Impact of feeding modified soy protein concentrate in the starter phase on growth performance and gastrointestinal responses in broiler chickens through to day 42 of age

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ABSTRACT Growth performance and physiological responses of feeding modified soy protein concentrate (**MSPC**, 72% CP) in the starter phase were investigated. A total of 1,216 d old male Ross x Ross 708 broiler chicks were placed in 32 floor pens based on BW, fed one of 4 (n = 8) corn-soybean meal-based diets formulated with 0, 7.7, 10.0 or 12.5% MSPC for 10 d and transitioned to common diets to d 42. Feed intake, BW, and mortality were measured. Samples of birds were bled on d 10 for plasma uric acid (**PUA**) and subsequently necropsied for organs weight and samples of pancreatic tissues for enzyme activity, jejunal tissues for enzyme activity and histomorphology and ceca digesta for microbial activity. Litter moisture was determined on d 36 and 42 and sample of birds were necropsied on d 42 for breast yield and ceca digesta sample for microbial activity. Feeding MSPC linearly ($P < 0.001$) increased starter growth performance. Overall (d 0−42), MSPC linearly ($P = 0.05$) improved FCR; The FCR was 1.566, 1.535, 1.488 and 1.527 for 0.0, 7.7, 10.0, and 12.5% MSPC, respectively. Feeding MSPC linearly ($P \leq 0.04$) increased breast yield and decreased small intestine length, gizzard digesta pH, and PUA. Breast yield was 230, 238, 246, and 252 g/kg BW for 0.0, 7.7, 10.0, and 12.5% MSPC, respectively. Pancreatic and jejunal chymotrypsin and trypsin activities and histomorphology were not ($P > 0.10$) influenced by the diets. On d 10, MSPC linearly ($P < 0.05$) reduced ceca digesta abundance of *Ruminococcaceae*, *E. Coli*, and *Clostridium* but increased abundance of *Bifidobacterium* and the ratio of Lactobacilli and *E. Coli*. Birds fed MSPC showed linear ($P = 0.01$) increase in abundance of *Bifidobacterium* on d 42. Feeding MSPC linearly increased ceca digesta acetic ($P = 0.01$) and reduced propionic ($P = 0.048$), and iso butyric ($P = 0.003$) in 10 d old broiler chicken. In conclusion, up to 12.5% MSPC inclusion in the starter phase increased growth performance through to d 42 linked to enhanced gut health through reduction of enteric pathogens.

Key words: broiler chicken, gastrointestinal physiology, growth performance, soy protein concentrate

INTRODUCTION

Soybean products are the major source of protein and amino acids in poultry diets around the world (NRC, 1994). However, factors such as variation in processing (oil extraction and heating), residual trypsin inhibitors, chemical constituents complexes, and allergenic proteins (glycinin and ß-conglycinin) among others have been implicated in blunting the nutritive value of soy products (Ravindran et al., 2014; Kiarie and Mills, 2019;

Kiarie et al., 2020). Newly hatched broiler chicks are particularly challenged by the components in soybean meal (**SBM**) (Batal and Parsons, 2002). This is because the gastrointestinal tract of neonatal monogastric animal is poorly developed in terms of (1) immature immune system, (2) limited endogenous enzyme secretory capacity, (3) sensitivity to allergenic feed components, and (4) unstable gut microbiota (Lindemann et al., 1986; Gilbert et al., 2007; Pluske, 2016; Kim et al., 2020). Soy protein concentrate (**SPC**) is produced by aqueous ethanol extraction at temperatures greater than 50°C resulting in deactivation of allergenic proteins and removal of water-soluble carbohydrates (Sissons et al., 1982). The SPC contains at least 65% crude protein and is well tolerated by animals with immature digestive tract (Li et al., 1991; Batal and Parsons, 2003; Lenehan et al., 2007; NRC, 2012; Vasconcelos et al., 2017).

Indeed, feeding SPC increased energy and amino acids utilization linked to enhanced pancreatic trypsin activity and indices of small intestine digestive function in broiler chickens (Batal and Parsons, 2003; Vasconcelos et al., 2017). In addition, further processed soy products have relatively low potassium concentration relative to SBM, an attribute that could impact litter quality (Swiatkiewicz et al., 2017).

Soy protein concentrate has been evaluated as a partial or complete replacement of regular SBM or other protein sources in practical starter feeding programs of monogastric animals, but growth performance responses have been variable. For example, inclusion of between 9.4 and 26% SPC in iso-nitrogenous and iso-caloric corn-SBM diet indicated that broiler fed the highest amount of SCP had lower feed intake and weight gain at the end of the 6 wk trial (Leske et al., 1995). Incorporation of 3 to 9% SPC in prestarter and starter diets (d 0−21) had no impact on broiler chicken growth performance through to d 40 of age (Vasconcelos et al., 2017). Broiler chickens were allocated 300 g feed for 12 d posthatch period; the control group was fed a corn-SBM diet and treated groups fed various amount (140, 160 and 180 g/bird) of a corn-SBM with 5% SPC diet (Zakaria and Ata, 2020). The data indicated that birds fed the highest amount of corn-SBM-5% SPC had lower d 35 body weight than control. Laying hens fed 18.7% SPC had lower feed intake and egg weight than control hens (Leske et al., 1995). Complete replacement of 40% regular SBM with 28% of 2 types of SPC (differing in processing) reduced growth and feed intake in piglets relative to piglets fed SBM (Lenehan et al., 2007). In contrast, improved growth performance was observed in piglets fed 25% SPC compared with piglets fed SBM from d 0 to 14 postweaning (Sohn et al., 1994).

Processing method can affect nutritive value of SPC; for example, comparative experimentation of protein efficiency ratios in broiler chickens indicated that commercially available SPC products were suboptimal and required further processing (Leske et al., 1995). Ethanol-extracted and moist-extruded SPC was demonstrated to have higher CP solubility and superior piglet growth performance than ethanol-extracted and dry heat-treated SPC (Lenehan et al., 2007). Majority of SBM fiber is insoluble and concentration in conventional SPC products is high (∼>8% neutral detergent fiber)(NRC, 2012). A new processing method based on incorporation of pH reduction step in SPC processing has been shown to produce a modified SPC (**MSPC**) with higher CP solubility and low concentration of fibrous components and antinutritional factors (Markedal et al., 2019). The combination of low pH and high (60°C) temperature in the processing of MSPC resulted in reduction of protein and nonprotein components complexes, e.g. phytate, phenolics, and saponins (Markedal et al., 2019). Furthermore, the soy products are also rich sources of bioactives with antimicrobial effects (Dhayakaran et al., 2016) that could be enhanced through further processing. Thus, application of further processed SPC in starter feeding programs may bolster establishment and maintenance of a healthy and functional gastrointestinal tract in young animals. A recent investigation of incorporating up to 12% of MSPC in broiler starter program (d 0−10) showed linear improvement of d 42 body weight and carcass yield and enhanced immune status (Zhang et al., 2021). However, more data were required to validate these recent findings and record the impact on indices on gastrointestinal health. Therefore, the objective of the present study was to evaluate the effects of incorporating MSPC in starter feeding program (from hatch to d 10) on growth performance, gastrointestinal physiology, and litter moisture of broiler chickens through to 42 d of age.

MATERIALS AND METHODS

Animal care and use protocols (#3521) were approved by the University of Guelph Animal Care and Use Committee and birds were cared for in accordance with the Canadian Council on Animal Care guidelines (CCAC, 2009).

Test Ingredient and Dietary Treatments

The sample of MSPC was procured from Triple A (Hornsyld, Denmark) and guaranteed chemical composition is presented in Table 1. The conventional SBM, corn, and wheat were procured from local feed mill (Floradale Feed Mill Ltd., Floradale, ON, Canada). The coefficients for standardized ileal digestibility of amino acids and AMEn for SPC (assumed to be equivalent to MSPC) and wheat were obtained from Evonik Aminodat 5.0 (Evonik industries, Essen,

Table 1. Chemical composition of modified soy protein concentrate[1], *as fed basis.*

Item	Amount
Dry matter, %	93.0
Crude protein, %	72.0
Crude fat, %	2.60
Crude fiber, %	3.60
Acid detergent fiber, %	1.90
Neutral detergent fiber, %	5.20
Total dietary fiber, %	13.0
Ash, %	3.30
Calcium, %	0.20
Total phosphorous, %	0.50
Available phosphorous, %	0.17
Sodium, %	0.50
AMEn, kcal/kg	2,930.14
Total Arg, %	5.10
Total Lys, %	4.30
Total Met, %	1.00
Total Cys, %	1.00
Total Met + Cys, %	2.00
Total Trp, %	1.00
Total His, %	1.90
Total Leu, %	5.70
Total Ile, %	3.40
Total Phe, %	3.90
Total Thr, %	2.80
Total Val, %	3.50
Total Tyr, %	2.80

[1]AX3 Advanced, TripleA A/S, Hornsyld, Denmark.

Table 2. Composition of the experimental diets, *as fed basis.*

MSPC[1], %	Starter d 0−10				Grower d 11−21	Finisher d 22−42
	0.0	7.7	10.0	12.5	0.0	0.0
Ingredient, %						
Corn	44.2	52.1	54.5	56.8	49.0	53.0
Wheat	10.0	10.0	10.0	10.0	10.0	10.0
Soybean meal 46% CP	37.7	24.2	20.1	15.8	32.5	28.7
L-Lysine HCl	0.23	0.26	0.27	0.28	0.23	0.21
DL-Methionine	0.31	0.30	0.30	0.30	0.29	0.24
L-Threonine	0.09	0.10	0.11	0.11	0.09	0.08
Limestone fine	0.96	0.98	0.98	0.99	0.72	0.71
Monocalcium phosphate	1.96	2.01	2.02	2.02	1.73	1.49
Salt	0.27	0.25	0.24	0.22	0.27	0.27
Sodium bicarbonate	0.12	0.02	0.00	0.00	0.13	0.13
Vitamin and mineral premix*	1.00	1.00	1.00	1.00	1.00	1.00
Soy oil	3.30	1.14	0.50	0.00	4.06	4.19
MSPC, 72% CP	0.00	7.70	10.0	12.5	0.00	0.00
Calculated provisions						
AMEn, kcal/kg	2,950	2,950	2,950	2,959	3,050	3,100
Crude protein, %	23.0	23.0	23.0	23.0	21.5	19.5
Crude fat, %	5.17	3.54	3.06	2.73	6.10	6.39
Linoleic acid, %	2.83	1.88	1.60	1.39	3.29	3.42
Crude fiber, %	2.93	2.79	2.75	2.71	2.81	2.74
Calcium, %	0.86	0.86	0.86	0.86	0.72	0.67
Available phosphorous, %	0.45	0.45	0.45	0.45	0.40	0.35
Sodium, %	0.16	0.16	0.16	0.16	0.16	0.16
Chloride, %	0.23	0.23	0.23	0.23	0.23	0.23
SID Arg, %	1.38	1.33	1.31	1.30	1.24	1.13
SID Lys, %	1.22	1.22	1.22	1.22	1.10	1.00
SID Met, %	0.61	0.61	0.61	0.60	0.56	0.50
SID Met + Cys, %	0.90	0.90	0.90	0.90	0.84	0.76
SID Trp, %	0.26	0.26	0.25	0.25	0.24	0.22
SID Ile, %	0.88	0.86	0.85	0.85	0.79	0.73
SID Thr, %	0.79	0.79	0.79	0.79	0.73	0.66
SID Val, %	0.92	0.92	0.92	0.93	0.83	0.77

[1]Modified soy protein concentrate.[2]Provided per kilogram of diet: vitamin A, 8,800.0 IU; vitamin D3, 3,300.0 IU; vitamin E, 40.0 IU; vitamin B12, 12.0 mg; vitamin K3, 3.3 mg; niacin, 50.0 mg; choline, 1,200.0 mg; folic acid, 1.0 mg; biotin, 0.22 mg; pyridoxine, 3.3 mg; thiamine, 4.0 mg; calcium pantothenic acid, 15.0 mg; riboflavin, 8.0 mg; manganese, 70.0 mg; zinc, 70.0 mg; iron, 60.0 mg; iodine, 1.0 mg; copper, 10 mg; and selenium, 0.3 mg (DSM Nutritional Products Canada Inc., Ayr, ON, Canada).

North Rhine-Westphalia, Germany) and for corn and SBM from Leung and Kiarie (2020). Four complete starter diets were formulated: control or control plus MSPC (at 7.7, 10 or 12.5% inclusion). Birds transitioned to common grower (d 11 to 24) and finisher (d 25 to 42) diets. All diets (Table 2) were formulated to meet the specification of Ross 708 (Aviagen, 2014). The starter feed was prepared in fine crumble, grower in course crumble, and finisher in pellet form. The temperature of the processing condition was 60−65°C and steam pressure of 30 psi. The feed was retained in the conditioner for 30 S.

Birds and Housing

A total of 1,216 d old (male) Ross x Ross 708 broiler chicks were procured from a commercial hatchery (Maple Leaf Foods, New Hamburg, ON, Canada), weighed and allocated to 32 floor pens (38 birds per pen) bedded with fresh wood shavings. Each pen measured 160 × 238 cm, had solid plastic white walls, and equipped with a round pan feeder (diameter = 33.75 cm) and 5 nipple drinkers. The room temperature was set at 32°C on d 0 and gradually decreased to 27°C by d 17. Birds were exposed to fluorescent lighting in a 23 h of light (20+ lux) for the

first 4 d and then a 16 light: 8 dark (10−15 lux) light cycle. Birds had free access to water via nipples and feed via feeders throughout the experiment.

Experimental Procedures, Measurements, and Sampling

The 4 diets were allocated in a completely randomized design based on pen average body weight (**BW**) on d 0 to give 8 replicates per diet. Birds had free access to feed and water; the BW and feed intake (**FI**) were recorded on d 10, 24, and 42, number and BW of dead birds were recorded. On d 10, 12 birds per pen were randomly selected for necropsy (Leung et al., 2019). Briefly, individual bird was weighed, bled via cardiac puncture (2 birds only), and euthanized via cervical dislocation. The pancreas, liver, spleen, and bursa were excised, gently blotted dry with paper towel, weighed, and discarded with exception of pancreas which was placed on ice and transported to the laboratory for storage at -80°C until required for analyses. Jejunal tissues were obtained from the bled birds as follows. Two portions of jejunal tissues (∼0.5 cm) were excised at 10 cm anterior to Meckel's diverticulum. One portion of jejunal tissue was stored in vials, placed on ice and stored at -80°C until required for

analyses. The other portion of jejunum was preserved in buffered formalin for histomorphology. Digesta from gizzard, duodenum, jejunum, and ileum luminal content were pooled on a pen basis into one bag and pH was measured immediately using Fisher Scientific Accumet AB 150 pH meter (Fisher Scientific, Toronto, ON, Canada) standardized with certified pH 4.0, 7.0, and 10.0 buffer solution (Fisher Scientific, Toronto, ON, Canada). The weight and length of small intestine segments (duodenum, jejunum, and ileum) were recorded for all birds. The ceca digesta was collected into one sterile bag on pen basis, mixed thoroughly, and separated in 2 portions. One portion was placed in biofreeze kits (Alimetric Diagnostics Ltd., Espoo, Finland) for the determination of microbiota population. The other portion was stored at -20°C for measuring concentration of short chain fatty acids (**SCFA**) (Leung et al., 2018). On d 36 and d 42, litter samples were collected from the center and midway between center and 4 corners of each pen (Leung et al., 2018) for litter moisture content determination. On d 42, 2 birds per pen were sacrificed to measure breast yield and to access ceca samples for microbial population and SCFA concentration as described for d 10 sampling.

Sample Processing and Analyses

Samples of MSPC along with SBM samples were submitted for analyses for trypsin inhibitors and oligosaccharides at commercial laboratory (Eurofins Steins Laboratorium A/S, Vejen, Denmark). Additional MSPC and SBM samples were submitted for glycinin, ß-conglycinin, and lectin analyses at commercial laboratory (Ducares B.V., Utrecht, The Netherlands). Diet samples were finely ground and submitted to a commercial lab (SGS Canada, Guelph) for dry matter, crude protein, crude fat, starch, and minerals analyses. Gross energy was determined using a bomb calorimeter (IKA Calorimeter System C 6000; IKA Works, Wilmington, NC). The diet pH was determined by suspending 0.5 gram of ground sample in 50 mL of deionized water under continuous stirring using a stir plate at room temperature and the pH of the solution was recorded after 3 min stabilization. Titrations were then performed by addition of acid (0.1 N HC1) until the pH reached 4. Total volume of acid added to each sample was recorded and then multiplied by the molarity to calculate titratable acidity. Titratable acidity was the milliequivalents of acid required to lower sample pH to 4. Acid binding capacity was calculated by dividing titratable acidity by the total change in pH units (Jasaitis et al., 1987; Lawlor et al., 2005).

Fixed jejunal tissues were cut into a longitudinal cross section and embedded in paraffin wax. The tissues were then sectioned (5 μm) and stained with hematoxylin and eosin for morphological measurements. A total of 5 villous-crypt structures were measured with a calibrated micrometer for each tissue using a Leica DMR microscope (Leica Microsystems, Wetzlay, Germany). Villous height and crypt depth ratio (VH:CD) were calculated.

Frozen pancreas and jejunal samples were ground using mortar and pestle in liquid nitrogen. For protein extraction, the jejunum free of digesta and pancreas samples (0.12 $\pm$ 0.022 g) were placed into free-standing microcentrifuge tube (02-682-558, Thermo Fisher, Waltham MA) followed by addition of Tissue Protein Extraction Reagent (**T-PER**; sample weight × 15; 78510, Thermo Fisher, Waltham MA) based on the described method by Akbari Moghaddam Kakhki et al. (2020). Then, 0.1 $\pm$ 0.01 acid-washed glass beads ($\leq$ 106 μm; G4649-100G, Sigma Aldrich, St. Louis, MO) were added and followed by homogenization with a bead mill for 2 cycles of 150-sec at 3 m/s (15-340-163; Fisher Brand bead mill-24, Thermo Fisher, Waltham MA). Homogenized samples were then centrifuged at 10,000 × g for 15 min at 4°C (Akbari Moghaddam Kakhki et al., 2020). Supernatants were analyzed for protein concentration based on the described method of Smith et al. (1985) using a Pierce BCA protein assay kit (23225, Thermo Fisher, Waltham MA) and kept at -80°C for further analyses. The concentration of total protease, trypsin, and chymotrypsin was measured in duplicate using ELISA kits that followed the recommended assay procedures (Total protease: EK19012; trypsin: EK18729; chymotrypsin: EK18728, Signalway Antibody, College Park, Maryland, USA.). The values were then expressed as a ratio to total protein concentration.

Microbial analyses were conducted using quantitative real-time polymerase chain reaction (**qPCR**) method (Amit-Romach et al., 2004; Agyekum et al., 2016) at a commercial laboratory (Alimetric Diagnostics Ltd., Espoo, Finland). Briefly, the samples were washed to remove solid particles and complex polysaccharides to improve subsequent DNA purification and the downstream qPCR applications. The liquid phase was subjected to differential centrifugation for collecting the bacterial cells. The cell microbial cell walls were disrupted, and the chromosomal DNA was quantitatively extracted and quantified using a Nanodrop 2000 spectrophotometer (ThermoScientific, Wilmington, DE, USA). The qPCR of microbial analyses were conducted with 16S rRNA gene targeted DistaMap analysis panel using SYBR Green chemistry method (Tajadini et al., 2014). Briefly, the method is based on the detection and quantification of a fluorescent reporter signal that increases in direct proportion to the amount of PCR product in the reaction. By recording the amount of fluorescence emission at each cycle, it is possible to monitor the PCR reaction during exponential phase where the first significant increase in the amount of PCR product correlates to the initial amount of target template. The present analyses targeted abundance of total bacteria, *Lachnospiraceae, Ruminococcaceae*, Bacteroides, *Bifidobacterium, Clostridium sensu stricto, Lactobacillus* and *Escherichia coli*. The primers for the target microbiota were previously reported (Kettunen et al., 2017). The data was reported as number of copies of 16S RNA per gram of sample.

The concentration of SCFA (lactic, acetic, propionic, iso-butyric, butyric, iso-valeric, and valeric) in the ceca

Table 3. Analyzed chemical composition of the experimental diets, as fed basis.

MSPC[1], %	Starter d 0−10				Grower d 11−21	Finisher d 22−42
	0.0	7.7	10.0	12.5		
Dry matter, %	87.5	86.8	86.9	86.9	88.2	88.3
Crude protein, %	22.3	22.8	22.7	22.8	20.5	19.3
Gross energy, kcal/kg	3,390	3,385	3,376	3,372	3,585	3,597
Crude fat, %	4.96	3.95	2.91	2.47	5.68	6.59
Starch, %	32.0	36.5	39.4	42.1	32.7	35.5
Neutral detergent fiber, %	7.91	7.53	7.48	7.40	7.87	8.23
Calcium, %	0.84	0.77	0.80	0.83	0.63	0.52
Phosphorus%	0.77	0.74	0.79	0.77	0.75	0.60
Potassium, %	1.02	0.80	0.76	0.71	1.00	0.76
Magnesium, %	0.18	0.16	0.15	0.14	0.18	0.14
Sodium, %	0.15	0.16	0.14	0.11	0.13	0.13
Acid binding capacity (ABC), mEq per kg	200	160	140	120	207	207

[1]Modified soy protein concentrate.

digesta was assayed according to Leung et al. (2018). Briefly, the digesta samples were thawed and approximately 0.1 g was resuspended with 1 mL 0.005N H_2SO_4 (1:10, wt/vol) in a microcentrifuge tube. The tube was vortexed vigorously until sample was completely dissolved, centrifuged at $11,000 \times g$ for 15 min and 400 μL supernatant transferred into a high- pressure liquid chromatography (**HPLC**) vial and 400μL of 0.005N H_2SO_4 buffer added. The resulting digesta fluid was then assayed for SCFA using HPLC (Hewlett Packard 1100, Germany) with Rezex ROA-Organic Acid LC column, 300×7.8mm from Phenomenex and Refractive Index detector at 40°C (Agilent 1260 Infinity RID from Agilent Technologies, Germany). Twenty μL of the resulting sample was injected into the column, with a column temperature of 60°C and mobile phase of 0.005 N H_2SO_4 buffer at 0.5 mL/min isocratic for 35 min. The detector was heated to 40°C. Litter moisture was determined by drying the samples at 60°C to constant weight.

Calculations and Statistical Analyses

Mortality corrected FCR, ADG, and ADFI for d 0 −10; d 11−21; d 22-42, and d 0−42 were calculated. The breast yield was standardized for BW. The microbial data were log transformed before statistical analyses. The data were subjected to statistical analyses using SAS 9.4 with pen as the experimental unit with diet as fixed factor in the model. Coefficients for linear and quadratic effects of MSPC were generated using IML procedures of SAS. An α level of $P \leq 0.05$ was used as the criteria for assessing for statistical significance and trends ($0.05 < P \leq 0.10$) were discussed.

RESULTS

The concentration of trypsin inhibitor activity, stachyose, and raffinose in SBM was 4,990 TIU/g, 4.71 g/100g, and 0.75 g/100g, respectively. The corresponding values for MSPC were 3,260 TIU/g, 0.31 g/100g, and 0.10 g/100g, respectively. The concentration of glycinin, ß-conglycinin, and lectin in SBM sample was 80,000, 20,000, and 70 ppm, respectively. The corresponding values for MSPC was 400, 2, and <1 ppm, respectively. The analyzed chemical composition and acid binding capacity data in the experimental starter diets and common grower and finisher diets is presented in Table 3.

In the starter phase (d 0−10), MSPC linearly ($P <$ 0.001) increased growth, feed intake and FCR (Table 4). As a result, chicks fed 7.7, 10.0, and 12.5% MSPC were 21.2, 38.1, and 41.70 g, respectively heavier ($P < 0.001$) than chickens fed control diet at the end of the starter phase. In the overall (d 0-42), MSPC linearly increased FCR corrected for mortality by 1.97, 4.99, and 2.48% for 7.7, 10.0, and 12.5% MSPC, respectively. Feeding MSPC resulted in tendency ($P = 0.09$) for heavier final BW. Specifically, birds fed 7.7, 10.0, and 12.5% MSPC, respectively were 90.0, 105.0, and 77.0g heavier than birds fed the control diet during the starter phase. Birds fed MSPC exhibited heavier ($P = 0.004$) breast. The breast was 230, 238, 246, and 252 g/kg BW for birds fed 0.0, 7.7, 10.0, and 12.5% MSPC, respectively (Table 4).

The diets had no effects ($P > 0.10$) on liver, pancreas, spleen, and bursa weight (Table 5). The pancreas was 4.65, 4.64, 4.30, and 4.49 g/kg of BW for 0, 7.7, 10.0, and 12.5% MSPC, respectively. There was a linear decrease in small intestine weight ($P = 0.08$) and length ($P = 0.001$) as MSPC increased in the diet. Jejunal histomorphology was not ($P > 0.10$) affected by the MSPC in 10-d old broiler (Table 5). The diets resulted in linear and nonlinear decrease ($P = 0.001$) on gizzard pH, however, there were no effects on duodenum, jejunum, and ileum pH (Table 6). Pancreatic and jejunal mucosal chymotrypsin and trypsin activities were not ($P > 0.10$) influenced by the diets (Table 6). There were a linear ($P = 0.04$) reduction on plasma uric acid (**PUA**) with increase of MSPC. The PUA concentration was 365, 262, 257, and 248 μmol/L for 0, 7.7, 10.0, and 12.5% MSPC, respectively.

Feeding MSPC had linear ($P < 0.001$) decrease in the abundance of *Ruminococcaceae*, *Clostridium sensu stricto*, and *E. Coli* (Table 7) in ceca digesta of 10-d old broiler chickens. A linear increase ($P < 0.001$) in the

Table 4. Growth performance and breast yield responses of broiler chickens fed starter diets supplemented with different doses of modified soy protein concentrate (MSPC).

| | | | | | | P value | |
MSPC, % Item	0.0	7.7	10.0	12.5	SEM	Linear	Quadratic
Body weight, g/bird							
d 0	47.13	47.06	47.03	47.10	0.050	-	-
d 10	230.9	252.1	269.0	272.6	4.030	<0.001	0.667
d 21	1,159	1,169	1,149	1,159	10.95	0.758	0.594
d 42	2,883	2,973	2,988	2,960	40.22	0.094	0.336
ADG, g/bird/d							
d 0−10	18.38	20.49	22.21	22.54	0.406	<0.001	0.673
d 11−21	83.49	82.55	78.90	79.68	1.068	0.004	0.506
d 22−42	82.10	85.89	87.61	85.79	1.948	0.084	0.418
d 0−42	67.51	69.65	70.05	69.36	0.956	0.091	0.333
ADFI, g/bird/d							
d 0−10	23.29	25.06	25.63	26.51	0.297	<0.001	0.653
d 11−21	111.2	116.1	106.1	110.1	1.754	0.214	0.061
d 22−42	140.1	141.1	140.5	141.4	1.629	0.625	0.977
d 0−42	105.7	106.9	104.2	105.9	1.050	0.727	0.400
FCR, g/g							
d 0-10	1.267	1.223	1.154	1.176	0.015	<0.001	0.826
d 11−21	1.332	1.406	1.345	1.382	0.027	0.493	0.472
d 22−42	1.706	1.643	1.604	1.648	0.040	0.147	0.381
d 0−42	1.566	1.535	1.488	1.527	0.023	0.047	0.333
Breast, g/kg BW	230.1	237.7	245.6	251.2	3.900	0.004	0.336

Data are least squares means of 8 replicate pens per treatment.

abundance of *Bifidobacterium* and *lactobacilli* and *E. Coli* ratio. The effects of MSPC on ceca microbiota in 42-d old broiler chickens was not as pronounced as in starter phase (Table 7), however, birds fed MSPC showed a linear increase ($P = 0.01$) in abundance of *Bifidobacterium*. The ceca digesta of 10-d old broiler chickens exhibited linear ($P = 0.001$) and quadratic ($P = 0.03$) increase in the concentration of acetic acid (Table 8). Feeding MSPC linearly reduced propionic ($P = 0.05$), iso-butyric ($P = 0.003$), and iso-valeric ($P = 0.08$) acid. There was tendency for linear ($P = 0.09$) and quadratic ($P = 0.07$) increase in ceca digesta acetic acid concentration in the ceca digesta of 10-d old broilers. Feeding MSPC in starter phase resulted in linear ($P = 0.01$) increase in ceca digesta butyric acid concentration in 42-d old broiler chickens (Table 8). There was no ($P > 0.10$) diet effect on litter moisture on d 36 or 42 (Table 8).

DISCUSSION

Given the importance of the nutritive value of soy products in enhancing animal protein production efficiency, several pretreatment and in-feed approaches have been applied to enhance utilization (NRC, 1994; NRC, 2012). Pretreatment through enzymatic and microbial treatments has been demonstrated to reduce the concentration of oligosaccharides and allergenic proteins with tremendous improvement in SBM utilization in monogastric animals (Cervantes-Pahm and Stein, 2010; Kim et al., 2018; Lu et al., 2018; Masey O'Neill et al., 2018; Yáñez et al., 2019). Application of exogenous feed enzymes has also been shown to improve amino acids and energy utilization in SBM products (Ayoade et al., 2012; Woyengo et al., 2016; Kiarie et al., 2020). Application of SPC has been studied in feeding programs for the young animals with immature

Table 5. Organ weights, small intestine length and jejunal histomorphology in 10-d old broiler chickens fed starter diets supplemented with different doses of modified soy protein concentrate.

| | | | | | | P value | |
MSPC, % Item	0.0	7.7	10.0	12.5	SEM	Linear	Quadratic
Organ weight, g/kg BW							
Liver	35.4	37.0	36.8	37.9	1.086	0.136	0.978
Pancreas	4.65	4.64	4.30	4.49	0.169	0.114	0.969
Spleen	0.85	0.84	0.88	0.76	0.051	0.454	0.331
Bursa	1.99	1.89	1.85	1.79	0.138	0.602	0.711
Small intestine weight	55.1	54.0	52.1	51.4	1.452	0.080	0.580
Small intestine length, cm	108.0	107.5	100.8	98.2	1.838	0.001	0.078
Histomorphology							
Villi height (VH), μm	1,186.2	1,066.7	1,100.1	1,076.5	47.46	0.103	0.406
Crypt depth (CD), μm	197.2	208.8	196.3	209.6	11.54	0.611	0.904
VH:CD ratio	6.154	5.173	5.679	5.267	0.350	0.109	0.401

Data are least squares means of 8 replicate pens per treatment.

Table 6. Digesta pH, and concentration of plasma uric acid in 10-d old broiler chickens fed starter diets supplemented with different doses of modified soy protein concentrate.

MSPC, % Item	0.0	7.7	10.0	12.5	SEM	P value	
						Linear	Quadratic
Digesta pH							
Gizzard	2.96	3.08	2.20	2.18	0.09	0.001	0.001
Duodenum	5.87	5.93	5.97	5.88	0.06	0.572	0.381
Jejunum	5.72	5.62	5.77	5.66	0.11	0.899	0.790
Ileum	6.04	5.73	5.79	5.81	0.21	0.403	0.523
Enzyme activity							
Pancreas							
Protein, μg/mL	2,680	2,858	2,910	2,770	131.7	0.405	0.381
Chymotrypsin, pg/μg of protein	27.3	30.0	30.5	30.3	1.90	0.200	0.677
Trypsin, pg/μg of protein	1.67	1.96	1.90	1.89	0.14	0.248	0.396
Jejunum							
Protein, μg/mL	2,692	2,693	2,759	2,844	94.5	0.302	0.437
Chymotrypsin, pg/μg of protein	40.43	39.40	40.05	37.43	1.95	0.404	0.656
Trypsin, pg/μg of protein	3.37	1.93	3.22	2.93	0.69	0.737	0.233
Plasma uric acid, μmol/L	364.8	262.4	256.8	247.5	41.9	0.039	0.530

Data are least squares means of 8 replicate pens per treatment.

gastrointestinal tract (Li et al., 1991; Batal and Parsons, 2003; Lenehan et al., 2007; NRC, 2012; Vasconcelos et al., 2017; Zakaria and Ata, 2020). However, growth performance in newly hatched chicks or pigs fed SPC has been variable. The general approach for producing SPC is a set of processes that involve oil extraction, washing of defatted soy flakes with ethanol for the extraction of soluble carbohydrates, and finally the thermal treatment for inactivation of antinutritional factors (Sissons et al., 1982). We evaluated further processed SPC (**MSPC**) with high CP solubility and low fibrous components and residual antinutritional factors (trypsin inhibitors, glycinin, ß-conglycinin, and lectin) (Markedal et al., 2019). The MSPC is produced by incorporating pH reduction step using citric acid or H_2SO_4 resulting in 7−10 % increase in CP solubility and reduction of total dietary

fiber from 24% to 10% (Markedal et al., 2019). Comparative analyses in the present study indicated that MSPC had lower concentration of trypsin inhibitor activity, glycinin, ß-conglycinin, and lectin than SBM used for the feed formulation, which would improve digestion of the MSPC.

The observed greater growth performance of birds fed MSPC in starter phase agreed with a recent evaluation of MSPC in broiler chickens (Zhang et al., 2021). Although, conventional SPC was not incorporated in the present study, improved growth performance of MSPC birds could be linked to differences in antinutritional factors, CP solubility, and fibrous components relative to SBM or SPC samples used in previous studies (Vasconcelos et al., 2017; Zakaria and Ata, 2020). The diets had no effects on liver, pancreas, spleen, and bursa

Table 7. Abundance of selected microbial population (Log10) in the ceca digesta of 10 and 42-d old broiler chickens fed starter diets supplemented with different doses of modified soy protein concentrate.

MSPC, % Item	0.0	7.7	10.0	12.5	SEM	P value	
						Linear	Quadratic
D 10							
Total bacteria	12.3	12.2	12.2	12.2	0.030	0.177	0.981
Lachnospiraceae	12.0	12.0	12.0	11.8	0.119	0.366	0.232
Ruminococcaceae	11.4	11.5	11.3	11.3	0.047	0.040	0.057
Bacteroides	4.82	5.25	5.88	4.97	0.448	0.391	0.373
Bifidobacteria	8.03	8.53	9.00	9.28	0.405	0.031	0.639
Clostridium sensu stricto	8.69	8.04	7.26	6.86	0.262	<0.001	0.232
Lactobacilli (LAB)	11.3	11.1	11.4	11.4	0.122	0.603	0.087
Escherichia coli	10.5	10.7	9.94	9.96	0.082	<0.001	<0.001
LAB: *E. coli*	1.08	1.04	1.14	1.15	0.014	<0.001	<0.001
D 42							
Total bacteria	12.3	12.3	12.3	12.3	0.048	0.940	0.749
Lachnospiraceae	11.8	11.9	11.9	11.9	0.063	0.546	0.895
Ruminococcaceae	11.8	11.9	11.8	11.9	0.057	0.117	0.677
Bacteroides	10.6	10.9	10.1	10.4	0.444	0.531	0.556
Bifidobacteria	9.48	10.2	10.5	11.0	0.362	0.006	0.701
Clostridium sensu stricto	5.14	5.85	5.53	5.57	0.348	0.354	0.337
Lactobacilli (LAB)	10.8	10.8	10.7	11.0	0.103	0.343	0.393
Escherichia coli	9.69	9.81	9.99	9.84	0.208	0.429	0.805
LAB: *E. coli*	1.12	1.11	1.08	1.12	0.027	0.569	0.552

Data are least squares means of 8 replicate pens per treatment.

Table 8. Concentration of short chain fatty acids (SCFA) (μmol/g) in the ceca digesta and litter moisture of broiler chickens fed starter diets supplemented with different doses of modified soy protein concentrate.

MSPC, % Item	0.0	7.7	10.0	12.5	SEM	P-value	
						Linear	Quadratic
D 10							
Lactic	20.0	18.3	16.7	18.6	2.816	0.564	0.725
Acetic	48.1	50.3	55.6	65.4	3.032	0.001	0.028
Propionic	5.08	4.22	4.14	4.07	0.381	0.048	0.574
Iso-butyric	7.04	6.04	6.09	5.44	0.334	0.003	0.965
Butyric	9.79	10.0	10.8	11.0	0.949	0.346	0.730
Iso-valeric	1.09	0.83	0.46	0.43	0.289	0.079	0.789
Valeric	3.35	3.35	2.67	2.94	0.324	0.207	0.699
Total SCFA*	94.4	93.0	96.5	107.9	4.362	0.088	0.067
D 42							
Lactic	14.3	17.2	19.1	15.9	1.955	0.275	0.314
Acetic	71.9	66.8	75.2	72.0	4.277	0.784	0.455
Propionic	7.37	7.51	7.40	7.21	0.661	0.906	0.768
Iso-butyric	9.11	8.87	8.24	8.47	0.513	0.257	0.935
Butyric	15.4	18.9	22.7	20.3	1.557	0.006	0.553
Iso-valeric	1.98	1.97	1.83	2.00	0.376	0.922	0.908
Valeric	6.71	6.92	5.89	5.28	0.783	0.211	0.301
Total SCFA*	126.8	128.2	140.3	131.1	4.649	0.187	0.878
Litter moisture, %							
D 36	25.02	25.16	24.59	24.25	1.157	0.649	0.689
D 42	29.65	30.49	30.02	30.11	1.307	0.804	0.750

*Summation of lactic, acetic, propionic, iso-butyric, butyric, iso-valeric and valeric acids.
Data are least squares means of 8 replicate pens per treatment.

weight, and pancreatic and intestinal digestive enzymes activities in 10-d old broiler chickens. Perhaps indicating residual antinutritional components in the SBM had no detrimental effects on visceral organs physiology in the present study. Similarly, incorporation of 3 to 9% SPC in prestarter and starter diets had no effects on pancreas weight but increased pancreatic trypsin activity in broiler chickens (Vasconcelos et al., 2017). Some feed ingredients bind more acid in the stomach resulting in a high gastric pH that is detrimental because it inhibits protein digestion (Lawlor et al., 2005). Thus, inclusion of MSPC reduced dietary acid binding capacity and reduced gizzard pH which may have improved amino acids digestibility in the present study. When birds were transitioned to common diet on d 11; the control birds appeared to express compensatory growth in the grower phase. Although feed intake was similar among the groups during the finisher phase, birds fed MSPC in the starter phase tended to grow better.

The small intestine is the major site of enzymatic digestion and absorption of nutrients and hence optimal growth performance is linked to functional intestinal mucosa (Kiarie et al., 2013; Kiarie and Mills, 2019). Birds fed MSPC showed lighter small intestine as well as shorter jejunum and ileum. Lower small intestine mass is associated with efficient nutrients utilization as the gut disproportionately consumes more nutrients and energy (Choct, 2009a). Although, we did not observe diet effects on jejunal histomorphology in the present study, feeding 3 to 9% SPC was shown to reduce small intestine weight and increased duodenum, jejunum, and ileum villi height (Vasconcelos et al., 2017). However, the same study indicated deeper small intestine crypt depth in birds fed SCP suggesting activated cell mitosis in the crypts to sustain larger villi (Goodlad et al.,

1991). Plasma uric acid is a key product of amino acids metabolism, and its concentration in the blood indicates less amino acids degradation and improved protein synthesis (Parenteau et al., 2020). It is, therefore, interesting that birds fed MSPC showed lower level of circulating PUA and subsequently better growth and higher breast yield. Given diets had no direct effects on digestive and absorptive capacity (as indicated by histomorphology and digestive enzymes), we hypothesize that the low antinutritional factors in MSPC might have benefitted the birds over the control fed birds. These benefits are linked to stimulation of protein denaturation in the proventriculus and gizzard through increased HCl production and subsequently improved amino acids balance for protein synthesis as indicated by low PUA and increased breast muscle yield.

A stable gut can help reduce the onset of enteric disease, improve nutrient utilization, and therefore growth performance. The ceca has the highest bacterial density and fermentation activity in poultry indicative of increased availability of undigested dietary components and endogenous inputs. Large flow of undigested protein in the ceca creates an imbalance in the resident commensal microbiota facilitating colonization and proliferation of the opportunistic pathogens such as *E. coli* and *clostridium* and suppression of gut health promoting bacteria such as *Bifidobacterium* and *lactobacilli* (Kiarie et al., 2013). The types of ingredients used in a diet can influence the microbiome diversity (Kiarie et al., 2013). We evaluated abundance of key microbial population in the ceca to evaluate the impact of feeding MSPC on ceca microbial activity (population and fermentation metabolites). *Ruminococcaceae* family comprises of fiber degrading bacteria through cellulosome-type enzyme complex (Flint et al., 2012). Thus, MSPC reduced abundance of

Ruminococcaceae perhaps suggesting reduced availability of fiber in MSPC compared to SBM as indicated by neutral detergent fiber concentration in the experimental diets. Different protein sources contribute varying amounts of soluble protein to the ileal digesta (Bryan et al., 2019). There is a concern regarding the types of ingredients used in feed because protein entering the hindgut of the bird can impact bird health (Choct, 2009b). *Clostridium sensu stricto* contains over 100 species, which are grouped around the type species *Clostridium butyricum* and belong to the *Clostridiaceae* 1 family. The genus contains many pathogenic species such as *Clostridium perfringens* a leading cause of necrotic enteritis in broiler chickens and therefore increased abundance is interpreted as indicator of a less healthy microbiota (Drew et al., 2004; Dahiya et al., 2006). It is therefore interesting that MSPC reduced abundance of *Clostridium sensu stricto*. Reduction of abundance of *E. coli* and increase of *Bifidobacterium* and *lactobacilli* and *E. Coli* ratio suggested MSPC improved indices of gut health. The effects of MSPC on ceca microbiota in 42-d old broiler chickens was not as pronounced as in starter phase, however, birds fed MSPC showed linear increase in abundance of *Bifidobacterium*.

Fermentation of carbohydrates by saccharolytic bacteria results in SCFA such as acetate, propionate, and butyrate and, H_2 and CO_2 as carbohydrates are preferred substrates for most microbes (Macfarlane and Macfarlane, 2003; Tiwari et al., 2019). On the other hand, fermentation of proteins and peptides that contain branched-chain amino acids results in SCFA such as 2-methylbutyrate, iso-butyrate, iso-valerate, and phenols, amines and CO_2 (Brestenský et al., 2017; Feng et al., 2018; Tiwari et al., 2019) some of which are considered harmful. Increase in acetic acid may be linked to increased abundance of *Bifidobacteria* in birds fed MSPC. Lactate can be produced by lactic acid bacteria (**LAB**) such as *Bifidobacteria*, and *Proteobacteria*, however, most get converted into different SCFA by other microbial species such as *Eubacteriumhallii* (Flint et al., 2015). Reduction in propionic acid concentration has been linked to decreased abundance of *Bacteroidaceae*. Reduction of branched SCFA such as iso-butyric and iso-valeric is an indication of reduced proteolytic fermentation. In the finisher phase, concentrations of butyrate increased in birds fed MSPC. The majority of butyrate is utilized by intestinal cells with positive health benefits (Bedford and Gong, 2018). Acetate, a 2-carbon SCFA which is the most abundant SCFA in the GIT can be used for lipogenesis or it can be absorbed in peripheral tissues where it acts as a direct source of energy by conversion to ATP (Jha and Berrocoso, 2015; Fernández et al., 2016). Propionate is a 3-carbon SCFA which gets drained into the portal vein and metabolized in gluconeogenesis in the liver (Aumiller et al., 2015; Fernández et al., 2016). Butyrate is a 4-carbon short-chain fatty acid, which is the major energy source for colonocytes and plays an important role in modulating immune and inflammatory responses and intestinal barrier function by increasing mucin production and tight junction integrity (Fernández et al., 2016).

Dietary K concentration has been associated with excessive water intake and excreta moisture; thus poor litter quality and higher risk of incidences foot pad dermatitis (Swiatkiewicz et al., 2017). Soybean meal contains high level of K and has been associated with inadvertently higher levels of K in commercial broiler diets. Although MSPC reduced K in the starter diet we did not observe impact on litter moisture. In contrast, broilers fed diet containing more than 1.25% K exhibited higher litter moisture compared to broiler fed less than 1% K (Koreleski et al., 2010; Fuhrmann and Kamphues, 2016). Perhaps suggesting that the K level in starter feed in the present study may not have had an impact on litter quality in the final days of grow-out period.

It is very important for nutritionists to pay particular attention to the types of ingredients used in feed so that they are aware of any possible impact of diet on immune function and microbiome diversity (Choct, 2009b). Arguably, given MSPC was fed in the first 10 d out of the 42 d birds were grown, it appears that the first 10 d are critical for the overall performance of broiler chickens. This is in line to the current thinking of early life nutrition. This developmental pattern is believed to reflect a survival strategy in which great importance is placed on the growth of nutrient supply functions early in life in order that postabsorptive growth functions can be maximized later in life cycle (Lilja, 1983; Ferket, 2012). The microorganisms living in the microbiome of the broiler chickens carry out 4 main classes of interactions including the exchange of nutrients, immune function, pathogen control, and the development of the digestive system (Kiarie et al., 2013). Bacteria such as *E. coli* and *Clostridium* are found at low levels in the gut of healthy birds throughout their life span (Amit-Romach et al., 2004). However, when provided with an ideal opportunity to proliferate and thrive, these bacteria can lead to significant disease challenges in poultry. One of the most problematic diseases in commercial poultry today is avian colibacillosis caused by enterotoxigenic *Escherichia coli* (Dziva and Stevens, 2008; Alber et al., 2020). Birds diagnosed with this disease often experience high mortality and are treated with antibiotics. The results presented here show that the microbiome can be pushed into a healthier composition by choosing the right ingredients for the starter phase. In conclusion, ≥ 7.7 to 12.5% MSPC inclusion in the starter phase improved growth performance through to d 42 of age linked to enhanced gut health through reduction of enteric pathogens.

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DISCLOSURES

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. M. H. M. is an employee of Triple A.

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