Modified dietary fiber from cassava pulp affects the cecal microbial population, short-chain fatty acid, and ammonia production in broiler chickens

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ABSTRACT The objective of this study was to investigate the effects of modified dietary fiber from cassava pulp (**M-DFCP**) supplementation in broiler diets on cecal microbial populations, short-chain fatty acids (**SCFAs**), ammonia production, and immune responses. A total of 336, one-day-old male broiler chicks (Ross 308) were distributed over 4 dietary treatments in 7 replicate pens (n = 12 chicks) using a completely randomized design. Chicks were fed the control diet and 3 levels of M-DFCP (0.5, 1.0, and 1.5%) for an experimental duration of 42 d. The M-DFCP contained total dietary fiber (**TDF**), soluble dietary fiber (**SDF**), insoluble dietary fiber (**IDF**), cello-oligosaccharides (**COS**), and xylo-oligosaccharides (**XOS**) of approximately 280.70, 22.20, 258.50, 23.93, and 157.55 g/kg, respectively. The 1.0 and 1.5% M-DFCP supplementation diets showed positive effects on stimulating the growth of *Lactobacillus* spp. and *Bifidobacterium* spp., enhancing SCFAs (acetic, propionic, butyric acid, and branched SCFAs) and lactic acid concentrations during growing periods. Broilers fed 1.0 and 1.5% M-DFCP also exhibited a significant increase in caecal *Lactobacillus* spp. and lactic acid concentrations during the finisher period as well. In addition, M-DFCP also reduced cecal digesta and excreta ammonia production in broilers over both periods (0−21 and 22−42 d of age). However, M-DFCP did not exhibit any effect on total serum immunoglobulin **(Ig)** or lysozyme activity. In conclusion, this study shows that M-DFCP can be used as a dietary fiber source in broiler diets, with a recommended level of approximately 1.0%.

Key words: modified dietary fiber, cassava pulp, microbial population, short-chain fatty acid, ammonia

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INTRODUCTION

After the use of antibiotics as growth promoters (**AGPs**) was banned in feed, managing nutrition became a crucial goal to improving poultry gut health and production. Namely, gut health improvement is key to ensuring animal well-being, as it is linked to a balanced microbiome and improved immune system (Jacquier et al., 2019; de Carvalho et al., 2021). Currently, there is a renewed interest in dietary fiber, with an emphasis on the main function of improving gut microbial activity. Previous research has shown that dietary fiber plays an important role in modulating the gastrointestinal tract (**GIT**) microbial host community, and consequently produces beneficial results for gut health (Yadav and Jha, 2019; Hou et al., 2020; Wang et al., 2021); thus, developing an improved understanding of the function of dietary fiber in poultry nutrition may contribute to improved animal health.

Dietary fibers are principally composed of plant cell walls, which consist mainly of nonstarch polysaccharides (**NSPs**) and lignin. The major NSPs present in plant cell walls are mostly cellulose, and a wide variety of non-cellulosic polysaccharides (arabinoxylans, mixed-linked ß-glucans, and xyloglucans) (Choct, 1997; Knudsen, 2014). Dietary fiber is classified into 2 categories according to water solubility: soluble dietary fiber (**SDF**), such as ß-glucans, pectins and gums; and insoluble dietary fiber (**IDF**), such as cellulose, some hemicellulose and lignin. (Dhingra et al., 2012; Choct, 2015). Dietary fiber passes through the small intestine into the lower GIT and is used as a substrate for fermentation by microorganisms. As a result, short-chain fatty acids (**SCFAs**), such as acetic, propionic, and butyric acids, as well as other metabolites (lactic and succinic acids) are the primary end products of dietary fiber fermentation (den Besten et al., 2013; Regassa and

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Nyachoti, 2018; Singh and Kim, 2021). SCFAs formation reduces the pH in a caecal environment, creating unfavorable conditions for pathogenic bacteria proliferation, while promoting the growth of beneficial bacteria, resulting in the improved microbial population balance and overall gut health of monogastric animals (Zduńczyk et al., 2015; Kheravii et al., 2018a). Additionally, SCFAs also appear to be associated with reduced levels of ammonia in excreta (Roberts et al., 2007), and enhanced immune function in broilers (Sabour et al., 2018).

Presently, there is a trend toward using modified dietary fiber derived from natural plant sources (e.g., agro-industrial byproducts) as feed supplements in poultry diets. Specifically, cassava pulp is a byproduct from cassava starch manufacturing, and contains a large quantity of NSPs (29%), mainly consisting of cellulose (20%) and xylan (4.2%) (Kosugi et al., 2009; Choct, 2015). Accordingly, dried cassava pulp (**DCP**) can be partially utilized as an energy feedstuff in poultry diets; however, its high fiber content necessitates that it should not exceed 10% in broiler diets (Khempaka et al., 2009), or 20% in laying hen diets (Khempaka et al., 2016). In addition, IDF (lignocellulose) has been shown to modulate gut microflora, SCFA production and gut health (Boguslawska-Tryk et al., 2015; Hou et al., 2020); thus, fiber extract from cassava pulp is likely to have a positive impact on poultry health and production. Our previous study found that supplementation with modified dietary fiber from cassava pulp **(M-DFCP)** in broiler diets can reduce meat cholesterol, abdominal fat and gizzard pH, as well as improve gizzard development and enhance nutrient digestibility without affecting growth performance (Okrathok and Khempaka, 2020). These observed effects are likely due to the dietary fiber extracts from cassava pulp, and the potential modification of crude dietary fibers via enzymatic treatment from their structure or degree of polymerization (**DP**). This result is in accordance with Ravn et al. (2017), who reported on the treatment of wheat bran with xylanase enzyme, and the assessment of its effects when used in an in vitro caecal fermentation assay, revealing a decrease in the DP of wheat bran arabinoxylan in arabinoxylo-oligosaccharides, and in turn, the enhanced the production of SCFAs by cecal bacteria fermentation. Singh and Kim (2021) similarly reported that the fermentable substrates of dietary fibers can vary from complex fragments to simple oligomers, which can act as prebiotics, serve as a selective nutrient for beneficial gut microbes, boost intestinal mucosal health, increase SCFA production and improve overall gut health. In general, the efficacy of dietary fiber in animal feed on gut microflora is related to fiber type, polymer chain composition, structure, and supplementation level.

In the present study, M-DFCP generated by extraction with NaOH, and subsequent hydrolyzing of the polymer chain into a mixture of cello-oligosaccharides (**COS**) and xylo-oligosaccharides (**XOS**) with cellulase and xylanase enzymes, were evaluated to investigate their effects on cecal microbial population, SCFAs, ammonia production and immune responses in broiler diets. We hypothesized that the inclusion of M-DFCP in broiler diets would improve the corresponding microbial population, increase SCFA and lactic acid concentrations, decrease ammonia excretion, and improve immunity.

MATERIALS AND METHODS

All experiments were conducted according to the principles and guidelines approved by the Animal Care and Use Committee of Suranaree University of Technology (**SUT**; Approval number: SUT3-303-60-24-09).

Dietary Fiber Sources of M-DFCP

Fresh cassava pulp from the Korat Flour Industry Co., Ltd (Nakhon Ratchasima, Thailand) was dried in a hot air oven at 55 to 60°C for 2 d. The DCP was ground to pass through a 1.0-mm mesh sieve and stored at 4°C until use. The M-DFCP was prepared according to the methods of Okrathok et al. (2019). It was analyzed for dry matter, ash, ether extract, crude protein, total dietary fiber (**TDF**), SDF, IDF, COS, and XOS as reported in our previous study (Okrathok and Khempaka, 2020), revealing that M-DFCP was composed of 58.6 g/kg moisture, 1.9 g/kg ether extract, 17.1 g/kg crude protein, 280.7 g/kg total dietary fiber (22.2 g/kg SDF and 258.5 g/kg IDF), 23.9 g/kg COS (DP 2-5), and 157.6 g/kg XOS (DP 2-5), based on an as-fed basis.

Birds, Experimental Design and Diets

A total of 336 one-day-old Ross 308 male broiler chicks were obtained from a local commercial hatchery, with an initial mean body weight of 40.0 ± 0.22 g. The chickens were randomly allocated to 4 groups of 84 (7 pen replicates, 12 birds/pen). The birds were housed in concrete floor pens (1.0 × 2.0 m) with rice husk as bedding material, and each pen was equipped with a tray feeder and 2 nipple drinkers. The housing temperature over the first week of life was 33°C, and was gradually decreased to 24°C over the course of the experiment. The light program consisted of 23 h during the first 10 d of age, followed by 18 h to the end of the trial. The experiment was conducted as a completely randomized design.

The four experimental diets included a control and those containing M-DFCP at levels of 0.5, 1.0 and 1.5%. All diets within the period were formulated to contain similar contents of ME and CP. Diets were also created to meet or exceed the minimum nutrient requirements of broiler chickens as recommended by the NRC (1994), and according to Ross 308 broiler nutrition specifications (Aviagen, 2014) for starter (0−10 d), grower (11−21 d) and finisher (22−42 d) periods. Cassava starch, meat meal, and rice bran oil were used to balance ME and CP contents at the expense of M-DFCP. Mash feed and water were provided ad libitum throughout the experimental period. The ingredient composition and calculations of the experimental diets are shown in

Table 1; whereas the analyzed values of the experimental diets are shown in Table 2.

Sampling Procedures

The weights of the chickens in each pen were measured at 21 d of age. Thereafter, 3 chickens with similar weights to the mean value of the flock were chosen from each pen and placed in metabolic cages for excreta collection and further determination of ammonia. Feed and water were supplied ad libitum throughout the trial. After a 4 d adaptation period (26 to 28 d of age), approximately 200 g of fresh excreta was collected in a clean plastic bag, and stored at −20°C for ammonia analysis.

The weights of the chickens were determined at 21 and 42 d of age, and 2 birds per replicate (14 birds per treatment) were randomly selected, individually weighed and used for blood and cecal digesta collection. Approximately 3.0 mL of blood samples from these chicks were collected in polypropylene tubes and separated by centrifugation at 1,000 × *g* for 15 min. The pure serum samples were then aspirated by pipette, transferred into 1.5 mL sterilized tubes, and stored at −20°C for further analysis. Subsequently, all chickens (14 birds per treatment) were euthanized, and the carcasses were immediately opened. Both ceca were removed, and the digesta contents were collected aseptically from each bird into empty 15 mL sterile conical tubes (right side for microbial analysis and left side for SCFA, lactic acid and ammonia analysis), and were frozen at −20°C until analysis.

Caecal Microbial Population Analysis by Quantitative Real-Time PCR

The contents of the caecal digesta were used for quantification of *Lactobacillus* spp., *Bifidobacterium* spp., *Enterobacte*r spp. and *E. coli*. Bacterial DNA was isolated using the QIAamp Fast DNA Stool kit (Qiagen Inc., Hilden, Germany) in accordance with the manufacturer's guidelines. The extracted DNA was quantified with a NanoVue Plus Nano Drop spectrophotometer (GE Healthcare, Chicago, IL) to evaluate purity and concentration, and then stored at −80°C until further analysis.

The populations of caecal microbes were analyzed by quantitative real-time PCR (**qPCR**). The extracted DNA was used as a template for PCR amplification, and the primers used to quantify different bacterial populations are detailed in Table 3. The qPCR assay was performed with a LightCycler 480 Instrument II (Roche Diagnostics, GmbH, Mannheim, Germany). PCR was performed in white LightCycler 480 Multiwell Plate 96 plates (Roche) in a final volume of 10 μL using the QuantiFast SYBR Green PCR kit (Qiagen Inc., Germantown, MD). Each reaction included 5.0 μL of 2 × SYBR Green Master Mix, 0.4 μL of 10 μM forward primer, 0.4 μL of 10 μM reverse primer, 2.0 μL of DNA samples and 2.2 μL of nuclease-free water. All samples were analyzed based on triplicate reactions. The reaction conditions for DNA amplification were a first denaturing at 94°C for 5 min, followed by 40 denaturing cycles at 94°C for 20 s, primer annealing at 50°C for

Table 1. Ingredients and calculated chemical composition of experimental diets on as-fed basis.

	Starter diets (1−10 days)				Grower diets (11−21 days)				Finisher diets (22−42 days)			
		M-DFCP[1]				M-DFCP[1]				M-DFCP[1]		
	Control	0.5%	1.0%	1.5%	Control	0.5%	1.0%	1.5%	Control	0.5%	1.0%	1.5%
Ingredients (g/kg diet)												
Corn	518.3	518.3	518.3	518.3	524.2	524.2	524.2	524.2	547.6	547.6	547.6	547.6
Soybean meal (44% CP)	315.5	315.5	315.5	315.5	280.5	280.5	280.5	280.5	248.2	248.2	248.2	248.2
Full-fat soybean	50.0	50.0	50.0	50.0	70.0	70.0	70.0	70.0	85.0	85.0	85.0	85.0
Meat meal (58% CP)	31.3	31.6	32.0	32.3	31.2	31.6	32.0	32.3	10.5	10.8	11.2	11.6
Cassava starch	25.3	16.9	8.4	0.0	25.3	16.8	8.4	0.0	25.5	17.0	8.5	0.0
Rice bran oil	26.4	29.5	32.6	35.7	36.0	39.1	42.1	45.2	48.2	51.4	54.5	57.6
M-DFCP[1]	0.0	5.0	10.0	15.0	0.0	5.0	10.0	15.0	0.0	5.0	10.0	15.0
Limestone	9.5	9.5	9.5	9.5	8.9	8.9	8.9	8.9	10.6	10.6	10.6	10.6
Monocalcium phosphate	10.2	10.2	10.2	10.2	9.8	9.8	9.8	9.8	11.2	11.2	11.2	11.2
DL- methionine	0.5	0.5	0.5	0.5	0.7	0.7	0.7	0.7	0.8	0.8	0.8	0.8
L- lysine	2.7	2.7	2.7	2.7	2.8	2.8	2.8	2.8	2.6	2.6	2.6	2.6
L- threonine	0.2	0.2	0.2	0.2	0.5	0.5	0.5	0.5	0.4	0.4	0.4	0.4
Sodium chloride	5.1	5.1	5.1	5.1	5.1	5.1	5.1	5.1	4.4	4.4	4.4	4.4
Premix[2]	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Calculated analysis (g/kg)												
ME (kcal/kg)	3,010	3,010	3,010	3,010	3,100	3,100	3,100	3,100	3,200	3,200	3,200	3,200
Crude protein	220.0	220.0	220.0	220.0	212.5	212.5	212.5	212.5	193.0	193.0	193.0	193.0
Calcium	9.0	9.0	9.0	9.0	8.7	8.7	8.7	8.7	7.9	7.9	7.9	7.9
Available phosphorus	4.6	4.6	4.6	4.6	4.5	4.5	4.5	4.5	3.9	3.9	3.9	3.9
Digestible lysine	11.8	11.8	11.8	11.8	11.5	11.5	11.5	11.5	10.3	10.3	10.3	10.3
Digestible methionine	5.7	5.7	5.7	5.7	5.8	5.8	5.8	5.8	5.3	5.3	5.3	5.3
Digestible methionine + cysteine	8.8	8.8	8.8	8.8	8.7	8.7	8.7	8.7	8.0	8.0	8.0	8.0
Digestible threonine	7.7	7.7	7.7	7.7	7.7	7.7	7.7	7.7	6.9	6.9	6.9	6.9

[1]Modified-dietary fiber from cassava pulp: composed of 58.6 g/kg moisture; 17.1 g/kg crude protein; 1.9 g/kg ether extract; 78.2 g/kg crude ash, 280.7 g/kg TDF (22.2 g/kg SDF and 258.5 g/kg IDF) 23.9 g/kg Cello-oligosaccharides, 157.6 g/kg Xylo-oligosaccharides (as-fed basis).

[2]Premix (5.0 g/kg) provided the following (per kg of diet): 15,000 IU of vitamin A; 3,000 IU of vitamin D3; 25 IU of vitamin E; 5 mg of vitamin K3; 2 mg of vitamin B1; 7 mg of vitamin B2; 4 mg of vitamin B6; 25 mg of vitamin B12; 11.04 mg of pantothenic acid; 35 mg of nicotinic acid; 1 mg of folic acid; 15 μg of biotin; 250 mg of choline chloride; 1.6 mg of Cu; 60 mg of Mn; 45 mg of Zn; 80 mg of Fe; 0.4 mg of I; and 0.15 mg of Se.

Table 2. Analyzed chemical composition of experimental diets containing different levels of modified-dietary fiber from cassava pulp (M-DFCP), g/kg as fed basis.

	Starter diets (0−10 days)				Grower diets (11−21 days)				Finisher diets (22−42 days)			
		M-DFCP[1]				M-DFCP[1]				M-DFCP[1]		
Items	Control	0.5%	1.0%	1.5%	Control	0.5%	1.0%	1.5%	Control	0.5%	1.0%	1.5%
Dry matter	902.3	904.1	907.1	908.9	899.8	900.1	898.7	899.8	912.7	905.0	909.3	909.0
Crude protein	233.9	228.6	222.5	222.7	214.5	217.9	213.6	222.3	198.8	199.0	194.4	199.0
Ether extract	47.5	53.8	48.4	58.9	72.7	79.7	84.2	81.5	90.5	93.0	94.5	95.6
Ash	64.0	64.5	65.5	59.1	58.6	59.7	60.1	59.6	56.3	59.3	58.9	58.3
Total dietary fiber	112.3	115.2	118.1	118.6	104.7	105.2	105.7	106.6	102.8	103.3	107.2	109.4
Soluble dietary fiber	26.0	26.2	26.4	26.6	24.9	25.8	25.6	24.9	23.6	23.4	24.1	24.3
Insoluble dietary fiber	86.3	89.0	91.7	92.0	79.8	79.4	80.1	81.7	79.2	79.9	83.1	85.1
Cello-oligosaccharides[2]	0.00	0.12	0.24	0.36	0.00	0.12	0.24	0.36	0.00	0.12	0.24	0.36
Xylo-oligosaccharides[2]	0.00	0.79	1.58	2.36	0.00	0.79	1.58	2.36	0.00	0.79	1.58	2.36

[1]Modified-dietary fiber from cassava pulp.
[2]Calculated values of COS and XOS (g/kg) in experimental diets.

E. coli, 58°C for *Lactobacillus* spp., 60°C for *Bifidobacterium* spp. and *Enterobacter* spp. over 30 s, and extended to 72°C for 20 s (Sharmila et al., 2015). To confirm the specificity of amplification, an analysis of the melting curve was carried out following the last cycle of each amplification.

Absolute quantification of the cecal microbial population was obtained using standard curves constructed by amplification of the known DNA content for the target bacteria. Standard bacterial strains used in this study, including *Lactobacillus acidophilus* (ATCC 4356), *Bifidobacterium bifidum* (ATCC 29521), *Enterobacter cloacae* (ATCC 13047) and *Escherichia coli* (ATCC 25922), were obtained from the American Type Culture Collection (ATCC; Manassas, VA).

SCFAs, Lactic Acid and Ammonia Analysis

The concentrations of SCFAs (acetic, propionic, isobutyric, butyric, isovaleric, and valeric acid) and lactic acid were analyzed using a modified procedure of Mookiah et al. (2014). The caecal digesta were treated with 24% meta-phosphoric acid in 1.5 M H_2SO_4 (sample to solution ratio was 1:1), and vortexed to mix. The samples were left overnight at room temperature, centrifuged at 10,000 × *g* and 4°C for 20 min, and the supernatant was used for analysis. The samples were assayed using gas chromatography (Agilent 7890B; Agilent Technologies, Santa Clara, CA) with flame ionization detection (**FID**) and nitrogen as a carrier gas. A fused silica capillary column (0.32 mm × 25 m; CP-Sil 5 CB column, J&W GC Column, Agilent) was used for analysis. SCFAs and lactic acid were analyzed using 4-methylvaleric acid (Alfa Aesar, Heysham, UK) and fumaric acid (Alfa Aesar) as internal standards, respectively. The external standards used to identify the peaks for SCFAs and lactic acid were a volatile acid mixture (C1−C7, 10 mM each in water, Supelco, Bellefonte, PA) and lithium L-lactate (Alfa Aesar), respectively.

The ammonia contents of the caecal digesta and excreta were determined via a modified procedure from Willis et al. (1996). A total of 250 mg of sample was added to a polypropylene test tube, followed by 50 mL of 5% lithium carbonate (Li_2CO_3, Sigma–Aldrich, St Louis, MO), vortexing to mix, and centrifuging at 10,000 × *g* and 4°C for 15 min. The supernatant up to 500 μL was then transferred to a 15 mL tube, added to 4 mL of salicylate reagent, followed by 1 mL of hypochlorite reagent and vortexed briefly. The mixture was incubated at room temperature for 30 min, the absorbance was then measured at 685 nm using a microplate reader (Multiskan GO, Thermo Fisher Scientific, Waltham, MA), and compared to a standard ammonia calibration curve.

Table 3. The sequence of primers used for determination of the *Lactobacillus* spp., *Bifidobacterium* spp., *Enterobacter* spp., and *E. coli* of chickens caecal digesta.

Target bacteria	Primer[1]
Lactobacillus spp.	F-5'-CATCCAGTGCAAACCTAAGAG-3'
	R-5'-GATCCGCTTGCCTTCGCA-3'
Bifidobacterium spp.	F-5'-GGGTGGTAATGCCGGATG-3'
	R-5'-TAAGCCATGGACTTTCACACC-3'
Enterobacter spp.	F-5'-CATTGACGTTACCCGCAGAAGAAGC-3'
	R-5'-CTCTACGAGACTCAAGCTTGC-3'
E. coli	F-5'-GTGTGATATCTACCCGCTTCGC-3'
	R-5'-AGAACGCTTTGTGGTTAATCAGGA-3'

[1]Data form reference Rezaei et al. (2015)

Serum Total Immunoglobulin and Lysozyme Analysis

Serum samples were employed for total immunoglobulin **(Ig)** analysis using a total protein kit (Micro Lowry, Peterson's Modification, Sigma, Product Codes: TP0300-1KT). Serum lysozyme activity was determined according to Kreukniet et al. (1995) using *Micrococcus lysodeikticus* cells as a substrate.

Statistical Analysis

All data were analyzed as a completely randomized design with 4 treatments, using one-way ANOVA in SPSS version 18.0 (SPSS Inc. 2010), where the pen served as the experimental unit for each parameter. Significant differences between means were separated by Tukey's post hoc test. Orthogonal polynomials were performed to study the linear, quadratic and cubic responses of the different

variables for the M-DFCP inclusion in the diets. A threshold level of $P < 0.05$ was used to determine significance.

RESULTS

Caecal Microbial Populations

The results of the microbial populations in the cecal contents of broiler chickens fed M-DFCP are shown in Figure 1. M-DFCP changed cecal *Lactobacillus* spp. of broilers at both periods (21 and 42 d of age). The populations of *Lactobacillus* increased linearly ($P < 0.01$) and cubically ($P < 0.01$) with increasing M-DFCP diet levels at 21 d. At 42 d of age, *Lactobacillus* spp. increased linearly ($P < 0.01$), quadratically ($P = 0.01$) and cubically ($P = 0.02$), as the values were significantly higher in broilers fed 1.0 and 1.5% than in the control group. In addition, at 21 days of age, the *Bifidobacterium* population also increased linearly ($P = 0.01$) and quadratically ($P = 0.01$) with increasing M-DFCP levels in diets, and a significant enhancement was observed across all M-DFCP diets (0.5 to 1.5%) compared to the control; however, no such significant differences were observed in broilers aged 42 d ($P > 0.05$). Furthermore, none of the M-DFCP dietary levels showed significant differences in the populations of *Enterobacter* spp. and *E. coli* compared to the control group ($P > 0.05$).

SCFAs and Lactic Acid Concentrations

The effects of M-DFCP supplementation in broiler diets on the concentrations of cecal SCFAs and lactic acid are shown in Table 4. At 21 d of age, the cecal SCFA contents increased linearly ($P < 0.01$) with increasing M-DFCP levels in the broiler diets. Acetic, propionic, butyric acid, and branched SCFAs increased linearly ($P < 0.01$) in response to M-DFCP supplementation as well. Linear and quadratic effects ($P < 0.01$) on butyric acid production were also observed. Indeed, all dietary levels of M-DFCP (0.5 to

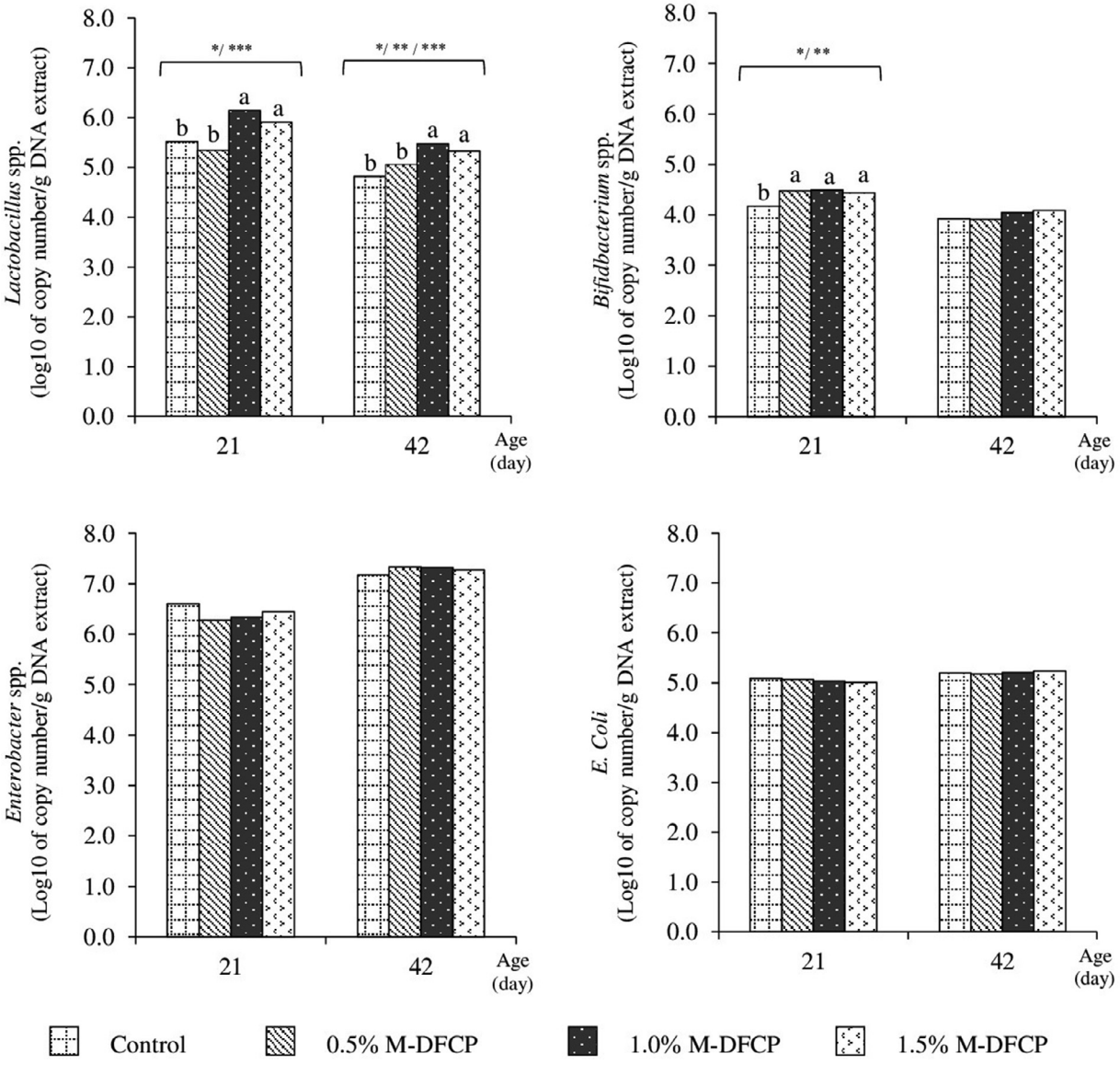

Figure 1. Effects of modified-dietary fiber from cassava pulp on cecal microbial population of broilers (log10 of copy number/g DNA extract). [a, b] Means with no common superscripts are significantly different at $P < 0.05$. * Significant linear effect; [**] Significant quadratic effect ($P < 0.05$). [***] Significant cubic effect ($P < 0.05$). N = 7.

Table 4. Effects of modified-dietary fiber from cassava pulp on the concentration of caecal SCFAs and lactic acid of broiler chickens at 21 and 42 days of age.

Items	Control	M-DFCP[1]			Pooled SEM	*P*-values		
		0.5%	1.0%	1.5%		Linear	Quadratic	Cubic
21 days of age								
Short-chain fatty acids (% of total SCFAs)								
Acetic acid	48.77[b]	51.44[b]	63.20[a]	63.19[a]	1.83	< 0.01	0.60	0.08
Propionic acid	2.94[b]	3.98[ab]	5.00[a]	4.78[ab]	0.28	0.01	0.21	0.58
Butyric acid	2.07[b]	3.67[a]	4.13[a]	4.17[a]	0.23	< 0.01	0.02	0.60
Branched SCFAs[2]	0.28[c]	0.46[bc]	0.74[ab]	0.95[a]	0.07	< 0.01	0.31	0.08
Valeric acid	45.94[a]	40.45[a]	26.93[b]	26.92[b]	2.17	< 0.01	0.86	0.68
Lactic acid (μmol/g of digesta)								
	15.58[c]	16.26[c]	19.46[b]	24.98[a]	0.84	< 0.01	< 0.01	0.93
42 days of age								
Short-chain fatty acids (% of total SCFAs)								
Acetic acid	77.07	77.60	77.15	78.77	0.46	0.29	0.57	0.47
Propionic acid	5.44	5.95	5.87	6.22	0.18	0.21	0.84	0.55
Butyric acid	4.17	4.40	4.44	4.44	0.16	0.60	0.74	0.92
Branched SCFAs[2]	0.93	0.92	1.01	1.10	0.06	0.15	0.75	0.45
Valeric acid	12.39	11.13	11.53	9.47	0.60	0.29	0.67	0.82
Lactic acid (μmol/g of digesta)								
	23.65[b]	24.29[b]	32.53[a]	32.50[a]	0.92	< 0.01	< 0.01	< 0.01

[a,b,c]Means with different superscripts in a row are significantly different at $P < 0.05$.
[1]Modified-dietary fiber from cassava pulp.
[2]Branched SCFAs = isobutyric acid + isovaleric acid.

Table 5. Effects of modified-dietary fiber from cassava pulp on total immunoglobulin and lysozyme activity in blood serum of broiler chickens.

Items	Control	M-DFCP[1]			Pooled SEM	*P*-values		
		0.5%	1.0%	1.5%		Linear	Quadratic	Cubic
Total Ig (mg/mL)								
21 days of age	18.35	18.41	18.45	18.42	0.21	0.89	0.93	0.98
42 days of age	18.21	18.43	18.52	18.53	0.22	0.63	0.82	0.97
Lysozyme (μg/ml)								
21 days of age	4.21	4.20	4.25	4.26	0.02	0.32	0.79	0.62
42 days of age	4.21	4.22	4.23	4.26	0.02	0.47	0.86	0.90

[1]Modified-dietary fiber from cassava pulp.

1.5%) improved butyric acid production compared to the control diet. Valeric acid decreased linearly ($P < 0.01$) with increased levels of M-DPCP. In contrast, at 42 d of age, cecal SCFAs (acetic, propionic, butyric, valeric acid, and branched SCFAs) contents were not significantly affected by the inclusion of M-DFCP in diets ($P > 0.05$).

Interestingly, a significant increase in the concentration of cecal lactic acid was observed in response to M-DFCP supplementation over both periods (21 and 42 d of age). The lactic acid concentration increased linearly ($P < 0.01$) and quadratically ($P < 0.01$) at 21 d of age, and increased linearly ($P < 0.01$), quadratically ($P < 0.01$) and cubically ($P < 0.01$) at 42 d of age. Significant differences were found in broilers fed 1.0 and 1.5% M-DFCP compared to the control group.

Total Serum Immunoglobulin and Lysozyme Concentrations

The effects of M-DFCP supplementation in broiler diets on total serum Ig and lysozyme activity are shown in Table 5. None of the dietary M-DFCP levels showed any significant effects on total Ig and lysozyme activity in broilers over either period ($P > 0.05$).

Table 6. Effects of modified-dietary fiber from cassava pulp on ammonia production of cecal digesta and excreta of broiler chickens.

Items	Control	M-DFCP[1]			Pooled SEM	*P*-values		
		0.5%	1.0%	1.5%		Linear	Quadratic	Cubic
Ammonia production (mg/g of cecal digesta)								
21 days of age	0.90[a]	0.73[b]	0.72[b]	0.72[b]	0.02	< 0.01	0.01	0.31
42 days of age	1.11[a]	0.95[b]	0.89[bc]	0.82[c]	0.03	< 0.01	0.19	0.48
Ammonia production (mg/g of excreta)								
28 days of age	1.96[a]	1.68[b]	1.63[b]	1.64[b]	0.04	< 0.01	0.01	0.50

[a,b,c]Means with different superscripts in a row are significantly different at $P < 0.05$.
[1]Modified-dietary fiber from cassava pulp.

Ammonia Production in Cecal Digesta and Excreta

The effects of the inclusion of M-DFCP in broiler diets on ammonia production are presented in Table 6. The concentration of ammonia in cecal digesta and excreta decreased significantly as M-DFCP diet levels increased ($P < 0.01$). Here, broilers fed M-DFCP revealed a significant decrease in the ammonia concentration of cecal digesta at 21 d (linear, $P < 0.01$ and quadratic, $P = 0.01$), and 42 d of age (linear, $P < 0.01$). In addition, ammonia production in excreta also showed a linear ($P < 0.01$) and quadratic ($P < 0.01$) response to M-DFCP diets. A significant reduction in ammonia concentration was observed in broilers fed 0.5 to 1.5% M-DFCP ($P < 0.05$) over both periods.

DISCUSSION

It is generally accepted that dietary fiber plays an important role in the shift of gut microbial populations and their metabolites (SCFAs and lactic acid) (Rinttilä and Apajalahti, 2013; Regassa and Nsyachoti, 2018; Röhe and Zentek, 2021). Based on our previous research, the inclusion of M-DFCP in diets has shown benefits regarding the reduction of meat cholesterol, abdominal fat and gizzard pH, including improvement of gizzard function, without producing any adverse effects on growth performance of broilers (Okrathok and Khempaka, 2020). In the present study, it was found that M-DFCP promoted the growth of *Lactobacillus* and *Bifidobacterium* populations, and consequently enhanced SCFA and lactic acid production. In addition, the M-DFCP also showed a reduction in the concentration of ammonia in cecal digesta and excreta. Accordingly, the recommended level of M-DFCP supplementation for broilers based on the current findings should be 1.0% in diets.

M-DFCP contains significant quantities of IDF, COS and XOS. The COS and XOS are generated as a result of cellulase and xylanase treatment of the raw material (respectively) and both of these low molecular weight oligossaccharides (DP 2-5) are easily fermented by *Lactobacillus* spp. and *Bifidobacterium* spp, to SCFAs in the cecum. This is in agreement with previous studies which revealed that bacterial strains such as *Lactobacillus* spp. and *Bifidobacterium* spp. were able to utilize COS and XOS (Moura et al., 2007; Hasunuma et al., 2011). De Maesschalck et al. (2015) reported that supplementing XOS in broiler diets resulted in increased populations of cecal *Lactobacillus* and butyrate-producing bacteria; whereas Song et al. (2013) also reported that broilers fed a diet supplemented with COS had higher and lower caecal populations of *Lactobacillus* and *E. coli*, respectively, because COS can be metabolized by microbes that generate SCFAs (e.g., butyric acid). Similarly, the findings of the present study revealed positive effects of COS and XOS in M-DFCP, and provided a suitable environment for beneficial microbes, while stimulating the production of SCFAs (acetic, propionic and butyric acid) and lactic acid in the caecum. In general, high SCFA and lactic acid concentrations create a lower pH cecal environment, leading to the inhibition of the viability of pathogenic bacteria in the GIT (Mookiah et al., 2014; Kheravii et al., 2018a); however, M-DFCP did not affect the cecal *Enterobacter* and *E. coli* populations of broilers here. This is likely derived from the chickens used in this study being raised in a suitable (stress-free) environment, which did not support the proliferation of pathogenic bacteria. For this reason, M-DFCP may not have had any significant effect on pathogen inhibition. Cao et al. (2003) reported that chickens fed 10% dietary cellulose showed significantly enhanced cecal *Lactobacillus* spp. and *Bifidobacterium* spp.; yet, there was no effect on *Clostridia* and *Enterobacteriaceae* populations. Rather, the maintenance of gut health and stability in the GIT ecosystem is a complex phenomenon. Previous studies have documented changes in microflora and demonstrated that the caecum contains the largest number of bacterial species in the chicken GIT. These microbial populations are influenced by multiple factors, such as diet composition, environment, health status, and age (Amit-Romach et al., 2004; Józefiak et al., 2004; Torok et al., 2009). Here, M-DFCP at levels of 1.0 to 1.5% in the diet significantly increased SCFA production in 21-d broilers, but not in 42-d broilers. It is likely that the cecal microbiota in GIT chickens is not static, but exhibits age-related temporal variation (Elling-Staats et al., 2022). Differences in cecal microbial composition were also observed in this study, and a significant alteration of *Lactobacillus* spp. was observed in 21- and 42-d broilers, but *Bifidobacterium* spp. was observed only at 21 . Overall, feeding broilers with M-DFCP resulted in a significant increase in COS and XOS intake over both periods. The cumulative intakes of COS and XOS in birds fed 1.0% M-DFCP at 21 days were 2.38 and 15.68 g/kg, and at 42 d were 9.45 and 62.20 g/kg, respectively (data not shown), which can lead to improved bacterial fermentation, as well as enhanced SCFA and lactic acid production.

Although the inclusion of M-DFCP in broiler diets showed positive impacts on the growth of *Lactobacillus* spp. and *Bifidobacterium* spp., in addition to SCFA concentrations, no significant effects were observed on serum total Ig or lysozyme activity. This too is likely due the suitable environment in which the broiler chickens were raised, with little risk of disease infection; therefore, the role of M-DFCP in immune responses in broiler diets remains unclear. Normally, SCFAs, mainly acetic, propionic and butyric acid, provide energy sources for epithelial cells in the gut, and have profound effects on immunomodulation (particularly butyric acid, which has also been reported to stimulate the immune system) (De Maesschalck et al., 2015; Pourabedin and Zhao, 2015). Indeed, intestinal mucosal barrier function provides vital immunity and defense against pathogen invasion (Huang et al., 2018; Kheravii et al., 2018b). In addition to serum total Ig or lysozyme activity, the measurement of immune responses at intestinal mucosal sites can better clarify the properties of M-DFCP on immune function.

In poultry housing, ammonia is a major aerial pollutant, and it can have adverse effects on animal health, productive performance and welfare (Rothrock et al., 2008). The present study found that feeding broilers with M-DFCP

resulted in a significant reduction in the production of ammonia in the caecum and excreta of broilers. This is likely because M-DFCP activates the metabolism and growth of *Lactobacillus* spp., and produces lactic acid bacteria, leading to the inhibition of bacterial enzymes involved in the breakdown of uric acid to ammonia. Indeed, the concentration of ammonia is proportional to the amount of uric acid excretion, where the conversion of uric acid to ammonia requires urease (McCrory and Hobbs, 2001). Additionally, Liu et al. (2007) have also reported that feeding a *Lactobacillus reuteri*-supplemented diet can noticeably reduce the concentration of ammonia in broiler excreta due to the reduction in urease activity produced by *L. reuteri*. It has been previously noted that the ammonia content in digesta and excreta can be associated with microbial population abundance, microbial fermentation and metabolite production. Poultry environmental factors such as temperature, litter pH, moisture content, etc., can substantially increase the growth of microbial urease, leading to increased ammonia production, and the resulting excreta will always contain more ammonia than cecal digesta. In addition, chicken excreta contains a high level of nitrogen, which starts to breakdown immediately after excretion, and releases ammonia from microbial urease activity (Ghaly and Alhattab, 2013; Elling-Staats et al., 2022). The present study indicates that M-DFCP can have a positive effect on the reduction of ammonia emissions to the environment.

In conclusion, this study revealed the benefits of supplemented dietary M-DFCP, which contains major portions of COS and XOS, to improve the gut health of broilers. The recommended inclusion of M-DFCP in broiler diets is 1.0%, as this level showed positive effects on improving caecal *Lactobacillus* and *Bifidobacterium* populations, enhancing SCFA and lactic acid production, and resulted in lower ammonia concentrations. Comparatively, M-DFCP did not show any effects on total Ig or lysozyme in the serum of broiler chickens. These beneficial effects of M-DFCP can provide a novel dietary fiber source for the broiler industry.

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DISCLOSURES

The authors declare that there are no conflicts of interest.

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