Aid Quality Report; FBF, fortified blended food; HFCS, high-fructose corn syrup; PER, protein efficiency ratio; SPI, soy protein isolate; SSB, sorghum–soy blend; SSB-15WPC, sorghum–soy blend with whey protein concentrate and 15% sucrose; SSB-0, sorghum–soy blend with 0% sucrose; SSB-5, sorghum–soy blend with 5% sucrose; SSB-10, sorghum–soy blend with 10% sucrose; SSB-15, sorghum–soy blend with 15% sucrose; WPC, whey protein concentrate.

Introduction

Fortified blended foods (FBFs) are food aid used for treatment and prevention of undernutrition and micronutrient deficiencies, including that of iron, the most prevalent micronutrient deficiency (1–3). Consumed primarily as complementary foods, FBFs are distributed as partially cooked, energy-dense, grain-legume dry blends fortified with micronutrients and may also contain sugar, oil, and additional protein. Recipients typically cook FBF powders with water to form a porridge for consumption. Corn–soy blend (CSB) FBFs, including CSB+ made

with corn, soybeans, and micronutrients, were the only FBFs provided as food aid by the United States Agency for International Development and partnering organizations in 2017 (4–6).

The 2011 Food Aid Quality Report (FAQR) recommended reformulation of FBFs to provide increased protein, fat, and total calories (7). Sorghum was recommended as an alternative, nongenetically modified cereal crop for use in FBFs. In addition, sorghum can be cultivated in hot, dry regions which experience low rainfall and is a familiar staple crop of many food aid–recipient countries in sub-Saharan Africa (7–9). Extruded FBFs made with sorghum, corn, soy, and/or

cowpea, formulated based on FAQR recommendations, improved iron bioavailability and protein digestibility compared with CSB+, a nonextruded FBF made with corn, soy, and micronutrients, in animal models (10, 11). Extrusion was selected for processing these FBFs in part because it utilizes heat, pressure, and mechanical stress to process and cook foods, reducing preparation time (12–14). These novel, extruded FBFs included 9.5% whey or soy protein, 9% vegetable oil, 3.2% micronutrients, and 15% sucrose (10). In Tanzanian children, significant improvements were observed from baseline in hemoglobin concentrations, anemia, and vitamin A deficiency in the novel, extruded FBF groups; however, these findings were not significantly different for the CSB+ group (15).

Whey protein concentrate (WPC) was included in the novel FBFs as a source of high-quality, animal-source protein based on the FAQR recommendation (7). However, 3% addition of WPC, as recommended in the FAQR, increases the total cost of FBFs by ~18% (16). Available research has led some to question whether the increased cost is justified to obtain desired growth improvements in children (16–18). Soy protein, which is approximately half the cost of whey protein, may serve as an alternative, plant-based, high-quality protein in FBFs (18).

In support of this possibility, we found that soy protein isolate (SPI) was a viable, cheaper alternative to WPC in sorghum–cowpea FBFs in broiler chicks (11). In addition to testing the efficacy of SPI, alterations in extrusion processing parameters allowed sorghum-based FBFs to meet viscosity requirements without the addition of sugar. This additional reformulated, “overprocessed” sorghum–cowpea blend resulted in similar outcomes to the blends with 15% added sugar (11).

In the present study with male, weanling Sprague Dawley rats, 2 previously developed WPC-containing FBFs were prepared with sorghum–soy or corn–soy and 15% sucrose. Based on an interest to test whether cheaper formulations may be equally efficacious and previous results from blends with SPI replacing WPC, 7 new sorghum–soy or corn–soy FBFs were formulated. These new blends aligned with FAQR nutrient recommendations and contained increased soy flour compared with the WPC-containing blends and varying amounts of sucrose (7).

The primary objective of this study was to determine if soy protein provided by soy flour is an efficacious, similarly perceived, and cheaper alternative to WPC in extruded corn–soy and sorghum–soy FBFs at several different sucrose concentrations as determined by evaluation of iron and protein outcomes. Secondary objectives were to evaluate feeding behaviors and outcomes as a result of different sucrose amounts, from 0% to 15%, in the FBFs and further compare sorghum–soy and corn–soy blends. A post hoc objective was to compare bone mineral density (BMD) of groups consuming FBFs with ≤10% sucrose with those consuming FBFs with 15% sucrose. We hypothesized that there would not be a difference between FBFs, and different sucrose concentrations.

Methods

Ethical standards

Animal procedures were approved by the Institutional Animal Care and Use Committee at Kansas State University (protocol 4016). Animals were assessed for well-being before and throughout the study for the duration of the experiment.

FBFs

Corn–soy blend and sorghum–soy blend FBFs were developed based on recommendations in the FAQR (7) and previous studies (10, 11). Corn–soy and sorghum–soy flour blends were extruded, milled, and mixed with the additional ingredients as detailed previously (10–12). Nine FBFs were formulated with different sucrose amounts and either WPC or adjusted amounts of soy flour to provide a similar amount of protein (Table 1). Four CSB FBFs were developed: 1 contained WPC and 15% sucrose (CSB-15WPC); 3 contained no WPC and varying amounts of sucrose: 0% (CSB-0), 5% (CSB-5), and 10% (CSB-10). A fourth non-WPC CSB FBF with 15% sucrose was planned; however, because of flow issues inside the extrusion barrel which locked the screw, it failed to extrude, and therefore was unable to be included in this study. Five SSB FBFs were developed: 1 contained WPC and 15% sucrose (SSB-15WPC); 4 contained no WPC and varying amounts of sucrose: 0% (SSB-0), 5% (SSB-5), 10% (SSB-10), and 15% (SSB-15). FBFs were evaluated for compliance with viscosity requirements for a previous USDA CSB FBF following procedures previously described (11, 12, 19). Vitamin and mineral contents of the FBFs have been described previously (10, 20).

Nutritional analyses

FBF iron concentrations were measured in duplicate by AIB International and macronutrient proximate analyses, amino acid profiles, and available lysine were assessed by the University of Missouri–Columbia Agricultural Experiment Station Chemical Laboratories as described previously (10, 11, 21).

Study design

The study duration was based on the preventative prophylactic (22) and protein efficiency ratio (PER) (23) methods that we had utilized previously (10). Male, weanling Sprague Dawley rats (Charles River) were randomly assigned into 10 diet groups ($n = 10$, 100 total), a group size following the United Nations University recommendation for the PER model. A control group was fed AIN-93G diet (Research Diets, Inc.) and the additional 9 groups were assigned to consume 1 of the dry FBFs. Because the diets varied in caloric content, caloric efficiency was also calculated. One rat (CSB-5 group) died and another (SSB-15 group) was euthanized; both deaths were attributed to pre-existing health conditions. A rat in the CSB-10 group acutely lost weight at study midpoint, never recovered, and as a result was excluded from analyses. Two additional animals, 1 from the SSB-0 group and 1 from the SSB-10 group, were excluded out of concern that FBFs may have been consumed beyond those assigned. Animals were individually housed in wire-bottom cages and were provided with a resting board, enrichment products, and ad libitum access to food and water for the 28-d study. The environment was temperature-controlled with 12-h alternating light-and-dark cycles. Feedings occurred every other day where remaining food was weighed and fresh food was provided. The rats were weighed upon arrival and weekly thereafter.

Data and sample collection

At study conclusion, animals were euthanized as described previously (10). Blood was drawn via cardiac puncture and collected in K2 EDTA-coated vacutette tubes for hemoglobin analysis. Tubes were held on ice and later transferred to a 4°C refrigerator where they were stored for

TABLE 1 Fortified blended food formulations¹

	CSB-15WPC	CSB-0	CSB-5	CSB-10	SSB-15WPC	SSB-0	SSB-5	SSB-10	SSB-15
Low-fat soy flour	15.2	32.0	33.0	34.0	16.0	32.0	33.0	34.0	35.0
Degermed coarse corn flour	48.1	55.8	49.8	43.8	—	—	—	—	—
Decorticated white sorghum flour	—	—	—	—	47.8	56.3	50.3	44.3	38.3
Whey protein concentrate	9.5	—	—	—	9.5	—	—	—	—
Sucrose	15.0	—	5.0	10.0	15.0	—	5.0	10.0	15.0
Vegetable oil	9.0	9.0	9.0	9.0	8.5	8.5	8.5	8.5	8.5
Vitamin and mineral premix	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2

¹Values are percentages. CSB, corn–soy blend; CSB-0, corn–soy blend with 0% sucrose; CSB-5, corn–soy blend with 5% sucrose; CSB-10, corn–soy blend with 10% sucrose; CSB-15WPC, corn–soy blend with whey protein concentrate and 15% sucrose; SSB, sorghum–soy blend; SSB-0, sorghum–soy blend with 0% sucrose; SSB-5, sorghum–soy blend with 5% sucrose; SSB-10, sorghum–soy blend with 10% sucrose; SSB-15, sorghum–soy blend with 15% sucrose; SSB-15WPC, sorghum–soy blend with whey protein concentrate and 15% sucrose; WPC, whey protein concentrate.

36 h before hemoglobin analysis. After blood collection, liver tissues were collected, weighed, and flash-frozen in liquid nitrogen. After flash-freezing, liver tissues were stored in a -80°C freezer until wet ashing. After tissue removal, body scans were performed on a DXA PIXImus to determine body composition and BMD as described previously (10).

Iron analysis

Hemoglobin.

A QuantiChrom Whole Blood Hb Kit (DWHB-250, BioAssay Systems) was used to determine hemoglobin concentrations. Samples were analyzed in duplicate according to the manufacturer's procedure. Duplicates for 11 samples had $>15\%$ variance and were assessed an additional time (triplicate).

Hepatic iron.

Samples were prepared as previously described (10) and analyzed at the Kansas State University Soil Testing Lab by inductively coupled plasma optical emission spectrometry (Varian 720-ES, Agilent Technologies). Duplicates for 14 samples had $>15\%$ variance and were assessed an additional time (triplicate).

Descriptive sensory analysis

FBFs were evaluated via descriptive sensory analysis by 6 highly trained panelists at the Center for Sensory Analysis and Consumer Behavior, Manhattan, KS. Similar panels have been used and described for analysis of novel FBFs in previous studies (24, 25). A total of 27 sensory attributes were analyzed including 1 for appearance, 6 for aroma, 13 for flavor, and 7 for texture/mouthfeel (see **Supplemental Table 1** for attributes, descriptions, and references). Samples were prepared with 1 part FBF and 2 parts water which were boiled and stirred continuously for 15 min. Porridges were cooled to 60°C and dished into Styrofoam containers for flavor and texture/mouthfeel analysis and medium sniffer glasses for evaluation of appearance and aroma. Samples were served on heated trays to preserve the temperature between 55 and 60°C . Panelists were provided with water, mozzarella cheese, and cucumber palate cleansers. All FBFs were evaluated in triplicate and samples were evaluated in randomly assigned groups of 4/d.

Cost analysis

Ingredient and production costs were estimated for cost of ingredient sourcing and production in the United States based on updated data and methods described previously (18).

Statistical analysis

Animal study data.

Results were assessed for normality using the Shapiro–Wilk test and for homogeneity of variance with Levene's test. Ln or square root transformations were used if assumptions for normality were not met. Group differences were assessed using 1-factor ANOVA with Tukey's test and significance at $P < 0.05$. Differences in BMD between groups based on the percentage of sucrose in the formulation ($\leq 10\%$ and 15% sucrose) were assessed with a t test and significance at $P < 0.05$. Data are reported as group means with SDs. Statistical analyses were performed in SAS Studio version 3.71 (SAS Institute Inc.).

Descriptive sensory analysis data.

A mixed-model ANOVA with treatments and replicates (fixed effects) as the main plots and panelists (random effects) as the subplots was used to determine significant differences for each attribute between the treatments. Tukey's test, significance at $P < 0.05$, was used to identify differences between treatments.

Results

Diet characteristics

On average, the FBFs provided 5.1% more energy, 3.7% more protein, and 26.2% more fat than AIN-93G (**Table 2**). The total energy and macronutrients were similar across all FBFs. The FBFs with increased soy protein contained on average 2.1% more protein, 53.2% more fiber, 16.9% less available lysine, and 21.8% less cysteine + methionine than the FBFs with WPC. Iron content of the FBF diets was on average 75.1% greater than that of the AIN-93G diet. The FBFs without WPC contained on average 8.1% more iron than the FBFs with WPC. The SSB FBF diets contained on average 7.4% more iron than the CSB diets.

All SSB FBFs had greater prepared porridge Bostwick viscosity flow rates (thinner) than the CSB FBFs (**Supplemental Table 2**) (12). CSB-15WPC, CSB-10, SSB-0, SSB-5, and SSB-10 were the only FBFs in this study that met the USDA preferred consistency quality specifications (9.0–21.0 cm/min Bostwick value) (19). The CSB-0 and CSB-5 were thicker whereas SSB-15WPC and SSB-15 were thinner than preferred.

Food intake and efficiencies

No significant differences were observed between FBF groups for food intake, with the exception of the SSB-0 group. The SSB-0 group

TABLE 2 Fortified blended food caloric, macronutrient, selected amino acid, and iron content¹

	CSB-15WPC	CSB-0	CSB-5	CSB-10	SSB-15WPC	SSB-0	SSB-5	SSB-10	SSB-15
Total energy, kcal/100 g	417.1	414.0	414.8	415.5	410.6	398.3	407.1	408.3	408.7
Carbohydrate									
g/100 g	62.5	60.8	61.0	60.8	62.7	59.3	60.1	60.4	60.0
% energy	60.0	58.8	58.8	58.6	61.1	59.6	59.0	59.1	58.8
Protein									
g/100 g	20.4	21.0	21.0	20.6	19.7	20.6	21.2	21.0	21.2
% energy	19.5	20.3	20.2	19.8	19.2	20.7	20.8	20.6	20.8
Fat									
g/100 g	9.5	9.6	9.7	10.0	9.0	8.7	9.1	9.2	9.3
% energy	20.5	20.9	21.0	21.6	19.7	19.7	20.1	20.3	20.5
Crude fiber, g/100 g	0.4	0.9	0.9	0.7	0.4	0.6	0.8	0.7	0.7
Ash, g/100 g	3.6	4.2	4.2	4.3	3.6	4.3	4.3	4.3	4.4
Moisture, g/100 g	4.0	4.3	4.2	4.3	5.0	7.1	5.3	5.1	5.0
Lysine, mg/g	13.8	11.0	11.1	11.3	13.3	10.9	11.6	12.0	12.2
Available lysine, mg/g	13.2	9.9	10.0	10.4	12.9	10.5	11.0	11.4	11.8
Cysteine and methionine, mg/g	7.6	5.9	5.8	6.0	7.4	6.0	6.2	6.2	6.1
Iron, mg/100 g	13.5	13.4	13.9	15.0	13.8	15.2	15.4	15.2	15.5

¹AIN-93G provides 390 kcal/100 g energy, 64.0 g/100 g carbohydrate, 20.0 g/100 g protein, 7.0 g/100 g fat, and 6.6 mg Fe/100 g. CSB, corn–soy blend; CSB-0, corn–soy blend with 0% sucrose; CSB-5, corn–soy blend with 5% sucrose; CSB-10, corn–soy blend with 10% sucrose; CSB-15WPC, corn–soy blend with whey protein concentrate and 15% sucrose; SSB, sorghum–soy blend; SSB-0, sorghum–soy blend with 0% sucrose; SSB-5, sorghum–soy blend with 5% sucrose; SSB-10, sorghum–soy blend with 10% sucrose; SSB-15, sorghum–soy blend with 15% sucrose; SSB-15WPC, sorghum–soy blend with whey protein concentrate and 15% sucrose; WPC, whey protein concentrate.

had significantly higher food intake than the CSB-15WPC group (Table 3, Figure 1). Both WPC-containing FBF groups had significantly increased caloric efficiency compared with all non-WPC-containing CSB FBF groups and significantly increased protein efficiency compared with all other diet groups (AIN-93G, non-WPC SSBs, and non-WPC CSBs). The CSB-5 group's caloric efficiency was significantly decreased compared with the AIN-93G, SSB-0, and SSB-5 groups, and protein efficiency was significantly decreased compared with the AIN-93G group.

Body weight, lean mass, and BMD

No significant differences were observed between FBF groups for total weight gain and final body weight, with the exception of the SSB-0 group. The SSB-0 group had significantly higher total weight gain and final weight than the CSB-5 group (Figure 2). No significant differences were observed between groups' lean mass content (Table 4). Whereas there were no significant differences between FBF groups for BMD, the 3 FBF groups which contained 15% sucrose had significantly lower BMD than the AIN-93G group (CSB-15WPC, $P = 0.02$; SSB-15WPC, $P = 0.02$; and SSB-15, $P = 0.01$).

Given that we found significantly reduced BMD in all 15% sucrose groups compared with the AIN-93G group, we further investigated a potential threshold effect among the FBF groups. FBF groups were organized into 2 new groups based on sucrose content: 1 with all $\leq 10\%$ sucrose groups and another with all 15% sucrose groups. The $\leq 10\%$ sucrose group mean BMD (83.2 ± 10.4) was significantly greater than the 15% sucrose group mean BMD (77.5 ± 10.1 , $P = 0.01$).

Iron outcomes

No significant differences were observed in hemoglobin concentrations (Table 4). Hepatic iron concentrations were significantly higher in all of the FBFs groups than in the AIN-93G group. No other differences were observed except the CSB-5 group had a significantly higher hepatic iron concentration than the SSB-15 group.

Descriptive sensory analysis

Of the 27 sensory attributes measured, 20 were found to be statistically different for ≥ 1 of the FBFs (Supplemental Table 3). No differences were detected for musty/dusty aroma; grain complex, soy, toasted, brown, and sour flavors; or oily mouthfeel. For the other attributes, the range of scores tended to be rather low (< 2.0 points difference on the 15-point scale). Only lightness/darkness of color and adhesiveness varied by ≥ 5.0 points. More importantly, there were few differences between the comparison FBFs with WPC and the corresponding FBFs without WPC except for color, which was darker in FBFs without WPC. Where other differences existed, they typically were small (< 1.0 on a 15-point scale), especially for the SSB FBFs. With CSB FBFs, some larger differences were noted in textural attributes such as stickiness, particle size uniformity, adhesiveness, and gumminess where the FBFs without WPC had less uniform particle size, and were thicker, more adhesive, and gummier.

Cost

Ingredient costs for all FBFs without WPC were much lower than for the FBFs with WPC (Supplemental Tables 4–6). The 0% sucrose FBFs were the least expensive and increased sucrose concentrations increased costs. Ingredient costs were similar for CSB and SSB FBFs with respect to sucrose content (e.g., CSB-5 and SSB-5 cost approximately the same per kilogram of FBF).

Discussion

CSB and SSB group comparisons

We hypothesized that protein provided from increased soy flour may serve as a suitable alternative to WPC in novel, extruded FBFs. In the present study, all SSB FBFs resulted in similar outcomes in weanling male rats. The only significant difference between SSB groups was a

TABLE 3 Food intake and food efficiencies¹

	AIN-93G	CSB-15WPC	CSB-0	CSB-5*	CSB-10*	SSB-15WPC	SSB-0*	SSB-5	SSB-10*	SSB-15*
Total food intake, g	502.4 ± 53.4 ^a	424.5 ± 38.8 ^b	439.2 ± 35.9 ^{ab}	436.0 ± 69.2 ^{ab}	472.8 ± 33.5 ^{ab}	447.0 ± 42.1 ^{ab}	501.6 ± 62.2 ^a	475.0 ± 59.6 ^{ab}	442.7 ± 34.4 ^{ab}	465.1 ± 46.4 ^{ab}
Final body weight, g	297.1 ± 29.4 ^{ab}	284.1 ± 25.3 ^{ab}	261.5 ± 18.1 ^{ab}	255.4 ± 38.5 ^b	286.7 ± 23.8 ^{ab}	291.0 ± 30.7 ^{ab}	304.2 ± 33.8 ^a	294.3 ± 39.1 ^{ab}	272.7 ± 22.7 ^{ab}	283.8 ± 30.6 ^{ab}
Caloric efficiency, g/100 kcal	12.7 ± 0.4 ^{ab}	13.3 ± 0.5 ^a	11.8 ± 0.6 ^{bc}	11.5 ± 0.7 ^c	12.1 ± 0.6 ^{bc}	13.2 ± 0.7 ^a	12.7 ± 0.3 ^{ab}	12.7 ± 1.5 ^{ab}	12.4 ± 0.3 ^{abc}	12.4 ± 0.4 ^{abc}
Protein efficiency, g/g	2.5 ± 0.1 ^{b†}	2.7 ± 0.1 ^a	2.3 ± 0.1 ^{bc}	2.3 ± 0.2 ^c	2.5 ± 0.1 ^{bc}	2.8 ± 0.1 ^a	2.5 ± 0.1 ^{bc}	2.5 ± 0.3 ^{bc}	2.4 ± 0.1 ^{bc}	2.4 ± 0.1 ^{bc}

¹Values are means ± SDs. *n* = 10, **n* = 9. Values with different letters are significantly different (*P* < 0.05) determined via 1-factor ANOVA with Tukey's test. Food intake: measured every other day by subtracting food remaining from food given. Caloric efficiency: total weight gained (g) divided by total energy (kcal) consumed. Protein efficiency: total weight gained (g) divided by total protein consumed (g). CSB-, corn-soy blend; CSB-0, corn-soy blend with 0% sucrose; CSB-5, corn-soy blend with 5% sucrose; CSB-10, corn-soy blend with 10% sucrose; CSB-15WPC, corn-soy blend with whey protein concentrate and 15% sucrose; SSB, sorghum-soy blend; SSB-0, sorghum-soy blend with 0% sucrose; SSB-5, sorghum-soy blend with 5% sucrose; SSB-10, sorghum-soy blend with 10% sucrose; SSB-15, sorghum-soy blend with 15% sucrose; SSB-15WPC, sorghum-soy blend with whey protein concentrate and 15% sucrose; WPC, whey protein concentrate.

[†]Based on label protein value rather than analyzed protein content.

13% increased protein efficiency for the SSB-15WPC group compared with the non-WPC SSB FBFs. Between the CSB groups, there were similarly few significant differences in outcomes. Although intake did not differ significantly, the CSB-15WPC group had somewhat lower intake and corresponding higher caloric and protein efficiencies than the CSB groups with increased soy flour.

Protein outcomes

The increased protein efficiency for both SSB-15WPC and CSB-15WPC groups compared with the respective non-WPC groups may in part be explained by similar growth and food intake among all groups combined with overall less protein consumed by the WPC groups. The SSB-15WPC FBF contained ~6.4% less protein, 1.3 g protein less per 100 g FBF, than the SSB FBFs with increased soy flour. Because PER is calculated based on total protein consumed, slightly more or less protein can magnify changes in protein efficiency. PER is also not a proportional measure of protein intake and corresponding growth because it does not account for protein used for maintenance (13, 26). The soy-based FBFs also contained limiting amounts of essential amino acids (lysine, cysteine + methionine), which were present at greater quantities in the WPC FBFs.

Another consideration is that soy protein is generally less digestible than protein from animal sources (27). Significantly lower body weight gain and protein energy efficiency for a soy-based group than for a whey-based group were observed in 5-wk-old male Sprague Dawley rats (28). In another study with Wistar rats, the soy group had significantly lower protein efficiency, body weight, total weight gain, and fat and lean mass gain than the whey group (29).

Although PER in rat models is an important outcome for evaluating the quality of FBFs, there are a few key differences between human and rat protein requirements that may further support the efficacy of soy flour-based SSB FBFs for humans. Protein used by weaning rats is predominantly for growth; in humans, even during phases of rapid growth, a higher proportion of protein is required for body maintenance (26, 30). Rats in addition require 50% more of the sulfur-containing amino acids, cysteine and methionine, to support fur growth (30). These were the most limiting amino acids in our soy flour-based FBFs. Differences in amino acid requirements result in lower protein digestibility-corrected amino acid scores for rats than for humans (SPI: 64 in rats, 100 in humans; skim milk powder: 74 in rats, 100 in humans) (23) and an overestimation of the quality of animal-source proteins compared with plant-source proteins for human growth (26).

In our 20-wk trial with Tanzanian children, we observed that our novel, extruded FBFs with WPC performed similarly to CSB+. However, this study was too short and underpowered to critically assess anthropometric outcomes (15). Despite limitations of also being underpowered and early termination, in unadjusted analyses, a 10-wk study did not find a significant difference in the proportion of Sierra Leone children who recovered from moderate-acute malnutrition with a CSB FBF (similar to CSB+), which contained no animal-source foods, compared with a CSB FBF with WPC (31, 32). Additional trials have demonstrated that animal-source protein in ready-to-use food aid products does not necessarily result in better anthropometric outcomes in malnourished children (33, 34). Furthermore, a recent systematic literature review was unable to identify a relation between animal-source foods and improved growth outcomes. This review was limited by a large

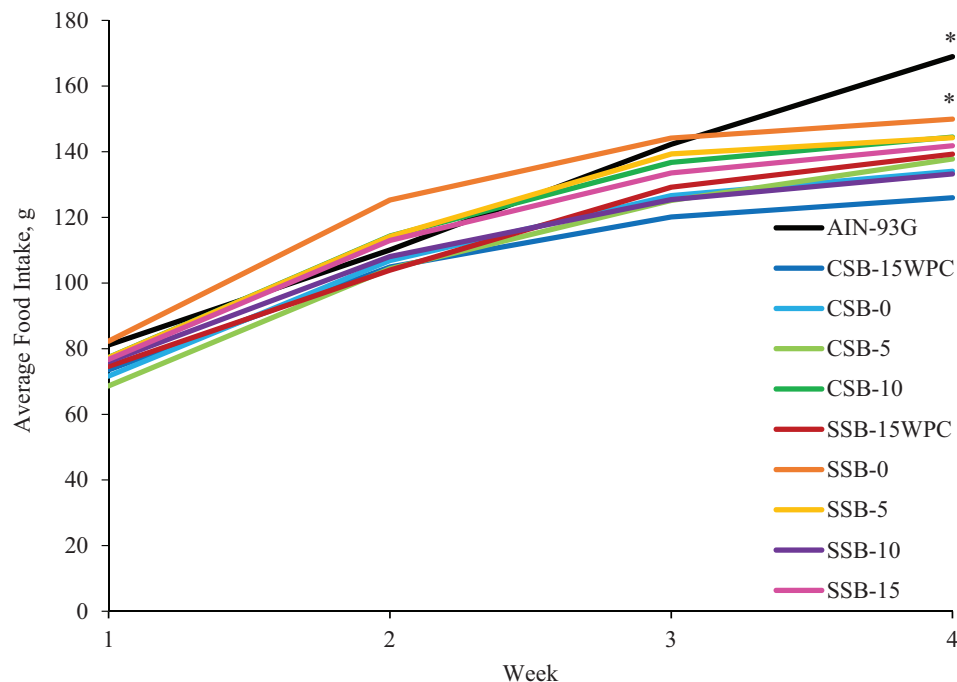


FIGURE 1 Mean weekly food intake ($n = 9-10$). *Total food intake for AIN-93G and SSB-0 significantly higher ($P < 0.05$) than for the CSB-15WPC group ($P < 0.05$) with no other significant differences between groups. CSB, corn-soy blend; CSB-0, corn-soy blend with 0% sucrose; CSB-5, corn-soy blend with 5% sucrose; CSB-10, corn-soy blend with 10% sucrose; CSB-15WPC, corn-soy blend with whey protein concentrate and 15% sucrose; SSB, sorghum-soy blend; SSB-0, sorghum-soy blend with 0% sucrose; SSB-5, sorghum-soy blend with 5% sucrose; SSB-10, sorghum-soy blend with 10% sucrose; SSB-15, sorghum-soy blend with 15% sucrose; SSB-15WPC, sorghum-soy blend with whey protein concentrate and 15% sucrose; WPC, whey protein concentrate.

heterogeneity between studies that included study design and the varieties of animal-source foods evaluated (35).

Iron outcomes

Our other main outcome of interest, in addition to protein performance, was iron status. Hepatic iron concentrations were significantly higher in all of the FBFs than in the AIN-93G group. This difference is most likely explained by the nearly doubled iron content of the FBFs compared with AIN-93G and differences in the iron fortificant used. Although AIN-93G is fortified with ferric citrate, which is less bioavailable than ferrous fumarate and NaFeEDTA, iron fortificants added to the FBFs, this is not likely the reason for the differences in iron outcomes (36). The hepatic iron concentration of the CSB-5 group was marginally higher than those of all the other FBF groups, and statistically higher than only that of the SSB-15 group. The CSB-5 group was on average smaller than all the other groups at the study conclusion. Lower growth and a likely lower blood volume of the rats in the CSB-5 group may have resulted in decreased demand for circulating iron, which allowed those animals to store more iron than the other groups (10).

We did not observe any differences in hepatic iron concentrations with increased sorghum content, unlike our previous FBF rat study (10). Soy has also been shown to inhibit iron absorption in humans (37), but no significant impacts on the iron outcomes were observed with the increased soy content of FBFs developed for this study.

Sucrose and sensory perceptions

In the previous extruded FBF rat study, the CSB+, which contains 0% sucrose, was poorly consumed. It was hypothesized that the 15% sucrose content, which was added in part to meet viscosity requirements, may have contributed to animal feeding behaviors (10). Addition of sugar, either from sucrose or fruit, has been observed to reduce or mask certain potentially unappealing flavors such as soy, grain, and bitter in cereals and porridges made from FBFs (24, 38). Rats have been observed to prefer the taste of sucrose (39), although there are differences in rat and human taste perceptions so it is unclear if rats perceive sucrose similarly to humans (40). As a result of changes in extrusion processing parameters, we were able to test formulations in the present study with varying amounts of sucrose. Although we cannot be certain how the rats perceived the FBFs, it does not appear that sucrose content affected feeding behaviors because both the CSB and SSB FBFs without sucrose were consumed equally compared with FBFs with sucrose at varying amounts. A 2018 study observed that children preferred SSB FBFs with 15% added sucrose over CSB+ and hypothesized it was due to their preference for sweeter foods and familiarity with sorghum (41). Perception of sweetness was minimal among SSB FBFs with the exception of SSB-0, which was perceived as significantly less sweet. SSB-0 was also perceived as significantly more bitter and sorghum-flavored than the other SSB FBFs.

The FBFs with added sucrose had thinner Bostwick viscosities than the respective blends without added sucrose, and each 5% increase in sucrose resulted in a slight thinning of the porridge

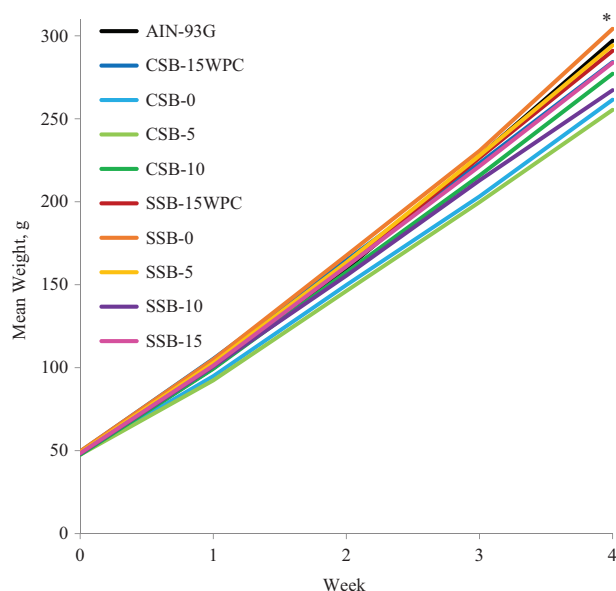


FIGURE 2 Mean weekly body weights ($n = 9-10$). *Mean final body weight and total weight gain significantly higher ($P < 0.05$) for SSB-0 than for CSB-5 with no other significant differences in total weight gain or final body weights between groups. CSB, corn-soy blend; CSB-0, corn-soy blend with 0% sucrose; CSB-5, corn-soy blend with 5% sucrose; CSB-10, corn-soy blend with 10% sucrose; CSB-15WPC, corn-soy blend with whey protein concentrate and 15% sucrose; SSB, sorghum-soy blend; SSB-0, sorghum-soy blend with 0% sucrose; SSB-5, sorghum-soy blend with 5% sucrose; SSB-10, sorghum-soy blend with 10% sucrose; SSB-15, sorghum-soy blend with 15% sucrose; SSB-15WPC, sorghum-soy blend with whey protein concentrate and 15% sucrose; WPC, whey protein concentrate.

compared with the previous blend. Notably, the SSB FBFs, including the blend with 0% sucrose, were all thinner than all the CSB FBFs, which may be a result of less starch accessibility and more protein interference from sorghum than from corn (12). With the exception of SSB-5, the trained panelists rated all texture attributes of the SSB FBFs similarly and, in general, found them to be thinner than the CSB-FBFs, similarly to the Bostwick results. The thinner prepared viscosities of the SSB FBFs are advantageous. Caregivers have been observed to thin porridges to their desired flow for infant feeding, which can result in insufficient caloric density of prepared FBFs (24, 42). The thinner nature of the SSB FBFs offers more nutrient density per volume of food consumed than CSB FBFs when thinned to similar viscosities, which is advantageous in ensuring adequate nutrient intake by recipient populations.

Sucrose and bone

In the present rat study, we observed similar BMDs for extruded FBFs compared with the formulations from our previous rat study, all of which contained 15% sucrose. The differences in BMD related to sucrose content were an interesting and unexpected finding. However, the BMD for the control group was higher in this study than in the previous rat study (95.4 compared with 87.4 g/cm²). The higher control group BMD in this study than in our previous study may

TABLE 4 Lean mass, BMD, and iron outcomes¹

	AIN-93G	CSB-15WPC	CSB-0	CSB-5*	CSB-10*	SSB-15WPC	SSB-0*	SSB-5	SSB-10*	SSB-15*
Lean mass, %	89.8 ± 1.4	89.7 ± 1.1	89.1 ± 1.4	88.5 ± 1.3	88.8 ± 1.6	89.6 ± 1.8	88.7 ± 1.6	89.5 ± 1.6	89.4 ± 1.2	89.4 ± 1.3
BMD, g/cm ² × 1000	95.4 ± 12.0 ^a	78.0 ± 9.6 ^b	86.1 ± 16.4 ^{ab}	81.9 ± 9.9 ^{ab}	84.7 ± 10.3 ^{ab}	77.8 ± 7.7 ^b	83.1 ± 6.1 ^{ab}	81.4 ± 7.9 ^{ab}	82.2 ± 10.3 ^{ab}	76.8 ± 13.8 ^b
Hemoglobin, g/dL	16.1 ± 1.3	15.8 ± 0.9	15.4 ± 1.1	15.3 ± 1.6	14.9 ± 0.6	14.8 ± 1.5	16.1 ± 2.5	15.0 ± 1.3	14.5 ± 1.1	14.7 ± 1.4
Hepatic iron, µg/g	9.5 ± 1.7 ^c	17.8 ± 2.5 ^{ab}	18.7 ± 3.1 ^{ab}	19.7 ± 4.4 ^a	17.2 ± 4.1 ^{ab}	15.9 ± 3.0 ^{ab}	15.9 ± 2.7 ^{ab}	16.4 ± 2.4 ^{ab}	15.5 ± 2.1 ^{ab}	15.0 ± 2.8 ^b

¹Values are means ± SDs. $n = 10$, * $n = 9$. Values with different letters are significantly different ($P < 0.05$) determined via 1-factor ANOVA with Tukey's test. Lean mass: total weight minus fat mass divided by total weight × 100. Liver weight per body weight: liver weight divided by body weight × 100. BMD, bone mineral density; CSB, corn-soy blend; CSB-0, corn-soy blend with 0% sucrose; CSB-5, corn-soy blend with 5% sucrose; CSB-10, corn-soy blend with 10% sucrose; CSB-15WPC, corn-soy blend with whey protein concentrate and 15% sucrose; SSB, sorghum-soy blend; SSB-0, sorghum-soy blend with 0% sucrose; SSB-5, sorghum-soy blend with 5% sucrose; SSB-10, sorghum-soy blend with 10% sucrose; SSB-15, sorghum-soy blend with 15% sucrose; SSB-15WPC, sorghum-soy blend with whey protein concentrate and 15% sucrose; WPC, whey protein concentrate.

explain why there were no significant differences comparing the extruded FBFs with 15% sucrose with the AIN-93G group previously (10).

Previously, we noted unexpected gait issues in broiler chickens consuming different prepared FBFs that were difficult to explain. In light of findings from the present study, 1 trend noted in our current findings is that the 4 FBF groups (including the same CSB-WPC and SSB-WPC FBFs as used in the present study) with significant gait issues all consumed 15% sucrose FBFs. However, a group consuming a sorghum-cowpea with SPI 15% sucrose FBF did not have these issues. BMD was not consistently significantly decreased in the groups affected (11). There has not been much research on the impact of sucrose and other caloric sweeteners on bone outcomes. AIN-93G is formulated with 10% sucrose (43) and it is possible that, in weanling rats, BMD is negatively affected by a sucrose content of >10% in the diet. This is consistent with findings of no significant difference in BMD in 2-mo-old Sprague Dawley rats fed diets with <10% sucrose (44). In weanling Wistar rats, a 43% sucrose diet resulted in tibia/femur densities and breaking strengths that were significantly decreased compared with the control diet with potato starch in place of sucrose (45). In weanling male rats fed either a 68% corn starch or a 68% sucrose diet, there were no differences in bone composition or mechanical properties. However, BMD, total intake, and weight gain for animals were not reported and it is unclear if the diets were fortified with micronutrients (46). Ad libitum access to AIN-93G and 1 solution—deionized distilled water or deionized distilled water with 13% sugar [from glucose, sucrose, fructose, or high-fructose corn syrup (HFCS)]—in 35-d-old female Sprague Dawley rats resulted in the sugar-solution groups' whole-femur BMDs not being significantly different compared with the control, but the glucose group had significantly reduced BMD compared with the sucrose, fructose, and HFCS groups (47).

Cost

Compared with CSB+, we believe our SSB FBFs without WPC offer superior protein quality, in part due to extrusion processing and because they offer more lysine and cysteine + methionine. Compared with Super Cereal *Plus*, which additionally includes skim milk powder, sugar, and oil compared with CSB+, we estimated total production costs were reduced for both SSB-5 and SSB-10. In addition, total estimated costs were decreased for the SSB-5 and SSB-10 FBFs compared with Super Cereal *Plus*, even if more were to be consumed by recipients to achieve similar anticipated outcomes. Considering all findings from this animal model and human food aid studies, SSB FBFs without WPC are an efficacious alternative to SSB-15WPC and likely Super Cereal *Plus* and may lead to similar anthropometric improvements in children.

Limitations

Several limitations for this study included the relatively short duration of 4 wk which took place during the rats' rapid growth period and the poor health of some of the animals, which resulted in the loss of 2 rats. In addition, the dry FBFs that the rats consumed were not typical of how humans consume the FBFs, as boiled porridges. We identified an interesting outcome of sucrose content related to BMD; however, owing to not anticipating this difference, we did not gather additional

data or samples that could have been used to better understand this outcome.

Conclusions

Evaluation of novel, extruded corn-soy and sorghum-soy FBFs was conducted including an animal study along with cost and sensory analyses. Similar outcomes were observed comparing the non-WPC-containing sorghum-soy FBF groups with both WPC-containing FBF groups. Despite differences observed in protein efficiencies, the sorghum-soy FBFs with increased soy flour may be a suitable, less expensive alternative to FBFs with WPC considering the overall similarities in all outcomes evaluated, including the sensory results that showed only minimal flavor and texture differences. Although protein quality is important and served as a main outcome of interest for this study, the observed differences in PER do not necessarily suggest that the FBFs without WPC will be inadequate to address the protein needs of food aid recipients.

In addition, ≤10% sucrose did not result in significantly different outcomes between SSB FBF groups. This study alone does not provide enough evidence to dismiss 15% added sucrose FBFs, but the negative impact on growing rat BMD observed is concerning. Further research on potential negative effects of high sucrose consumption on bone health is warranted. Collectively, results from this study support that SSB FBFs with 5%–10% added sucrose, to increase acceptability, and increased soy flour are efficacious and cost-effective alternatives to FBFs with animal-source protein.

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