Multi-Carbohydrase Addition Into a Corn-Soybean Meal Diet Containing Wheat and Wheat By Products to Improve Growth Performance and Nutrient Digestibility of Broiler Chickens

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Primary Audience: Poultry Feed Manufacturers, Producers, Nutritionists, Researchers

SUMMARY

Feed enzymes are used in poultry diets to enhance nutrient availability and thereby improve growth performances of the birds. The objective of the present study was to investigate the effect of dietary Multi-Carbohydrase (**MC**) supplementation on growth performance, blood metabolites, visceral organ weights, gut morphology, and nutrient digestibility in broiler chickens. A total of 168 one-day-old broiler chicks (47.5 ± 0.20 g) were randomly allocated into one of four dietary treatments in a 2 × 2 factorial arrangement with 6 replications per treatment. Dietary treatments were as follows: (1) positive control (**PC**; energy sufficient, 3,200 ME, kcal/kg); (2) negative control (**NC**; energy deficient, 3,100 ME, kcal/kg); (3) PC with MC (MC; Superzyme-CS^{TM}; 0.05%); (4) NC with MC. Greater ADG ($P = 0.022$) was observed with the birds fed MC for the entire period (1–35 d) compared to birds fed a diet without MC. Birds fed the NC diet supplemented with MC showed improved FCR ($P = 0.037$) compared to birds fed the NC diet during the starter period (1–21 d). MC supplementation increased dry matter ($P = 0.029$), crude protein ($P = 0.015$), and energy digestibility ($P = 0.015$) of the birds compared to those fed a diet without MC on day 21. Moreover, birds fed a diet with MC had increased ($P = 0.037$) dry matter digestibility on day 35 compared to its counterpart, regardless of dietary energy level. Therefore, our study indicated that MC improved growth performance along with

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nutrient digestibility in broiler chickens fed corn soybean-meal-based diets containing wheat and wheat by-products from hatch to 35 d of age, regardless of dietary energy level.

Key words: broiler, growth performance, gut morphology, Multi-Carbohydrase, nutrient digestibility

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DESCRIPTION OF PROBLEM

Digestive enzymes from exocrine glands and epithelial cells play pivotal roles in depolymerizing polymeric macromolecules into their component parts and are known to be critical for cell metabolism along with life support of host animals [1]. The addition of exogenous enzymes in poultry diets have been applied and/or discussed for the last few decades as a method for phenotype improvements in broiler chickens [1, 2]. Exogenous enzymes such as beta-glucanase, xylanase, alpha-galactosidase, and phytase have been suggested for use in poultry diets due to limited endogenous production of these enzymes in the gastrointestinal tract (i.e., exocrine glands and epithelial cells) [1, 3]. Additionally, exogenous enzymes in poultry diets have been demonstrated to increase energy availability, improve gut health, and even further, reduce the amount of phosphorus excretion into the environment [3–5].

Plant-derived feed ingredients such as wheat and wheat by-products generally have higher amounts of indigestible components, such as non-starch polysaccharides (**NSP**) and resistant starch (**RS**), compared to corn. These and other indigestible components tend to escape digestion by endogenous enzymes in the small intestine, which can contribute to impaired growth performance in broiler chickens [6, 7]. Furthermore, undigested fractions of these indigestible components flow past the small intestine and undergo microbial fermentation in the large intestine by microbiota. This process can cause digestive disturbances and microbial imbalance. Thus, dietary supplementation of poultry diets with exogenous enzymes can support growth performance by increasing the utilization of complex nutrient molecules in broiler chickens that lack of sufficient intestinal digestive enzymes [2, 4].

With this in mind, many studies have been conducted to evaluate the effects of different exogenous enzymes with various levels of dietary energy on growth performance and nutrient digestibility in broiler chickens [5, 8]. Furthermore, it has been demonstrated that the addition of exogenous enzymes to cereal-based broiler diets improves nutrient digestibility and leads to higher growth performance in broilers compared to those fed control diets [2, 9]. However, Slominski [3] reported that diets supplemented exclusively with xylanase, beta-glucanase, or both may not lead to performance improvements in broiler chickens fed cereal-based diets. Therefore, it follows that the correct product of exogenous carbohydrase enzymes mixture is critical for realizing better outcomes under commercial conditions due to the fact that many indigestible fractions are present within the feed matrix [3].

The present study was therefore conducted to investigate the effects of a multi-enzyme blend (i.e., MC) on growth performance, blood metabolites, visceral organ weights, intestinal architecture, and nutrient digestibility in broiler chickens fed with corn–soybean-meal-based diets containing wheat and wheat by-products. We hypothesized that feeding a diet with MC would improve intestinal architecture, nutrient digestibility, and thus growth performance of broiler chickens.

MATERIALS AND METHODS

All procedures were approved, and birds were cared for according to the guidelines of the Animal Care and Use Committee (Protocol No. CNU-00751), Chungnam National University, South Korea.

Birds and Housing

An experiment was conducted using one-day-old 168 Ross 308 broiler chickens for 35 d. Birds were allocated into 4 dietary treatments in a 2 × 2 factorial treatment arrangements.

Each dietary treatment contained 6 replicate-pens. Seven birds were housed in each raised wire-floor pen (0.85 × 0.55 × 0.35 m^3), with similar body weights (47.5 ± 0.20 g). Birds were offered the experimental diets on an ad libitum basis and had free access to fresh clean drinking water via nipple drinkers throughout the experimental period.

Experimental Design, Diets, and Treatments

The experiment was conducted using a completely randomized design, with respective factors being (1) two different energy levels [Nutrient sufficient; 3200 ME, kcal/kg (positive control (**PC**)) vs. nutrient deficient; 3100 ME, kcal/kg (negative control (**NC**)], (2) without and with exogenous enzyme (0.05%) were formulated based on corn and soybean meal together with wheat and wheat bran (Table 1), to meet or exceed recommended specifications [10]. In addition, Cr_2O_3 [11] was added as an internal indigestible marker for digestibility analysis in a proportion of 0.3% to all 4 experimental diets. In this study, commercially available Superzyme^{TM}-CS [12] MC was used, which supplied 1,200 U of xylanase, 150 U of glucanase, 700 U of invertase, 5,000 U of protease, 500 U of cellulase, 12,000 U of amylase, and 60 U of mannase per kilogram of diet.

Growth Performance Evaluation

Body weight was recorded at the start and on day 7, 14, 21, 28, and 35 of the experimental period, and feed consumption was recorded

Table 1. Composition (g/kg, as-fed basis) of the Experimental Diets.

	Positive control		Negative control	
Ingredients	1–21 d	22–35 d	1–21 d	22–35 d
Corn	45.08	56.75	44.35	58.35
Wheat	5.90	–	5.90	–
Wheat bran	6.22	5.95	8.15	7.75
Soybean meal 48%	36.00	30.60	36.00	28.40
Vegetable oil	2.80	2.80	1.60	1.60
Limestone	1.50	1.50	1.50	1.50
Monocalcium phosphorus	1.70	1.60	1.70	1.60
Salt	0.30	0.30	0.30	0.30
Vit-Min premix[1]	0.30	0.30	0.30	0.30
DL-Methionine	0.20	0.20	0.20	0.20
Calculated values[2],				
ME, kcal/kg	3200	3200	3100	3100
Crude protein, %	23	20	23	20
Calcium, %	1.1	1.0	1.1	1.0
Available phosphorus, %	0.4	0.4	0.4	0.4
Total lysine, %	1.3	1.1	1.3	1.1
Total methionine, %	0.5	0.5	0.5	0.5
Total Met+Cys, %	0.9	0.9	0.9	0.9
Total threonine, %	0.9	0.7	0.9	0.7
Total tryptophan, %	0.3	0.2	0.3	0.2
Total valine, %	1.1	1.0	1.1	1.0
Total arginine, %	1.5	1.3	1.5	1.3
Analyzed values				
Gross energy, kcal/kg	4163	4149	4156	4088
Crude protein, %	22.8	20.0	22.7	20.1

[1]Supplied per kilogram of total diets: Fe ($FeSO_4.H_2O$), 80 mg; Zn ($ZnSO_4 \cdot H_2O$), 80 mg; Mn ($MnSO_4 \cdot H_2O$) 80 mg; Co ($CoSO_4 \cdot H_2O$) 0.5 mg; Cu ($CuSO_4 \cdot H_2O$) 10 mg; Se (Na_2SeO_3) 0.2 mg; I, ($Ca(IO_3) \cdot 2H_2O$) 0.9 mg; Vitamin A, 24,000 IU; Vitamin D_3, 6000 IU; Vitamin E, 30 IU; Vitamin K, 4 mg; Thiamin, 4 mg; Riboflavin, 12 mg; Pyridoxine, 4 mg; Folacine, 2 mg; Biotin, 0.03 mg; Vitamin B_8, 0.06 mg; Niacin, 90 mg; Pantothenic acid, 30 mg.

[2]The values were calculated according to the values of feedstuffs in NRC (1994).

weekly to calculate average daily gain, average daily feed intake, and feed conversion ratio.

Post-mortem Procedure and Sample Collection

Blood sample collections were carried out on days 21 and 35 of the experiment. Six birds per treatment were selected randomly and euthanized by cervical dislocation for sample collection. Blood samples were collected from the brachial vein into vacutainer [13] before euthanizing the birds. Collected blood samples were quickly transferred to a laboratory for plasma separation.

Abdominal incisions were made on each sacrificed bird to separate the ileum from the gastrointestinal track. The ileum was defined as the segment of small intestine that extended from Meckel's diverticulum to the ileocecal junction. A 3 cm piece of ileum were removed and flushed with ice-cold phosphate-buffered saline (PBS saline) at pH 7.4. The sample was placed into plastic containers that contained 10% formaldehyde for fixation and stored until mucosal morphology measurements analysis. Following the separation of morphological samples, the remaining digesta within each ileal segment was gently collected by finger stripping into labeled plastic containers for ileal nutrient digestibility analysis. Samples were stored in a −20°C freezer until further analysis.

On day 35, both caeca and large intestine were removed separately. Remain contents removed manually and the weights were recorded.

Sample Preparation and Laboratory Analysis

Collected blood samples were centrifuged [14] at 3000 × g for 10 min at 4 °C. Plasma samples were separated and stored at −20 °C until analysis. Plasma urea nitrogen levels were analyzed by urease enzymatic kinetic method and plasma creatinine concentration analyzed with Jaffe assay [15] using auto analyzer [16]. Meantime, plasma glucose level also analyzed glucose oxidase method using the auto analyzer [16].

Ileal samples fixed in 10% formaldehyde were prepared according to process described by Pelicano et al. [17]. Briefly, ring-shaped lengths of ileum were excised, dehydrated, and embedded in paraffin wax. From each of these, six transverse sections (4–6 μm) were cut, stained with haematoxylin and eosin, and mounted on glass slides. The height of 10 well-oriented villi and their associated crypts were measured using live microscope viewer software [18] with an inverted microscope [19] using a calibrated eyepiece graticule.

The digesta samples were pre-dried at 55°C for 24 h, ground through a 0.75-mm sieve [20], and analyzed for levels of dry matter, crude protein [21], ether extract, and gross energy according to the methodologies of AOAC [22]. Chromium oxide concentration of the feed and digesta samples were also analyzed according to Fenton and Fenton [23]. The digestibility coefficient of nutrients was calculated as described by Huang et al. [24] using following equation:

$$\text{Digestibility coefficient} = 1 - [(\text{ID} \times \text{AF}) / (\text{IF} \times \text{AD})]$$

where ID is the concentration of an indigestible marker in the diet; IF is the indigestible-marker concentration in ileal digesta; AF is the nutrient concentration in ileal digesta and AD is the nutrient concentration in the diet.

Calculations and Statistical Analysis

Data were analyzed as completely randomized design, using general linear model (**GLM**) procedure of 2-way ANOVA of SPSS software [25]. Pen was used as the experimental unit for all growth performance measurements. Selected individual birds were considered the experimental unit for blood parameters, proportion of intestinal weights, gut morphology, and nutrient digestibility values, respectively. Dietary energy level and enzyme supplementation were considered as 2 main effects. Mean differences were considered significant at $P < 0.05$, and P-value $0.05 < P < 0.1$ considered as a trend**.** When treatment effects were significant ($P < 0.05$), means were separated using Duncan multiple range test procedures of SPSS software [25].

RESULTS

Growth Performance

Multi-carbohydrase (MC) supplementation improved BW on day 7 (201.16 vs. 209.10 g;

$P = 0.050$), day 14 (529.89 vs. 552.44 g; $P = 0.020$) and day 35 (1992.30 vs. 2070.72 g; $P = 0.020$), regardless of dietary energy level. A tendency for dietary energy level and MC interaction ($P = 0.053$) on BW was observed on day 14.

Supplementation of MC showed a tendency to improve ADG on day 7 (23.56 vs. 22.49 g/day; $P = 0.059$), during the starter period (48.52 vs. 47.06 g/day; 1 to 21 d of age; $P = 0.059$) and during the overall period (58.60 vs. 56.40 g/day; 1 to 35 days; $P = 0.022$), regardless of dietary energy level. On day 14, birds fed NC diet supplemented with MC had increased ADG ($P = 0.045$) compared to those fed the other treatment diets (Table 2).

An interaction was observed between dietary energy level and MC on ADFI in broiler chickens on day 28 ($P = 0.033$), within the grower period (22 to 35 d of age; $P = 0.047$) and within the entire period (1 to 35 d of age; $P = 0.042$). Birds fed the NC diet consumed more feed (68.5

Table 2. Effect of Dietary Energy Level and Multi-Carbohydrase Supplementation in Diet on Growth Performance of Broiler Chickens.[1]

	Dietary Treatment					*P*-value		
	Positive		Negative					
Period	Enzyme −	Enzyme +	Enzyme −	Enzyme +	SEM[2]	Energy[3]	Enzyme[4]	E × E
BW, g								
Day 7	198.91	206.26	203.41	211.93	1.91	0.197	0.050	0.880
Day 14	526.64	530.88	533.13	574.00	4.46	0.012	0.020	0.053
Day 21	1036.07	1052.79	1028.05	1073.19	7.61	0.688	0.056	0.361
Day 28	1595.09	1664.51	1599.74	1642.21	14.81	0.769	0.073	0.654
Day 35	1977.28	2052.24	2007.32	2089.19	15.46	0.291	0.020	0.912
ADG, g/d								
Day 7	22.17	23.21	22.80	23.91	0.27	0.225	0.059	0.942
Day 14	46.82[a]	46.37[a]	47.10[a]	51.73[b]	0.59	0.027	0.093	0.045
Day 21	72.78	74.56	70.70	71.32	1.07	0.229	0.582	0.788
Day 28	79.86	87.39	81.67	81.29	1.99	0.596	0.379	0.332
Day 35	57.94	62.39	62.23	63.86	0.98	0.157	0.136	0.479
Day 1–21	47.25	48.05	46.87	48.99	0.36	0.706	0.059	0.373
Day 22–35	68.90	74.89	71.95	72.57	0.98	0.855	0.107	0.186
Day 1–35	55.91	58.78	56.90	58.42	0.44	0.727	0.022	0.453
ADFI, g/d								
Day 7	27.7	27.1	27.3	27.3	0.38	0.921	0.724	0.711
Day 14	64.1	65.9	67.2	69.9	0.72	0.023	0.141	0.742
Day 21	99.3	104.3	106.7	99.8	2.00	0.719	0.812	0.149
Day 28	138.3[a]	150.9[b]	142.8[a,b]	137.6[a]	1.95	0.275	0.352	0.033
Day 35	114.0	120.7	121.3	125.0	1.85	0.126	0.173	0.690
Day 1–21	63.7	65.8	67.1	65.7	0.73	0.272	0.827	0.243
Day 22–35	126.1[a]	135.8[b]	132.1[a,b]	131.3[a,b]	1.23	0.770	0.086	0.047
Day 1–35	88.7[a]	93.8[b]	93.1[a,b]	91.9[a,b]	0.72	0.385	0.185	0.042
FCR, g/g								
Day 7	1.25	1.17	1.20	1.14	0.02	0.226	0.039	0.656
Day 14	1.37	1.42	1.44	1.35	0.02	0.963	0.581	0.069
Day 21	1.37	1.40	1.51	1.40	0.02	0.193	0.452	0.172
Day 28	1.74	1.74	1.76	1.71	0.03	0.935	0.588	0.684
Day 35	1.97	1.94	1.95	1.96	0.01	0.620	0.664	0.428
Day 1–21	1.33	1.33	1.38	1.30	0.01	0.612	0.037	0.062
Day 22–35	1.86	1.84	1.86	1.83	0.02	0.920	0.483	0.960
Day 1–35	1.54	1.54	1.57	1.51	0.01	0.929	0.088	0.143

[1]Values are the mean of 6 replicates per treatment; [a,b]Values in a row with different superscripts differ significantly ($P < 0.05$).

[2]Pooled standard error of mean.

[3]Dietary energy level.

[4]Enzyme supplementation.

vs. 65.0 g/day; $P = 0.023$) compared to birds fed PC diets on day 14. Birds fed diets supplemented with MC showed a tendency of higher ($P = 0.086$) ADFI within the grower period (22 to 35 d of age) compared to birds fed diets without MC regardless of dietary energy level.

A tendency for dietary energy level and MC interaction on FCR was observed on day 14 ($P = 0.069$) and during the starter period (1 to 21 d of age; $P = 0.062$). Further, improved FCR was observed in birds fed diet supplemented with MC compared to those fed diets without MC on day 7 (1.16 vs. 1.23; $P = 0.039$), during the starter period (1.31 vs. 1.36; $P = 0.037$) and marginally during for entire period (1.56 vs. 1.52; 1 to 35 d of age; $P = 0.088$).

Blood Metabolites

There were no interaction effects ($P > 0.05$) between dietary energy level and MC supplementation with respect to blood metabolites in broiler chickens at day 21 and 35 (Table 3). Moreover, both dietary energy level and MC supplementation did not affect ($P > 0.05$) blood metabolites in broiler chickens at 21 and 35 d of age.

Intestinal Organ Weights

The proportion of caeca weight was increased by 12.5% (0.36 vs. 0.32; $P = 0.027$) in the birds fed PC diet compared to the birds fed NC diets, regardless of MC supplementation on day 35 (Table 4). Nevertheless, the proportion of the large intestine weight was not affected ($P > 0.05$) by dietary energy level nor MC supplementation.

Ileal Digestibility of Nutrients

The addition of MC increased dry matter ($P = 0.029$), crude protein ($P = 0.015$), and energy digestibility coefficient ($P = 0.015$) by 2%, 4%, and 3%, respectively, in these birds compared to those fed a diet without MC on day 21 (Table 5). Moreover, dry matter digestibility coefficient was higher (0.82 vs. 0.81; $P = 0.037$) in MC-supplemented diet fed birds compared to birds fed diet without MC on day 35, regardless of energy level. For the main effect of dietary energy level, birds fed the PC diet showed increased crude protein (0.71 vs. 0.68; $P = 0.011$) and energy digestibility coefficient (0.79 vs. 0.76; $P = 0.001$) on day 21 along with higher energy digestibility coefficient (0.84 vs. 0.82; $P = 0.030$) on day 35, independent of MC supplementation.

Table 3. Effect of Dietary Energy Level and Multi-Carbohydrase Enzyme Supplementation in Diet on Blood Metabolites of Broiler Chickens.[1]

	Dietary Treatment					*P*-value		
	Positive		Negative					
Period	Enzyme −	Enzyme +	Enzyme −	Enzyme +	SEM[2]	Energy[3]	Enzyme[4]	E × E
Plasma urea nitrogen, mg/dL								
Day 21	0.48	0.41	0.48	0.55	0.02	0.135	0.989	0.132
Day 35	0.46	0.39	0.40	0.44	0.04	0.992	0.824	0.467
Creatinine, mg/dL								
Day 21	0.26	0.27	0.26	0.27	0.01	0.918	0.539	0.999
Day 35	0.23	0.22	0.22	0.23	0.01	0.927	0.989	0.186
Glucose, mg/dL								
Day 21	273.00	268.35	269.58	261.52	3.08	0.416	0.316	0.785
Day 35	270.15	263.92	269.28	284.03	5.31	0.379	0.694	0.338

[1] Values are the mean of 6 replicates per treatment.

[2] Pooled standard error of mean.

[3] Dietary energy level.

[4] Enzyme supplementation.

Table 4. Effect of Dietary Energy Level and Multi-Carbohydrase Enzyme Supplementation in Diet on Intestinal Organ Weight of Broiler Chickens on Day 35.[1]

	Dietary Treatment					*P*-value		
	Positive		Negative					
Item	Enzyme −	Enzyme +	Enzyme −	Enzyme +	SEM[2]	Energy[3]	Enzyme[4]	E × E
Caeca weight, %[5]	0.37	0.34	0.33	0.31	0.008	0.027	0.135	0.541
Large intestine weight, %	0.17	0.15	0.14	0.14	0.006	0.183	0.561	0.561

[1]Values are the mean of 6 replicates per treatment; organ weights as a percentage of full body weight.
[2]Pooled standard error of mean.
[3]Dietary energy level.
[4]Enzyme supplementation.
[5]Percentages as proportion of live body weight.

Table 5. Effect of Dietary Energy Level and Multi-Carbohydrase Enzyme Supplementation in Diet on Nutrient Digestibility Co-efficient of Broiler Chickens.[1]

	Dietary Treatment					P-value		
	Positive		Negative					
Period	Enzyme −	Enzyme +	Enzyme −	Enzyme +	SEM[2]	Energy[3]	Enzyme[4]	E × E
Dry matter								
Day 21	0.79	0.80	0.78	0.80	0.003	0.281	0.029	0.704
Day 35	0.81	0.82	0.81	0.82	0.002	0.451	0.037	0.496
Crude protein								
Day 21	0.70	0.72	0.67	0.70	0.005	0.011	0.015	0.859
Day 35	0.80	0.82	0.79	0.80	0.004	0.051	0.061	0.683
Energy								
Day 21	0.79	0.80	0.75	0.77	0.004	0.001	0.015	0.497
Day 35	0.84	0.84	0.81	0.83	0.003	0.030	0.192	0.202

[1]Values are the mean of 6 replicates per treatment.
[2]Pooled standard error of mean.
[3]Dietary energy level.
[4]Enzyme supplementation.

Ileal Morphology

No interaction was observed ($P > 0.05$) between energy level and MC on villus height and crypt depth in section of the ileum on days 21 and 35 (Table 6). Furthermore, neither energy level nor MC supplementation affected ($P > 0.05$) ileal morphological architecture in broiler chickens on days 21 and 35.

DISCUSSION

Increased usage of legumes and non-conventional cereals as feed ingredients in monogastric diets has led to the use of exogenous dietary enzymes to mitigate the negative growth effects associated with anti-nutritional factors in these ingredients. The efficacy of exogenous carbohydrase supplementation has been evaluated in many studies [2, 9] for corn–soybean-meal-based poultry diets rich in indigestible components such as NSP and RS, which can potentially impair growth performance of broiler chickens [6, 7]. In this light, many studies have attempted to improve growth performance through increased nutrient digestibility of broiler chickens fed a diet with many types of exogenous enzymes, including carbohydrase [26] and multi-exogenous-enzyme complexes [2, 27].

Table 6. Effect of Dietary Energy Level and Multi-Carbohydrase Enzyme Supplementation in Diet on Gut Morphology of Broiler Chickens on Day 21 and 35.[1]

	Dietary Treatment					*P*-value		
	Positive		Negative					
Item	Enzyme −	Enzyme +	Enzyme −	Enzyme +	SEM[2]	Energy[3]	Enzyme[4]	E × E
Day 21								
Villus height, μm	343.57	365.79	438.18	474.46	35.74	0.198	0.695	0.924
Crypt depth, μm	55.56	68.03	65.97	77.91	6.07	0.431	0.348	0.983
V:C ratio	6.16	5.51	6.61	6.14	0.20	0.215	0.206	0.823
Day 35								
Villus height, μm	487.37	539.80	481.05	502.03	35.33	0.766	0.622	0.831
Crypt depth, μm	73.54	81.82	73.92	68.23	5.00	0.533	0.901	0.511
V:C ratio	6.60	6.60	6.56	7.39	0.22	0.429	0.384	0.375

[1]Values are the mean of 6 (10 measurements per each) replicates per treatment.

[2]Pooled standard error of mean

[3]Dietary energy level.

[4]Enzyme supplementation.

In the present study, birds fed a diet containing corn, soybean meal, wheat, and wheat bran supplemented with MC showed significantly higher growth performance compared to birds fed diets without MC, regardless of dietary energy level. It could be possible that the improved growth performance observed in birds fed a diet with MC in the present study is due to significantly increased nutrient digestibility within the starter period. The enzyme supplement used in the present study demonstrates a variety of fibrolytic activities, which could be able to degrade indigestible cell wall polysaccharides, thereby allowing endogenous enzymes (i.e., pancreatic- and epithelial-enzymes) access to nutrients entrapped within the feed matrix. In this manner, disruption of the cell walls in feed grains could have improved nutrient digestibility, thereby improving growth performance of broiler chickens in the present study, which is consistent with the findings of the previous study by Luo et al. [28].

In addition, young broiler chickens less than 14 d of age, respond more prominently to commercial exogenous enzymes due to lack of endogenous digestive enzyme secretions [9, 29]. In support of this notion, improved growth performances under MC supplementation were observed in the starter phase in the present study. The fact that there were no differences in growth performance observed during the grower phase could, in part, be due to sufficient amounts of endogenous enzyme activities present within the gastrointestinal tract in birds during this phase. Moreover, starter diets contained more wheat and wheat bran than did grower diets in which provide more substrate for MC to liberated more nutrient for birds during starter phase compered to grower phase. It is known that wheat and wheat by-products contain a considerable amount of non-starch polysaccharides [3, 30] that are not readily digestible by broiler chickens.

In general, feed intake of poultry species is inversely related to dietary energy level as birds will attempt to consume more low energy feeds in order to maintain a consistent energy balance [31–33]. This phenomenon has been clearly demonstrated when dietary energy levels are sufficiently different [31]. In this light, the limited extent of observed ADFI differences in present study could be due to an insufficient difference in dietary energy levels (i.e., 100 kcal/kg) between NC and PC dietary treatments.

Improved feed efficiency with MC supplementation without altering feed intake in the starter phase of the present study corresponded to daily gain and may have been due to improved nutrient digestibility in the starter period. Identically, improved feed efficiency without altering feed intake was observed with MC supplementation in previous studies [34, 35]. In line with our notion, Zhang et al. [34] attributed this improved feed efficiency with no corresponding increase in feed intake to improved nutrient utilization.

In the present study, MC supplementation significantly enhanced dry matter, crude protein, and energy digestibility of birds regardless of energy level. As mentioned previously, enhanced nutrient digestibility may result from degradation of the non-starch polysaccharides in cell walls by various carbohydrases present in MC preparation, which allow endogenous digestive enzymes to access encapsulated nutrients [36, 37]. Delineating the same mechanism, MC preparation was found to improve the nutritional value of flaxseed and canola [27]. Increased energy digestibility associated with increased carbohydrate availability via xylanase activity is one of the main reasons for the addition of supplemental enzymes to wheat-based poultry diets [1, 38]. Consistent with our results, earlier studies have reported that the addition of exogenous enzymes, especially xylanases, to wheat-based diets, increased ileal and total track digestibility of nutrients [34, 39, 40].

Blood urea nitrogen was used in the present study as an indirect indicator of nitrogen utilization efficiency [41, 42], while blood creatinine and glucose were used as indicators of muscle metabolism. Friesen et al. [43] reported that, enzyme supplementation improved starch digestion that was absorbed into blood in the form of glucose and subsequently increased blood sugar content [44]. Nevertheless, responses of all blood metabolites in the present study did not differ between the treatments at any stage of the study even though protein and energy digestibility were significantly affected by dietary energy level and MC supplementation. These observed results may have ascribed the consequence of the interaction between metabolism and absorption of the nutrients with a different mechanism. It has been suggested that a proper balance of amino acid levels in dietary treatments allow for constant amino acid utilization [28]. This may be the reason for similar plasma urea nitrogen levels being observed across dietary treatments in this study, as our dietary treatments maintain the ideal amino acid profile [10]. Similarly, although energy digestibility was affected by dietary energy level and enzyme supplementation, blood creatinine and glucose levels were not affected in the present study. An explanation for this observation could be that dietary fiber digestion was improved with MC although glucose absorption was not affected directly. This postulation is supported by Gao [45] and Luo et al. [28], who reported that xylanase supplementation did not affect glucose concentration excepting creased glucose digestibility. Consistent with our results, Corduk et al. [46] and Rabie et al. [47] reported that dietary energy level did not significantly affect blood creatinine and glucose levels in broiler chickens. Perhaps the observation can be ascribed to the fact that, birds regulate level of glucose absorption to meet the needs of metabolism without maintaining high blood glucose [28, 48]. In agreement with our results, previous studies found that various types of commercial enzymes did not affect blood parameters [47, 49]. Moreover, Józefiak et al. [27] demonstrated that the same MC preparation used in our study had no effect on blood glucose.

In the present study, birds fed diets supplemented with MC had no difference in caeca or large intestine weights compared to birds fed diets without MC, regardless of dietary energy level. Similar to our results, Brenes et al. [50] reported that enzyme supplementation did not significantly affect the relative colon weight in broilers at 42 d of age when fed wheat-based diets. Moreover, the observation of no effect of MC on viscera organ weights is in agreement with previous broiler studies [7, 51].

Ileal morphology is an indicator of gut health and nutrition absorption capacity in broiler chickens and can also be an indicator of the response of the intestinal tract to dietary ingredients [52]. Our results show that neither energy level nor MC supplementation affected ileal morphological parameters in the present study. In contrast, Sharifi et al. [2] and Zhu et al. [7] reported exogenous enzyme supplementation improved ileal morphological parameters in broiler chickens at 40 d and 21 d, respectively. The paradox that morphological parameters were the same across the all treatments is difficult to explain, but improved feed efficiency and nutrient digestibility in this study may not be related to intestinal villus height and crypt depth structure. Perhaps other mechanisms not measured in this study, such as villus width related surface area and ileal adherent mucin layer thickness can result in higher absorption capacity. The findings of Ayoola et al. [53] support this notion, where higher villus surface area was observed when

beta-mannanase was supplemented in diets fed to turkeys without any difference in villus height or crypt depth. Differences between the results of the present study and those of previous studies may be due to differences in diet ingredients (different NSP fractions), compositions of the enzyme complex and supplementation levels of the enzyme complex.

CONCLUSIONS AND APPLICATIONS

1. Multicarbohydrase supplementation in broiler chickens fed diets containing wheat and wheat by-products improved growth performance and feed efficiency along with increased nutrient digestibility during the starter period from 1 to 21 d of age, regardless of dietary energy level.
2. The effect of MC supplementation was less pronounced on blood metabolites, intestinal organ weights, and ileal morphology throughout the entire study period.
3. Further investigation to determine the best MC dosage level is warranted.

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