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 of carbohydrases and acidifier (XG + A) improves FCR in broiler chickens.

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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...
phytate complexes in plant-derived ingredients. There are a number of mechanisms involved in the positive impacts of glycansases 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; Zaeferian et al., 2013). In a recent study, Zeller et al. (2015) found that xylanase may enhance the accessibility of phytate for dietary phytate in broiler chickens. Moreover, the exogenous carbohydrates 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 (Effekhari et al., 2015), nutrient utilization (Ragaa and Korany, 2016), intestinal morphometric indices (Effekhari 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 1.25 mg I; 0.3 mg Se.

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 washed with cold saline to remove the intestinal content. The intestinal segment was then cut into 10 cm long segments. Each segment was then cut open by scissors and placed in 50 ml of 0.9 % saline solution. The solution was mixed by shaking and then filtered using 280-μm mesh size. The filtrate was used to examine the number of live microbiota using methyltetrazolium method with a correlation coefficient of 0.8, as recommended by the National Research Council (2012).

### Table 1

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

| Item                      | Starter d 1 to 10 | Grower d 11 to 24 | Finisher 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           | 8.6               | 7.2               | 5.5                 |
| Mineral premix           | 2.5               | 2.5               | 2.5                 |
| DL-Met                   | 3.0               | 2.5               | 2.5                 |
| 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 D3; 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 B12; 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 D3; 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 B12; 18 mg pantothenic acid; 1.9 mg folic acid; 0.25 mg biotin; 1,600 mg choline chloride; and 8.5 mg antioxidant.). (Finisher: 10,000 IU vitamin A; 4,000 IU vitamin D3; 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 B12; 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 steriley. 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, sharpie 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 on body 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.

#### 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 multienzyme 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

| Item                          | Treatments | SEM² | *P*-value |
|-------------------------------|------------|------|-----------|
| 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                    | 194.98¹,²  | 187.32¹,²| 183.46²  | 186.32¹,²| 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                    | 1.65¹      | 1.60² | 1.57²    | 1.57² | 0.007| 0.001|
| d 29 to 35                    | 1.99¹      | 1.87¹,²| 1.90¹,²  | 1.80²  | 0.02 | 0.02 |
| d 1 to 35                     | 1.74¹      | 1.67² | 1.65²,³  | 1.64³  | 0.006| 0.005|

¹, ² Means not sharing the same superscripts are significantly different (*P* < 0.05).

Table 3

| Item                             | Treatments | SEM² | *P*-value |
|----------------------------------|------------|------|-----------|
| Carcass eviscerated              |            |      |           |
| Breast                           | 66.16      | 64.82| 64.84     | 65.61 | 0.342| 0.47 |
| Liver                            | 26.31      | 25.54| 25.93     | 25.91 | 0.303| 0.85 |
| Spleen                           | 19.07      | 18.50| 18.14     | 18.72 | 0.168| 0.30 |
| Intestine                        | 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 |
| Kidney                           | 0.151²     | 0.128²| 0.178²    | 0.133²| 0.007| 0.045|
| Kidney                           | 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).

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2. Hashemi, S., et al. (2012). The impact of organic acids on the broilers performance has been investigated in the several studies.
3. Ragaa, A. and Korany, R. (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.
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5. Esmaeilipour, A. et al. (2011). demonstrated that no effective interaction between xylanase and citric acid was observed in any measured responses.
6. Li, R. et al. (1999). The findings of Li et al. (1999) support these results which indicated that feeding organic acids and a multienzyme 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...
Means not sharing the same superscripts are significantly different (P < 0.05).

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

| Item                      | Treatments          | SEM  | P-value |
|---------------------------|---------------------|------|---------|
| Lactobacillus, log10 cfu/g | Control            | 8.13 |         |
| E. coli, log10 cfu/g      | XG + P              | 8.45 |         |
|                           | XG + A              | 7.87 |         |
|                           |                     | 7.92 |         |
|                           |                     | 0.129| 0.41    |

Note: Data represent the mean of 6 replicate pens of 10 broiler chickens per pen.

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

| Item                      | Treatments          | SEM  | P-value |
|---------------------------|---------------------|------|---------|
| Villus length, μm          | Control             | 3.60 |         |
|                           | XG + P              | 3.22 |         |
|                           | XG + A              | 3.15 |         |
|                           |                     | 4.06 |         |
|                           |                     | 0.03 |         |
| Villus width, μm           | Control             | 3.22 |         |
|                           | XG + P              | 3.60 |         |
|                           | XG + A              | 3.15 |         |
|                           |                     | 4.06 |         |
|                           |                     | 0.03 |         |
| Crypt depth, μm            | Control             | 3.60 |         |
|                           | XG + P              | 3.22 |         |
|                           | XG + A              | 3.15 |         |
|                           |                     | 4.06 |         |
|                           |                     | 0.03 |         |
| Villus length/Crypt depth  | Control             | 3.60 |         |
|                           | XG + P              | 3.22 |         |
|                           | XG + A              | 3.15 |         |
|                           |                     | 4.06 |         |
|                           |                     | 0.03 |         |

Note: Data represent the mean of 6 replicate pens of 10 broiler chickens per pen.

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). Nduo 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 microbital 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 Li 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; Rebolé et al., 1999). However, the beneﬁcial effect of exogenous carbohyd rase 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.

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