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Original Research Article

Influence of dietary carbohydrases, individually or in combination with phytase or an acidifier, on performance, gut morphology and microbial population in broiler chickens fed a wheat-based diet

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ABSTRACT

The objective of this study was to examine the effects of dietary carbohydrases (xylanase and β-glucanase; XG), individually or in combination with phytase or acidifier on the growth performance, carcass attributes, intestinal microbial counts and morphology in broiler chickens fed a wheat-based diet. A total of 240 one-day-old male broiler chicks were randomly allocated into 4 treatment groups with 6 replicates of 10 birds each. The dietary treatments included a basal diet, the basal diet with an enzyme complex containing XG, XG plus a microbial phytase (XG + P) and XG plus acidifier (XG + A). The results indicated that feed conversion ratio (FCR) was improved in broiler chickens which received XG + A during the entire production period (1 to 35 d) of the trial ($P < 0.05$). The broiler chickens fed XG + P had lower feed intake compared with the control group at 29 to 35 d of age. The experimental treatments had no effect on the body weight gain of broiler chickens. In carcass traits, except for spleen ($P < 0.05$), the dietary treatments had no effects on the carcass characteristics of broiler chickens. The birds which received diets supplemented with XG and XG + A had a lower weight of the spleen compare with the control. Addition of XG in combination with phytase (XG + P) resulted in a decrease in ileal enumeration of *Escherichia coli* at 35 d of age ($P < 0.05$). However, dietary treatments did not alter the population of ileal *Lactobacilli* in broiler chickens. Supplementing carbohydrases with phytase and acidifier (XG + P and XG + A) significantly increased the intestinal villus length at 35 d of age ($P < 0.05$). In conclusion, the present study demonstrated that supplementation of the wheat-based diet with a combination with carbohydrases and acidifier (XG + A) improves FCR in broiler chickens. Furthermore, combinations of carbohydrases with phytase (XG + P) and with acidifier (XG + A) decrease the *E. coli* counts and increase the villus length in broiler chickens.

1. Introduction

Because of the ban of in-feed antibiotics, there is a clear need for safe alternatives feed additives in poultry industry. To date, a number of alternatives for antibiotics have been proposed (Seal et al., 2013). Several studies have been conducted on the ability of exogenous enzymes (Bedford and Morgan, 1996), oligosaccharides (Iji and Tivey, 1998) and organic acids (Patten and Waldroup, 1988) to act as growth promoters in broiler chickens.

Enzymes, which are commonly used in the poultry feed industry, are the glycanases (xylanases and β-glucanases) which can hydrolyze the non-starch polysaccharides (NSP) in cereal grains such as wheat, barley and microbial phytases, and hydrolyze

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phytate complexes in plant-derived ingredients. There are a number of mechanisms involved in the positive impacts of glycanases to improve energy and nutrient availability in wheat-based diets (Bedford and Schulze, 1998). Firstly, degradation of NSP in the cell wall matrix and the release of captured nutrients; secondly, lowering digesta viscosity in the digestive tract and increasing digestion; thirdly, enhancing the availability of nutrients to digestive enzymes secreted in digestive tract; and lastly, increasing the intestinal motility and feed passage rate (Wu et al., 2004).

The ability of exogenous phytase to improve the bioavailability of phytate-bound phosphorus, metabolizable energy and other nutrients such as calcium, amino acids and fatty acids to sustain the performance and skeletal health of poultry is now clearly established (Camden et al., 2001; Cowieson et al., 2009; Selle et al., 2009; Zaefarian et al., 2013). In a recent study, Zeller et al. (2015) found that xylanase may enhance the accessibility of phytate for dietary phytase in broiler chickens. Moreover, the exogenous carbohydrases may also enhance the efficacy of phytase due to elimination of phytic acid-chelating influences of NSP (Woyengo et al., 2010). Selle et al. (2009) observed greater growth response in broilers fed combination of phytase and xylanase compared with phytase individually in wheat-based diets.

The use of organic acids has been reported to have positive effects on growth and feed efficiency (Eftekhari et al., 2015), nutrient utilization (Ragaa and Korany, 2016), intestinal morphometric indices (Eftekhari et al., 2015) and microbiota activity (Chaveerach et al., 2004) in broiler chickens. Several mechanisms have been proposed to explain the beneficial effects of organic acids in improving energy and nutrient utilization in broiler including: 1) penetrate to the bacteria cell wall and disrupt the normal physiology of certain types of bacteria (Hashemi et al., 2012); 2) reduce the acidity of digesta and increase the pancreatic secretion (Dibner and Buttin, 2002).

According to the Ellis et al. (2002), the supplementation of feed acidifiers and enzyme individually to broiler diets improved growth performance, but when used in combination these improvements were decreased. However, Owens et al. (2008) found that combination of xylanase, acidifier and yeast extract in wheat-based diets had a beneficial influence on feed efficiency. *In vitro* study showed that the activity of the galactosidase and protease increased by acidification with citric acid (Ao et al., 2010). However, limited information exists on the effect of combination of exogenous carbohydrases with phytase or acidifier on intestinal morphology and microbial population in broilers fed wheat-based diets.

Therefore, the aim of the present study was to examine the influence of exogenous carbohydrases, individually or in combination, with phytase or a blend acidifier, on performance, carcass traits, gut morphology and microbiota counts in broilers fed on wheat-soy diets.

2. Materials and methods

2.1. Birds, housing and diets

The experiment was conducted in a commercial farm (Sari, Mazandaran, Iran) and was approved by the animal welfare commissioner of the Department of Animal Science, Islamic Azad University, Qaemshar branch (Qaemshahr, Iran).

Two hundred and forty one-day-old male broiler chicks (Arbor Acres Plus) were purchased from a commercial hatchery and randomly distributed across 4 treatments with 6 replicates of 10 birds each. The broiler chickens were raised in floor pens bedded with a layer of wood shaving with constant lighting program for the experimental period of 35 d. In this trial, pens with dimensions of 1.80 m × 0.6 m were used. Each pen was equipped with a separate feeder and a nipple drinker. The experimental diets (in mash form) were a basal diet and the basal diet with anexogenous carbohydrase (XG) as Natugrain TS in single (100 g/t) or combined with phytase (XG + P) as 100 g/t PhyzymeXP 5000 (Danisco Animal Nutrition, UK) with 500 FTU/g or an acidifier (XG + A) as 3,000 g/t BioAcid Ultra (Biochem, Germany). Natugrain TS was purchased from a commercial company (BASF, Germany). The enzyme contained 2 enzyme activities including endo-1,4 β-xylanase (5,600 TXU/g) and endo-1,4 β-glucanase (2,500 TGU/g). The acidifier product contained formic acid, lactic acid, propionic acid, ammonium formate and ammonium propionate. The broiler chicks received their feed and water *ad libitum* at 20 ± 3 °C. The ingredients and chemical compositions of basal diets are shown in Table 1.

2.2. Performance and carcass characteristics

Body weight gain and feed consumption of each replicate pen was recorded at 7, 14, 21, 28 and 35 d of age. Feed conversion ratio (FCR) for each pen was calculated by dividing feed intake by body weight gain. In order to evaluate of the carcass components, at the end of the experiment (35 d of age), 2 male broiler chickens from each replicate were randomly selected, individually weighed and sacrificed after 4 h feed deprivation. After the removing of viscera manually, the weight of the breast, thigh, liver, pancreas, spleen and the length of the intestine were recorded and then calculated as a percentage of live body weight.

2.3. Microbiota activity

At 35 d of age, 6 broiler chickens per treatment were chosen and sacrificed. The intestinal segment was removed immediately and

Table 1
The ingredients and chemical composition of basal diets (as-fed basis).

Item	Starter	Grower	Finisher
	d 1 to 10	d 11 to 24	d 25 to 35
Ingredients, g/kg			
Wheat grain	527.1	597.8	664.1
Soybean meal (410 g CP/kg)	384.2	318.1	256.7
Soybean oil	40.0	40.0	40.0
Calcium carbonate	13.8	12.6	11.6
Dicalcium phosphate	13.5	11.8	10.2
Common salt	1.5	1.4	0.5
Sodium bicarbonate	2.4	2.5	3.6
Vitamin premix[1]	8.6	7.2	5.5
Mineral premix[2]	2.5	2.5	2.5
DL-Met	3.0	2.5	2.1
L-Lys	2.5	2.4	2.4
L-Thr	1.0	1.0	0.6
Chemical composition (calculated), g/kg			
ME, kcal/kg	2,810	2,891	2,966
CP	225	207	190
Ca	9.00	8.10	7.32
Available P	4.50	4.05	3.66

[1] Provides per kilogram of diet: (Starter:13,000 IU vitamin A; 5,000 IU vitamin D_3; 80 IU vitamin E; 3.2 mg menadion; 3.2 mg thiamine; 8.6 mg riboflavin; 65 mg niacin; 5.4 mg pyridoxine; 17 μg vitamin B_{12}; 20 mg pantothenic acid; 2.2 mg folic acid; 0.3 mg biotin; 1,700 mg choline chloride; and 9.4 mg antioxidant.), (Grower: 11,000 IU vitamin A; 4,500 IU vitamin D_3; 65 IU vitamin E; 3 mg menadion; 2.5 mg thiamine; 6.5 mg riboflavin; 60 mg niacin; 4.3 mg pyridoxine; 17 μg vitamin B_{12}; 18 mg pantothenic acid; 1.9 mg folic acid; 0.25 mg biotin; 1,600 mg choline chloride; and 8.85 mg antioxidant.), (Finisher: 10,000 IU vitamin A; 4,000 IU vitamin D_3; 55 IU vitamin E; 2.2 mg menadion; 2.2 mg thiamine; 5.4 mg riboflavin; 45 mg niacin; 3.2 mg pyridoxine; 11 μg vitamin B_{12}; 15 mg pantothenic acid; 1.6 mg folic acid; 0.2 mg biotin; 1,500 mg choline chloride; and 8.25 mg antioxidant.).

[2] Provides per kilogram of diet: 120 mg Mn; 110 mg Zn; 20 mg Fe; 16 mg Cu; 1.25 mg I; 0.3 mg Se.

3 g of fresh digesta from ileum was collected sterilely. The samples were put on ice until they were transported to the laboratory for enumeration of microbial populations. Each sample was serially diluted from 1/10 to $1/10^7$ in sterilized physiological saline solution (NaCl 85%). Then 0.1 mL of each diluted sample was plated onto the following media. *E. coli* was cultured on eosin methylene blue agar (Merck, Darmstadt, Germany) at 37 °C for 24 h. *Lactobacilli* bacteria were counted on de Man, Rogosa, sharpe agar (Merck, Darmstadt, Germany) after incubation for 48 to 72 h at 37 °C.

2.4. Intestinal morphology

A 2-cm piece of the middle of the jejunum from 6 broiler chickens per treatment was excised for morphometric analysis. The samples were flushed clean with phosphate buffered saline to avoid damage to the tissues. A 0.5-cm section was processed and embedded in paraffin. Then, the paraffin-embedded samples were stained by eosin blue. The 10 longest and straightest villi and associated crypts were measured in each segment (Eftekhari et al., 2015).

2.5. Statistical analysis

Data were analyzed based on a completely randomized design using one-way analysis of variance in PROCGLM of SAS (SAS, 1999). The means were compared using the Duncan's multiple range tests at $P < 0.05$.

3. Results

3.1. Growth performance and carcass characteristics

As presented in Table 2, the experimental treatments had no significant effect onbody weight gain. However, feed intake (at 29 to 35 d of age) and FCR (at 22 to 28, 29 to 35 and 1 to 35 d of age) were statistically influenced by the experimental treatments ($P < 0.05$). The broiler chickens fed XG + P had a lower feed intake compared with the control birds. According to these results, supplemental carbohydrases in combination with an acidifier or phytase improved FCR in broiler chickens.

All carcass parameters, except for the spleen, were not affected by the experimental treatments (Table 3). The results revealed that the birds which received diets supplemented with XG and XG + A had a lower relative weight of the spleen ($P < 0.05$).

3.2. Microbial population and jejunum morphology

Data for ileal population of *Lactobacilli* and *E. coli* are presented in Table 4. There was no significant difference among treatments on the population of *Lactobacilli* in broiler chickens at 35 d of age. However, the ileal population of *E. coli* in broiler chickens was affected by the dietary treatments at 35 d of age ($P < 0.05$). According to these results, the diet with XG in combination with phytase (XG + P) had an inhibitory effect on the *E. coli* population.

The results of the effects of dietary treatments on intestinal morphology in broiler chickens are shown in Table 5. The Villus width, crypt depth, the ratio of villus length to villus width and crypt depth was not affected by the dietary treatments. The villus length was greater in broiler chickens fed XG + P diet ($P < 0.05$).

4. Discussion

It was observed that broiler chickens received XG + A diet had a better FCR from 1 to 35 d of age than the other groups. The impact of organic acids on the broilers performance has been investigated in the several studies (Eftekhari et al., 2015; Hashemi et al., 2012; Ragaa and Korany, 2016). However, the possible interaction and/or additive effect of an acidifier supplement in combination with a carbohydrase on the broiler performance have not been fully investigated. In line with current findings, Ao et al. (2009) found that acidification of broiler diets improved the efficacy of α-galactosidase. According to these authors, dietary inclusion of organic acid could reduce the pH of the diet and crop digesta and enhance the activity of exogenous α-galactosidase. Similarly, it has also been shown that the activity of α-galactosidase and protease was enhanced by addition of citric acid (Ao et al., 2010). On the other hand, Esmaeilipour et al. (2011) demonstrated that no effective interaction between xylanase and citric acid was observed in any measured responses. The findings of (Li et al., 1999) support these results which indicated that feeding organic acids and a multi-enzyme either alone or together produced no significant improvement in growth performance of the pigs. Therefore, keeping in view the mentioned properties of organic acids, it can be noted that the better growth performance in broiler chickens fed

Table 2
Effects of dietary treatments on weight gain, feed intake and feed conversion ratio (FCR) in broiler chickensa[4].

Item	Treatments				SEM[8]	*P*-value
	Control	XG[5]	XG + P[6]	XG + A[7]		
Weight gain, g/(bird·d)						
d 1 to 14	32.05	32.23	32.93	34.01	0.56	0.61
d 15 to 21	60.76	61.31	61.81	60.26	0.43	0.62
d 22 to 28	91.15	91.31	93.80	94.76	0.62	0.13
d 29 to 35	97.71	100.00	97.31	103.23	1.73	0.62
d 1 to 35	70.00	71.13	71.38	72.75	0.67	0.57
Feed intake, g/(bird·d)						
d 1 to 14	48.79	49.54	48.39	49.79	0.59	0.83
d 15 to 21	93.42	93.93	92.17	93.19	0.77	0.88
d 22 to 28	150.56	146.50	147.68	148.90	0.94	0.48
d 29 to 35	194.98[1]	187.32[1,2]	183.46[2]	186.32[1,2]	1.71	0.04
d 1 to 35	121.94	119.32	117.93	119.55	0.87	0.45
FCR						
d 1 to 14	1.52	1.54	1.47	1.46	0.01	0.10
d 15 to 21	1.54	1.53	1.49	1.54	0.008	0.13
d 22 to 28	1.65[1]	1.60[2]	1.57[2]	1.57[2]	0.007	0.001
d 29 to 35	1.99[1]	1.87[1,2]	1.90[1,2]	1.80[2]	0.02	0.02
d 1 to 35	1.74[1]	1.67[2]	1.65[2,3]	1.64[3]	0.006	0.005

[1, 2, 3] Means not sharing the same superscripts are significantly different ($P < 0.05$).
[4] Data represent the mean of 6 replicate pens of 10 broiler chickens per pen.
[5] XG = xylanase and β-glucanase (100 g/t; Natugrain, BASF, Germany).
[6] P = phytase (100 g/t; Phyzyme XP 5000, Danisco Animal Nutrition, UK).
[7] A = acidifier (3,000 g/t; BioAcid Ultra, Biochem, Germany).
[8] SEM = standard error of the mean.

Table 3
Effects of dietary treatments on carcass characteristics (g/100 g body weight of bird) and intestine length (cm) in broiler chickens at 35 days of age[3].

Item	Treatments				SEM[7]	*P*-value
	Control	XG[4]	XG + P[5]	XG + A[6]		
Carcass eviscerated	66.16	64.82	64.84	65.61	0.342	0.47
Breast	26.31	25.54	25.93	25.91	0.303	0.85
Thigh	19.07	18.50	18.14	18.72	0.168	0.30
Liver	2.58	2.87	2.67	2.71	0.091	0.74
Pancreas	0.243	0.235	0.240	0.263	0.006	0.45
Spleen	0.153[1,2]	0.128[2]	0.178[1]	0.133[2]	0.007	0.045
Intestine	224.50	215.50	222.25	217.50	2.472	0.57

[1, 2] Means not sharing the same superscripts are significantly different ($P < 0.05$).
[3] Data represent the mean of 6 replicate pens of 10 broiler chickens per pen.
[4] XG = xylanase and β-glucanase (100 g/t; Natugrain, BASF, Germany).
[5] P = phytase (100 g/t; Phyzyme XP 5000, Danisco Animal Nutrition, UK).
[6] A = acidifier (3,000 g/t; BioAcid Ultra, Biochem, Germany).
[7] SEM = standard error of the mean.

Table 4
Effects of dietary treatments on ileal microbial counts in broiler chickens at 35 days of age[4].

Item	Treatments				SEM[8]	*P*-value
	Control	XG[5]	XG + P[6]	XG + A[7]		
Lactobacillus, $\log_{10}$ cfu/g	8.13	8.45	7.87	7.92	0.129	0.41
E. coli, $\log_{10}$ cfu/g	7.22[1,2]	7.45[1]	6.29[3]	6.73[2,3]	0.221	0.03

[1, 2, 3] Means not sharing the same superscripts are significantly different ($P < 0.05$).
[4] Data represent the mean of 6 replicate pens of 10 broiler chickens per pen.
[5] XG = xylanase and β-glucanase (100 g/t; Natugrain, BASF, Germany).
[6] P = phytase (100 g/t; Phyzyme XP 5000, Danisco Animal Nutrition, UK).
[7] A = acidifier (3,000 g/t; BioAcid Ultra, Biochem, Germany).
[8] SEM = standard error of the mean.

Table 5
Effects of dietary treatments on intestinal morphology indices in broiler chickens at 35 days of age[3].

Item	Treatments				SEM[7]	*P*-value
	Control	XG[4]	XG + P[5]	XG + A[6]		
Villus length, μm	1,360.21[2]	1,442.72[1,2]	1,574.20[1]	1,531.11[1]	40.6	0.03
Villus width, μm	232.27	232.71	232.15	223.87	9.69	0.99
Crypt depth, μm	236.52	248.72	253.64	263.60	7.37	0.62
Villus length/Villus width	5.85	6.30	7.02	6.88	0.25	0.41
Villus length/Crypt depth	5.81	5.87	6.32	5.86	0.27	0.93

[1, 2]Means not sharing the same superscripts are significantly different ($P < 0.05$).
[3] Data represent the mean of 6 replicate pens of 10 broiler chickens per pen.
[4] XG = xylanase and β-glucanase (100 g/t; Natugrain, BASF, Germany).
[5] P = phytase (100 g/t; Phyzyme XP 5000, Danisco Animal Nutrition, UK).
[6] A = acidifier (3,000 g/t; BioAcid Ultra, Biochem, Germany).
[7] SEM = standard error of the mean.

XG + A in the present experiment may be due to the change in the pH of the gut by the acidifier supplement resulting to make an optimum acidity condition for exogenous enzyme activity.

The gastrointestinal microbiota plays important roles in nutrition, immunity and physiological systems of the chickens. It is well documented that the pathogenic microbes such as *E. coli* enhance infections and decline the growth performance of poultry. In the present experiment, the addition of phytase supplement to the wheat-based diet in combination with exogenous carbohydrases (XG + P) had an inhibitory effect on the ileal population of the *E. coli* in broiler chickens. The effects of exogenous carbohydrases such as xylanase and glucanase on the *Lactobacilli* and *E. coli* enumeration have been observed in other studies (Bedford and Cowieson, 2012; Munyaka et al., 2016; Wang et al., 2005). Ndou et al. (2015) reported that the diet type and its digestibility may affect the efficacy of NSP enzymes and intestinal microbiota activity in broiler chickens. Similar to this finding, Józefiak et al. (2010) showed that the exogenous enzyme supplementation altered the microbial population in broiler chickens and declined potentially pathogenic populations. It is well demonstrated that the oligosaccharides resulting from the action of xylanases may also modulate the microbial population in the hindgut of broiler chickens (Bedford and Cowieson, 2012). According to the existing literature, not many studies are available on the impact of phytase supplementation on the microbial profile of the broiler chickens. Recently, it has been observed that phytase inclusion (5,000 phytase units FTU/kg) may alter the ileal microbial population in broiler chickens (Ptak et al., 2015). Furthermore, Sharma et al. (2016) found that the addition of higher levels of phytase decreased the undesirable microbiota activity and thus the amount of the inflammation and size of the gastrointestinal tract. The mode of action of phytase supplementation on the hindgut microbial population in broiler chickens was demonstrated by Ptak et al. (2015). According to the authors, changes in the digesta pH may result in shifts of endogenous microbiota profiles. Therefore, the effects of phytase have been related to a reduction in the buffering capacity of a diet with subsequent effect on microbiota profiles.

The results of the present experiment indicated that the addition of phytase or acidifier in combination with exogenous carbohydrases supplementation increased the jejunal villus length in broiler chickens. However, these findings contradict with findings of Wu et al. (2004) and Iji et al. (2001) who indicated that the addition of xylanase and the phytase supplements had no influence on villi characteristics in broilers fed wheat-based diets. Similarly, it is reported that use of xylanase and β-glucanase to the diets had no impact on the villi and microvilli measurements (Kalmendal and Tauson, 2012; Parsaie et al., 2007; Rebole et al., 2010; Rebolé et al., 1999). However, the beneficial effect of exogenous carbohydrase such as xylanase and glucanase on the intestinal morphology of broiler chickens has been well known in several reports (Sun et al., 2015; Wang et al., 2005).

In the present experiment, the broiler chickens fed XG + A diet also showed a higher intestinal villus length. These results are in accordance with the observations of Ragaa and Korany (2016) who indicated that dietary organic acids in broiler diets had a significant effect on gut health. Besides, the findings of a previous study revealed that the villus width, crypt depth and the villus length to crypt depth ratio were greater in broilers that received acidified drinking water than those with normal drinking water (Eftekhari et al., 2015). In contrast with the current study, it has been reported that the intestinal morphology of broiler chickens was not influenced by the dietary organic acids (Vieira et al., 2008). The mode of action of organic acids on the intestinal morphology of the broiler chickens was demonstrated by García et al. (2007). According to the authors, acidifiers may have a positive effect on the microbial load, which in turn reduces the presence of toxins that are related with alterations in gut morphology of broiler chickens. In the reviewed literature, no information is available on the impact of acidifiers in combination with exogenous carbohydrases on the intestinal morphology of broiler chickens.

5. Conclusions

In conclusion, the addition of exogenous carbohydrases in combination with an acidifier to diets had a positive effect on FCR in broiler chickens. The diet supplemented with XG + P or XG + A decreased the population of *E. coli* in the hindgut of the broiler chickens.

Conflicts of interest

The authors hereby certify that they have no conflict of interest.

References

Ao T, Cantor AH, Pescatore AJ, Ford MJ, Pierce JL, Dawson KA. Effect of enzyme supplementation and acidification of diets on nutrient digestibility and growth performance of broiler chicks. Poult Sci 2009;88:111–7.
Ao T, Cantor AH, Pescatore AJ, Pierce JL, Dawson KA. Effects of citric acid, alpha-galactosidase and protease inclusion on in vitro nutrient release from soybean meal and trypsin inhibitor content in raw whole soybeans. Anim Feed Sci Technol 2010;162:58–65.
Bedford M, Morgan A. The use of enzymes in poultry diets. World's Poult Sci J 1996;52:61–8.
Bedford M, Schulze H. Exogenous enzymes for pigs and poultry. Nutr Res Rev 1998;11:91–114.
Bedford MR, Cowieson AJ. Exogenous enzymes and their effects on intestinal microbiology. Anim Feed Sci Technol 2012;173:76–85.

Camden B, Morel P, Thomas D, Ravindran V, Bedford M. Effectiveness of exogenous microbial phytase in improving the bioavailabilities of phosphorus and other nutrients in maize-soya-bean meal diets for broilers. Anim Sci 2001;73:289–97.
Chaveerach P, Keuzenkamp DA, Lipman LJA, Van Knapen F. Effect of organic acids in drinking water for young broilers on campylobacter infection, volatile fatty acid production, gut microflora and histological cell changes. Poult Sci 2004;83:330–4.
Cowieson A, Bedford M, Selle P, Ravindran V. Phytate and microbial phytase: implications for endogenous nitrogen losses and nutrient availability. World's Poult Sci J 2009;65:401–18.
Dibner JJ, Buttin P. Use of organic acids as a model to study the impact of gut microflora on nutrition and metabolism1. J Appl Poult Res 2002;11:453–63.
Eftekhari A, Rezaeipour V, Abdullahpour R. Effects of acidified drinking water on performance, carcass, immune response, jejunum morphology, and microbiota activity of broiler chickens fed diets containing graded levels of threonine. Livest Sci 2015;180:158–63.
Ellis S, McCracken K, Collins M. The effects of a feed acidifier or an oligosaccharide, with and without enzyme, on the gut microflora and performance of broiler chickens. Br Poult Sci 2002;43:35–6.
Esmaeilipour O, Shivazad M, Moravej H, Aminzadeh S, Rezaian M, van Krimpen MM. Effects of xylanase and citric acid on the performance, nutrient retention, and characteristics of gastrointestinal tract of broilers fed low-phosphorus wheat-based diets. Poult Sci 2011;90:1975–82.
García V, Catalá-Gregori P, Hernández F, Megías MD, Madrid J. Effect of formic acid and plant extracts on growth, nutrient digestibility, intestine mucosa morphology, and meat yield of broilers. J Appl Poult Res 2007;16:555–62.
Hashemi SR, Zulkifli I, Davoodi H, Zunita Z, Ebrahimi M. Growth performance, intestinal microflora, plasma fatty acid profile in broiler chickens fed herbal plant (Euphorbia hirta) and mix of acidifiers. Anim Feed Sci Technol 2012;178:167–74.
Iji P, Tivey D. Natural and synthetic oligosaccharides in broiler chicken diets. World's Poult Sci J 1998;54:129–43.
Iji PA, Saki AA, Tivey DR. Intestinal development and body growth of broiler chicks on diets supplemented with non-starch polysaccharides. Anim Feed Sci Technol 2001;89:175–88.
Józefiak D, Rutkowski A, Kaczmarek S, Jensen BB, Engberg RM, Højberg O. Effect of β-glucanase and xylanase supplementation of barley- and rye-based diets on caecal microbiota of broiler chickens. Br Poult Sci 2010;51:546–57.
Kalmendal R, Tauson R. Effects of a xylanase and protease, individually or in combination, and an ionophore coccidiostat on performance, nutrient utilization, and intestinal morphology in broiler chickens fed a wheat-soybean meal-based diet. Poult Sci 2012;91:1387–93.
Li D, Liu SD, Qiao SY, Yi GF, Liang C, Thacker P. Effect of feeding organic acid with or without enzyme on intestinal microflora, intestinal enzyme activity and performance of weaned pigs. Asian Aus J Anim Sci 1999;12:411–6.
Munyaka PM, Nandha NK, Kiarie E, Nyachoti CM, Khafipour E. Impact of combined β-glucanase and xylanase enzymes on growth performance, nutrients utilization and gut microbiota in broiler chickens fed corn or wheat-based diets. Poult Sci 2016;95:528–40.
Ndou SP, Kiarie E, Agyekum AK, Heo JM, Romero LF, Arent S, et al. Comparative efficacy of xylanases on growth performance and digestibility in growing pigs fed wheat and wheat bran- or corn and corn DDGS-based diets supplemented with phytase. Anim Feed Sci Technol 2015;209:230–9.
Owens B, Tucker L, Collins MA, McCracken KJ. Effects of different feed additives alone or in combination on broiler performance, gut microflora and ileal histology. Br Poult Sci 2008;49:202–12.
Parsaie S, Shariatmadari F, Zamiri M, Khajeh K. Influence of wheat-based diets supplemented with xylanase, bile acid and antibiotics on performance, digestive tract measurements and gut morphology of broilers compared with a maize-based diet. Br Poult Sci 2007;48:594–600.
Patten J, Waldroup P. Use of organic acids in broiler diets. Poult Sci 1988;67:1178–82.
Ptak A, Bedford MR, Świątkiewicz S, Żyła K, Józefiak D. Phytase modulates ileal microbiota and enhances growth performance of the broiler chickens. PLos One 2015;10, e0119770.
Ragaa NM, Korany RMS. Studying the effect of formic acid and potassium diformate on performance, immunity and gut health of broiler chickens. Anim Nutr 2016;2:296–302.
Rebole A, Ortiz LT, Rodriguez ML, Alzueta C, Trevino J, Velasco S. Effects of inulin and enzyme complex, individually or in combination, on growth performance, intestinal microflora, cecal fermentation characteristics, and jejunal histomorphology in broiler chickens fed a wheat- and barley-based diet. Poult Sci 2010;89:276–86.
Rebolé A, Rodrıguez ML, Alzueta C, Ortiz LT, Treviño J. A short note on effect of enzyme supplement on the nutritive value of broiler chick diets containing maize, soyabean meal and full-fat sunflower seed. Anim Feed Sci Technol 1999;78:153–8.
SAS. SAS statistics user's guide. Statistical analytical system. 5th revised ed. Carry, NC: SAS Institute Inc; 1999.
Seal BS, Lillehoj HS, Donovan DM, Gay CG. Alternatives to antibiotics: a symposium on the challenges and solutions for animal production. Anim Health Res Rev 2013;14:78–87.
Selle PH, Ravindran V, Partridge GG. Beneficial effects of xylanase and/or phytase inclusions on ileal amino acid digestibility, energy utilisation, mineral retention and growth performance in wheat-based broiler diets. Anim Feed Sci Technol 2009;153:303–13.
Sharma NK, Choct M, Wu S-B, Smillie R, Morgan N, Omar AS, et al. Performance, litter quality and gaseous odour emissions of broilers fed phytase supplemented diets. Anim Nutr 2016;2:288–95.
Sun Q, Liu D, Guo S, Chen Y, Guo Y. Effects of dietary essential oil and enzyme supplementation on growth performance and gut health of broilers challenged by Clostridium perfringens. Anim Feed Sci Technol 2015;207:234–44.
Vieira SL, Oyarzabal OA, Freitas DM, Berres J, Peña JEM, Torres CA, et al. Performance of broilers fed diets supplemented with sanguinarine-like alkaloids and organic acids. J Appl Poult Res 2008;17:128–33.
Wang ZR, Qiao SY, Lu WQ, Li DF. Effects of enzyme supplementation on performance, nutrient digestibility, gastrointestinal morphology, and volatile fatty acid profiles in the hindgut of broilers fed wheat-based diets. Poult Sci 2005;84:875–81.
Woyengo TA, Slominski BA, Jones RO. Growth performance and nutrient utilization of broiler chickens fed diets supplemented with phytase alone or in combination with citric acid and multicarbohydrase. Poult Sci 2010;89:2221–9.
Wu Y, Ravindran V, Thomas D, Birtles M, Hendriks W. Influence of phytase and xylanase, individually or in combination, on performance, apparent metabolisable energy, digestive tract measurements and gut morphology in broilers fed wheat-based diets containing adequate level of phosphorus. Br Poult Sci 2004;45:76–84.
Zaefarian F, Romero L, Ravindran V. Influence of a microbial phytase on the performance and the utilisation of energy, crude protein and fatty acids of young broilers fed on phosphorus-adequate maize-and wheat-based diets. Br Poult Sci 2013;54:653–60.
Zeller E, Schollenberger M, Kühn I, Rodehutscord M. Effect of diets containing enzyme supplements and microwave-treated or untreated wheat on inositol phosphates in the small intestine of broilers. Anim Feed Sci Technol 2015;204:42–51.