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Effects of Different Levels of Resistant Starch on Growth Performance and Ileum Morphology in Broilers: A Comparison to Fructooligosaccharide and Zinc Bacitracin

Research Article

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ABSTRACT

This study was conducted to examine effects of resistant starch compared to fructooligosaccharide (FOS) and zinc bacitracin (ZnB), on performance and ileum morphology in broilers. The experiment groups included a control (basal diet with no additive; CON), four groups receiving different levels of resistant starch type 2 (1, 2, 3, and 4% added to basal diet; RS), a group receiving fructooligosaccharide (0.4% in basal diet; FOS), and a group receiving Zinc bacitracin (50 mg/kg basal diet; ZnB). The findings on the day 35 indicated that the groups that received 2% and 3% RS were not significantly different from the FOS group and the ZnB group in terms of feed intake. The largest body weight and the smallest feed conversion ratio was found in the ZnB group (P<0.05). Body weights in the groups treated with 3% and 4% RS were not significantly different from the FOS group. The 3% and 4% RS groups had a greater villus height (P<0.05) and a smaller crypt depth compared to the FOS group. These results demonstrated that while the ZnB group had a better performance than other groups, it seems that adding 3% and 4% RS resulted in a performance similar to adding FOS.

KEY WORDS antibiotic, broiler, prebiotic, resistant starch.

INTRODUCTION

Antibiotic growth promoters (AGPs) are among the feed additives used to enhance growth performance and inhibit the growth of intestinal pathogens in poultry nutrition (Suresh *et al.* 2017). In fact, AGPs enhance growth performance in birds by reducing the number and diversity of normal bacteria in the intestine, thereby increasing bioavailability of nutrients and decreasing deleterious microbial metabolites (Gadde *et al.* 2017). However, recent concerns over antibiotic resistance in human body and tendency of consumers to eat healthy food have resulted in a ban on application of AGPs into poultry feed (Castanon,

2007; Khan and Naz, 2013; Alagawany *et al.* 2018). Thus, a large number of studies are being conducted in an attempt to find substitutes for AGPs, recommending probiotics, prebiotics, synbiotics, organic acids, or herbs as replacements for AGPs (Dibner and Richards, 2005; Gadde *et al.* 2017). Recent years have witnessed greater focus on prebiotics as a feed additive for monogastric animals such as human, poultry, and pigs, resulting in application of prebiotics in poultry with enhanced immune response and resistance against pathogens, which can further develop digestive system and increase intake of nutrients (Ganguly *et al.* 2013). In fact, prebiotics can be regarded as indigestible components of feed which resist digestion, and are fer-

mented by cecum or intestinal bacteria to selectively promote or increase bacterial activity in hosts (Griggs and Jacob, 2005; Hume, 2011; Ricke, 2015). Prebiotics come in various types including fructooligosaccharides, mannanoligosaccharides, isomaltooligosaccharides, and glucooligosaccharides (Iji and Tivey, 1998). In line with properties described for prebiotics such as fructooligosaccharide, another highly fermentable feed additive with potential prebiotics properties emphasized by researchers is resistant starch (Clark and Slavin, 2013; Zaman and Sarbini, 2016). "Resistant starch" refers to a part of consumed starch left undigested by digestive enzymes in the small intestine, which then escapes to the colon (Ashwar *et al*. 2016). Resistant starch comes in 5 types discussed in details by Ashwar *et al*. (2016) and Lockyer and Nugent (2017). Short-chain fatty acids (SCFAs) are among the final products of resistant starch fermentation that can inhibit pathogen growth (Topping *et al*. 2003). Resistant starch has been recommended as an intestinal health factor that can change microflora community of the digestive system (Raigond *et al*. 2015; Ashwar *et al*. 2016). An experiment by M'Sadeq *et al*. (2015) examining impacts of acetylated and butyralated high-amylose maize starch on broilers showed that acetylated high-amylose maize starch reduced luminal pH and increased SCFAs, thereby improving intestinal health and growth performance in broilers challenged with *Eimeria* and *C. perfringens*. In addition, experiments on pigs and rats also confirmed enhanced health against pathogens as well as modified intestinal morphology (Kleessen *et al*. 1997; Bhandari *et al*. 2009; Zhou *et al*. 2017). It seems therefore that feed additives such as resistant starch and prebiotics can be used to promote useful bacteria growth and to reduce growth of pathogens, leading to modified intestinal morphology which affects growth performance in poultry (Yang *et al*. 2009). Few studies have examined effects of resistant starch on growth performance and intestinal morphology compared to the effects of prebiotics and AGPs in broilers. Therefore, the present study attempts to examine how different levels of resistant starch type 2 (high-amylose maize starch) affects growth performance and ileum morphology in broilers and to compare these effects with those of fructooligosaccharide as a prebiotic and zinc bacitracin as an antibiotic growth promoter.

MATERIALS AND METHODS

Experiment groups, diet, and management

The experiment has been approved by the Semnan University Committee of Animal Ethics and complied with Iranian guidelines for animal welfare. In this experiment, 350 one-day old Ross 308 male chickens were randomly assigned to 7 groups with 5 replicates. The experiment groups included a control group (basal diet with no additive; CON), four groups receiving different levels of resistant starch type 2 (1, 2, 3, and 4% added to basal diet; RS), a group receiving fructooligosaccharide as prebiotic (0.4% in basal diet; FOS), and a group receiving the antibiotic zinc bacitracin (50 mg per kg basal diet; ZnB). The RS type 2 (high-amylose maize starch) used in this study was obtained from Ingredion ANZ Pty Ltd, New South Wales (Australia) and the fructooligosaccharide (Raftilose® P95) was obtained from Beneo-Orafti (Belgium). Raftilose, the source of fructooligosaccharide used in this study, was produced through partial enzymatic hydrolysis of chicory inulin. Zinc bacitracin (Albac 150) was obtained from Ridley AgriProducts (Tamworth, NSW, Australia). We used MEGAZYME kit (Megazyme, Bray, Ireland) to measure total starch content in broiler feed. The broiler diets were formulated based on Ross 308 nutrient specifications, using UFFDA software for the three periods starter (days 1-10), grower (days 11-24), and finisher (days 25-35). Table 1 presents ingredient and chemical composition of the basal diet. Birds were placed in 150 cm × 100 cm pens. Each pen was equipped with wood shavings. Vaccination was scheduled based on veterinary recommendations. The broilers were fed *ad libitum* and received lighting under a 23 light/1-hour dark program. The temperature was set at 32 ˚C which gradually reduced according to breeding standards.

Measuring growth performance parameters

Feed intake (FI), body weight (BW), and feed conversion ratio (FCR) for each pen were recorded for starter (days 1-10), grower (days 11-24), finisher (days 25-35), and the entire experiment (days 1-35) periods. FCR was calculated as FI divided by BW. During these periods, the number and weights of mortalities in broiler were recorded to adjust the growth performance parameters. To assess dressing percentage (DP), two birds were randomly selected from each replicate on day 35 and slaughtered by bleeding for 90 sec caused by a single cut to sever the carotid artery and jugular vein.

Measuring ileum morphology

Villus height, crypt depth, and villus height: Crypt depth ratios were measured in order to investigate ileum morphology. On day 35, two birds from each replicate were randomly selected and slaughtered by cervical dislocation. A 1.5 cm section was cut from the middle ileum and washed using NaCl 0.9%. We used a buffered 10% formol solution for 8 hours to clean ileum samples that were later washed three times in a 70% ethanol solution prior to storage at 4 ˚C. Then following the Feulgen method (Feulgren and Rossenbeck, 1924), Schiff reagent was used to stain 0.5 cm^2 sections from each ileum sample.

Table 1 Ingredients and chemical composition of the experimental basal diets

Ingredients (g/kg)	Starter (1-10 d)	Grower (11-24 d)	Finisher (25-35 d)
Wheat	568.8	630	661.1
Soybean meal	302.2	208.15	139
Meat meal	28	48	68
Canola solvent	30	45	70
Canola oil	33.2	40	43.9
Limestone	11.9	7.4	4
Dicalcuim phosphate	9.24	5.32	0
Sodium bicarbonate	2	2	2
Salt	1.91	1.45	1
Vitamin premix[1]	2.5	2.5	2.5
Mineral premix[2]	2.5	2.5	2.5
L-lysine HCl	2.31	2.95	2.55
DL-methionine	3.46	3.15	2.31
L-threonine	1.98	1.58	1.14
Calculated composition			
Metabolizable energy (kcal/kg)	2950	3050	3100
Crude protein (%)	24.15	21.10	20.50
Available phosphorous (%)	0.48	0.47	0.45
Met + Cys (%)	1.10	0.8	0.7
Lysine (%)	1.45	1.31	1.17
Total starch (%)	33.4	35.2	36.8

[1] Vitamin premix provided per kilogram of diet: vitamin A (transretinyl acetate): 10000 IU; vitamin D_3 (cholecalciferol): 5000 IU; vitamin E (DL-α-tocopherol acetate): 50 IU; vitamin K_3 (bisulphate menadione complex): 3 mg; Thiamine (thiamine mononitrate): 3 mg; Riboflavin: 9 mg; Nicotinic acid: 50 mg; Pantothenic acid (D-calcium pantothenate): 15 mg; vitamin B_6: 4 mg; D-biotin: 0.1 mg; Folic acid: 2 mg; vitamin B_{12} (cyanocobalamin): 0.02 mg and Choline (choline chloride): 1000 mg.

[2] Mineral premix provided per kilogram of diet: Iron ($FeSO_4 \cdot 7H_2O$): 55 mg; Iodine (Ca $(IO_3)_2$): 1.3 mg; Manganese ($MnSO_4 \cdot H_2O$): 120 mg; Zinc (ZnO): 100 mg; Copper ($CuSO_4 \cdot 5H_2O$): 16 mg and Selenium (Na_2SeO_3): 0.3 mg.

Photographs were taken from at least 10 villi and 10 crypts taken from each section under a stereo microscope. We then utilized Visilog 6.3 Viewer Lite (Noesis, Saint Aubin, France) (Catalá-Gregori *et al.* 2007) to measure villus height and crypt depth.

Statistical analysis

The experiment was conducted based on a completely random design with 7 groups, 5 replicates each containing 10 broilers. The data obtained from the experiment were analyzed using the ANOVA in SAS (SAS, 2008). The comparison of means was done through Duncan's multiple range test at the level of 0.05. Probability values of less than 0.05 (P<0.05) were considered significant. Before performing statistical analysis of data, all data were tested by a normality test.

RESULTS AND DISCUSSION

Growth performance
Feed intake

Table 2 presents the results for effects of different levels of RS on FI in broilers compared to FOS and ZnB.

The results indicate no significant difference between the experiment groups during the starter period (days 1-10). However, the smallest and the greatest FI were respectively observed in the CON group and the 4% RS group. For the grower period (days 11-24), the FI values for the 3% and 4% RS groups were not significantly different from the FOS and the ZnB groups. In addition, broilers receiving 1% RS showed no significant difference from the CON group. During the finisher period (days 25-35), again the greatest FI was found in the 4% RS group (P<0.05). The FOS group had the lowest level of FI over the finisher period, showing a significant difference from the 2% and 4% RS groups. The results for the entire experiment period (days 1-35) demonstrated the highest level of FI for the 4% RS group (P<0.05) while the smallest FI was found in the CON group with a significant difference from all other groups except for the 1% RS group (P<0.05). No significant difference was observed between the 2% and 3% RS groups, the FOS group, and the ZnB group.

Body weight

Table 2 presents the results on effects of different levels of RS, compared to FOS and ZnB, on the broilers' BW.

Table 2 Effect of resistant starch, fructooligosaccharide and zinc bacitracin on feed intake (g), body weight (g) and feed conversion ratio of broilers[1]

Item	Dietary treatment								
	CON	1% RS	2% RS	3% RS	4% RS	FOS	ZnB	SEM	P-value
Feed intake									
Starter, 1-10 d	277.6	285.0	285.2	286.8	288.6	285.8	287.2	3.47	0.585
Grower, 11-24 d	1219.0^c	1213.6^c	1255.6^b	1288.2^a	1299.8^a	1298.0^a	1299.0^a	6.68	< 0.001
Finisher, 25-35 d	1622.2^{bc}	1630.8^{bc}	1639.2^b	1630.0^{bc}	1670.2^a	1599.4^c	1613.4^{bc}	4.92	0.001
Total period	3118.8^c	3129.4^c	3180.0^b	3205.0^b	3258.6^a	3183.2^b	3199.6^b	8.11	< 0.001
Body weight									
Starter, 1-10 d	245.6^d	253.0^d	270.4^c	280.8^{bc}	293.4^{ab}	273.2^c	297.6^a	1.48	< 0.001
Grower, 11-24 d	810.0^{cd}	793.2^d	826.6^{bc}	843.8^b	850.2^b	893.6^a	896.6^a	7.21	< 0.001
Finisher, 25-35 d	960.2^d	994.0^d	1055.0^c	1132.8^{ab}	1096.0^{bc}	1094.2^{bc}	1148.4^a	12.49	< 0.001
Total period	2015.8^d	2040.2^d	2152.0^c	2257.4^b	2239.6^b	2261.0^b	2342.6^a	19.98	< 0.001
Feed conversion ratio									
Starter, 1-10 d	1.13^a	1.13^a	1.06^b	1.02^{bc}	0.99^{bc}	1.05^b	0.97^c	0.013	< 0.001
Grower, 11-24 d	1.51^{ab}	1.53^a	1.52^a	1.53^a	1.53^a	1.45^b	1.45^b	0.009	0.016
Finisher, 25-35 d	1.69^a	1.64^a	1.56^b	1.44^d	1.53^{bc}	1.46^{cd}	1.41^d	0.018	< 0.001
Total period	1.55^a	1.53^a	1.48^b	1.42^c	1.46^b	1.41^c	1.37^d	0.011	< 0.001

[1] Values are the means of 5 pens of 10 birds per pen.
CON: control; RS: resistant starch; FOS: fructooligosaccharide and ZnB: zinc bacitracin.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

The results for the starter period (days 1-10) showed the largest BW in the ZnB group, with significant differences from other groups except for the 4% RS group. The 2% and 3% RS group were not significantly different from the FOS group. The smallest BW for the grower period (days 11-24) was found in the 1% RS group, showing no significant difference from the CON group. During the grower period, the greatest BW was observed in the ZnB group with no significant difference from the FOS group. The greatest BW during the finisher period (days 25-35) was observed in the ZnB group with significant difference (P<0.05) from all other groups except for the 3% RS group. Furthermore, the FOS group showed no significant difference from the 2%, 3%, and 4% RS groups. The CON group had the smallest BW during the finisher period, showing no significant difference from the 1% RS group. For the entire experiment period, the greatest BW was found in the ZnB group (P<0.05). The broilers in the FOS group had greater BW than the broilers in the 1% and 2% RS groups (P<0.05). However, the FOS group showed no significant difference from the 3% and 4% RS groups. Although the smallest BW was that of the CON group, it was not significantly different from that of the 1% RS group.

Feed conversion ratio
Table 2 shows the result on effects of the different levels of RS, compared to FOS and ZnB, on FCR in broilers. Over the starter period (days 1-10), the smallest FCR was observed in the ZnB group, showing no significant difference from the 3% and 4% RS groups. No significant difference was found between the FOS group and the 2%, 3%, and 4% RS groups in terms of FCR. The CON group and the 1% RS group had the largest FCR.

During the grower period (days 11-24), the ZnB and the FOS groups had the smallest FCR, showing significant difference (P<0.05) from all other groups except for the CON group. The RS groups were not significantly different in terms of FCR.

Over the finisher period (days 25-35), the ZnB group had the smallest FCR, showing no significant difference from the FOS and the 3% RS groups. Over the entire experiment period, the smallest and the greatest FCR values were found in the ZnB and the CON group, respectively. The FOS group showed no significant difference from the 3% RS group.

Dressing percentage
Table 3 presents the results on the effects of different levels of RS, compared to FOS and ZnB, on DP in broilers. The ZnB group had the greatest DP (P<0.05). The 4% RS group and the FOS group showed significant gain in DP compared to the 1%, 2%, and 3% RS group and the CON group (P<0.05). The smallest DP was found in the CON group, showing no significant difference from the 1%, 2%, and 3% RS groups.

Ileum morphology
Table 4 presents the results for effects of different levels of RS, compared to FOS and ZnB, on ileum morphology in broilers. The greatest villus height was observed in the ZnB group (P<0.05). The broilers treated with RS, except for the 1% RS group, had significant increase in villus height compared to the CON group. Moreover, among the groups that received RS, the 3% and 4% RS groups had significant increase in villus height (P<0.05) compared to the FOS group.

Table 3 Effect of resistant starch, fructooligosaccharide and zinc bacitracin in dressing percentage of broilers[1]

Item	Dietary treatment								
	CON	1% RS	2% RS	3% RS	4% RS	FOS	ZnB	SEM	P-value
Dressing percentage	68.34[c]	68.82[c]	69.02[c]	69.14[c]	70.12[b]	70.18[b]	71.80[a]	0.18	< 0.001

[1] Values are the means of 5 pens of 10 birds per pen.
CON: control; RS: resistant starch; FOS: fructooligosaccharide and ZnB: zinc bacitracin.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 4 Effect of resistant starch, fructooligosaccharide and zinc bacitracin on ileum morphology of broilers[1]

Item	Dietary treatment								
	CON	1% RS	2% RS	3% RS	4% RS	FOS	ZnB	SEM	P-value
Villus height, μm	842.07[e]	861.84[de]	898.18[cd]	927.66[bc]	954.10[b]	882.01[de]	1005.10[a]	12.41	< 0.001
Crypt depth, μm	178.23[a]	169.38[ab]	147.96[c]	146.89[c]	148.32[c]	158.46[bc]	128.26[d]	3.67	< 0.001
Villus height:crypt depth	4.72[e]	5.08[e]	6.08[bc]	6.32[b]	6.44[b]	5.57[cd]	7.84[a]	0.26	< 0.001

[1] Values are the means of 5 pens of 10 birds per pen.
CON: control; RS: resistant starch; FOS: fructooligosaccharide and ZnB: zinc bacitracin.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

The smallest villus height was found in the CON group with no significant difference from the 1% RS group and the FOS group. The ZnB group had the smallest crypt depth (P<0.05). The 2%, 3%, and 4% RS groups were not significantly different from the FOS group in terms of crypt depth. The greatest crypt depth was found for the CON group (P<0.05), showing significant difference from all groups, except for the 1% RS group. As far as villus height: crypt depth ratio is concerned, our results found the greatest ratio in the ZnB group (P<0.05). Of the groups that received RS, the 2%, 3%, and 4% RS groups had significant increase in this ratio (P<0.05) compared to the CON and the 1% RS groups. In addition, a significant increase in this ratio was observed in the 3% and 4% RS groups compared to the FOS group. The smallest ratio was observed in the CON group.

Growth performance

Results found for different periods showed greater FI in the RS groups than in the CON group, although the FI difference between the CON group and the 1% RS group was smaller compared to other groups. In line with our findings, M'Sadeq *et al*. (2015) reported a greater FI in broilers receiving acetylated and butyralated high-amylose maize starch compared to their control group. In addition, in an experiment on the effects of potato's retrograded resistant starch (*S. Tuberosum* and *S. Phureja*) and the prebiotic mannan-oligosaccharide, Ariza-Nieto *et al*. (2012) reported no significant difference between the experiment groups in terms of FI. In this experiment, over the entire experiment period (35 days), the groups receiving 2% and 3% RS did not show any significant difference from the FOS and the ZnB groups. However, M'Sadeq *et al*. (2015) reported a smaller FI in broilers treated with antibiotic than broilers receiving acetylated and butyralated high-amylose maize starch over a period of 35 days.

Clark and Slavin (2013) noted that resistant starch and fructooligosaccharide as feed additives did not reduce feed intake, and their experiment as well as the one conducted by M'Sadeq *et al*. (2015) found that the broilers that received acetylated and butyralated high-amylose maize starch did not show decreased FI compared to their respective control group. As resistant starch is indigestible and has been referred to as fiber by several authors, it has been suggested that birds that receive resistant starch consume more feed to compensate for loss of their energy while it has also been suggested that high fiber levels can increase FI (M'Sadeq *et al*. 2015).

Regarding the observations on FI for broilers treated with FOS in this experiment, it should be noted that previous studies on effects of fructooligosaccharide on broilers have produced inconsistent results, as adding 2, 4, and 8 grams of fructooligosaccharide per kilogram (Xu *et al*. 2003), 5 g/kg fructooligosaccharide (Shang *et al*. 2015), and 500 mg/kg fructooligosaccharide (Emami *et al*. 2012) produced no significant difference between the fructooligosaccharide groups and the control groups in terms of FI, while Williams *et al*. (2008) reported a significant drop in FI for broilers treated with 0.6 g fructooligosaccharide per kg.

However, in this experiment we did not observe any reduction in FI for broilers receiving FOS similar to those receiving 2%, 3%, and 4% RS compared to the control group over the entire experiment period (35 days), although the smallest FI over the finisher period was observed in the FOS group.

Regarding BW, the results suggested that the 2%, 3%, and 4% RS groups as well as the FOS group had a greater BW than the CON group, while no considerable difference was found between the 1% RS group and the CON group in terms of BW and even in terms of DP. The greater BW may be attributed to increased FI in the 2%, 3%, and 4% RS groups.

M'Sadeq *et al.* (2015) also reported a greater BW in broilers receiving acetylated and butyralated high-amylose maize starch than the broilers in the control group, although the difference was not significant. In addition, challenged broilers treated with acetylated and butyralated high-amylose maize starch had a significant body weight gain compared to the control group. Huff *et al.* (2015) examined three types of resistant starch (HI Maize 260, unmodified potato starch, and fresh raw russet potato) in broilers and reported a higher body weight in *E. coli* challenged broilers receiving unmodified potato starch under cold stress compared to the other experiment groups, although the body weights of broilers that received HI-Maize 260 was not significantly different from those of the broilers treated with unmodified potato starch. Ariza-Nieto *et al.* (2012) observed no significant difference in terms of daily body weight gain in broilers receiving retrograded resistant starch (*S. Tuberosum* and *S. Phureja*) and the mannanoligosaccharide compared to the control group.

However, this improved growth performance in the broilers receiving resistant starch and those receiving fructooligosaccharide can be attributed to enhanced innate immune function as well as increased production of SCFAs which can be absorbed through hindgut and act as a source of energy for tissues (Adhikari and Kim, 2017). SCFAs can also actively induce changes in the intestinal mucus (Montagne *et al.* 2003). Authors of some previous studies have reported no change in body weights of broilers receiving fructooligosaccharide (Emami *et al.* 2012; Shang *et al.* 2015), while Xu *et al.* (2003) found significant body weight gain in broilers receiving 4% fructooligosaccharide in their feed compared to the broilers in the control group.

In this experiment, the antibiotic group always had a greater BW and DP compared to the other groups. Several studies reported body weight gain in broilers that received AGPs like zinc bacitracin (Engberg *et al.* 2000; Yang *et al.* 2007). In fact, this improved growth is attributable to modified bacterial community in the intestine, as Engberg *et al.* (2000) suggested zinc bacitracin can reduce coliform count in ileum and boost amylase and lipase activity.

The findings regarding FCR indicated that the RS groups had smaller FCR than the CON group while the FOS group had a better FCR than the RS groups. However, as noted in the results section, the FCR of the ZnB group was smaller than the other groups. But M'Sadeq *et al.* (2015) found no significant difference between the experiment groups (acetylated and butyralated high-amylose maize starch) in both cases of disease-challenged (*Eimeria* and *C. Perfringens*) and unchallenged broilers. In addition, while they observed no significant difference between the experiment groups in terms of FCR, the antibiotic group had the smallest FCR.

Ileum morphology

Ileum morphology can be used as an important index for intestinal health and nutrient absorption (Xu *et al.* 2003; Shang *et al.* 2015). Carbohydrates that pass through the gastrointestinal tract can be fermented by bacteria (Shang *et al.* 2015) and since resistant starch remains unaffected by digestive enzymes, it can be expected to get fermented by bacteria (Fuentes Zaragoza *et al.* 2011). As the final product of resistant starch fermentation, SCFAs (acetate, propionate, butyrate, and lactate) can reduce pH and inhibit pathogen growth (Ma and Boye, 2017), and resistant starch seems to be more capable of producing butyric acid than other SCFAs (Leeson *et al.* 2005; Fuentes Zaragoza *et al.* 2011), and like fructooligosaccharides, it can be butyrogenic (Pryde *et al.* 2002). However, the host must have certain bacteria to degrade resistant starch and increase butyric acid production (Yang *et al.* 2017). Kleessen *et al.* (1997) suggested that increased amount of SCFAs caused by intake of resistant starch type 2 is attributable to promoting the growth of *Bifidobacterium* and *Lactobacillus*. This experiment showed an increase in *Lactobacillus* count for the groups that received RS, particularly at 4% RS (data not shown). SCFAs, and in particular butyric acid, have been suggested to play a role in growth and development of the small intestine through epithelial cell proliferation. These changes therefore can affect the intestinal mucus (Xu *et al.* 2003).

Increased crypt depth or reduced villus height may suggest the presence of toxic agent in the body while reduced villus may inhibit nutrient absorption. Although the ZnB group in this study had a greater villus height than the other groups, our results for the RS groups shows that the 3% and 4% RS group had a greater villus height and smaller crypt depth compared to the FOS and the CON groups, which can indicate that, similar to fructooligosaccharide, resistant starch can also undergo bacterial fermentation in the ileum and this can increase villus height and surface area for nutrient absorption (Shang *et al.* 2015). As noted above, the 3% and 4% RS groups outperformed the CON group in terms of growth performance.

In addition, M'Sadeq *et al.* (2015) reported greater villus height and smaller crypt depth in the jejunum of broilers treated with acetylated and butyralated high-amylose maize starch on day 24 in comparison to the broilers of the control group.

Deeper crypts indicate faster tissue turnover which, in turn, results in greater need for nutrients and slower growth, as observed in the CON group and the 1% RS group. In fact, deeper crypts and shorter villi in the control group suggest lowered nutrient absorption, reduced resistance against pathogen, and thus poorer growth performance.

Similarly, M'Sadeq *et al.* (2015) observed the smallest villus height and the greatest crypt depth on day 24 in the control group. Consistent with our findings, they also found that on days 15 and 24, the antibiotic group had greater villus height and smaller crypt depth.

Our experiment revealed a significant (P<0.05) increase in villus height: crypt depth ratio for the ZnB group when compared to the other experiment groups, similar to the findings of M'Sadeq *et al.* (2015) who reported a significant increase in this ratio in the jejunum of the antibiotic group on day 24.

The RS groups in this experiment exhibited a significant increase in villus height: crypt depth ratio in the ileum compared to the control group, which is in line with Ariza-Nieto *et al.* (2012) who noted a significant increase in villus height: crypt depth ratio in the jejunum of broilers treated with potato's retrograded resistant starch (*S. Tuberosum* and *S. Phureja*) compared to the control group. Furthermore, M'Sadeq *et al.* (2015) observed an insignificant increase in the villus height: crypt depth ratio on day 24 in the jejunum of broilers that received acetylated and butyralated high-amylose maize starch compared to the control group. However, Ariza-Nieto *et al.* (2012) noted a significant increase in the villus height: crypt depth ratio in the duodenum of broilers that received the resistant starch *S. Tuberosum* potato compared to broilers that received mannanoligosaccharide.

In this experiment, we found a significant (P<0.05) increase in the villus height: crypt depth ratio in the ileum of the broilers that received 3% and 4% RS compared to the group treated with FOS.

CONCLUSION

Our results indicated that supplementing broiler basal diet with 2%, 3%, and 4% RS leads to enhanced growth performance compared to the CON group while on the other hand, the 3% and 4% RS groups had also better growth performance than the group that received the FOS. This enhanced growth performance in the broilers that received RS can be attributed to modified ileum morphology (increased villus height and reduced crypt depth). The broilers treated with ZnB showed a better growth performance than the broilers in the RS groups, but one should also note improved intestinal health of broilers that received RS, which could result from modified intestinal microflora and morphology. It should also be noted that it is not clear whether resistant starch has effects similar to those of fructooligosaccharide, and therefore further studies are need in this area. Future research can compare distinct levels of prebiotics to various types of resistant starch supplemented to different basal diets used for different strains of broilers.

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