

Original Research Article

Effect of probiotic, carbohydrase enzyme and their combination on the performance, histomorphology and gut microbiota in broilers fed wheat-based diets

Abstract

The effect of supplementation of probiotic and/or carbohydrase on performance and gut health in broiler chickens was studied.

Diet 1 (~~Negative control, NC~~) was the basal diet with no supplement while diets 2 (~~Positive control, PC~~), 3, 4 and 5 were supplemented with antibiotic, probiotic, carbohydrase and combination of probiotic and carbohydrase, respectively in a randomized complete block design.

The experiment was carried out at the Poultry Unit of the Teaching and Research Farm, ~~of the~~ University of Ibadan, Nigeria between March and April, 2013. 240 one-day-old ~~unsexed~~ broiler chickens (~~Arbor acre strain~~) were allotted to 5 diets with 6 replicates of 8 birds each in a 35-day experiment. On day 35, birds were sacrificed and digesta samples from the duodenum, ileum, caecum and colon were collected for microbial load count. Digesta viscosity and pH from the ileum were also determined. Sections of the ileum (~~5cm posterior to Meckel's diverticulum~~) were obtained for morphological measurements.

Result showed that diet had no effect on the feed intake, dry matter intake and feed conversion ratio of birds at starter (d 0-21), grower phases (d 22-35) and overall period (d 0-35). Body weight gain was significantly ($P = .05$) improved at d 0-21 and 22-35 with the addition of probiotic and/or carbohydrase but not at d 0-35. Diet had no effect on the microbiota in the various segments of the GIT of birds. Coliform counts of birds fed diet supplemented with carbohydrase were significantly ($P = .05$) higher (5.64×10^6 cfu/ml) than the values in other diets. The crypt depth, villus height, villus width and villus height: crypt depth ratio were not affected by diet. Highest digesta viscosity (1072mPa) was recorded in birds on the negative control while the least digesta viscosity (529mPa) was observed in birds on diet supplemented with carbohydrase enzyme.

It is concluded that probiotic and/or carbohydrase are viable feed supplements in wheat based-diets for broilers.

Keywords: Probiotic, carbohydrase, growth response, gut integrity, broiler chickens

1. INTRODUCTION

Antibiotics have been used for decades in animal production. At minimal levels of inclusion in diets, antibiotics have proven to enhance growth and to increase feed conversion in livestock. However, the prolonged use of antibiotics as feed supplement in poultry has been a serious concern because of the potential development of antimicrobial resistance and transfer of antibiotic resistance genes from animal to human [1]. Though the EU has banned the use of antibiotics in animal feeds [2,3], antibiotic is still used in several other countries where a similar legislative legislation is yet to take effect. Consequently, the focus in research has shifted to other feed supplements such as probiotics, prebiotics, organic acids, acidifiers, enzymes, plant extracts as alternatives to antibiotics in poultry diets. Several possible mechanisms of these feed additives have been suggested such as altering of the gut pH [4,5], maintaining protective gut mucins [6], selecting beneficial intestinal organisms or ones antagonistic to pathogens [7], enhancing nutrient uptake [8,9] or increasing the immune response [10,11]. Apparently, probiotics and prebiotics have been shown to reduce serum cholesterol and abdominal fat in broiler chickens [12,13], increase performance, improve resistance to pathogenic bacteria colonization and enhance host mucosa immunity thus resulting in a reduced pathogen load, and improved health status of broilers [14,15].

Wheat is an important source of energy in broiler diets because of its high starch and crude protein contents [16], but arabinoxylans, the predominant non-starch polysaccharide (NSP) in wheat can form highly viscous solutions thus increasing the intestinal digesta viscosity. Increased gut digesta viscosity usually limits contact between digestive enzymes and substrates, as well as contact between nutrients and absorption sites in the intestinal mucosa. [17] observed abnormal villi in the ileum of laying hens fed wheat middling-based diets without xylanase supplementation but these adverse effects were reversed with enzyme addition. Carbohydrase is a commercial multi-enzyme complex that contains β -glucanase and β -xylanase, amylase and cellulase. Its addition to appropriate relevant poultry diets usually results in numerous beneficial effects, such as increased utilization of nutrients, improved apparent metabolizable energy values and decreased viscosity of intestinal digesta and improved growth performance [18,19,20,21]. It was therefore the aim of this study to investigate the effect of probiotic (Grow Up[®] -grow-up), carbohydrase and their combination on the performance, gut morphology, intestinal microbiota and digesta viscosity of broiler chickens fed wheat-based diets.

2. MATERIAL AND METHODS

2.1 Experimental diets and management of birds

Two hundred and forty one-day-old unsexed broiler chicks used for this experiment were obtained from a local commercial hatchery (CHI Farms, Ajanla, Lagos - Ibadan express way). The birds were tagged, weighed, and randomly allotted to 5 dietary treatments sorted by body weight in a completely randomized block design. Each diet had 6 replicates of 8 birds per replicate. The experimental diets and fresh water were supplied *ad libitum* during the study period that lasted for 35 days.

The basal diet was a wheat-soyabean meal diet formulated to meet the nutrient requirements [22] for starter (0 to 21 days) and grower (22 to 35 days) broilers. Diet 1 was the negative control, NC (basal without antibiotic); Diet 2 was the positive control, PC (basal with oxytetracycline

hydrochloride added at the rate 0.105g/kg). Diets 3 and 4 were the NC plus either 10g/kg probiotic (*Lactobacillus sporogenes* + *Saccharomyces cerevisiae*) or 0.5g/kg carbohydrase (amylase -80000U.A/kg, protease -80000000U.P/kg, xylanase -500000U.X/kg, glucanase -300000U.BG/kg, cellulose -700000U.C/kg, lipase 20000U.L/kg. Diet 5 was the NC together with both probiotic and carbohydrase. The gross compositions of the experimental diets are as shown in Tables 1 and 2. The analyzed proximate compositions of the diets are shown in Tables 3 and 4.

At day 35, two birds from each replicate were sacrificed and eviscerated. From each of the birds, the transverse and longitudinal sections of the ileal tissue were cut and tissues from each group of birds in replicate were preserved in pre-labeled polyvinyl bottle filled formalin and later processed for histological examination according to the methods of [23,24].

Table 1. Gross composition (g/ kg) of experimental starter diets (d 0-21)

Ingredient	Diet ¹				
	NC	PC	NC+Probiotic	NC+Carbohydrase	NC+Probiotic +Carbohydrase
Wheat	550	549.895	540	549.5	559.5
Soybean meal	270	270	270	270	250
Fish meal	50	50	50	50	50
Soybean oil	98.5	98.5	98.5	98.5	98.5
Dicalcium phosphate ²	15	15	15	15	15
Limestone ³	10	10	10	10	10
DL-meithionine	1	1	1	1	1
L-lysine	1	1	1	1	1
Salt	2	2	2	2	2
Vit-Mineral premix ⁴	2.5	2.5	2.5	2.5	2.5
Antibiotic	0	0.105	0	0	0
Probiotic	0	0	10	0	10
Carbohydrase	0	0	0	0.5	0.5
Total	1000	1000	1000	1000	1000
Calculated nutrients and energy					
Crude protein, g/kg	228	228	227	228	221
ME Kcal/kg	3153	3153	3124	3152	3136
Crude fiber, g/kg	35.9	35.9	35.6	35.9	34.8

Ca, g/kg	10.0	10.0	10.0	10.0	9.98
Total Phosphorus, g/kg	7.71	7.71	7.67	7.71	7.61
Non-phytate P, g/kg	4.14	4.14	4.13	4.14	4.10
Ca:P	1.30	1.30	1.31	1.30	1.31
Ca:NPP	2.42	2.42	2.43	2.42	2.43
Amino acid, g/kg					
Ile	9.24	9.24	9.20	9.24	8.89
Leu	16.5	16.5	16.4	16.5	15.9
Lys	12.6	12.6	12.6	12.6	12.1
Met	4.80	4.80	4.78	4.80	4.70
Cys	3.76	3.76	3.73	3.75	3.65
Thr	8.20	8.20	8.16	8.20	7.89
Trp	3.27	3.27	3.25	3.27	3.13
Val	10.4	10.4	10.4	10.4	10.1

103 ¹NC = Negative control, PC = Positive control; ²Contains 22% Ca and 18% P

104 ³Contains 38% Ca; ⁴Supplied the following per kg DIET: Vit. A, 5484 IU; Vit. D3, 2643 ICU; Vit E,11 IU; Menadione sodium
105 bisulfite,4.38 mg; Riboflavin, 5.49 mg; d-Vpantothenic acid, 11 mg; Niacin, 44.1 mg; Choline chloride, 771 mg; Vit B12, 13.2
106 ug; Biotin, 55.2 ug; Thiamine mononitrate, 2.2 mg; Folic acid, 990 ug; ; Vit. D3, 881 ICU; Vit E,3.67 IU; Menadione sodium
107 bisulfite,1.46 mg; Riboflavin, 1.83 mg; d pantothenic acid, 3.67 mg; Niacin, 4.69 mg;

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110 Table 2. Composition of experimental grower diets (d 22-35)

Ingredient	Diet ¹				
	NC	PC	NC+Probiotic	NC+Carbohydase	NC+Probiotic +Carbohydase
Wheat	620	628.395	618.5	628	618
Soybean meal	238.5	230	230	230	230
Fish meal	40	40	40	40	40
Soybean oil	70	70	70	70	70
Dicalcium phosphate ²	15	15	15	15	15
Limestone ³	10	10	10	10	10
DL-meithionine	1	1	1	1	1
L-lysine	1	1	1	1	1
Salt	2	2	2	2	2
Vit-Mineral premix ⁴	2.5	2.5	2.5	2.5	2.5
Antibiotic	0	0.105	0	0	0
Probiotic	0	0	10	0	10
Carbohydase	0	0	0	0.5	0.5
Total	1000	1000	1000	1000	1000
Calculated nutrients and energy					
Crude protein, g/kg	218	215	214	215	214
ME Kcal/kg	3021	3026	3000	3025	2996
Crude fiber/g/kg	35.7	35.3	35.1	35.3	35.0
Ca, g/kg	9.60	9.58	9.58	9.58	9.58

Total P, g/kg	7.52	7.49	7.46	7.49	7.46
Non-phytate P, g/kg	4.15	4.14	4.13	4.14	4.12
Ca:P	1.28	1.28	1.28	1.28	1.28
Ca:NPP	2.31	2.32	2.32	2.32	2.32
Amino acid, g/kg					
Ile	8.63	8.50	8.45	8.50	8.45
Leu	15.6	15.4	15.3	15.4	15.3
Lys	10.7	10.5	10.5	10.5	10.5
Met	3.56	3.53	3.50	3.52	3.50
Cys	3.69	3.66	3.63	3.66	3.63
Thr	7.65	7.53	7.50	7.53	7.49
Trp	3.07	3.02	3.00	3.02	3.00
Val	9.85	9.73	9.67	9.72	9.67

¹NC = Negative control, PC = Positive control; ²Contains 22% Ca and 18% P

³Contains 38% Ca; ⁴Supplied the following per kg DIET: Vit. A, 5484 IU; Vit. D3, 2643 ICU; Vit E, 11 IU; Menadione sodium bisulfite, 4.38 mg;

Riboflavin, 5.49 mg; d-Vpantothenic acid, 11 mg; Niacin, 44.1 mg; Choline chloride, 771 mg; Vit B12, 13.2 ug; Biotin, 55.2 ug; Thiamine

mononitrate, 2.2 mg; Folic acid, 990 ug; ; Vit. D3, 881 ICU; Vit E, 3.67 IU; Menadione sodium bisulfite, 1.46 mg; Riboflavin, 1.83 mg; d-pantothenic

acid, 3.67 mg; Niacin, 4.69 mg;

Table 3. Proximate composition of experimental starter diets (%)

Variables	NC	PC	NC + P	NC+ C	NC+ P + C
Dry matter	89.83	90.58	91.20	86.76	93.46
Crude protein	23.18	20.56	23.84	24.28	21.00
Ether extract	11.00	13.00	22.00	22.00	13.00
Ash	7.50	6.00	6.00	5.50	9.00
Crude fibre	4.20	3.50	2.70	1.10	3.00
NFE	54.12	56.94	45.46	47.12	54.00
GE (Kcal/g)	4.54	4.53	4.53	4.53	4.53

NC- Negative control, PC- Positive control, P- Probiotic, C- Carbohydrase, NFE- Nitrogen free extract, GE- Gross energy.

Table 4. Proximate composition of experimental grower diets (%)

Variables	NC	PC	NC + P	NC + C	NC + P + C
Dry matter	91.46	91.16	91.02	91.38	90.97
Crude protein	24.19	23.63	22.53	21.87	21.87
Ether extract	22.00	21.00	23.00	22.00	23.00
Ash	5.50	6.50	8.50	5.50	8.50
Crude fibre	1.30	0.90	2.30	4.20	3.30
NFE	47.01	47.97	43.67	46.43	43.33
GE (Kcal/g)	4.52	4.53	4.54	4.53	4.54

NC- Negative control, PC- Positive control, P- Probiotic, C- Carbohydase, NFE- Nitrogen free extract, GE- Gross energy.

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180 **2.2 Gut Histomorphometry.**

181 The slides of tissues of the gastrointestinal tract were examined under the light microscope at
182 x400 magnification and villus height, villus width and crypt depth were measured with the aid of
183 the graticle in micrometers (μm) and the multiplication factor of 0.138305755.

184 **2.3 Microbial count**

185 Digesta was collected from the duodenum, ileum, caecum and colon of each bird for microbial
186 count and stored in a sterile container and refrigerated at 4°C [25]. Digesta was mixed in a 10ml
187 pre-reduced salt medium and serially diluted according to the procedure described by [26] to
188 examine the count of Lactobacilli (Rogosa, CM 0627, incubated anaerobically 48h) and
189 coliforms (Mackonkey, CM 0115, incubated aerobically 24h). Gut tissue samples were serially
190 diluted from 10^{-7} to 10^{-3} . From each dilution, 0.1ml of the sample was plated onto the
191 appropriate medium for enumeration of bacteria.
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193 **2.4 Viscosity measurements**

194 Ileal digesta was diluted (1:1) with distilled water and homogenized for 20 min at room
195 | temperature before ~~centrifugation~~ centrifuging at 3500 RPM for 15 min. The viscosity of the
196 supernatant was measured at 29.1°C and at a shear rate of 60rpm using a viscometer with coaxial
197 cylinders and a mobile rotor (model NDJ-8S).

198 **2.5 Digesta pH**

The pH of fresh ileal digesta was obtained using a pH meter (Model E-HI98128) immediately after collection from the specified sections.

2.6 Organs weights

At day 35, two birds per replicate were sacrificed through cervical dislocation. They were subsequently opened and the liver, heart, gizzard and pancreas harvested and weighed.

2.7 Chemical and statistical analyses

The proximate composition of diets was determined by the methods of AOAC [27]. Data obtained were analysed using ANOVA of SAS [28] and significant level of $P = .05$ was used. The treatment means were compared using Tukey HSD.

RESULTS AND DISCUSSION

The performance of broilers fed wheat based-diets supplemented with antibiotic, probiotic and/or carbohydrase enzyme at the end of starter and grower phases is shown in Table 5. The dietary treatments had no effect on the feed intake, dry matter intake and feed conversion ratio of birds at the two phases or overall. This result is in agreement with observations of [29] and [30] who reported that supplementation of probiotics had no effect on the performance of broilers in any of the breeding phase. Similar results were reported for turkey poults by [31] and [32]. Furthermore, [33] also reported that dietary mannanoligosaccharides and probiotic addition did not affect the feed intake of young turkeys from 0 to 8 wks of age and Bronze bronze turkeys from 7 to 21 weeks of age. Supplementation with antibiotic, probiotic and carbohydrase resulted in significant ($P = .05$) body weight gain (BWG) at d 0-21 and d 22-35 over the NC diet. Birds on the probiotic, carbohydrase and probiotic + cabohydrase diets had significantly ($P = .05$) higher BWG than those on the NC diet. For the overall period small significance ($P = 0.06$) was obtained for BWG in birds on the diets with antibiotic, probiotic, carbohydrase and probiotic + carbohydrase over those NC and PC diets. The gain: feed was significantly ($P = .05$) higher in birds on diets with antibiotic, probiotic, carbohydrase and combination of probiotic and carbohydrase than those on the NC diets only at d 0-21. There was no effect of diet on the parameter at d 22-35 and for the overall period. There was no effect of diet on the feed conversion ratio at any of the phases. [34] has reported similar results for broiler chickens on days 22 and 42 when enzyme was included in their diets. The report of [20] on the positive effect of Roxazyme 2G2 on the final weight and weight gain of birds fed 10% or 20 % wheat diets shows the positive effects of feed enzymes on broiler growth as reported in the current study. Though [35] did not observe improvement in weight by feed enzyme beyond the brooding, the

improved BWG of birds even at the grower phase could be due to the ability of birds to carry the advantage of enzyme supplementation on weight gain from the early starter to grower phase. [36] has also reported that such improved body weight gain in broilers could be attributed to the effect of probiotics in improving absorption of nutrients and reducing harmful bacteria that cause growth depression. Similar to the results of the present study for FCR, [37] reported that probiotic had no effect on the FCR in broiler chickens. [38] posited that dietary inclusion of prebiotic and probiotic had no significant effect on feed conversion ratio in broiler chickens while [9] observed no remarkable effect of diets supplemented with probiotic or symbiotic on final weight, feed conversion ratio, protein efficiency ratio and protein intake in turkey poults. Results of the present study showed that gain:feed ~~ratio~~ of birds on the experimental starter diets was significantly improved. The improvement in gain to feed ratio by probiotic and carbohydrase is due to improved growth caused by the two feed additives. Probiotic ultimately enhanced the efficiency of nutrient utilization by reducing the competition between the host and its intestinal microbial inhabitants, thereby conferring a beneficial effect on the animal health by improving its intestinal microbial balance. The results of relative ~~weights of organs~~ ~~organs-weights~~ and digesta pH are shown in Table 6. The organ weights and digesta pH were not significantly affected by supplementation with antibiotic, probiotic, carbohydrase or a combination of probiotic and carbohydrase. These results are similar to the findings of [39] who reported that relative internal organ weights of chickens were not influenced by dietary probiotics. Similar to the findings of [9], the relative weights of the gizzard, heart, liver and pancreas were unaffected by the supplementation of probiotic and symbiotic to the diets of turkey poults.

Table 5. Performance of birds on experimental diets

Phase/Diet	Parameter				
	Feed intake, g/bird	BWG, g/bird	Dry matter intake, g/bird	Gain:Feed, g/kg	Feed conversion ratio
Day 0-21					
Negative control	1063	482 ^c	960	472 ^c	1.94
Positive control	1084	617 ^b	974	592 ^b	1.84
NC + Probiotic	979	697 ^a	894	670 ^a	1.69
NC + Carbohydrase	1083	670 ^a	967	586 ^b	1.77
NC + Probiotic + Carbohydrase	1091	642 ^a	991	561 ^b	1.88
SEM	49.9	45.1	45.1	18.3	0.08
<i>P value</i>	0.4946	0.0401	0.6108	0.0005	0.3515
Day 22-35					
Negative control	1724	620 ^c	1570	409	2.37
Positive control	1708	837 ^b	1556	491	2.15
NC + Probiotic	1740	901 ^a	1588	503	2.20
NC + Carbohydrase	1769	964 ^a	1617	518	1.95
NC + Probiotic + Carbohydrase	1904	881 ^a	1735	452	2.47
SEM	87.8	55.2	80.1	37.1	0.20
<i>P value</i>	0.5333	0.0197	0.5383	0.4098	0.4355
Day 0-35					

Negative control	2787	987	2531	449	2.17
Positive control	2792	1431	2530	515	2.01
NC + Probiotic	2777	1471	2482	526	2.04
NC + Carbohydase	2852	1610	2584	542	1.88
NC + Probiotic + Carbohydase	2996	1504	2726	489	2.18
SEM	126.1	147.2	118.0	31.4	0.15
<i>P value</i>	0.7183	0.0618	0.6429	0.3543	0.5822

^{a,b,c}Means with different superscript on same column and in each growth phase are significantly different (P<0.05).

Table 6. Organ weight (g/100 g live weight) and digesta pH in broilers on experimental diets

Parameter					
Diet	Heart	Liver	Gizzard	Pancreas	Digesta pH
Negative control	0.55	2.70	3.71	0.28	5.03
Positive control	0.66	2.62	4.21	0.31	5.10
NC + Probiotic	0.66	3.13	4.49	0.32	4.80
NC + Carbohydase	0.63	3.04	3.67	0.34	5.33
NC + Probiotic + carbohydase	0.69	3.68	4.72	0.34	4.95
SEM	0.058	0.257	0.405	0.033	0.210
<i>P value</i>	0.5393	0.0800	0.2501	0.7519	0.5148

Means with the same superscripts on each column are similar significantly

According to [40] the weights of liver, spleen, and heart were not considerably affected by addition of enzyme to the diet of broilers.

Results of the **Lactic Acid Bacteria (LAB)** ~~lactic acid bacteria~~, coliform and total bacteria count in the duodenum, ileum, caecum and colon of birds are presented in Table 7. There was no effect of diet on the microbiota in the various **segments** ~~segment~~ of the GIT of birds except the coliform in the ileum of birds on the diet supplemented with carbohydrase, which was significantly ($P = .04$) higher than that in the other diets. However the LAB in ileum was numerically higher in the probiotic, carbohydrase and probiotic + carbohydrase diets than the NC and PC diets. Several workers have reported the effects carbohydrase [41] and probiotics [42,43] have on microbial population of poultry. In this study, inclusion of probiotics and/or carbohydrase did not affect gut microbiota in different sections of the GIT of broilers with the exception of coliform in the ileum of the birds. Coliform count is used as an indicator for determining the presence and population of pathogen in the intestine. The coliform count was reduced in birds on supplementation with antibiotic and probiotic as similarly reported by [5], who observed a lowered coliform count in the ileum of turkey poult fed probiotic and synbiotic diets. This is similar to the report of [7] that Lactobacilli reduce coliform by competitive exclusion. The results of coliform count in this study showed an effective modulation of intestinal microflora and significant inhibition of pathogenic microbes in the antibiotic, probiotic and/or carbohydrase diets. According to [41], enzymes simply reduce the number of bacteria by increasing the rate of digestion and limiting the amounts of substrates available to the microflora in the ileum. In contrast to the present study, coliform count in the ileum of birds on the carbohydrase diet increased significantly when compared to other diets. This high coliform count on the carbohydrase diet was unexpected. It could be that in the gut microbiota of normal birds, the results of probiotics or carbohydrase supplementation are variable because of the difference in origin, strain as well as species of probiotics or enzyme. The reports of [44] and [45] showed that reduced caecal coliform populations were noticed in chickens given a diet supplemented with **Lactobacilli** ~~lactobacilli~~ strains, isolated from chicken intestine, but the populations of other kinds of bacteria were not affected.

The pH level in specific areas of the gastrointestinal tract (GIT) is a factor, which establishes a specific microbial population, and also affects the digestibility and absorptive value of most nutrients. Most of the pathogens grow in a pH close to 7 or slightly higher. In contrast, beneficial microorganisms live in an acidic pH (5.8-6.2) and compete with pathogens [46]. [26] reported that the addition of xylanase reduced the pH in the contents of duodenum and jejunum from pH 5.68 to 5.61, ($P = 0.048$) and in the contents of the caecum from pH 5.63 to 5.52 ($P = 0.017$). However the pH values of 4.80-5.33 obtained in this study were lower than those of [26]. The intestinal pH recorded in birds fed with dietary supplements was similar to those on the negative control. This suggests that the inclusion of enzyme to wheat diets reduced the digesta pH to that of the control diet leading to cleaving of the arabinoxylan in wheat to enhance the production of more lactic and other volatile fatty acids, thereby causing a reduction in the intestinal pH. This is supported by the report of [47,48,4]. Similarly, the pH recorded in the probiotic group could be a result of the fermentative action of lactic acid bacteria on carbohydrate to produce more lactic acid, resulting in the decrease of pH level. This indicates the germicidal effect of LAB at lower pH as postulated by [49], who admitted that fermentation of food provides characteristic taste profiles and lowers the pH, which prevents contamination by potential pathogens.

318 Table 7. Gut microbiota ($\times 10^6$ CFU) in broilers on experimental diets

Microbiota/Diet	Gut section			
	Duodenum	Ileum	Caecum	Colon
Lactic acid bacteria				
Negative control	2.87	0.47	1.68	1.77
Positive control	1.37	0.52	0.23	1.33
NC + Probiotic	1.98	1.42	1.27	1.50
NC + Carbohydrase	0.86	2.80	1.19	1.24
NC + Probiotic + Carbohydrase	1.98	3.06	0.75	0.25
SEM	1.19	1.05	0.77	1.10
<i>P value</i>	0.8270	0.3963	0.7327	0.8928
Coliform				
Negative control	3.07	3.10 ^b	1.97	3.26
Positive control	2.78	1.25 ^c	1.74	1.16
NC + Probiotic	1.55	1.10 ^c	1.60	2.81
NC + Carbohydrase	4.33	5.64 ^a	3.87	1.53
NC + Probiotic + Carbohydrase	2.43	1.37 ^c	2.74	0.75
SEM	1.12	1.05	1.31	0.97
<i>P value</i>	0.5233	0.0367	0.7251	0.3253
Total bacteria count				
Negative control	1.87	0.52	1.66	2.65
Positive control	0.73	2.17	0.25	0.40
NC + Probiotic	2.17	1.07	2.85	0.53
NC + Carbohydrase	1.20	0.90	2.59	3.06
NC + Probiotic + Carbohydrase	1.62	1.33	0.85	1.47
SEM	0.98	0.92	0.99	1.10
<i>P value</i>	0.8554	0.5470	0.3114	0.3385

319 ^{a,b,c}Means with different superscript in same column and for each microbial specie
320 are significantly different (P<0.05).
321

The results of gut morphology and digesta viscosity are shown in Table 8. The crypt depth, villus height, villus width and villus height: crypt depth ratio of birds on experimental diets were not affected by the supplementation. As expected, the addition of probiotic and carbohydrase significantly ($P < 0.05$) reduced digesta viscosity compared to the NC diet. There was a 46.8 and 50.6 percentage point reduction in digesta viscosity by addition of probiotic and carbohydrase, respectively. According to [50] and [51], high gut viscosity decreases the rate of diffusion of substrates and digestive enzymes and hinders their effective interaction at the mucosal surface. Inclusion of cereals which are rich in NSP increases the digesta viscosity, reduces apparent nutrient digestibility, alters bacterial profiles and gut physiology. [52] considered increased digesta viscosity to be one of the greatest influences of NSP on broiler chicken productivity. Through enzyme supplementation, as shown in the current study the digesta viscosity is reduced and nutrient uptake and animal performance are improved [53]. [21] found that enzyme supplementation of wheat-based diets significantly reduced foregut digesta viscosity of birds. The reduction in viscosity was achieved primarily by reducing the molecular weight through hydrolysis of xylan backbone by endo-xylanase into smaller compounds and thus reduction in viscous effects of the feed because foregut digesta viscosity is directly proportional to the molecular weight of wheat arabinoxylans [54]. This agrees with the report of [41] and [18] on the benefits of exogenous enzyme. Thus, the reduced digesta viscosity observed in this study can be attributed to the effectiveness of carbohydrase in degrading the xylan backbone found in arabinoxylan, which is the predominant non starch polysaccharide in wheat. The effects of enzyme supplementation to wheat or barley-based diets on gut morphology have been reported in turkeys [55]; layers [17] and broilers [23]. The use of exogenous enzymes that promote hydrolysis of NSPs can increase the height of villi and the proportion height-to-depth of the crypts, and lower the bacterial activity of the GIT as well [56]. [57] reported that the jejunum of birds fed a diet containing 60% barley without β -glucanase showed shortening, thickening and trophy of the villi and decreased number of goblet cells compared to those fed a corn-soy diet. Similarly, significant improvement in the gut morphology in turkey poults fed corn-soybean meal supplemented with probiotic or synbiotic was reported by [5]). According to [58], an increased villus height is paralleled by an increased digestive and absorptive function of the intestine due to increased absorptive surface area, expression of brush border enzymes and nutrient transport systems. The villus crypt is considered as the villus factory and deeper crypts indicate fast tissue turnover to permit renewal of the villus as needed in response to normal sloughing or inflammations from pathogens or their toxins and high demands for tissue [59].

Crypt depth is implicated in the production of enterokinase – a proteolytic enzyme that changes inactive trypsinogen to trypsin, which helps in digestion of protein. Increased crypt depth indicates high mucosal secretion [60] and cell turnover [59]. [61] and [5] observed deeper crypt depth in the gut mucosa of broiler chickens and turkey poults respectively when fed with probiotic and **mannanoligosaccharide** **mannaoligosaccharide**, as compared to those fed antibiotic supplemented diet. Increase in villus height to crypt depth ratio is related to an increase in digestion and absorption as reported by [62]. Similarly, [56] reported a lower villus length-to-crypt depth ratio in broiler chickens fed the rye-based diet than in those fed the corn- or rye-based diet supplemented with xylanase and β -glucanase. Supplementation of diets with probiotic and/or carbohydrase had no substantial effect on the villi to crypt ratio in this study.

Table 8. Gut morphology (mm) and digesta viscosity (mPa) in broilers on experimental diets

Parameter					
Diet	Crypt depth	Villus height	Villus width	Villus height: crypt depth	Digesta viscosity
Negative control	1.03	9.23	1.67	8.86	1072 ^a
Positive control	1.48	10.6	1.67	7.28	661 ^c
NC + Probiotic	1.32	12.7	2.05	10.1	570 ^{cd}
NC + Carbohydase	1.06	8.66	1.48	8.67	529 ^d
NC + Probiotic + carbohydase	1.15	8.90	1.39	8.33	772 ^b
SEM	0.201	1.38	0.287	1.11	108.6
<i>P</i> value	0.4790	0.2354	0.5536	0.5300	0.0341

^{a,b,c,d} Means with different superscript in same column are significantly different ($P < 0.05$).

CONCLUSION

The present study showed that inclusion of probiotic and/or carbohydase to wheat based-diets in 35-day-old broilers did significantly improve feed intake, dry matter intake and feed conversion ratio. Probiotic and carbohydase enzyme supplementation resulted in improved BWG at starter and grower phases and the gain:feed at starter phase over the diets with or without antibiotic.

Organ weights and gut microflora were not affected by diet. The digesta viscosity was reduced by probiotic and carbohydase enzyme supplementation. The villus height, crypt depth and their ratio were not affected by diet.

It can be concluded that probiotic and/or carbohydase are viable feed supplements in wheat based-diets for broilers.

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