

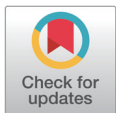
Multi-carbohydase application into energy and amino acid deficient broiler diets: A strategy to enhance performance of broiler chickens

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Abstract

The effect of Multi-Carbohydase (MC) supplementation on growth performance, visceral organ weights, blood metabolites, jejunum morphology, nutrient digestibility, and carcass parameters of broiler chickens fed nutrient-deficient corn soybean-meal based diets containing high levels of non-starch polysaccharides from wheat and wheat by-products was investigated. A total of 378 one-day-old Ross 308 broiler chickens were randomly assigned to one of seven dietary treatments to give six replicates per treatment (nine birds per pen). Dietary treatments were as follows: (1) positive control (PC; commercial standard diet); (2) negative control 1 (NC-1; PC-120 kcal/kg metabolizable energy); (3) NC-2 (PC-3% standardized ileal digestibility [SID] amino acids). The remaining four dietary treatments were formulated with the addition of MC (MC; Superzyme-CS™) into two negative controls along with two supplementation levels of MC (i.e., 0.025% and 0.05%, respectively). Improved body weight, average daily gain, and feed conversion ratio ($p < 0.05$) were observed in broiler chickens fed a reduced energy diet supplemented with MC compared to birds fed NC-1 diet from days 1-35. Additionally, birds fed a reduced energy diet with 0.05% MC showed comparable ($p > 0.05$) growth performance with birds fed PC for 35-day post-hatch. Furthermore, the addition of MC into reduced amino acid diets improved ($p < 0.05$) growth performance. Broiler chickens fed MC supplemented nutrient-deficient diets showed a greater ($p < 0.05$) villus height to crypt depth ratio than birds fed diets without MC on days 21 and 35. Similarly, improved ($p < 0.05$) nutrient digestibility was observed in birds fed reduced energy diets supplemented with MC compared to birds fed NC-1 on days 21 and 35. Our results suggest that MC supplementation into reduced energy or reduced amino acid diets containing wheat and wheat by-products has the potential to improve growth performance and nutrient digestibility while maintaining healthier gut morphology in broiler chickens from 1 to 35 days of age.

Keywords: Broiler, Growth performance, Gut morphology, Multi-Carbohydase, Nutrient digestibility

Competing interests

No potential conflict of interest relevant to this article was reported.

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Availability of data and material

Upon reasonable request, the datasets of this study can be available from the corresponding author.

Authors' contributions

Conceptualization: Patterson R, Heo JM.
Data curation: Wickramasuriya SS, Heo JM.
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Writing - original draft: Wickramasuriya SS, Macelline SP.
Writing - review & editing: Wickramasuriya SS, Macelline SP, Patterson R, Heo JM.

Ethics approval and consent to participate

The complete experimental procedure was prior approved by the animal ethics committee of the Chungnam National University, Korea (Protocol No. CNU - 01155).

INTRODUCTION

The accumulative demand for feed ingredients and the burdensome price of conventional corn-soybean diets compels feed manufacturers and scientists in the field of animal nutrition to find alternatives and substitutions. Several alternatives such as wheat, oats, barley, rye, and cereal byproducts have been considered as energy sources to replace corn in dietary formulations. The key problem with those alternatives is underestimating their digestibility and specific fiber fractions including non-starch polysaccharide (NSP) contents [1].

The use of exogenous dietary enzymes has become widespread in most regions of the world where poultry diets are primarily formulated using alternative plant materials. Recently, carbohydrase blends that combine different exogenous enzymes have become more popular than single or double carbohydrase preparations [2]. A recent study reported that performance in broilers and layer chickens was not enhanced using single carbohydrase activity produced by single fermentation [3]. In contrast, complex indigestible fiber fractions present in the feed matrix respond favourably to the correct blend of exogenous carbohydrase mixtures, which improves growth performance under commercial conditions [2]. With these conventional cereal-based formulations, the use of Multi-Carbohydrase (MC) technology has routinely yielded improvements in growth and feed efficiency about 3%–5% on average [4,5]. The running mode of action behind such improvements is the efficient hydrolysis of indigestible components present in feedstuffs such as NSP, phytate, and oligosaccharides which leads to enhanced nutrient digestibility, and subsequently improve growth performance of host animals [6].

Wheat has a higher crude protein content, more lysine, and superior pelleting capacity than corn in broiler diets. However, factors that limit its inclusion in broiler diet formulations are the lower metabolizable energy (ME) and higher NSP (i.e., arabinoxylans) contents compared to corn [7]. The use of wheat and wheat by-products in broiler diets has previously been tested together with various exogenous carbohydrase enzymes [5,8].

According to our previous study [6], broilers fed a corn soybean-meal-based diet containing wheat and wheat bran along with MC supplementation resulted in approximately 3% improvement in growth performance. Similarly, others [5] observed a 3.2% improvement in the growth performance of broilers fed a wheat-based diet with another multi-enzyme supplement containing different carbohydrase activities. Moreover, MC technology for the improvement of growth performance was tested in broilers fed a diet containing full-fat rapeseed [9], canola meal [10], and barley [11]. Most of these studies were conducted with a 100 kcal dietary energy deficit to test the ability of the MC to compensate for the reduced energy level in the diet. Nevertheless, findings related to digestible amino acid (AA) compensation *per se* are scarce.

Consequently, the present study was designed to investigate the effects of MC supplementation in the reduction of nutrient density diets (i.e., ME or AA) on growth performance, blood metabolites, visceral organ weights, intestinal architecture, nutrient digestibility, and carcass parameters in broilers fed with a corn-soybean-meal based diet containing wheat and wheat by-products. It was hypothesized that feeding a reduced energy or AA diet containing wheat and wheat byproducts with MC would improve intestinal architecture, nutrient digestibility than their counterparts, and subsequently improve the growth performance of broiler chickens.

MATERIALS AND METHODS

Experimental design, birds, and housing

A total of 378 one-day-old broiler chicks (Ross 308) were utilized in a 35-day experiment. Birds

were allocated into seven dietary treatments in a completely randomized design. Each dietary treatment contained six replicate pens. Nine birds were housed in each raised wire-floor pen ($0.85 \times 0.55 \times 0.35 \text{ m}^3$), with similar body weights ($45.6 \pm 0.20 \text{ g}$). All the management practices were followed by Ross 308 broiler management guidance [12]. Birds were offered the experimental diets on an ad-libitum basis and had free access to fresh clean drinking water.

Diets and treatments

The experiment contained three control diets which were 1) positive control (PC) with sufficient nutrient levels [13], 2) Negative control 1 (NC-1) with reduced ME (PC-4% ME [-120 kcal/kg]) and 3) NC-2 with reduced digestible AA [PC-3% AA]. The remaining four dietary treatments were formulated with the addition of MC (Superzyme™-CS, Canadian Bio-System, Calgary, AB, Canada) into two negative controls in two different dosages of 0.025% and 0.05%, respectively. A two-phase feeding protocol was followed with a starter period (days 1–21) and a grower period (days 22–35). All diets were formulated based on corn and soybean meal together with wheat and wheat bran (Tables 1 and 2), to meet or exceed Ross broiler 308 broiler nutrient specification [13]. Moreover, all the diets followed the 0.15% reduction of Ca and available P and were supplemented with 0.01% phytase (Bio-Phytase 5000 G, Canadian Bio-System) to achieve 500 phytase units (FTU/kg) to emulate the commercial practice. NSP fractions and other enzyme substrates were balanced across and between dietary treatments (Table 3) using the dietary NSP profile estimator (iNSPect: inspect.canadianbio.com; Canadian Bio-system). Chromium (III) oxide (> 99.9%, Sigma-Aldrich, St. Louis, MO, USA) was added as an internal marker for digestibility analysis in a proportion of 0.3% to all seven experimental diets. The MC preparation (Superzyme™-CS; Canadian Bio-Systems) which was used, contained 3,300 XYL μg xylanase, 650 GLU μg glucanase, 1,500 INV μg invertase, 12,000 HUT μg protease, 2,600 CMC μg cellulase, 23,000 FAA μg amylase, and 60 MAN μg mannase as calculated enzyme activities. Xylanase activity of the final diets was determined (Table 4) according to the method of Józefiak et al. [9] using Xylazyme AX (Megazyme International Ltd., Ireland) tablets. Phytase activity across each treatment diet was assayed according to the methodology outlined by Slominski et al. [14].

Growth performance evaluation

Body weight and feed disappearance of the cages were recorded on days 7, 14, 21, 28, and 35 of the experimental period. The average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR) were calculated and corrected for mortalities.

Post-mortem procedure and sample collection

Six birds per treatment (one bird from each cage) were removed randomly on days 21 and 35, and euthanized by cervical dislocation. Before euthanization, live body weights were recorded and blood samples were drawn from the brachial vein into BD Vacutainer® coated with K2 EDTA (BD Biosciences, Franklin Lakes, NJ, USA). Plasma separation was carried soon after transferring the blood samples to the laboratory.

Selected birds were sacrificed and the abdomen dissected to separate the gastrointestinal tract and visceral organs. The ileum was defined as the segment of the small intestine that extended from Meckel's diverticulum to the ileocecal junction [15,16]. Contents from the ileal segments were gently collected by finger stripping into labelled plastic containers and stored in a -20°C for nutrient digestibility analysis. A 3–4 cm piece of jejunum was removed and flushed with ice-cold phosphate-buffered saline (PBS saline at pH 7.4). Gently cleaned jejunum samples were placed into a 10% formaldehyde container for fixation and stored until further processing and analyses of mucosal

Table 1. Composition (% , as-fed basis) of the experimental diets for starter period

Item	Starter period - (1–21 days) ¹⁾						
	PC	NC-1	NC-2	NC-1+1X	NC-2+1X	NC-1+2X	NC-2+2X
Corn	46.84	50.54	46.75	50.52	46.73	50.49	46.70
Wheat	8.40	8.40	8.40	8.40	8.40	8.40	8.40
Wheat bran	5.68	5.20	5.68	5.20	5.68	5.20	5.68
Soybean meal (48%)	32.27	31.77	32.47	31.77	32.47	31.77	32.47
Vegetable oil	3.38	0.65	3.40	0.65	3.40	0.65	3.40
Limestone	1.04	1.05	1.04	1.05	1.04	1.05	1.04
Monocal phosphorus	0.77	0.76	0.76	0.76	0.76	0.76	0.76
Salt	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Vit-Min premix ²⁾	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Lysine-HCl	0.29	0.30	0.24	0.30	0.24	0.30	0.24
DL-Methionine	0.20	0.20	0.18	0.20	0.18	0.20	0.18
L-Threonine	0.12	0.12	0.08	0.12	0.08	0.12	0.08
L-Cysteine	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Phytase	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Superzyme	0.00	0.00	0.00	0.025	0.025	0.05	0.05
Cr ₂ O ₃	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Calculated values ³⁾							
ME (kcal/kg)	3,056	2,934	3,055	2,934	3,054	2,933	3,053
Crude prote (%)	22.08	22.08	22.08	22.08	22.07	22.08	22.07
Crude fibre (%)	3.65	3.69	3.65	3.69	3.65	3.69	3.65
NSP (%)	9.93	9.95	9.95	9.95	9.95	9.94	9.95
Calcium (%)	0.73	0.73	0.73	0.73	0.73	0.73	0.73
Available phosphorus (%)	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Lysine (%)	1.34	1.34	1.31	1.34	1.31	1.34	1.31
Methionine (%)	0.54	0.54	0.51	0.54	0.51	0.54	0.51
Methionine+cysteine (%)	0.91	0.91	0.88	0.91	0.88	0.91	0.88
dLysine	1.15	1.15	1.12	1.15	1.12	1.15	1.12
dMethionine	0.48	0.48	0.45	0.48	0.45	0.48	0.45
dThreonine	0.77	0.77	0.74	0.77	0.74	0.77	0.74
dMethionine+Cysteine	0.87	0.87	0.84	0.87	0.84	0.87	0.84

¹⁾PC, nutrient sufficient diet; NC-1, 120 kcal/kg, reduce energy diet; NC-2, 3%, digestible amino acid reduced diet; 1X, 0.025% superzyme-CS addition; 2X, 0.05% superzyme-CS addition.

²⁾Supplied per kilogram of total diets: Fe (FeSO₄·H₂O), 80 mg; Zn (ZnSO₄·H₂O), 80 mg; Mn (MnSO₄·H₂O), 80 mg; Co (CoSO₄·H₂O), 0.5 mg; Cu (CuSO₄·H₂O), 10 mg; Se (Na₂SeO₃), 0.2 mg; I (Ca[IO₃]₂·2H₂O), 0.9 mg; vitamin A, 24,000 IU; vitamin D₃, 6,000 IU; vitamin E, 30 IU; vitamin K, 4 mg; thiamin, 4 mg; riboflavin, 12 mg; pyridoxine, 4 mg; folacine, 2mg; biotin, 0.03 mg; vitamin B₈, 0.06 mg; niacin, 90 mg; pantothenic acid, 30mg.

³⁾The values were calculated according to the values of feedstuffs in NRC [43].

PC, positive control; NC, negative control; ME, metabolizable energy; NSP, non-starch polysaccharides.

morphology. Following this, both the caeca and gizzard were removed separately the contents of which were removed manually, and the weights were recorded to calculate the proportion of weight as compared to the whole body. To analyze the carcass parameters, breast muscle including pectoralis major and pectoralis minor muscle, skin-less right leg, and drumstick weight were recorded.

Table 2. Composition (% as-fed basis) of the experimental diets for grower period

Item	Grower diet (22–35 days) ¹⁾						
	PC	NC-1	NC-2	NC-1+1X	NC-2+1X	NC-1+2X	NC-2+2X
Corn	55.50	58.87	55.60	58.85	55.58	58.82	55.55
Wheat	8.34	8.71	8.34	8.71	8.34	8.71	8.34
Wheat bran	2.98	2.50	2.74	2.50	2.74	2.50	2.74
Soybean meal (48%)	26.25	25.68	26.51	25.68	26.51	25.68	26.51
Vegetable oil	3.78	1.07	3.78	1.07	3.78	1.07	3.78
Limestone	0.95	0.96	0.95	0.96	0.95	0.96	0.95
Monocal phosphorus	0.57	0.56	0.57	0.56	0.57	0.56	0.57
Salt	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Vit-Min premix ²⁾	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Lysine-HCl	0.31	0.32	0.27	0.32	0.27	0.32	0.27
DL-Methionine	0.19	0.18	0.15	0.18	0.15	0.18	0.15
L-Threonine	0.12	0.13	0.08	0.13	0.08	0.13	0.08
L-Cysteine	0.11	0.11	0.10	0.11	0.10	0.11	0.10
Phytase	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Superzyme	0.00	0.00	0.00	0.03	0.03	0.05	0.05
Cr ₂ O ₃	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Calculated values ³⁾							
ME (kcal/kg)	3,200	3,079	3,201	3,078	3,200	3,077	3,199
Crude protein (%)	19.50	19.50	19.50	19.50	19.50	19.50	19.50
Crude fibre (%)	3.42	3.48	3.40	3.48	3.40	3.48	3.40
NSP (%)	8.82	8.84	8.79	8.84	8.79	8.84	8.79
Calcium (%)	0.64	0.64	0.64	0.64	0.64	0.64	0.64
Available phosphorus (%)	0.25	0.24	0.25	0.24	0.25	0.24	0.25
Lysine (%)	1.19	1.19	1.16	1.19	1.16	1.19	1.16
Methionine (%)	0.48	0.48	0.45	0.48	0.45	0.48	0.45
Methionine+Cysteine (%)	0.81	0.81	0.78	0.81	0.78	0.81	0.78
dLysine	1.03	1.03	1.00	1.03	1.00	1.03	1.00
dMethionine	0.43	0.43	0.40	0.43	0.40	0.43	0.40
dThreonine	0.69	0.70	0.66	0.70	0.66	0.70	0.66

¹⁾PC, nutrient sufficient diet; NC-1, 120 kcal/kg, reduce energy diet; NC-2, 3%, digestible amino acid reduced diet; 1X, 0.025% superzyme-CS addition; 2X, 0.05% superzyme-CS addition.

²⁾Supplied per kilogram of total diets: Fe (FeSO₄·H₂O), 80 mg; Zn (ZnSO₄·H₂O), 80 mg; Mn (MnSO₄·H₂O), 80 mg; Co (CoSO₄·H₂O), 0.5 mg; Cu (CuSO₄·H₂O), 10 mg; Se (Na₂SeO₃), 0.2 mg; I (Ca[IO₃]₂·2H₂O), 0.9 mg; vitamin A, 24,000 IU; vitamin D₃, 6,000 IU; vitamin E, 30 IU; vitamin K, 4 mg; thiamin, 4 mg; riboflavin, 12 mg; pyridoxine, 4 mg; folacin, 2mg; biotin, 0.03 mg; vitamin B₆, 0.06 mg; niacin, 90 mg; pantothenic acid, 30 mg.

³⁾The values were calculated according to the values of feedstuffs in NRC [43].

PC, positive control; NC, negative control; ME, metabolizable energy; NSP, non-starch polysaccharides.

Sample preparation and laboratory analysis

Blood samples were centrifuged (Micro 12, Hanil Science, Gimpo, Korea) at 3,000×g for 10 min at 4°C to separate plasma. Plasma samples were stored at -80°C until further analysis. Plasma glucose level was analyzed glucose oxidase method using the Hitachi 7180 chemistry analyzer (Hitachi, Tokyo, Japan). Meanwhile, plasma uric acid level was analyzed by urease enzymatic kinetic method and plasma creatinine concentration analyzed with Jaffe assay [17] using Hitachi 7180 chemistry analyzer (Hitachi).

Jejunum samples fixed in 10% formaldehyde were further processed according to the process

Table 3. NSP fractions in the treatment diets

Item	Non-starch polysaccharides fractions ¹⁾					
	Arabinose	Xylose	Mannose	Galactose	Cellulose & β -glucans	Pectins
Starter ²⁾						
PC	19.08	21.03	3.91	13.27	30.92	11.9
NC-1	19.13	21.25	3.95	12.91	31.16	11.73
NC-2	19.07	20.99	3.91	13.32	30.89	11.93
NC-1+1X	19.13	21.25	3.95	12.91	31.16	11.73
NC-2+1X	19.07	20.99	3.91	13.32	30.89	11.93
NC-1+2X	19.13	21.25	3.95	12.91	31.16	11.73
NC-2+2X	19.07	20.99	3.91	13.32	30.89	11.93
Grower						
PC	19.29	21.97	4.06	11.71	32.00	11.07
NC-1	19.34	22.18	4.09	11.37	32.24	10.88
NC-2	19.27	21.89	4.07	11.78	31.99	11.11
NC-1+1X	19.34	22.18	4.09	11.37	32.24	10.88
NC-2+1X	19.27	21.89	4.07	11.78	31.99	11.11
NC-1+2X	19.34	22.18	4.09	11.37	32.24	10.88
NC-2+2X	19.27	21.89	4.07	11.78	31.99	11.11

¹⁾Values are proportionate to total NSP content.

²⁾PC, nutrient sufficient diet; NC-1, 120 kcal/kg, reduce energy diet; NC-2, 3%, digestible amino acid reduced diet; 1X, 0.025% superzyme-CS addition; 2X, 0.05% superzyme-CS addition.

NSP, non-starch polysaccharides; PC, positive control; NC, negative control.

Table 4. Xylanase and phytase recovery in the feed

Item	Treatment ¹⁾						
	PC	NC-1	NC-2	NC-1+ 1X	NC-2+ 1X	NC-1+ 2X	NC-2+ 2X
Xylanase activity (U/kg)							
Starter	736	731	630	2,954	3,148	6,532	6,350
Grower	889	981	853	3,186	3,537	6,003	6,844
Phytase activity (FTU/kg)							
Starter	739	601	841	739	560	715	638
Grower	763	826	720	512	749	594	734

¹⁾PC, nutrient sufficient diet; NC-1, 120 kcal/kg, reduce energy diet; NC-2, 3%, digestible amino acid reduced diet; 1X, 0.025% superzyme-CS addition; 2X, 0.05% superzyme-CS addition.

PC, positive control; NC, negative control.

described by Wickramasuriya et al [6]. Briefly, ring-shaped and longitudinal lengths of jejunum were excised, dehydrated, and embedded in paraffin wax. From each of these, six transverse sections (4–6 μ m) were cut, periodically stained with haematoxylin and eosin, and mounted on glass slides. The height of 10 well-oriented villi and their associated crypts were measured using NIS-Elements Viewer software (Version: 4.20; NIS Elements, Nikon, Tokyo, Japan) with an inverted microscope (Eclipse TE2000, Nikon Instrument, Melville, NY, USA) using a calibrated eyepiece graticule. Villus height to crypt depth ratio (V: C) was calculated.

The digesta samples were pre-dried at 55 °C for 24 hours, ground through a 0.75-mm sieve (ZM 200 Ultra-Centrifugal Mill, Retsch GmbH & Co. KG, Haan, Germany), and analyzed for levels of dry matter, crude protein (N \times 6.25, macro-Kjeldahl), and gross energy according to the methodologies of AOAC [18]. Chromium oxide concentration of the samples was also analyzed

[19]. The ileal digestibility of nutrients was calculated using the following equation:

$$\text{Digestibility} = [1 - (F_{Cr} / D_{Cr}) \times (D_N / F_N)] \times 100$$

Where F_{Cr} is the concentration of Chromium (III) oxide in the feed; D_{Cr} is the Chromium (III) oxide concentration in ileal digesta; D_N is the nutrient concentration in ileal digesta and F_N is the nutrient concentration in the feed.

Calculations and statistical analysis

Data were separately pooled to evaluate the MC supplementation effect on energy and AA-reduced diets and overall. Pooled data were analyzed using the general linear model (GLM) procedure of one-way Analysis of variance (ANOVA) of SPSS software (Version 24; IBM SPSS 2012, IBM, Armonk, NY, USA) in a completely randomized design. The pen was used as the experimental unit for all growth performance measurements. Selected individual birds were considered as the experimental unit for all other measures. Mean differences were considered significant at $p < 0.05$. When treatment effects were significant ($p < 0.05$), means were separated using Tukey's multiple comparison test.

RESULTS

Growth performance

MC supplementation in broilers fed a 120 kcal/kg reduced energy and 3% reduced AA diet improved ($p < 0.05$) body weight on days 7, 14, 21, 28, and 35 (Table 5). Nevertheless, only broilers fed an energy-deficient diet supplemented with 0.05% MC showed comparable ($p > 0.05$) body weight to birds fed the PC diet. However, MC supplementation improved ($p < 0.05$) body weight of broilers fed reduced AA diets from 22–35 days post-hatch.

ADG, ADFI, and FCR of broilers fed reduced-nutrient diets supplemented with MC are presented in Table 6. The addition of MC into a reduced energy diet improved ($p < 0.05$) the ADG of the broilers during the entire period of study. Moreover, 0.05% MC into reduced energy diets improved ADG in the broilers ($p < 0.05$) to a level commensurate with the PC throughout the experimental period. However, 0.05% MC supplementation into a reduced AA diet improved ($p < 0.05$) daily gain only in the starter period (days 0–21).

MC supplementation in the reduced energy diets did not affect ($p > 0.05$) the feed intake of the

Table 5. Superzyme addition into reduced energy and reduced amino acid diet on body weight of the broiler chickens

Item	Treatment ¹⁾							SEM	p-value ²⁾		
	PC	NC-1	NC-2	NC-1 + 1X	NC-2 + 1X	NC-1 + 2X	NC-2 + 2X		Overall	Energy	AA
Day 7	142.02 ^a	114.37 ^{cd}	105.37 ^d	126.00 ^{bc}	115.65 ^{cd}	137.11 ^{ab}	115.07 ^{cd}	2.159	0.001	0.001	0.024
Day 14	378.03 ^a	304.11 ^{cd}	252.24 ^e	328.37 ^{bc}	268.78 ^{de}	356.10 ^{ab}	288.10 ^{de}	7.265	0.001	0.007	0.016
Day 21	796.55 ^a	647.37 ^c	532.18 ^e	700.71 ^{bc}	555.92 ^{de}	736.50 ^{ab}	623.58 ^{cd}	15.457	0.001	0.032	0.002
Day 28	1,311.64 ^a	1,044.74 ^{cd}	959.30 ^d	1,136.35 ^{bc}	1,051.96 ^{cd}	1,287.39 ^{ab}	1,161.12 ^{abc}	22.580	0.001	0.001	0.005
Day 35	1,913.44 ^a	1,615.71 ^b	1,541.03 ^b	1,729.46 ^{ab}	1,606.48 ^b	1,924.26 ^a	1,764.50 ^{ab}	28.380	0.001	0.001	0.066

¹⁾PC, nutrient sufficient diet; NC-1, 120 kcal/kg, reduce energy diet; NC-2, 3%, digestible amino acid reduced diet; 1X, 0.025% superzyme-CS addition; 2X, 0.05% superzyme-CS addition.

²⁾Overall, p-value for all treatment effect; Energy, p-value for enzyme effect on reduced energy diets; AA, p-value for enzyme effect on reduced amino acid diets.

^{a-e}Means within a same column with no common superscript differ significantly ($p < 0.05$).

PC, positive control; NC, negative control.

Table 6. Superzyme addition into reduced energy and reduced amino acid diet on growth performance of the broiler chickens

Item	Treatment ¹⁾							SEM	p-value ²⁾		
	PC	NC-1	NC-2	NC-1 + 1X	NC-2 + 1X	NC-1 + 2X	NC-2 + 2X		Overall	Energy	AA
Average daily gain (g/d)											
Day 0–21	35.76 ^a	28.66 ^c	23.17 ^e	31.20 ^{bc}	24.30 ^{de}	32.90 ^{ab}	27.52 ^{cd}	0.736	0.001	0.032	0.002
Day 22–35	79.78 ^{ab}	69.17 ^b	72.06 ^{ab}	73.49 ^{ab}	75.04 ^{ab}	84.84 ^a	81.49 ^{ab}	1.354	0.011	0.001	0.250
Day 0–35	53.36 ^a	44.86 ^b	42.73 ^b	48.11 ^{ab}	44.60 ^b	53.67 ^a	49.11 ^{ab}	0.811	0.001	0.001	0.067
Average daily feed intake (g/d)											
Day 0–21	54.72 ^a	52.04 ^a	45.57 ^b	53.88 ^a	46.19 ^b	55.18 ^a	50.57 ^{ab}	0.714	0.001	0.296	0.012
Day 22–35	138.36 ^{ab}	137.33 ^{ab}	130.16 ^{ab}	136.12 ^{ab}	126.28 ^b	145.02 ^a	141.61 ^{ab}	1.743	0.055	0.287	0.056
Day 0–35	88.18 ^{ab}	86.16 ^{abc}	79.40 ^{bc}	86.78 ^{abc}	78.23 ^c	91.11 ^a	86.98 ^{abc}	1.019	0.001	0.268	0.027
Feed conversion ratio (g/g)											
Day 0–21	1.56 ^d	1.89 ^{abc}	2.07 ^a	1.78 ^{bc}	1.96 ^{ab}	1.69 ^{cd}	1.89 ^{abc}	0.030	0.001	0.005	0.165
Day 22–35	1.74 ^{ab}	2.04 ^a	1.8 ^{ab}	1.88 ^{ab}	1.71 ^b	1.71 ^b	1.75 ^{ab}	0.029	0.018	0.001	0.638
Day 0–35	1.63 ^c	1.95 ^a	1.97 ^a	1.82 ^{abc}	1.86 ^{ab}	1.70 ^{bc}	1.83 ^{ab}	0.024	0.001	0.001	0.278

¹⁾PC, nutrient sufficient diet; NC-1, 120 kcal/kg, reduce energy diet; NC-2, 3%, digestible amino acid reduced diet; 1X, 0.025% superzyme-CS addition; 2X, 0.05% superzyme-CS addition.

²⁾Overall, p-value for all treatment effect; Energy, p-value for enzyme effect on reduced energy diets; AA, p-value for enzyme effect on reduced amino acid diets.

^{a–e}Means within a same column with no common superscript differ significantly ($p < 0.05$).

PC, positive control; NC, negative control.

broilers compared with their counterparts from hatch to 35 days of age. MC supplementation in a reduced AA diet increased ($p < 0.05$) feed intake of broiler during the starter period (days 0–21) and overall period (days 0–35).

Improved ($p < 0.05$) FCR commensurate with an improved daily gain was observed in broiler fed a reduced energy diet supplemented with MC during the overall period of study (days 0–35). MC supplementation into a reduced AA diet did not affect ($p > 0.05$) the feed efficiency of broilers from hatch to 35 days.

Visceral organ weights

The addition of MC to a reduced energy diet decreased ($p < 0.05$) the proportion of gizzard weight in the broiler on day 35 (Table 7). MC supplementation into reduced energy diets did not affect ($p > 0.05$) the proportion of gizzard in the broiler on day 21. A reduced AA diet supplemented with MC did not affect ($p > 0.05$) the proportion of gizzard weight either on days 21 or 35. No effect ($p > 0.05$) of MC supplementation into reduced energy or reduced AA diets was observed concerning the proportion of the ceca weights found on days 21 and 35.

Jejunum morphology

Supplementation of MC into a reduced energy diet did not affect ($p > 0.05$) villus height, crypt depth, villus width, or the V: C ratio of broilers on day 21 (Table 8). Similarly, no effect ($p > 0.05$) was observed on morphology in broilers fed reduced energy diets supplemented with MC on day 35, except for the V: C ratio. Supplementation of MC into a reduced energy diet increased ($p < 0.05$) the jejunum V: C ratio of broiler compared to birds fed a reduced energy diet without MC on day 35. Furthermore, broilers fed a reduced AA diet with MC showed a longer ($p < 0.05$) villus height and higher V: C ratio compared to birds fed a reduced AA diet without MC on day 21. On day 35, MC supplementation into a reduced AA diet decreased ($p < 0.05$) the crypt depth but increased the associated V: C ratio.

Table 7. Superzyme addition into reduced energy and reduced amino acid diet on visceral organ weight of the broiler chickens

Item	Treatment ¹⁾							SEM	p-value ²⁾		
	PC	NC-1	NC-2	NC-1+1X	NC-2+1X	NC-1+2X	NC-2+2X		Overall	Energy	AA
Gizzard (%)											
Day 21	2.09	2.31	2.55	2.25	2.29	2.26	2.09	0.048	0.151	0.925	0.066
Day 35	1.43	1.68	1.70	1.50	1.50	1.41	1.54	0.036	0.193	0.044	0.437
Caeca (%)											
Day 21	0.51	0.57	0.58	0.52	0.51	0.56	0.45	0.013	0.067	0.347	0.073
Day 35	0.41	0.41	0.45	0.39	0.38	0.37	0.37	0.012	0.671	0.700	0.156

¹⁾PC, nutrient sufficient diet; NC-1, 120 kcal/kg, reduce energy diet; NC-2, 3%, digestible amino acid reduced diet; 1X, 0.025% superzyme-CS addition; 2X, 0.05% superzyme-CS addition.

²⁾Overall, *p*-value for all treatment effect; Energy, *p*-value for enzyme effect on reduced energy diets; AA, *p*-value for enzyme effect on reduced amino acid diets.

PC, positive control; NC, negative control.

Table 8. Superzyme addition into reduced energy and reduced amino acid diet on jejunum morphology of the broiler chickens

Item	Treatment ¹⁾							SEM	p-value ²⁾		
	PC	NC-1	NC-2	NC-1+1X	NC-2+1X	NC-1+2X	NC-2+2X		Overall	Energy	AA
Day 21											
Villus height (um)	877.72	789.23	729.36	852.35	836.28	834.42	815.14	13.701	0.053	0.463	0.014
Crypt depth (um)	88.09	90.50	84.51	89.63	75.21	76.87	81.31	2.035	0.262	0.151	0.435
Villus width (um)	95.19	100.12	87.74	97.01	93.08	98.13	94.42	1.170	0.128	0.778	0.167
V:C ratio	10.40 ^{ab}	9.03 ^b	9.02 ^b	9.76 ^{ab}	12.02 ^a	11.36 ^{ab}	9.47 ^{ab}	0.269	0.005	0.052	0.003
Day 35											
Villus height (um)	1,077.62	989.87	974.93	998.75	1,017.56	1,135.55	1,047.20	19.359	0.284	0.061	0.699
Crypt depth (um)	99.68	115.08	121.31	79.72	82.66	114.95	109.09	4.446	0.062	0.199	0.002
Villus width (um)	109.14	110.00	109.26	105.67	101.80	112.38	105.83	1.229	0.317	0.381	0.353
V:C ratio	11.58 ^{abc}	9.41 ^{bc}	8.22 ^c	13.09 ^a	12.68 ^{ab}	12.04 ^{ab}	10.19 ^{abc}	0.378	0.001	0.018	0.001

¹⁾PC, nutrient sufficient diet; NC-1, 120 kcal/kg, reduce energy diet; NC-2, 3%, digestible amino acid reduced diet; 1X, 0.025% superzyme-CS addition; 2X, 0.05% superzyme-CS addition.

²⁾Overall, *p*-value for all treatment effect; Energy, *p*-value for enzyme effect on reduced energy diets; AA, *p*-value for enzyme effect on reduced amino acid diets.

^{ab}Means within a same column with no common superscript differ significantly ($p < 0.05$).

PC, positive control; NC, negative control.

Blood metabolites

MC supplementation into a reduced nutrient diet did not affect ($p > 0.05$) the blood metabolites of broilers on days 21 and 35 (Table 9).

Ileal digestibility of nutrients

Supplementation of MC into a reduced energy diet improved ($p < 0.05$) digestibility of dry matter, crude protein, and energy in the broiler compared to birds fed a reduced energy diet without MC on day 21 (Table 10). Moreover, birds fed a reduced energy diet supplemented with MC showed greater ($p < 0.05$) digestibility of crude protein and energy than their counterparts on day 35. Improved digestibility of crude protein and energy ($p < 0.05$) were observed in broiler fed reduced AA diets supplemented with MC on day 21. Ileal nutrient digestibility was not affected ($p > 0.05$) due to MC supplementation into a reduced AA diet on day 35.

Carcass parameters

No differences ($p > 0.05$) were observed in carcass parameters on days 21 and 35 among dietary treatments, respectively (Table 11).

Table 9. Superzyme addition into reduced energy and reduced amino acid diet on blood metabolites of the broiler chickens

Item	Treatment ¹⁾							SEM	p-value ²⁾		
	PC	NC-1	NC-2	NC-1+1X	NC-2+1X	NC-1+2X	NC-2+2X		Overall	Energy	AA
Creatinine (mg/dL)											
Day 21	0.21	0.23	0.21	0.22	0.20	0.19	0.22	0.004	0.558	0.179	0.511
Day 35	0.19	0.20	0.21	0.21	0.20	0.20	0.21	0.004	0.787	0.538	0.938
Glucose (mg/dL)											
Day 21	301.73	294.01	319.59	300.48	300.94	296.98	303.12	3.438	0.588	0.872	0.214
Day 35	303.39	311.76	297.38	312.98	311.58	299.62	296.79	2.709	0.459	0.427	0.176
Uric acid (mg/dL)											
Day 21	6.33	9.10	10.11	10.03	8.45	8.88	8.33	0.414	0.227	0.714	0.492
Day 35	4.30	5.20	5.50	6.28	5.06	5.56	4.27	0.228	0.199	0.485	0.186

¹⁾PC, nutrient sufficient diet; NC-1, 120 kcal/kg, reduce energy diet; NC-2, 3%, digestible amino acid reduced diet; 1X, 0.025% superzyme-CS addition; 2X, 0.05% superzyme-CS addition.

²⁾Overall, p-value for all treatment effect; Energy, p-value for enzyme effect on reduced energy diets; AA, p-value for enzyme effect on reduced amino acid diets.

PC, positive control; NC, negative control.

Table 10. Superzyme addition into low energy and low amino acid diet on ileal digestibility of the nutrient in broiler chickens

Item	Treatment ¹⁾							SEM	p-value ²⁾		
	PC	NC-1	NC-2	NC-1+1X	NC-2+1X	NC-1+2X	NC-2+2X		Overall	Energy	AA
Dry matter (%)											
Day 21	63.73 ^a	60.81 ^{ab}	59.19 ^b	63.94 ^a	60.63 ^{ab}	63.58 ^a	63.52 ^a	0.399	0.001	0.001	0.052
Day 35	73.68	71.19	72.77	70.89	72.40	73.66	73.15	0.329	0.114	0.119	0.787
Crude protein (%)											
Day 21	81.26 ^{ab}	80.38 ^b	80.31 ^b	82.93 ^a	81.85 ^{ab}	83.02 ^a	82.88 ^a	0.239	0.001	0.001	0.011
Day 35	85.54 ^a	83.92 ^b	84.82 ^{ab}	85.07 ^{ab}	85.55 ^a	85.98 ^a	85.09 ^{ab}	0.155	0.007	0.014	0.134
Energy (%)											
Day 21	66.98 ^a	64.34 ^{ab}	61.07 ^b	64.74 ^{ab}	64.07 ^{ab}	67.50 ^a	68.38 ^a	0.499	0.001	0.029	0.003
Day 35	74.95 ^{ab}	72.44 ^b	73.56 ^{ab}	72.93 ^b	72.77 ^b	76.06 ^a	74.25 ^{ab}	0.306	0.006	0.001	0.519

¹⁾PC, nutrient sufficient diet; NC-1, 120 kcal/kg, reduce energy diet; NC-2, 3%, digestible amino acid reduced diet; 1X, 0.025% superzyme-CS addition; 2X, 0.05% superzyme-CS addition.

²⁾Overall, p-value for all treatment effect; Energy, p-value for enzyme effect on low energy diets; AA, p-value for enzyme effect on low amino acid diets.

^{ab}Means within a same column with no common superscript differ significantly ($p < 0.05$).

PC, positive control; NC, negative control.

Table 11. Superzyme addition into low energy and low amino acid diet on carcass parameters of the broiler chickens

Item	Treatment ¹⁾							SEM	p-value ²⁾		
	PC	NC-1	NC-2	NC-1+1X	NC-2+1X	NC-1+2X	NC-2+2X		Overall	Energy	AA
Breast (%)											
Day 21	21.62	21.02	21.79	20.99	21.03	22.43	22.05	0.285	0.778	0.227	0.671
Day 35	25.45	25.07	24.27	25.69	24.64	26.73	23.97	0.264	0.077	0.330	0.662
Whole leg (%)											
Day 21	8.35	7.80	8.74	8.21	8.79	8.95	8.51	0.130	0.241	0.083	0.836
Day 35	8.90	8.65	9.57	9.17	8.98	8.73	9.26	0.130	0.534	0.348	0.558
Drumstick (%)											
Day 21	3.93	3.89	4.31	4.08	4.22	3.95	4.09	0.059	0.451	0.741	0.662
Day 35	4.06	4.15	4.28	4.18	4.11	4.22	4.27	0.046	0.877	0.905	0.581

¹⁾PC, nutrient sufficient diet; NC-1, 120 kcal/kg, reduce energy diet; NC-2, 3%, digestible amino acid reduced diet; 1X, 0.025% superzyme-CS addition; 2X, 0.05% superzyme-CS addition.

²⁾Overall, p-value for all treatment effect; Energy, p-value for enzyme effect on low energy diets; AA, p-value for enzyme effect on low amino acid diets.

PC, positive control; NC, negative control.

DISCUSSION

Wheat and wheat by-products are commonly used as a substitute for corn in broiler diets, although wheat has a lower apparent ME compared to corn. Moreover, higher fiber contents, including NSP, in wheat and wheat byproducts are often problematic in their full replacement [20]. Nevertheless, wheat has a superior protein profile, pelleting properties, and a higher lysine content compared to corn which partially offsets the aforementioned negative nutritional characteristics. Recent studies have shown that wheat contains soluble NSP cell wall components with anti-nutritive effects, such as xylans and β -glucans, which can obstruct nutrient digestion and absorption in broiler chickens. To overcome the NSP issues in feed ingredients, exogenous carbohydrases have been evaluated in many studies and found to be effective in broilers in terms of improving nutrient digestibility [21,22]. The addition of single or double carbohydrases containing xylanase, β -glucanase, and β -mannanase, especially in corn soybean-meal-based diets, has been well documented and demonstrated to improve growth performance and nutrient utilization in broilers [23,24]. Nevertheless, little is known about the effect of carbohydrase combination in a wheat-based diet for broilers and the subsequent effects in a diet with reduced nutrient density. Hence, this study evaluated the effect of two levels (i.e., 0.025% or 0.05%) of MC supplementation on growth performance along with nutrient digestibility, intestinal responses, and carcass parameters of broilers fed a reduced nutrient density diet containing wheat and wheat by-products.

In the present study, broilers fed an MC-supplemented reduced energy diet had a higher body weight, daily weight gain, and feed efficiency compared to those fed diets without MC from hatch to 35 days. Despite the fact that MC improved growth performance in a dose-dependent manner in the reduced energy diets, 0.05% supplementation equalled the growth performance of the birds fed a PC diet. Additionally, a recent study [6], demonstrated an improvement in growth performance in broilers fed an MC supplemented diet containing wheat and wheat byproducts. The results obtained in this study may prove that exogenous carbohydrase eliminates the nutrient-encapsulating effect of NSP and allows endogenous enzymes to digest and absorb more nutrients from the feed matrix and subsequently enhance the growth performance of broilers. In support of this notion, improved nutrient digestibility in the current study led to higher growth performance and feed efficiency in broilers fed reduced energy diets supplemented with MC. However, MC supplementation into digestible AA deficient diets generated significant and numerical increment of CP digestibility at days 21 and 35 post-hatch, respectively. Nevertheless, these improvements of CP digestibility coefficients had not supported growth performance but compromised FCR by 12% (1.63 *versus* 1.85) in comparison to PC diets at 35 days post-hatch. In the present study, soybean meal and corn approximately represent 70% and 20% of CP levels in the diets, respectively. Therefore, outcomes of protein digestibility results may be attained from the impact of MC supplementation on aforesaid feedstuffs in AAs deficient diets. Soybean meal and corn are considered as the feedstuffs which are limiting on lysine, and sulfur-containing AAs; consequently, increasing digestibility of those feedstuffs may generate adverse effects to broiler chickens such as AAs imbalances at the sites of protein synthesis or further increase of the deficiency of most limiting AAs in broiler chickens. Therefore, MC supplementation to energy deficient diets is more favourable for optimize growth performance in broiler chickens rather than digestible AAs deficient diets.

Previous studies have tested the physiological effects of exogenous MC on gut morphology, nutrition absorption, blood profiles, and microbial populations of broiler, although they may be less tangible from a commercial and practical nutritional perspective [5,25]. Nonetheless, the relevance of the aforementioned metrics in broilers should be taken into consideration in so much,

as understanding the underlying mechanism of how body weight and feed efficiencies are affected by dietary enzyme supplementation. In the present study, no difference in gizzard weight was observed among treatments on day 21. Zhu *et al.* [26] reported similar findings that carbohydrase enzyme supplementation had no significant effect on the relative weight of the gizzard at day 21. Interestingly, this indicates that supplementation with MC significantly lowers the proportion of the gizzard weight on day 35 in a dose-dependent manner when broiler fed reduced energy diets. This effect was not prominent when MC was supplemented in reduced AA diets. In agreement with the current study, Wu *et al.* [5] reported that MC enzyme supplementation reduced the relative gizzard weight of broilers by day 42. The exact mechanism for the decreased gizzard weight is not clear and has not been documented previously, although observation indicates that it can be ascribed to the incidence of gastric reflexes and grinding actions [27]. However, it has been found that indigestible plant cell wall structures make coarse digesta and increase digesta retention time in the gizzard, thus increasing gizzard weight due to increased functionality [28]. In the present study, the NC-1 diet resulted 6% reduction in feed intake than the NC-1+2X diets in the grower phase. This may indicate a slower digesta passage rate in broilers offered NC-1 diet probably because of accumulation of undigested plant cell wall structures in gizzard which may also result in increased gizzard weight in broilers offered NC-1 diet at grower phase.

The intestinal mucosa is intended for nutrient digestion and absorption and as such has a high cellular turnover rate [26], therefore, small intestinal morphology was assessed to evaluate the impact of dietary enzyme supplementation on the gut health of broilers. Previous studies showed both improvement [26,29] and no effect [6] in small intestinal morphology with exogenous carbohydrase supplementation. Even though in the current study, no significant effects on villus height and crypt depth were observed, an improved V: C ratio was observed in broilers fed a reduced energy and AA diets supplemented with MC on days 21 and 35, respectively. Consistent with the higher V: C ratio, previously reported [29] that carbohydrase supplementation in corn soybean meal diets containing flaxseed significantly increased the V: C ratio of the duodenum and jejunum of 22 days old Ross broiler chickens. It has been speculated that a higher V: C ratio is evidence of a well-differentiated intestinal mucosa with an enhanced digestive and absorptive capacity [29,30]. Hence, the improved nutrient digestibility in this study may be ascribed to the improved V: C ratio. According to Wang *et al.* [31], these significant modifications of the intestinal structure may interrelate with the gut viscosity. Inspire of the fact that the gut morphology of the broilers improved with carbohydrase supplementation, the underline mechanism is yet to be discovered.

The current results showed improved dry matter, crude protein, and energy digestibility of broilers fed reduced ME and reduced AA diets supplemented with MC by day 21. However, the effect was persistent for crude protein and energy digestibility on day 35. Consistent with the present results, our previous study showed [6] improved nutrient digestibility in broilers fed reduced energy diets containing wheat and wheat byproducts supplemented with MC. It is reasonable to attribute improved digestibility to the action of the carbohydrases on the cell wall, facilitating action by endogenous digestive enzymes. Other studies [32,33,34] have also noted improved energy and protein digestibility in broiler fed diets supplemented with different carbohydrase combinations. The present study demonstrated that the addition of 0.05% MC into both energy and AA-deficient diets improved the nutrient digestibility in the broilers over the control.

Biochemical and hematological parameters can indicate the efficacy of feed additives in broiler nutrition [35,36]. The addition of carbohydrase improves starch digestion commensurate with absorption in the form of glucose, which subsequently increases blood glucose level [37]. However, broilers fed diets supplemented with MC were similar in respect of blood creatinine, blood

glucose, and blood uric acid levels compared to broilers fed a diet without MC in the present study. Similarly, a previous study [6] also detected similarities in blood metabolites with the addition of MC into corn soybean-meal based diet with wheat and wheat by-products. Others [9,38,39] also found that the addition of MC did not affect blood glucose, a result which could be account for by homeostatic regulation of blood and metabolite requirements.

Although growth parameters are the key interest in the broiler industry, carcass yield and consumer demand for commercial cuts are equally important when considering an operation's profitability. Nevertheless, studies examining the effects of enzyme combinations on carcass characteristics are scarce [40]. In the present study, birds fed a reduced nutrient diet supplemented with MC were similar in the proportion of breast meat, whole leg meat, and drumstick yield compared to broilers fed a reduced nutrient diet without MC on days 21 and 35. Roofchaei et al. [41] also reported that the addition of exogenous carbohydrase containing beta-glucanase and beta xylanase to wheat soybean meal-based diets did not affect breast and thigh meat yield by day 35. In another study [40] it was demonstrated that the addition of xylanase, amylase, or a combination of these with phytase in corn soybean-meal diets did not affect carcass, leg, and breast meat weight. In contrast, Dalólio et al. [42] reported that supplementation with exogenous enzymes in corn soybean-meal diets improved breast meat yield in broilers on day 42. Observed dissimilarities, between these studies on carcass yield, may be attributed to the different factors such as feed formulations, enzyme supplementation, and the methods used to measure the carcass yields. Assuring the carcass yield in proportion to live body weight may indicate no significant effect.

In conclusion, MC supplementation in reduced energy or reduced AA diets containing wheat and wheat byproducts has a positive effect on growth performance and nutrient digestibility, along with improved gut morphology in broiler from 1 to 35 days of age. Furthermore, 0.05% MC supplementation restored growth performance in broiler fed a reduced energy diet to equal that of the nutrient sufficient diets.

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