

Vitamins and minerals

# Novel Formulated Fortified Blended Foods Result in Improved Protein Efficiency and Hepatic Iron Concentrations Compared with Corn-Soy Blend Plus in Broiler Chickens

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## Abstract

**Background:** Corn- and soybean-based fortified blended foods (FBFs) have been the primary food aid product provided by the United States. Sorghum and cowpea have been suggested as alternative FBF commodities because they are drought-tolerant, grown in food aid-receiving areas, and not genetically modified. Extrusion processing has also been suggested to improve the quality of these FBFs.

**Objectives:** The aim of this study was to determine the protein quality and iron and vitamin A bioavailability of novel FBFs in broiler chickens.

**Methods:** Whey protein concentrate (WPC)-containing FBFs corn-soy blend 14, sorghum-soy, and sorghum-cowpea (SC); a soy protein isolate (SPI)-containing SC FBF (SC+SPI); 2 reformulated, overprocessed SC FBFs (O-SC+WPC, O-SC+SPI); and a nonextruded WPC-containing SC FBF were developed. Nonextruded corn-soy blend plus (CSB+), a currently used FBF, and a gamebird starter/grower diet were used as comparison diets. In the prepared FBF study, 9 groups of 8-d-old broiler chicks ( $n = 10$ ) consumed prepared FBFs for 21 d. In the dry study, 8 groups of 4-d-old broiler chicks ( $n = 24$ ; control:  $n = 23$ ) consumed dry FBFs for 14 d. Results were analyzed by 1-factor ANOVA with least-significant-difference test.

**Results:** In the prepared study, novel formulated FBFs significantly increased caloric and protein efficiency and nonsignificantly increased body weight gain, despite similar food intake compared with CSB+. In the dry study, novel formulated FBFs, except for O-SC+SPI, significantly increased food intake, caloric efficiency, and protein efficiency and nonsignificantly increased body-weight gain compared with CSB+. Novel formulated FBFs nonsignificantly and significantly increased hepatic iron concentrations compared with all FBFs in the prepared and dry studies, respectively.

**Conclusion:** Novel formulated FBFs, apart from O-SC+SPI, resulted in improved protein efficiencies and hepatic iron concentrations compared with CSB+, suggesting that they are of higher nutritional quality. *Curr Dev Nutr* 2018;2:nzy073.

## Introduction

Protein-energy malnutrition, iron, and vitamin A continue to be the most common nutritional deficiencies globally (1–3). Fortified blended foods (FBFs) and partially precooked grain-legume blends that are micronutrient fortified have traditionally been used to combat malnutrition (4). Corn-soy blend plus (CSB+) is an FBF that has been widely used by the US Agency for International Development (USAID) (5). Since their introduction into food aid programs in the 1980s, there has been little research on the efficacy of FBF formulations even as they have been updated. Therefore, recommendations have been made to improve FBFs, including using



**Keywords:** iron bioavailability, protein quality, fortified blended foods, broiler chicken, cereal and legume fortification

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Abbreviations used: BMD, bone mineral density; CSB+, corn-soy blend plus; CSB14, corn-soy blend 14; FBF, fortified blended food; ICP-OES, inductively coupled plasma optical emission spectrometry; N-SC, nonextruded sorghum-cowpea; O-SC, overprocessed sorghum-cowpea; PER, protein-efficiency ratio; SC, sorghum-cowpea; SPI, soy protein isolate; SS, sorghum-soy; USAID, US Agency for International Development; WPC, whey protein concentrate.

drought-tolerant commodities that are locally available in food aid-receiving countries, as well as using processing methods such as extrusion to improve FBF nutritional quality (6). Sorghum and cowpea may be suitable FBF commodities because of their complementary amino acids and availability in food aid-receiving countries (7–9). The latter may allow local and regional procurement of FBFs, thereby improving agricultural markets while allowing food aid countries to provide more cost-effective FBFs (6). Extrusion processing, which involves moisture, high pressure, temperature, and mechanical shear to quickly cook food, has been shown to decrease antinutritional factors and thus may improve FBF protein and iron bioavailability (10). This process precooks FBFs, meaning that they require less time and resources to prepare. It has also been suggested that animal-source protein, such as whey protein concentrate (WPC), be included in FBFs (6); however, its costly inclusion has been questioned (11, 12). Plant protein alternatives, such as soy protein isolate (SPI), may be a less-expensive option that leads to similar product and nutritional outcomes (13).

The broiler chicken has been suggested to be a good *in vivo* model for assessing iron bioavailability because its iron outcomes are consistent with the widely used *in vitro* digestion/Caco-2 cell model (14). Rats have traditionally been the primary *in vivo* model for this application, and pigs have been utilized as well. However, due to the former model's more efficient iron absorption (due to large differences in energy expenditure for body size, life span, body proportion, and gastrointestinal morphology) and the latter model being more costly (15), the chicken model may be more advantageous because of its anatomy, size, growth rate, and low cost (14). To the best of our knowledge, although a protein-efficiency ratio (PER) model has been established (16), the chicken model has not been used to assess protein quality of foods for a direct human nutrition application.

This study is a follow-up to the Micronutrient Fortified Food Aid Pilot Project, which investigated the use of sorghum and cowpea FBFs and led up to an efficacy study in Tanzania (17). Our previous study found that sorghum and cowpea were suitable alternatives to corn and soy FBFs based on vitamin A, iron, and growth outcomes in rats fed dry FBFs for 4 wk, and all FBFs were of better nutritional quality than CSB+ (18). In this study, the previously developed WPC-containing sorghum-cowpea (SC) and sorghum-soy (SS) FBFs and an SPI-containing SC FBF were evaluated along with 3 novel FBFs to determine the importance of extrusion, and if overprocessed FBFs without sugar, less oil, and less WPC or SPI (overprocessed SC FBFs) are equally efficacious, less-expensive options.

The primary objective of the 2 studies in this article was to determine the protein quality and iron and vitamin A bioavailability of new FBFs compared with a current USAID FBF, CSB+. Extruded sorghum, cowpea, corn, and soy FBFs were formulated according to USAID recommendations (6), along with a nonextruded SC (N-SC) group, to assess if sorghum and cowpea can be used as alternative commodities to corn and soy and if extrusion processing is needed to result in similar or improved protein, iron, and vitamin A outcomes. Another objective was to compare the protein quality of WPC FBFs to SPI FBFs. In addition, 2 reformulated, overprocessed less-expensive FBFs were developed to determine if more cost-effective formulations have similar protein quality and iron and vitamin A concentrations compared with other FBFs.

## Methods

### Animal safety and ethics

The Kansas State University Institutional Animal Care and Use Committee approved all animal procedures (protocols 3717.2 and 3790).

### Diets

Seven FBFs were formulated on the basis of USAID food aid recommendations (6) and our previous studies (17, 18; Table 1). Three white sorghum with cowpea blends FBFs (SC, SC+SPI, and N-SC), 1 SS blend, and 1 corn-soy blend (CSB14) were developed. Two additional white SC FBFs were similarly produced; however, they were overprocessed and reformulated with decreased WPC or SPI (3%) and no sugar (O-SC and O-SC+SPI). CSB+ was purchased from a USDA producer (Bunge Milling) and is prepared from heat-treated corn and soybeans, with added micronutrients. A 22% gamebird starter/grower diet (Country Lane; Orscheln Farm and Home) was fed to the control group in both studies to compare outcomes of FBFs with a normal chicken diet.

Iron forms and concentrations among CSB+, novel formulated FBFs, and the control chicken diet were different. The gamebird starter/grower diet contained ferrous sulfate (41.5 mg/100 g) almost 4 times higher than CSB+ and an average of 2.5 times higher than new FBFs. CSB+ and novel formulated FBFs contained sodium iron EDTA (NaFeEDTA) and ferrous fumarate at different concentrations (17, 18). NaFeEDTA was included due to its superior bioavailability compared with ferrous fumarate; therefore, the combination of the 2 forms was expected to enhance iron bioavailability from FBFs (6).

### FBF food production

FBFs were produced by extruding grain and legume flours (with the exception of N-SC) and milling them to a powder. SC, SS and corn-soy grain and legume flours were extruded on a single screw extruder X-20 (Wenger Manufacturing Co.) at the Kansas State University Extrusion Lab. Extrusion of SC, SC+SPI, SS, and CSB14 FBFs was completed at an in-barrel moisture of 24%, a motor load of 74%, and a specific mechanical energy of 299 kJ/kg. O-SC and O-SC+SPI FBFs had an in-barrel moisture of 21%, a motor load of 78%, and a specific mechanical energy of 370 kJ/kg. Steam and water were added in the preconditioner at an average of 14% and 16%, respectively, for normally processed and at 18% and 6%, respectively, for overprocessed FBFs. Preconditioner discharge temperature was maintained >85°C, and the die had a single circular opening of 4.1 mm for all FBFs. After cutting, extrudates were dried using a double-pass dryer/cooler (Series 4800; Wenger Manufacturing Co.) operating at 107°C, where they were retained for 10 min before being cooled for 5 min at room temperature. Cooled extrudates were hammer milled (Schutte) fitted with a 315- $\mu$ m screen and collected directly into 50-pound 3-walled paper bags and sealed until further use. The vitamin and mineral premix (3.2%; Repco), WPC 80% (Davisco Food International, Inc.), or SPI 90% (ARDEX F Dispersible 066-921; ADM) and sugar (15%) were mixed into the extruded flours in steps to ensure mixing uniformity. Once dry ingredients were combined through this process, soybean oil (9%) was added and mixed thoroughly to produce the final FBF product.

**TABLE 1** Composition of FBFs<sup>1</sup>

	Sorghum flour, %	Cowpea flour, %	Soy flour, %	Corn flour, %	Sugar, %	Whey protein concentrate, %	SPI, %	Soybean oil, %	Micronutrient premix, %
SC, N-SC	24.7	38.6	0	0	15	9.5	0	9.0	3.2
SC+SPI	24.7	38.6	0	0	15	0	9.5	9.0	3.2
O-SC	31.5	54.0	0	0	0	3.0	0	8.3	3.2
O-SC+SPI	31.5	54.0	0	0	0	0	3.0	8.3	3.2
SS	47.6	0	15.7	0	15	9.5	0	9.0	3.2
CSB14	0	0	15.2	48.1	15	9.5	0	9.0	3.2

<sup>1</sup>CSB+: whole corn (78.4%), whole roasted soy (20%), vitamins-minerals (0.2%), tricalcium phosphate (1.16%), potassium chloride (0.17%). Gamebird starter/grower diet based on label ingredients: grain products, plant-protein products, processed grain by-products, roughage products, vitamin supplements, minerals. CSB+, corn-soy blend plus; CSB14, corn-soy blend 14; FBF, fortified blended food; N-SC, nonextruded sorghum-cowpea; O-SC, overprocessed sorghum-cowpea; SC, sorghum-cowpea; SPI, soy protein isolate; SS, sorghum-soy.

### Diet macronutrient and iron concentrations

Diet total calories, protein, fat, carbohydrates, and amino acids were analyzed by AOAC official methods by the University of Missouri–Columbia Agricultural Experiment Station Chemical Laboratories or calculated from these results as described previously (14). Diet iron concentrations were analyzed in duplicate ( $n = 9$ ) (AACC method 40.70.01; AIB International).

### Water iron concentrations

Duplicate-facility water samples were collected by turning on water faucets for 5 min before taking the sample. Samples were taken in duplicate, and iron was assessed by both flamed atomic absorption spectrometry and inductively coupled plasma optical emission spectrometry (ICP-OES) at the Kansas State University Soil Testing Lab. Neither technique was able to detect iron; atomic absorption spectrometry minimum detection concentration was 0.11  $\mu\text{g}/\text{mL}$  and ICP-OES minimum detection concentration was 0.2  $\mu\text{g}/\text{mL}$ . Water iron concentrations were much lower than the  $0.379 \pm 0.012 \mu\text{g}/\text{mL}$  (14) reported previously in a similar broiler chicken study.

### Viscosity

Prepared FBF viscosity was assessed in duplicate using a Bostwick Consistometer (CSC Scientific Company, Inc.). Novel formulated FBFs were prepared at 20% solids; CSB+ was prepared at 13.79% solids as directed (5). Water was brought to a boil and the FBF was slowly mixed in and left to boil for 1 min with constant stirring. CSB+ and N-SC were boiled for 5 min with constant stirring due to their partially and non-precooked characteristics, respectively. After 1 or 5 min, the FBFs were removed from the heat source and stirred for another 30 s before being covered with aluminum foil and set in a water bath for 10 min at 30°C. After 10 min, the FBF was weighed and the lost water, due to evaporation, was added back. The FBF sample was put back in the water bath for 1 h at 30°C. Then, the FBF sample was weighed once more and water was added if there was any loss. The FBF was stirred and poured into the Bostwick Consistometer chamber, leveled off, and allowed to settle for 30 s. Then, the gate was opened and the FBF was allowed to flow for 1 min and the distance traveled was recorded.

### Study design

For both studies, 1-d-old male broiler chicks were obtained from a commercial hatchery (Cobb-Vantress, Inc.) and arrived at the Kansas

State University Poultry Unit the same day. Male chicks were chosen because they have been used in previous similar studies (14, 19–21).

**Prepared FBF study.** Ninety 1-d-old male broiler chicks were placed in 5- × 13-foot floor pens covered in absorbent bedding material in a temperature-controlled facility with 24-h light provided (16). Initial temperature was 34°C and by the end of the study was decreased to 24°C. Chicks were fed a basic broiler starter diet (OH Kruse Feed Technology Innovation Center) that consisted of corn and soybean meal with micronutrients for 1 wk. On day 8, chicks were randomized into 9 groups on the basis of body weight, with two 5-chick floor pen replicates/group ( $n = 10$ ; 90 total) (14). Pen positions for replicates were assigned to best control for environmental impacts by spreading them strategically throughout the facility. Food and water were provided ad libitum. Water was supplied by a uniform water source, and each pen had its own hanging tube nipple waterers. From days 8 to 23, chicks were fed using small round plastic containers with half lids (to keep chicks out of food) due to the small volume of food provided. On day 24 through the end of the study, larger feeders that consisted of a one-third-size foil steam-table pan inside a wooden base and half covering were used. Normally, CSB+ is directed to be prepared at 13.79% solids and new FBFs at 20% solids. However, due to the limited stomach capacity of the chickens, solids percentages were increased to make FBFs more nutrient dense, to better meet daily feed requirements outlined by the Cobb-Vantress, Inc., hatchery (22). Therefore, CSB+ was prepared at 1:2.55 solids to water, and novel FBFs and the control diet were prepared at 1:2 solids toward this goal but also maintained the same difference between the solid composition of CSB+ and novel FBFs. Due to their partially and non-precooked characteristics, CSB+ and N-SC were boiled with water and stirred for 10 min in large turkey fryers to ensure complete cooking. For other FBFs, water was boiled in a large turkey fryer, then was divided out and mixed into FBFs; room-temperature water was added and mixed into the control chicken diet. Chicks were fed twice daily and food was prepared each afternoon; two-thirds was fed to chicks for the afternoon feeding (1600 h), and one-third was refrigerated overnight in plastic containers, then fed to chicks for the morning feeding (0730 h). Food intakes were calculated by weighing food left at the end of feeding periods. Chickens were weighed weekly as a replication group until the study end when they were weighed individually. Chickens were fed for 21 d, so they were 28 d

old at termination; study length and sample size were based on a similar published study (18).

Four days before the study end, blood was found in chicks' feces; the poultry unit staff believed it was due to coccidiosis (a protozoan infection) and treatment was started immediately. CORID (amprolium) 9.6% oral solution was given to chicks in their drinking water according to dosage and administration instructions. Some chickens were displaying gait issues by the study end; thus, before termination, the degree of impairment was assessed using criteria (0 = none to 5 = complete impairment) from a modified gait scoring system (23).

**Dry FBF study.** Two hundred 1-d-old male broiler chicks were placed in 3.25- × 1.1- × 0.8-foot wire-bottomed battery brooder units. Unit temperatures ranged from 26° to 29°C with 24-h light provided (16). Chicks were fed basic broiler starter diet (OH Kruse Feed Technology Innovation Center) for 4 d before beginning experimental diets (an acclimation period used for previous unpublished PER protocols conducted by one of the authors, RSB). On day 4, chicks were weighed and allocated on the basis of body weight into 8 groups of 4 replicates of 6 chicks/unit ( $n = 24$ ; control:  $n = 23$ ; 191 total) (16). In each battery brooder unit, replicates were assigned to different locations to control for environmental effects. The control group originally contained 24 chicks; however, 1 chick was killed a few days before study end due to an unexplained physical injury unrelated to the study regimen. Chicks were provided food and water ad libitum. Before study termination, units were randomized to select 6 chicks from each diet group to kill for sample collection. Feed intakes and body weights were measured weekly as a replication group except at the study end when the killed subset were weighed individually. Treatment diets were fed for 14 d so chicks were 18 d old at the study end; study duration and sample size were based on a previous PER study (16).

Control replication-group mean food intake and body weights were readjusted to account for killing of the chick early. Food intake was averaged per chick; then, the total intake for the 5 chicks in that replication was calculated and added together for each week. For body weights, because the removed chick was 12% smaller than the average of the other chicks in its group, we subtracted the proportional amount of body weight from the initial and week 1 replication body weights.

#### Data and sample collection

At termination of both studies, final individual body weights were recorded. For hemoglobin analysis in the prepared study, blood was collected via the wing vein into 4-mL EDTA-K2 vacuolized tubes (BD and Company) and in the dry study via cardiac puncture into 2-mL EDTA-K2 vacuolized tubes. EDTA-K2 vacuolized tubes were immediately placed on ice and subsequently stored at 4°C for 6–7 or 2 d before analysis in the prepared and dry studies, respectively. After blood collection, chickens were killed by cervical dislocation. Liver tissue was collected, weighed, flash-frozen in liquid nitrogen, and stored at –80°C. In the prepared study, both legs of chickens were collected and stored at –20°C for future assessment.

**Hemoglobin concentrations.** Hemoglobin was assessed in duplicate (prepared,  $n = 10$ ; dry,  $n = 6$ ) using the QuantiChrom Hemoglobin Assay Kit (DIHB-250; BioAssay Systems) following the manufacturer's

instructions. Whole blood was diluted 100-fold with deionized distilled H<sub>2</sub>O (20 to 1980 μL). If duplicates were >25% different, a triplicate sample was analyzed.

**Hepatic iron and retinol concentrations.** Hepatic iron concentrations (prepared,  $n = 10$ ; dry,  $n = 6$ ) were determined in duplicate as described previously (18) and stored at room temperature before quantification by ICP-OES (Varian 720-ES; Agilent Technologies) at the Kansas State University Soil Testing Lab. If duplicates were >25% different, a triplicate sample was analyzed. Hepatic retinol was determined in duplicate ( $n = 5$ ) in a subset of prepared-study chickens as described previously (18).

**Tibia bone mineral densities.** Prepared-study ( $n = 10$ ) right legs were brought to room temperature before tibiae were dissected, and bone mineral density (BMD) was measured via Lunar PIXImus Densitometer (GE Medical Systems). Four BMD measurements were taken due to reports of BMD varying across the tibia regions (24). The first measurement analyzed BMD of the entire tibia (total BMD). The second measurement analyzed the diaphysis region (diaphysis BMD), which was defined as the middle-50% region (25). The third and fourth measurements analyzed the BMD of the proximal (proximal BMD) and distal (distal BMD) epiphyses, defined as the top- and bottom-25% regions, respectively (25). Total lengths of the tibiae were first measured, and regions were calculated from lengths and visibly marked. Tibiae were placed in the scanning area, and metal references were placed adjacent to marks so the region of interest could be adjusted on the PIXImus once scanned to obtain each BMD measurement.

#### Statistical analysis

Group differences were assessed with the use of 1-factor ANOVA and least-significant-differences post hoc test at a significance level of  $P < 0.05$  using SAS Studio 3.6 (SAS Institute). Natural-log transformation was used if the assumption of normality was violated.

## Results

#### FBF composition and viscosity

**Composition.** Novel formulated FBFs contained, on average, 6.5% more energy and 15.6% more protein than CSB+ (Table 2). CSB+ contained similar fat content to both overprocessed FBFs (O-SC and O-SC+SPI), and collectively these FBFs contained 20.7% less fat than the other FBFs. N-SC provided less energy, and O-SC and O-SC+SPI also contained less available lysine than other FBFs but more than CSB+. CSB+ contained 36.5% less iron than the other FBFs; the control-diet iron content was markedly higher than the novel formulated FBFs and CSB+. WPC and SPI FBFs had comparable macronutrient and micronutrient compositions.

**Viscosity.** The required USAID Bostwick consistency for corn-soy blend is 9–21 cm (27). N-SC did not meet viscosity requirements; SC and SS slightly exceeded requirements. Extruded sorghum-containing FBFs (SC, SC+SPI, and SS), on average, were 43.5% less viscous (higher Bostwick consistency values) than corn-containing FBFs (CSB14 and



**TABLE 2** Analyzed macronutrients, selected amino acids, iron content, and Bostwick measurements<sup>1</sup>

	SC	SC+SPI	N-SC	O-SC	O-SC+SPI	SS	CSB14	CSB+
Total energy, kcal/100 g	412.8	418.4	402.7	403.0	401.7	411.7	429.5	384.8
Carbohydrate, g/100 g	62.6	61.2	59.4	64.3	63.8	62.7	60.6	61.3
Protein, <sup>2</sup> g/100 g	19.1	19.8	18.1	17.9	18.4	19.5	18.4	15.8
Fat, g/100 g	9.6	10.5	10.3	8.3	8.1	9.2	12.7	8.5
Ash, g/100 g	3.9	3.7	3.6	3.9	4.2	3.5	3.2	3.6
Crude fiber, g/100 g	0.6	0.6	0.7	0.8	0.9	0.6	0.5	2.1
Moisture, g/100 g	4.3	4.3	7.9	4.8	4.7	4.5	4.8	8.8
Lysine, mg/g	14.0	11.8	13.5	10.8	10.4	13.3	12.6	8.5 <sup>2</sup>
Cysteine + methionine, <sup>2</sup> mg/g	6.3	4.8	6.0	4.7	4.4	7.0	6.5	5.2
Available lysine, mg/g	13.4	11.2	13.1	9.7	9.5	12.8	12.0	8.2 <sup>2</sup>
Iron, mg/100 g	17.2	16.8	16.2	16.5	16.8	15.9	16.0	10.5
Bostwick measurement, <sup>3</sup> cm	21.5	18.75	5	11	11.25	21.5	13.25	10

<sup>1</sup>The gamebird starter/grower diet is formulated to provide 230.2 kcal, 22 g protein, and 41.5 mg Fe per 100 g diet. Macronutrient and micronutrient contents were analyzed in duplicate (macronutrients and amino acids: AOAC official methods, University of Missouri-Columbia Agricultural Experiment Station Chemical Laboratories; iron: AIB International). New FBFs were prepared at 20% solids. CSB+, corn-soy blend plus; CSB14, corn-soy blend 14; FBF, fortified blended food; N-SC, nonextruded sorghum-cowpea; O-SC, overprocessed sorghum-cowpea; SC, sorghum-cowpea; SPI, soy protein isolate; SS, sorghum-soy.

<sup>2</sup>Values are lower than NRC requirements for 0- to 42-d-old broiler chickens (26).

<sup>3</sup>New FBFs prepared at 20%, CSB+ at 13.79% solids.

CSB+; Table 2). Overprocessed sorghum and cowpea (O-SC and O-SC+SPI) formulations met viscosity requirements.

### Food intake, body weight, food efficiency, iron, vitamin A, and anthropomorphic outcomes

**Prepared FBF study.** The control group had significantly higher food intake, weight gain, final body weight, and caloric efficiency compared with all FBF groups (Table 3, Figures 1 and 2). The SS group had significantly higher food intake than the N-SC, O-SC+SPI, and SC groups. The SS group had significantly increased body-weight gain compared with all FBFs, except for the CSB14 and O-SC groups. The O-SC+SPI group had significantly reduced body-weight gain and final body weights compared with SS, CSB14, O-SC, and SC+SPI groups. The CSB+ group had reduced body-weight gain compared with all FBF groups, with the exception of the SC and O-SC+SPI groups, despite having a higher food intake than most FBF groups. The SS group had significantly increased final body weight compared with all FBF groups, except for CSB14; other novel formulated FBFs performed similarly.

The CSB+ group had significantly decreased caloric and protein efficiencies compared with all groups (−24% and −15%, respectively, compared with the next-least-efficient group, O-SC+SPI). The SS group had significantly improved caloric and protein efficiencies compared with all novel formulated FBF groups, except for N-SC (caloric), N-SC, and CSB14 (protein) groups. The N-SC group had significantly improved caloric efficiency compared with O-SC, O-SC+SPI, and CSB+ groups and improved protein efficiency compared with SC, O-SC, O-SC+SPI, and CSB+ groups. The O-SC+SPI group had significantly decreased food efficiencies compared with all other novel FBF groups.

There were no significant differences in hemoglobin concentration between groups (Table 3). Control and CSB+ groups had significantly reduced hepatic iron concentrations compared with all novel formulated FBF groups. The O-SC+SPI group had significantly higher hepatic iron concentrations compared with all groups, except for the O-SC and

SC+SPI groups. The SC+SPI group had significantly decreased hepatic retinol concentrations compared with CSB14 and CSB+ groups.

Some chickens developed gait issues halfway through the study, and thus gait scores were collected (23). N-SC and SC groups had significantly increased gait scores (indicating impairment) compared with all other groups (Table 4). CSB14 and SS groups also had gait scores that were significantly greater than the other groups that did not have any impairment.

Due to gait issues identified in the prepared study, FBFs were compared with NRC requirements for protein, amino acid, and certain minerals. FBFs did not meet protein, certain amino acid, calcium, or phosphorus requirements for broiler chickens 0- to 21-d-old; however caloric and iron requirements were exceeded by all FBFs (26) (Table 5). CSB+ contained less lysine than other FBFs, which may have contributed to the lower growth seen in the dry study; however, O-SC+SPI had comparable nutrient content to other new FBFs and its consumption resulted in outcomes similar to CSB+.

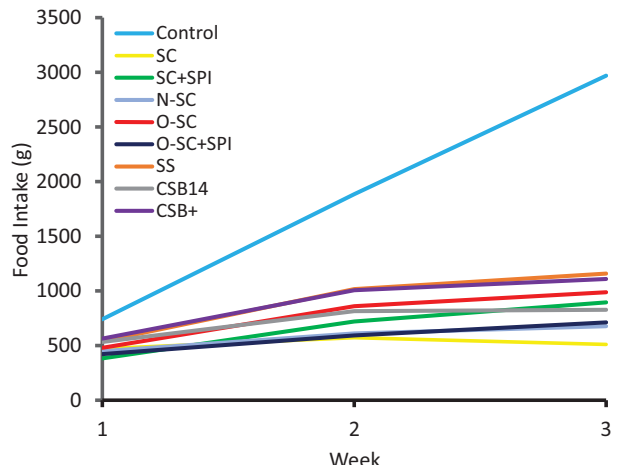
To determine if the gait issues were due to bone weakness in their legs, BMDs were collected on right tibias. Control-group total, diaphysis, proximal, and distal BMDs were significantly higher than all other groups. SS, SC+SPI, and O-SC groups had significantly higher total BMD than all FBF groups, except for the CSB14 group. SC, N-SC, and CSB+ groups had significantly lower total BMD than all FBF groups, except for the O-SC+SPI group. Diaphysis, proximal, and distal BMD differences followed similar trends as seen in total BMD.

**Dry FBF study.** The control group had significantly increased food intake, weight gain, and final body weights compared with all other groups (Table 6, Figures 3 and 4). CSB+ and O-SC+SPI groups had significantly decreased total food intake, total weight gain, and final body weight compared with all other groups. The SS group's total food intake was significantly greater than all other FBF groups, except for the CSB14 group. The SC group's total food intake was significantly greater than the SC+SPI and O-SC groups' intake. The SS group also had

**TABLE 3** Prepared FBF study food intake, body weight, food efficiencies, hemoglobin, hepatic iron, and retinol<sup>1</sup>

	Control	SC	SC+SPI	N-SC	O-SC	O-SC+SPI	SS	CSB14	CSB+
Total food intake, g	6120.2 ± 82.7 <sup>a</sup>	1759.2 ± 103.9 <sup>b</sup>	2217.8 ± 53.3 <sup>b,c,d</sup>	1946.6 ± 58.1 <sup>b,c</sup>	2592.4 ± 61.3 <sup>c,d</sup>	1928.4 ± 177.6 <sup>b</sup>	3023.3 ± 131.2 <sup>d</sup>	2445.9 ± 36.4 <sup>b,c,d</sup>	2989.3 ± 66.3 <sup>d</sup>
Total weight gained, g	1065.0 ± 16.4 <sup>a</sup>	292.3 ± 23.6 <sup>b,d</sup>	404.9 ± 10.9 <sup>b,c</sup>	354.9 ± 19.0 <sup>b,c,d</sup>	418.9 ± 13.3 <sup>b,c,e</sup>	265.6 ± 32.5 <sup>d</sup>	623.0 ± 22.4 <sup>e</sup>	459.6 ± 15.1 <sup>e</sup>	295.6 ± 8.3 <sup>b,d</sup>
Final body weight, g	1165.6 ± 15.3 <sup>a</sup>	390.8 ± 23.5 <sup>b,c</sup>	505.4 ± 11.8 <sup>b,e</sup>	456.2 ± 19.8 <sup>b,c,e</sup>	518.6 ± 12.6 <sup>b,e</sup>	363.7 ± 32.9 <sup>c</sup>	723.7 ± 21.5 <sup>d</sup>	561.1 ± 14.3 <sup>d,e</sup>	396.3 ± 7.9 <sup>b,c</sup>
Caloric efficiency, g/kcal × 1000	16.5 ± 0.0 <sup>a</sup>	9.1 ± 0.2 <sup>b,c</sup>	9.7 ± 0.0 <sup>b,c</sup>	10.1 ± 0.2 <sup>b,e</sup>	8.9 ± 0.1 <sup>c</sup>	7.6 ± 0.2 <sup>d</sup>	11.2 ± 0.1 <sup>e</sup>	9.8 ± 0.2 <sup>b,c</sup>	5.7 ± 0.0 <sup>f</sup>
Protein efficiency, g/g × 100	17.3 ± 0.0 <sup>a,2</sup>	19.6 ± 0.4 <sup>b</sup>	20.5 ± 0.0 <sup>b,d</sup>	22.5 ± 0.5 <sup>c,d</sup>	20.1 ± 0.2 <sup>b</sup>	16.5 ± 0.5 <sup>a</sup>	23.6 ± 0.2 <sup>c</sup>	23.0 ± 0.4 <sup>c</sup>	14.0 ± 0.1 <sup>e</sup>
Hemoglobin, g/dL	8.2 ± 0.5	10.0 ± 0.7	8.7 ± 0.6	9.4 ± 0.9	8.7 ± 0.5	8.3 ± 0.4	8.9 ± 0.7	8.9 ± 1.1	8.5 ± 1.4
Hepatic iron, µg/g	13.5 ± 1.4 <sup>a</sup>	22.6 ± 3.6 <sup>b,c</sup>	27.5 ± 4.0 <sup>c,d</sup>	21.3 ± 1.6 <sup>b,c</sup>	30.1 ± 3.6 <sup>c,d</sup>	35.3 ± 4.2 <sup>d</sup>	22.2 ± 2.8 <sup>b,c</sup>	24.5 ± 4.0 <sup>b,c,3</sup>	18.9 ± 4.0 <sup>a,b</sup>
Hepatic retinol, ng/mg	95.5 ± 16.2 <sup>a</sup>	221.1 ± 69.4 <sup>a,b,c</sup>	127.7 ± 50.0 <sup>a</sup>	216.3 ± 64.9 <sup>a,b,c</sup>	136.2 ± 25.9 <sup>a,b</sup>	147.1 ± 46.1 <sup>a,b</sup>	161.1 ± 42.7 <sup>a,b,c</sup>	259.4 ± 83.0 <sup>b,c</sup>	374.4 ± 98.3 <sup>c</sup>

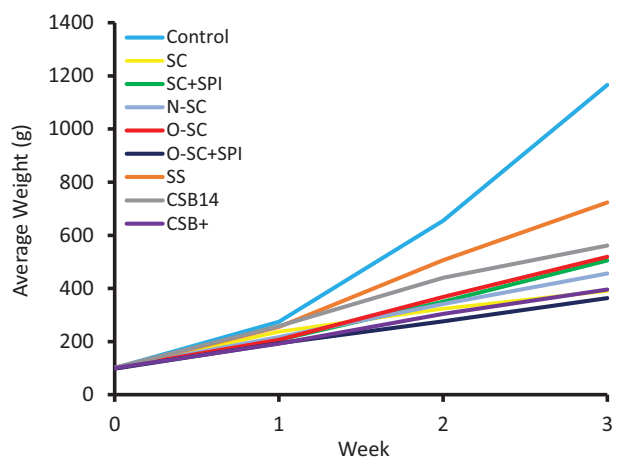
<sup>1</sup>Values are means ± SEMs, n = 10. Means without a common superscript letter differ, P < 0.05. CSB+, corn-soy blend plus; CSB14, corn-soy blend 14; FBF, fortified blended food; N-SC, nonextruded sorghum-cowpea; O-SC, overprocessed sorghum-cowpea; SC, sorghum-cowpea; SPI, soy protein isolate; SS, sorghum-soy.  
<sup>2</sup>Based on label protein values rather than analyzed protein values.  
<sup>3</sup>n = 9.



**FIGURE 1** Prepared FBF study mean weekly food intake (n = 10). Control group compared with all FBF groups (P < 0.05); SS and CSB+ groups compared with N-SC, O-SC+SPI, and SC groups (P < 0.05); O-SC compared with O-SC+SPI group (P < 0.05). CSB+, corn-soy blend plus; CSB14, corn-soy blend 14; FBF, fortified blended food; N-SC, nonextruded sorghum-cowpea; O-SC, overprocessed sorghum-cowpea; SC, sorghum-cowpea; SPI, soy protein isolate; SS, sorghum-soy.

significantly higher total weight gain and final body weight compared with all other FBF groups, except for the SC group.

The CSB+ group had significantly reduced caloric and protein efficiencies compared with all groups, except the O-SC+SPI group. O-SC, SS, SC, and SC+SPI groups had significantly increased caloric



**FIGURE 2** Prepared FBF study mean weekly body weights (n = 10). Body-weight gain: control group compared with all FBF groups (P < 0.05); SS group compared with all groups except for CSB14 and O-SC groups (P < 0.05); CSB14 group compared with SC, O-SC+SPI, and CSB+ groups (P < 0.05); SC+SPI and O-SC groups compared with O-SC+SPI group (P < 0.05); CSB14 and SC groups compared with SC+SPI and O-SC groups (P < 0.05). CSB+, corn-soy blend plus; CSB14, corn-soy blend 14; FBF, fortified blended food; N-SC, nonextruded sorghum-cowpea; O-SC, overprocessed sorghum-cowpea; SC, sorghum-cowpea; SPI, soy protein isolate; SS, sorghum-soy.

TABLE 4 Prepared FBF study gait scores and BMD outcomes<sup>1</sup>

	Control	SC	SC+SPI	N-SC	O-SC	O-SC+SPI	SS	CSB14	CSB+
Gait score (+1)	1.0 ± 0.0 <sup>a</sup>	3.4 ± 0.6 <sup>b</sup>	1.0 ± 0.0 <sup>a</sup>	3.9 ± 0.6 <sup>b</sup>	1.0 ± 0.0 <sup>a</sup>	1.0 ± 0.0 <sup>a</sup>	1.8 ± 0.6 <sup>a,c</sup>	2.1 ± 0.6 <sup>c</sup>	1.0 ± 0.0 <sup>a</sup>
Total BMD, g/cm <sup>2</sup> × 100	21.1 ± 0.5 <sup>a</sup>	8.4 ± 0.4 <sup>b</sup>	11.7 ± 0.3 <sup>c</sup>	9.1 ± 0.5 <sup>b</sup>	11.6 ± 0.7 <sup>c</sup>	9.6 ± 0.5 <sup>b,d</sup>	12.0 ± 0.6 <sup>c</sup>	10.6 ± 0.3 <sup>c,d</sup>	9.2 ± 0.3 <sup>b</sup>
Diaphysis BMD, g/cm <sup>2</sup> × 100	25.6 ± 0.7 <sup>a</sup>	9.3 ± 0.4 <sup>b</sup>	12.1 ± 0.3 <sup>c</sup>	9.3 ± 0.4 <sup>b</sup>	12.1 ± 0.6 <sup>c</sup>	10.3 ± 0.6 <sup>b,c</sup>	11.8 ± 0.6 <sup>c</sup>	11.0 ± 0.4 <sup>c</sup>	9.7 ± 0.3 <sup>b</sup>
Proximal BMD, g/cm <sup>2</sup> × 100	17.3 ± 0.3 <sup>a</sup>	7.9 ± 0.5 <sup>b</sup>	11.1 ± 0.4 <sup>c</sup>	9.0 ± 0.6 <sup>b</sup>	11.0 ± 0.6 <sup>c</sup>	8.8 ± 0.5 <sup>b</sup>	12.3 ± 0.5 <sup>d</sup>	10.8 ± 0.3 <sup>c</sup>	8.5 ± 0.2 <sup>b</sup>
Distal BMD, g/cm <sup>2</sup> × 100	19.1 ± 0.5 <sup>a</sup>	8.0 ± 0.6 <sup>b</sup>	11.8 ± 0.3 <sup>c</sup>	8.8 ± 0.6 <sup>b,d</sup>	11.7 ± 0.8 <sup>c</sup>	9.5 ± 0.6 <sup>b,d</sup>	12.1 ± 0.6 <sup>c</sup>	9.8 ± 0.4 <sup>d</sup>	9.3 ± 0.3 <sup>b,d</sup>

<sup>1</sup>Values are means ± SEMs, *n* = 10. Means without a common superscript letter differ, *P* < 0.05. Gait scores: increased by 1 to be able to analyze data in SAS. 1 = no impairment, 6 = complete lameness (25). BMD, bone mineral density; CSB+, corn-soy blend plus; CSB14, corn-soy blend 14; FBF, fortified blended food; N-SC, nonextruded sorghum-cowpea; O-SC, overprocessed sorghum-cowpea; SC, sorghum-cowpea; SPI, soy protein isolate; SS, sorghum-soy.

efficiency compared with the CSB14 and O-SC+SPI groups. The O-SC+SPI group's caloric efficiency was significantly decreased compared with all novel FBF groups, except the CSB14 group. CSB+ contained less lysine than other FBFs (Table 2), which may have contributed to lower intake, efficiencies, and weights; however, O-SC+SPI had comparable nutrient content to the novel FBFs, and it had similar outcomes. The O-SC, SS, and SC group protein efficiencies were significantly increased compared with the other FBF groups.

The CSB+ group had significantly increased hemoglobin concentrations compared with all other groups (Table 6). There were no other significant differences between novel formulated FBF group hemoglobin concentrations. The CSB+ group had significantly reduced hepatic iron concentrations compared with all other FBF groups. The O-SC+SPI group had significantly higher hepatic iron concentrations compared with all other novel formulated FBF groups, except the SC+SPI and O-SC groups.

## Discussion

In these studies, with the exception of O-SC+SPI, novel FBFs resulted in improved protein efficiency and hepatic iron concentrations compared with CSB+. Novel formulated FBFs resulted in similar protein, hepatic iron, and hemoglobin concentrations, with the exception of O-SC+SPI, suggesting that sorghum and cowpea are suitable replacements for corn and soy. SPI is an equally efficacious alternative to WPC in some formulations; and reformulated, overprocessed FBFs with WPC can be considered as a less-expensive FBF option.

Overall, CSB+ trended toward reduced food efficiency outcomes compared with all novel formulated FBFs, except for O-SC+SPI. Although CSB+ contained lower caloric and protein content than the new FBFs, all FBFs did not meet protein but exceeded calorie and fat recommendations for 0- to 21-d-old broiler chickens; therefore, it is not likely that this slight decrease in protein content resulted in the significantly reduced food efficiency outcomes observed. The lower solids content of CSB+ likely contributed to the reduced food efficiencies. However, this is also a limitation of CSB+ in treating children for malnutrition in food aid programs due to their limited stomach capacity, and thus the need for a more nutrient-dense FBF (6) like the novel formulated FBFs researched in this study.

In a study with similar novel formulated FBFs and CSB+ fed to rats, CSB+ resulted in decreased food intake, growth suppression, and reduced caloric and protein efficiencies compared with other groups (18). In addition, CSB+ inhibited growth in week 1 despite similar food intake with other groups (18), suggesting poorer nutritional quality and digestibility than novel formulated FBFs. In broiler chickens, an energy-sufficient but decreased lysine diet content resulted in significantly lower weight gain compared with other groups (28). Similarly, CSB+ contained sufficient energy but lower lysine than the new FBFs, and significantly lower weight gain was observed in the prepared study compared with SS and CSB14 and in the dry study compared with all groups except for O-SC+SPI; this suggests lower protein quality compared with new FBFs. Extrusion processing has been often cited to improve cereal and legume starch and amino acid digestibility (29–32); therefore, the fact that CSB+ is not extruded was hypothesized previously to explain these outcomes in rats (18).

**TABLE 5** Comparison of NRC broiler chicken nutrient requirements to 100-g FBF contents<sup>1</sup>

	NRC <sup>2</sup>		SC	SC+SPI	N-SC	O-SC	O-SC+SPI	SS	CSB 14	CSB+
	0–21 d	22–42 d								
Kilocalories	320	320	412.8	418.4	402.7	403.02	401.7	411.7	429.5	384.8
Protein and amino acids, g										
Crude protein	23	20	19.1	19.8	18.1	17.9	18.4	19.5	18.4	15.8
Arginine	1.25	1.1	0.89	1.31	0.87	1	1.14	0.92	0.82	0.98
Glycine + serine	1.25	1.14	1.44	1.65	1.35	1.38	1.46	1.54	1.37	1.29
Histidine	0.35	0.32	0.47	0.53	0.46	0.48	0.51	0.45	0.42	0.41
Isoleucine	0.8	0.73	0.99	0.9	0.95	0.81	0.8	1.06	0.98	0.66
Leucine	1.2	1.09	1.87	1.68	1.78	1.61	1.58	2.06	1.86	1.43
Lysine	1.1	1	1.34	1.18	1.35	1.08	1.04	1.33	1.2	0.82
Methionine	0.5	0.38	0.33	0.27	0.31	0.28	0.26	0.34	0.32	0.25
Methionine + cysteine	0.9	0.72	0.63	0.48	0.6	0.47	0.44	0.7	0.65	0.52
Phenylalanine	0.72	0.65	0.89	1.08	0.86	0.94	1.02	0.9	0.82	0.78
Phenylalanine + tyrosine	1.34	1.22	1.43	1.68	1.38	1.47	1.58	1.51	1.37	1.29
Proline	0.6	0.55	0.93	0.9	0.83	0.78	0.78	1.17	1.04	0.92
Threonine	0.8	0.74	0.97	0.7	0.91	0.71	0.65	0.98	0.92	0.57
Tryptophan	0.2	0.18	0.3	0.25	0.28	0.24	0.22	0.32	0.31	0.2
Valine	0.9	0.82	1.04	1	1	0.93	0.93	1.09	0.98	0.77
Selected minerals, mg										
Iron	8	8	17.2	16.8	16.2	16.5	16.8	15.9	16.0	10.5
Calcium <sup>3</sup>	1000	900	279.1	279.1	279.1	279.1	279.1	279.1	279.1	452
Nonphytate phosphorus <sup>3</sup>	450	350	291	291	291	291	291	291	291	290

<sup>1</sup>Control chicken diet based on label values: 230.2 (kcal/100 g), crude protein (22%), lysine (0.87%), methionine (0.43%), crude fat (2.5%), crude fiber (7.0%), calcium (1.0–1.1%), phosphorus (0.78%), salt (0.15–0.4%), and sodium (0.01–0.3%); iron (41.5 mg/100 g). FBF protein, amino acid, and iron contents were analyzed in duplicate. CSB+, corn-soy blend plus; CSB14, corn-soy blend 14; FBF, fortified blended food; N-SC, nonextruded sorghum-cowpea; O-SC, overprocessed sorghum-cowpea; SC, sorghum-cowpea; SPI, soy protein isolate; SS, sorghum-soy.

<sup>2</sup>Data from reference 26.

<sup>3</sup>FBF calcium and nonphytate phosphorus values based on formulation.

However, N-SC, which was also not extruded, performed similarly to novel formulated, extruded FBFs and nonsignificantly improved weight gain while significantly improving food efficiencies compared with CSB+, despite not being precooked and thus requiring the same preparation procedures as CSB+. This suggests that the nutritional quality of the N-SC formulation is greater than that of CSB+, although N-SC did not meet viscosity requirements, making it not a viable FBF option.

Along with CSB+, O-SC+SPI resulted in poorer growth and efficiency outcomes in both the prepared and dry studies. O-SC, a formulation similar to WPC, consumption resulted in growth and efficiency outcomes similar to the other novel formulated, extruded FBFs. In a study performed with similar FBFs in rats, the SC+SPI group resulted in reduced protein digestibility, caloric efficiency, and weight gain compared with the SC+WPC group (18). In the case of O-SC+SPI, cowpea flour provides a majority of the protein therefore, it might be that SPI is not sufficient to make up for its lower protein quality in the amounts provided, unlike how WPC did in O-SC. However, the other SPI-containing group, SC+SPI, performed similarly in both studies to its WPC-containing formulation, SC, suggesting that, in certain formulations of FBFs, SPI is an effective alternative to WPC. This supports the conclusion of a review that found that isocaloric, isonitrogenous animal-source proteins were not superior to plant-source proteins in enhancing linear growth, suggesting that the costly inclusion of animal-source proteins is not needed in FBFs (11). Considering these outcomes, further research on protein sources in FBFs is warranted.

Considering the relative protein and caloric efficiency results in this study along with the results from our previous rat study (18) compared with CSB+, SS was the blend that best-supported weight gain across the 3 different models. However, the magnitude of the relative difference between CSB+ and SS caloric efficiencies ranged from 2.34 (rat) to 1.96 (prepared study chicken) and 1.33 (dry study chicken). Given that limitations of the PER in rats are well recognized (33), our results suggest that broiler chicken efficiencies have similar protein quality assessment utility as results obtained from rats.

Hemoglobin concentrations were not significantly different in the prepared study between all groups, but the CSB+ group's hepatic iron concentrations were lower than the novel formulated FBF groups. In the dry study, the CSB+ group had significantly increased hemoglobin and decreased hepatic iron concentrations compared with all novel formulated FBFs. The CSB+ group's higher hemoglobin, but lower hepatic iron, concentrations may have been due to increased oxidative stress, which increased the tissue demand for oxygen, and thus increased iron mobilization as hemoglobin. This would be consistent with low-temperature-induced oxidative stress increasing hemoglobin concentrations in broiler chickens (34). In our previous rat study, the CSB+ group had the highest hemoglobin and hepatic iron concentrations, which was not observed in these chicken studies; inhibited growth rates of rats may explain the difference in outcomes between the different species (18). In both studies, the O-SC+SPI group had significantly increased hepatic iron concentrations compared with other novel formulated FBF groups, except for the O-SC and SC+SPI groups. This was most likely due to markedly slower growth rates,

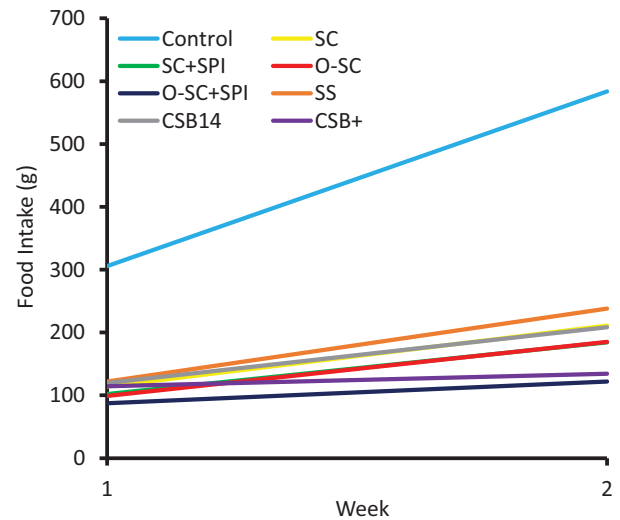


**TABLE 6** Dry FBF study food intake, body weight, food efficiencies, hemoglobin, and hepatic iron<sup>1</sup>

	Control (n = 23)	SC (n = 24)	SC+SPI (n = 24)	O-SC (n = 24)	O-SC+SPI (n = 24)	SS (n = 24)	CSB14 (n = 24)	CSB+ (n = 24)
Total food intake, g	889.5 ± 3.3 <sup>a</sup>	325.1 ± 2.5 <sup>b</sup>	286.5 ± 5.5 <sup>c</sup>	284.1 ± 2.7 <sup>c</sup>	209.4 ± 3.0 <sup>d</sup>	360.0 ± 6.4 <sup>e</sup>	327.9 ± 6.1 <sup>b,e</sup>	248.9 ± 2.4 <sup>f</sup>
Total weight gained, g	575.3 ± 2.9 <sup>a</sup>	141.4 ± 1.0 <sup>b,d</sup>	125.9 ± 3.4 <sup>b</sup>	126.9 ± 2.8 <sup>b</sup>	75.6 ± 1.7 <sup>c</sup>	163.1 ± 4.0 <sup>d</sup>	131.2 ± 4.6 <sup>b</sup>	79.0 ± 2.2 <sup>c</sup>
Final body weight, g	663.6 ± 2.9 <sup>a</sup>	229.7 ± 1.1 <sup>b,d</sup>	212.8 ± 3.6 <sup>b</sup>	215.1 ± 2.6 <sup>b</sup>	163.0 ± 2.1 <sup>c</sup>	251.3 ± 4.2 <sup>d</sup>	221.3 ± 4.8 <sup>b</sup>	167.4 ± 2.4 <sup>c</sup>
Caloric efficiency, g/kcal × 1000	49.3 ± 1.3 <sup>a,2</sup>	17.6 ± 0.2 <sup>b</sup>	17.5 ± 0.1 <sup>b</sup>	18.4 ± 0.2 <sup>b</sup>	14.9 ± 0.1 <sup>c,d</sup>	18.3 ± 0.2 <sup>b</sup>	15.5 ± 0.3 <sup>c</sup>	13.7 ± 0.3 <sup>d</sup>
Protein efficiency, g/g × 100	51.6 ± 1.3 <sup>a,2</sup>	38.0 ± 0.3 <sup>b,c</sup>	37.0 ± 0.3 <sup>b,e</sup>	41.5 ± 0.5 <sup>c</sup>	32.7 ± 0.3 <sup>d</sup>	38.7 ± 0.5 <sup>b,c</sup>	36.2 ± 0.8 <sup>b,d</sup>	33.4 ± 0.6 <sup>d,e</sup>
Hemoglobin, g/dL	9.2 ± 0.9 <sup>a</sup>	9.4 ± 0.1 <sup>a</sup>	8.5 ± 0.6 <sup>a</sup>	8.8 ± 0.3 <sup>a</sup>	8.6 ± 0.3 <sup>a</sup>	9.6 ± 0.4 <sup>a</sup>	9.5 ± 0.5 <sup>a</sup>	12.4 ± 0.4 <sup>b</sup>
Hepatic iron, µg/g	7.9 ± 1.1 <sup>a</sup>	17.6 ± 3.2 <sup>b</sup>	27.1 ± 4.5 <sup>c,d</sup>	21.9 ± 2.0 <sup>b,c</sup>	32.9 ± 4.1 <sup>c</sup>	20.7 ± 2.1 <sup>b,d</sup>	16.4 ± 4.0 <sup>b</sup>	8.0 ± 0.7 <sup>a</sup>

<sup>1</sup>Values are means ± SEMs. Means without a common superscript letter differ,  $P < 0.05$ . CSB+, corn-soy blend plus; CSB14, corn-soy blend 14; FBF, fortified blended food; N-SC, nonextruded sorghum-cowpea; O-SC, overprocessed sorghum-cowpea; SC, sorghum-cowpea; SPI, soy protein isolate; SS, sorghum-soy.

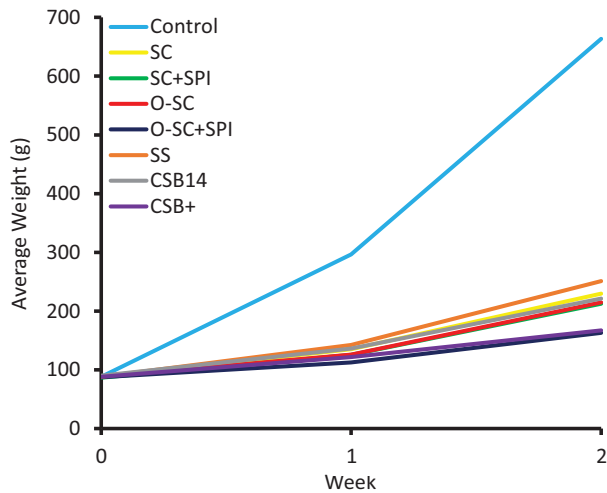
<sup>2</sup>Based on label protein values rather than analyzed protein values.



**FIGURE 3** Dry FBF study mean weekly food intake ( $n = 24$ ; control:  $n = 23$ ). Total food intake: control group compared with all FBF groups ( $P < 0.05$ ); SS group compared with all groups except for CSB14 ( $P < 0.05$ ); CSB+ group compared with all groups ( $P < 0.05$ ); O-SC+SPI group compared with all groups ( $P < 0.05$ ); CSB14 and SC groups compared with SC+SPI and O-SC groups ( $P < 0.05$ ). CSB+, corn-soy blend plus; CSB14, corn-soy blend 14; FBF, fortified blended food; O-SC, overprocessed sorghum-cowpea; SC, sorghum-cowpea; SPI, soy protein isolate; SS, sorghum-soy.

and thus less demand for iron; similar outcomes were observed in the CSB+ group in our previous rat study (18). The SS group had increased growth but similar hepatic iron concentration compared with other novel formulated FBF groups, which is different than the decreased hepatic iron concentrations in rats (18). The CSB+ group's significantly increased hepatic retinol concentration was most likely due to higher vitamin A levels in the diet as well as slower growth rates than in the other groups. Similar but less significant outcomes were seen in our previous rat study (18).

In the prepared study, N-SC, SC, CSB14, and SS groups had gait issues. SC and N-SC groups had lower BMD measurements, but they were similar to those in the CSB+ and O-SC+SPI groups, which did not have gait issues. The primary factors cited for occurrence of leg disorders, including locomotion issues represented by high gait score, are rapid growth and weight gain and decreased locomotor activity (35–37). However, in the prepared study, the impacted chickens had slow growth and weight gain and large pens with food and water sources spread apart that required locomotion. In addition, the control group had significantly increased growth and weight gain, which were more comparable to commercial broilers, but no gait issues. BMD is affected by age, sex, type of production, diet, and management (38); and tibia BMD has been cited to linearly increase with increasing levels of nonphytate phosphorus and a constant calcium content at 1.0% of diet (39). All FBFs did not meet NRC calcium (1000 mg/100 g) or phosphorus (450 mg/100 g) requirements, and both mineral levels were the same across all new FBFs. It is not clear what caused gait issues in some FBF groups and not others; however, low calcium and phosphorus content in FBFs may have contributed.



**FIGURE 4** Dry FBF study mean weekly body weights ( $n = 24$ ; control:  $n = 23$ ). Body weight gain: control group compared with all FBF groups ( $P < 0.05$ ); SS group compared with all groups except for SC ( $P < 0.05$ ); CSB+ group compared with all groups ( $P < 0.05$ ); O-SC+SPI group compared with all groups ( $P < 0.05$ ). CSB+, corn-soy blend plus; CSB14, corn-soy blend 14; FBF, fortified blended food; O-SC, overprocessed sorghum-cowpea; SC, sorghum-cowpea; SPI, soy protein isolate; SS, sorghum-soy.

### Limitations

The locomotion impairment (gait issues) observed in some chickens and the potential coccidiosis infection and corresponding treatment in the prepared study may have affected food intake and activity, and thus had an effect on overall outcomes. Both of these studies were short in duration; therefore, results from this rapid growth period have to be translated with caution to humans. FBFs are normally meant to be consumed along with other foods; therefore, complementary feeding of FBFs might result in different outcomes than observed in this study.

### Conclusions

In conclusion, sorghum and cowpea FBFs performed similarly to corn and soy FBFs, suggesting that these commodities are suitable replacements for corn and soy. SPI (SC+SPI) was an effective alternative to WPC (SC) in certain formulations, suggesting that SPI can be a less-expensive protein source in FBFs. However, O-SC+SPI resulted in poorer outcomes than other FBFs, suggesting that there may be limitations to the ability of SPI to complement cowpea protein in FBFs. Reformulated, overprocessed FBFs with WPC can be considered a less-expensive FBF option. Surprisingly, N-SC was equally efficacious as extruded SC, suggesting that extrusion is not necessary to improve FBF protein and iron bioavailability in that formulation. However, it should be noted that N-SC did not meet viscosity requirements, preventing it from being a viable FBF. Overall, new FBFs, with the exception of O-SC+SPI, resulted in improved growth and hepatic iron outcomes compared with CSB+, suggesting that they are of higher nutritional quality. Further research is needed to refine and optimize FBF formulations.

### Acknowledgments

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