Productive performance, nutrient digestibility and intestinal morphometry in broiler chickens fed corn or wheat-based diets supplemented with bacterial- or fungal-originated xylanase

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ABSTRACT
An experiment was conducted to evaluate effects of dietary supplementation of a corn- or a wheat-based diet with two sources of exogenous xylanase (fungal or bacterial originated) on productive performance, ileal nutrients digestibility, nitrogen-corrected apparent metabolisable energy (AMEn) and digestive organs morphometry in broiler chickens. Broilers were fed with one of the six dietary treatments consisting of two basal diets (based on corn or wheat) each with or without xylanase (from bacterial or fungal origin) supplementation. Compared with corn-based diet, feeding birds with the wheat-based diet improved weight gain and feed intake by 4.71 and 4.81%, respectively, and ileal digestibility of dry matter and crude protein were greater in birds fed wheat-based diets compared with the birds grown on corn-based during Days 1–21 of age ($p<.05$). Villus length and villus length to crypt depth ratio increased ($p<.05$) in birds received wheat-based diets compared with those fed on corn-based diets. Gizzard weight was greater in birds fed with corn-based diets on days 21 and 42 of age by 11.5% and 31.8%, respectively, compared with those received wheat-based diets ($p<.05$). Supplementing diets with fungal xylanase increased liver weight in birds grown on wheat-based diets compared with those grown on control and corn-based diets, respectively, on Days 21 and 42 of age ($p<.05$). It was concluded that supplementing a corn- or wheat-based diet with a xylanase of bacterial or fungal origin had no effect on productive performance or AMEn of diets in broiler chickens during the starter and growing periods.

Introduction
Implementation of exogenous xylanase in poultry diets has considerably increased due to promising research finding concerning possible benefits of utilising wheat instead of corn in poultry diets when corn supply is limited or cost benefit diets with corn are problematic. Effects of dietary inclusion of exogenous xylanase in corn or wheat-based diets are mainly inconsistent due to several factors such as inherent variation in nature of dietary non-starch polysaccharides (NSPs), in particular arabinoxylans (AX; Choct & Annison 1990) and origin of added enzyme (Polizeli et al. 2005). It was shown that in broilers fed with a corn-based diets low in water soluble NSPs, the positive response in performance due to xylanase supplementation was lower compared with birds received diets formulated based on viscous grains such as wheat (Slominski 2011). Corn and wheat contains 9 and 28 of soluble and 60 and 87 g/kg of insoluble NSPs, respectively (Amerah 2015). Therefore, the entrapment of nutrients caused by the insoluble AX fraction in both wheat and corn-based poultry diets should be taken into consideration (Cowieson et al. 2006). Courtin et al. (2001) reported that inclusion of a xylanase preparation with a high affinity for insoluble AX diminished the depressing effects of insoluble AX on nutrients digestibility in a common corn-based diet.

There are many different commercially available xylanases differing in certain biochemical properties due to diverged metabolism of microorganisms used as their sources. The Aspergillus fungi and Bacillus bacteria are the known sources for xylanase production.
Xylanases prepared from different origins may vary in their impacts on production performance of broilers through certain features including susceptibility of xylanase to proteolytic enzymes, mode of action, optimal pH for activity (Beg et al. 2001), end products produced by xylanase and their ability to attach to soluble as well as insoluble AXs structures. Despite of huge literature on xylanase effects in poultry diets, information concerning comparative effects of fungal or bacterial originated xylanase on nutrient digestibility and gut morphology in poultry species is scarce. Therefore, the current study was carried out to examine the effects of dietary inclusion of xylanase from two origins (bacterial and fungal) in a corn or wheat-based diet on production performance, nutrients digestibility and certain gut morphometric measures in broiler chicken.

Materials and methods

Animal handling and experimental procedures were approved by ethical committee of Lorestan University and performed according to the Guides for the Care and Use of Laboratory Animals by the National Institutes of Health (Bethesda, MD).

Enzyme preparation

The commercial *Bacillus subtilis* (Bac, s) xylanase (Nutrex NV., Achrerenhoek 5, 2275 Lille, Belgium) was used and its activity (1500 units per g of product) was measured according to the method described by Bailey et al. (1992). The fungal xylanase was produced by solid-state fermentation method using non-genetically modified *Aspergillus niger* (CCUG 33991) and it was prepared in Department of Animal Science, Isfahan Research Center for Agriculture and Natural Resources, Isfahan, Iran. The fungal enzyme preparation consisted of 1700 U/g xylanase, as the primary enzyme, and 809 U/g cellulase and 950 U/g proteases as the minor residual fractions. All enzymes were supplemented to provide 1000 unit of xylanase per kg of diet (as-fed basis). One unit of xylanase was defined as the quantity of enzyme which liberated one micromole of xylose from birch wood xylan (Sigma X4252) per minute at pH = 4.8 and 50°C. One unit of cellulase was defined as the quantity of enzyme that released one micromole cellulose from carboxymethyl cellulose (Merk, K3912364) at 40°C, pH = 5, per minute. One unit of protease was defined as the quantity of enzyme which released one micromole peptide from casein at 37°C, pH = 3, per minute. In a preliminary study, optimal pH for the activity of xylanase with fungal and bacterial origin was determined as described by Gomes et al. (2013), and it was 4–5 for bacterial and 6–7 for fungal originated xylanase, respectively. Furthermore, these enzymes showed more than 80% recovery in the test of proteolytic stability against pepsin at pH = 2 and 3 according to the method of Ndou et al. (2015).

Experimental flock and treatments

A total of 510 one-day-old Ross 308 male broiler chicks were provided from a commercial hatchery, weighted and randomly allocated to one of the 30 floor pens. Each pen was equipped with one hanging feeder and a nipple drinker. House temperature was kept at 31°C for days 1–3 and then gradually decreased to 21°C by the end of sixth week. Light was continuous during the first two days and a 23 h:1 h lightening:darkness regimen was employed afterwards. The birds were vaccinated against infectious bronchitis disease (at 2 and 7 d), Newcastle disease (ND live B1 at 7 d and La Sota at 18 d) and infectious bursal disease (at 14 and 25 d). In a factorial arrangement of treatments with five replicates of 17 birds each, effects of dietary supplementation with 1000 U/kg of diet xylanases from two origins (no-supplementation and supplementation with bacterial or fungal originated xylanase) in either a corn or a wheat based broiler diet were examined. Experimental diets were wheat-based diet supplemented with 1000 U/kg of fungal xylanase, wheat-based diet supplemented with 1000 U/kg of bacterial xylanase, corn-based diet supplemented with 1000 U/kg of fungal xylanase and corn-based diet supplemented with 1000 U/kg of bacterial xylanase. Diets were offered to birds in two-phases including starter (1–21 d) and grower (22–42 d) periods (Table 1). All experimental diets were iso-caloric and iso-nitrogenous and formulated to meet or slightly exceed the recommended requirements for broilers (NRC 1994). Experimental diets provided to the birds ad libitum in mash form. To determine coefficients of apparent ileal nutrient digestibility, acid insoluble ash (as Celite; AIA) (20 g/kg) was added to all diets as an indigestible marker during the last week of the starter (d 14–21) and grower (d 35–42) periods.

Growth performance, digestive organ morphometry and nutrient digestibility

Body weights and feed intake were recorded weekly for all birds in a pen in Day 1–21, 22–42 and 1–42 of age and data were used for calculating feed
conversion ratio (FCR; as gram of feed intake per gram of weight gain) for the same periods. Mortality was recorded daily throughout the experimental course. On day 21 and 42, four intentionally selected birds from each pen with the closest weight to the mean body weight were killed by cervical dislocation and their digestive tracts was quantitatively excised from gizzard to caeca. The empty wet weight of small intestine (from pancreatic loop to ileocecal junction) as well as the length and weight of caeca were recorded. Moreover, the proportional weights of liver, pancreas and the length and weight of gizzard were calculated. The contents of the distal half segment of ileum were collected (Ravindran et al. 2005). Digesta from all birds in each pen was pooled, resulting in five samples per each dietary treatment. The digesta samples were immediately frozen at –18°C prior to drying at 60°C for 48 h, ground to pass through a 0.5 mm screen and analysed for nitrogen (AOAC 1995; method 954.01), AIA (Siriwan et al. 1993) and gross energy (GE) (Wu et al. 2004).

Moreover, morphometric measurements (villus height, villus width and crypt depth) were made on a specimen with 5 cm in length taken from the midpoint of jejunum.

To determine apparent metabolisable energy corrected for nitrogen retention (AMEc) of the experimental diets, two birds per each replicate (10 birds per treatment with body weight close to the average body weight of the relevant pen) were selected on days 14 and 35 of age. Birds were randomly placed in individual metabolic cages (60 × 30 × 30 cm, length–width–height) located in a temperature controlled room with the ambient temperature of 21°C and relative humidity of 60%. The birds received 24 h lightening provided by fluorescent bulbs. Each cage was equipped with an individual confined feeder and a nipple drinker. Birds in each cage had free access to water and feed. The trial was conducted from days 14 to 21 and 35 to 42 of age and consisted of a four days of preliminary adaptation period, followed by three days of excretion collection in triplicate. For excreta collection, clean trays were placed under the cages. Contamination (e.g. feathers and down) was carefully removed, and the collected excreta per cage during three days were pooled and stored at −20°C. Before analysis, samples of excreta were thawed and dried at 60°C for 48 h, ground to pass through a 0.5 mm sieve and stored in airtight plastic containers. Celite (AIA) as an indigestible marker, was added at 20g/kg into the diets for determination of AMEn during Days 14–21 and 35–42 of experimental period.

**Laboratory analysis**

All chemical analyses were performed in duplicate and their results were reported on a DM basis. Samples of feeds, ileal digesta and excreta were analysed for dry matter (AOAC, 934.01), total ash (AOAC, 930.05) and crude protein (AOAC, 954.01). Gross energy was measured using an adiabatic calorimeter bomb (Gallenkamp, London, UK), standardised with benzoic acid. The AIA contents of dried diets, ileal digesta and excreta samples were determined according to the method of Siriwan et al. (1993).

The following equations were used for calculating AMEn (Kaczmarek et al. 2014) and coefficient of apparent ileal digestibility of experimental diets (Kiarie et al. 2014):

$$\text{AMEn (kcal/kg of diet)} = \frac{\text{GE kcal of excreta} - \text{[GE kcal of excreta} \times \frac{\text{AIA % diet}}{\text{AIA % excreta}}\text{]} - 8.22}{\text{N% diet} - \frac{\text{N% excreta}}{\text{AIA% diet}} \times \frac{\text{AIA% excreta}}{\text{AIA % diet}}}$$

where GE is given in kcal per kilogram, and feed intake and excreta output in kilograms per day and 8.22 is the energy equivalent of 1 g of uric acid N.

$$\text{Apparent ileal digestibility coefficient} = \frac{(\text{NT/AIA})_d - (\text{NT/AIA})_i}{(\text{NT/AIA})_d}$$

where (NT/AIA)d is the ratio of nutrient and insoluble marker in the diet and (NT/AIA)i is the ratio of nutrient and insoluble marker in the ileal content.

Morphological measures were taken on jejunal samples according to the method of Iji et al. (2001) with minor modifications. Samples from each bird were fixed in a 10% buffered formalin solution, dehydrated in ethanol with incremental concentrations of 70, 80, 95 and 100%, cleared by two changes of xylene, and embedded in paraffin. Each sample was then sectioned at a thickness of 7 μm, stained with haematoxylin–eosin and examined for villus height, villus width and crypt depth microscopically. The measurements were averaged on 10 villi and crypts per cross-section.

**Statistical analysis**

For performance, nutrient digestibility and AMEn, data, pen mean and for digestive organs and jejunal morphometrical measurements data from individual birds were considered as the experimental unit. Data were analysed in a 2 × 3 factorial arrangement of treatments in a completely randomised design using the GLM procedure of SAS 9.2 (Cary, NC). The statistical model
supplemented diets showed no improvement in assessed performance variables compared to those fed on the control diet during Days 1–21 and 22–42 of age (Table 2).

Apparent ileal digestibility of dry matter and crude protein were better in birds fed wheat-based diets compared with those fed corn-based diets during Days 1–21 of age (Table 3, \( p < .05 \)). Supplementation of diet with either bacterial or fungal xylanase did not improve apparent ileal digestibility of dry matter, crude protein and GE. The AMEn of diet was not affected by either the type of predominant grain in the basal diet (corn or wheat) or the origin of supplemented xylanase (\( p > .05 \); Table 3).

Villus length and villus length to crypt depth ratio increased by 18.5 and 16.4%, respectively (Table 4, \( p < .05 \)) in the birds grown on wheat-based diets compared with those fed with corn-based diets.

At Day 21 of age, supplementing corn-based diets with fungal and bacterial xylanase decreased llum weight by 9.9 and 12.2%, respectively (Table 5, \( p < .05 \)) compared with not-supplemented diets. Moreover, dietary supplementation of corn-based diets with bacterial xylanase decreased small intestine weight on Day 21 of age by 13.2%, compared with not-supplemented diets. A significant increase in caeca weight was observed in the birds fed wheat-based diets compared with those fed with the control diet (Tables 5 and 6, \( p < .05 \)).

In addition, feeding birds with corn-based diets supplemented with fungal xylanase increased liver weight by 18.9% compared with control birds on Day 42 of age (Table 6, \( p < .05 \)). The birds grown on wheat-based diets had greater liver weight on Days 21 and 42 of age by 10.2 and 13.2%, respectively of age compared with the birds grown on corn-based diets (Tables 5 and 6, \( p < .05 \)).

Discussion

In the present study, the primary objective was to examine the possible effects of supplementing a corn

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**Table 1.** Ingredient composition and nutritive values (as fed basis) of the experimental diets.

| Item                              | Wheat | Corn | Wheat | Corn |
|-----------------------------------|-------|------|-------|------|
| **Ingredient, g/kg**              |       |      |       |      |
| Corn                              | 0.00  | 540  | 0.00  | 568  |
| Wheatb                           | 610   | 645  | 0.00  | 0.00 |
| SBM (440 g CP/kg)                 | 325   | 394  | 273   | 348  |
| Soybean oil                       | 20    | 40   | 4.0   | 4.0  |
| \( \alpha \)-Methionine           | 2.4   | 2.1  | 2.9   | 2.9  |
| \( \gamma \)-Lysine HCl            | 2.5   | 1.7  | 0.9   | 0.9  |
| \( \alpha \)-Threonine             | 0.9   | 0.7  | 0.6   | 0.6  |
| Salt                              | 2.3   | 2.7  | 1.4   | 2.1  |
| NaHCO₃                            | 1.8   | 1.8  | 2.5   | 1.8  |
| CaCO₃                             | 15.4  | 16.2 | 15.1  | 16.0 |
| Monocalcium phosphate             | 16.0  | 16.1 | 14.3  | 14.3 |
| Choline Cl (600 g/kg)             | 2     | 2    | 2     | 2    |
| Vitamin-mineral Premixc           | 2     | 2    | 2     | 2    |

Calculated analysis

| Item                              |       |      |       |      |
|-----------------------------------|-------|------|-------|------|
| ME, kcal/kg                       | 2893  | 2900 | 3075  | 3084 |
| Crude protein, g/kg               | 220   | 220  | 200   | 200  |
| Lysine, g/kg                      | 13    | 13   | 11    | 11   |
| Methionine, g/kg                  | 6     | 6    | 5     | 5    |
| Met + Cys, g/kg                   | 10    | 10   | 9     | 9    |
| Threonine, g/kg                   | 9     | 9    | 8     | 8    |
| Calcium, g/kg                     | 10    | 10   | 10    | 10   |
| Available phosphorus, g/kg        | 5     | 5    | 5     | 5    |
| Sodium, g/kg                      | 2     | 2    | 2     | 2    |
| DCAB, mEq/kg                      | 240   | 247  | 230   | 230  |

Determined analysis

| Item                              |       |      |       |      |
|-----------------------------------|-------|------|-------|------|
| DNL, g/kg                         | 901   | 865  | 905   | 865  |
| GE, kcal/kg                       | 4062  | 4062 | 4133  | 4133 |
| CP, g/kg                          | 217   | 218  | 198   | 199  |
| Total ash, g/kg                   | 30    | 29   | 27    | 26   |

*Celete (20 g/kg) was added to all diets as an indigestible marker in the weekend of starter and grower period at the expense of either corn or wheat for experiment.

Wheat analysis (% as fed): Dry matter, 93.3; CP, 11.6; Fat, 1.6; Ash, 1.77; Ca, 0.1; TP, 0.28; ADF, 3.06; NDF, 12.5; CF, 2.41; GE, 4300 kcal/kg.

Mineral combination (mg provided per kilogram of diet): manganese, 120; iron, 70; zinc, 110; copper, 16; iodine, 1.3; selenium, 0.35. Vitamin combination (U or mg provided per kilogram of diet): vitamin A, 12,000 U; vitamin D₃, 4500 U; vitamin E, 70 U; vitamin K, 3.5 mg; vitamin B₁₂, 0.3 mg; vitamin B₆, 7.5 mg; vitamin B₁₂, 30 mg; vitamin B₅, 65 mg; vitamin B₆, 4.3 mg; vitamin B₃, 2 mg; vitamin B₇, 0.025 mg; biotin, 0.3 mg; choline chloride, 800 mg.

included the fixed effects of predominant grain in the diet (corn or wheat), the origin of supplemental xylanase (fungal or bacterial) and their interactions. When a significant F-test was detected (\( p < .05 \)) for the main effects, corresponding means were separated by the Tukey test and interactions between treatments were analysed using a LS means test adjusted for the Tukey test.

Results

Weight gain was 4.7% greater in birds fed wheat-based diets compared with those received corn-based diets during Days 1–21 of age (Table 2, \( p < .05 \)). Feeding birds with the wheat-based diets improved feed intake by 4.8% compared with those receiving corn-based diet during the same experimental period (Table 2, \( p < .05 \)). Birds fed with xylanase-
or wheat-based diet with xylanase from two origins on production performance, nutrient digestibility and certain gut morphometric parameters in broiler chicken. From nutritional point of view, bacterial xylanases are advantageous to fungal xylanases as their optimal acting pH are close to that of the intestine, they are thermostable, they have greater aggression towards xylans due to high tenacity of carbohydrate domains, and they are superior to fungal xylanase in degradation of insoluble AX which are the majority of the AX fraction (Courtin et al. 2001; Chakdar et al. 2016). All these have made xylanases from bacterial origin more amenable to be included in poultry diets.

In general, no noticeable influence was found for supplemented xylanase from either source on growth performance parameters, the results which are in the line with many previous reports (Meng & Slominski 2005; Amerah et al. 2008). Supplementation of xylanase in

Table 2. Effects of supplementing corn and wheat-based diets with xylanase of microbial or fungal origin on performance of broiler chickens (0–42 d).

| Item       | Diet         | Xylanase | BW gain, g/bird | Feed intake, g/bird | Feed per gain, g/g |
|------------|--------------|----------|-----------------|---------------------|--------------------|
|            |              | d 0–21   | d 22–42 | d 42–64 | d 0–21 | d 22–42 | d 42–64 | d 0–21 | d 22–42 | d 42–64 | d 0–21 | d 22–42 | d 42–64 |
| Corn       | Bacterial    | 35.8     | 98.9    | 67.4    | 46.2   | 168    | 107    | 1.28   | 1.69    | 1.58    | 1.28   | 1.69    | 1.58    |
| Fungus     | 35.7        | 101.0    | 68.4    | 44.9    | 163    | 104    | 1.25   | 1.61    | 1.52    | 1.28   | 1.66    | 1.56    |

Table 3. Effects of supplementing corn and wheat-based diets with xylanase of microbial or fungal origin on coefficient apparent ileal digestibility of dietary components and AMEn (kcal/kg) in broiler chickens (1–21 d and 22–42 d).

| Item       | Xylanase | DM | CP | GE | AMEn |
|------------|----------|----|----|----|-------|
|             |           |    |    |    |       |
| Corn        | Bacterial | 0.787 | 0.767 | 0.689 | 3082 |
| Fungus      | 0.772     | 0.739 | 0.660 | 2987 |
| Wheat       | Bacterial | 0.811 | 0.777 | 0.700 | 3082 |
| Fungus      | 0.803     | 0.781 | 0.702 | 3035 |
| Bacteria    | 0.799     | 0.772 | 0.695 | 3082 |

AMEn: AME of the diets corrected by zero N retention; SEM: standard error of means.

**a,b**Means with different letters within a factor of analysis (diet, xylanase and their interactions) are significantly different ($p < .05$). SEM: standard error of means.
broiler diets was often accompanied by inconsistent responses in birds performance mainly due to variations in structure and composition of cell wall carbohydrates in diet ingredients (Kim et al. 2005), architecture of xylanase molecule (Beg et al. 2001), form of feed (Attia et al. 2012, 2014), and genetic background as well as age of birds (Amerah et al. 2011). Our results failed to confirm that the selected bacterial xylanase is superior to the fungal xylanase with regard to the breakdown and solubilisation of insoluble AX fraction. The efficacy of exogenous xylanase varies very much depending on feedstuffs used in the diets, because nutrient and energy released by xylanase inclusion is depended upon structure of feedstuff per se. In practical application, supplementary xylanase must be contest with the diet ingredients, in other words, user must know which enzyme is suitable for a specific diet. However, the present study could verify no difference between the tested exogenous xylanases when implemented in either a corn or a wheat-based diet. Vandeplas et al. (2010) reported that xylanase supplementation from

| Item | Xylanase | Villus length, µm | Villus width, µm | Crypt depth, µm | Ratio, vil/cry |
|------|----------|-------------------|------------------|----------------|---------------|
| Diet |          |                   |                  |                |               |
| Corn | Bacillus | 826               | 195              | 270            | 3.06          |
|      | Fungus   | 849               | 217              | 292            | 2.91          |
| Wheat| Bacillus | 785               | 219              | 269            | 2.92          |
|      | Fungus   | 918               | 233              | 272            | 3.38          |
|      | –        | 1061              | 230              | 304            | 3.49          |
|      | –        | 937               | 231              | 269            | 3.48          |
| Main effect | |                   |                  |                |               |
| Diet | Corn     | 820b              | 231              | 277            | 2.96b         |
|      | Wheat    | 972a              | 231              | 282            | 3.45a         |
| Xylanase |          |                   |                  |                |               |
| Corn | Bacillus | 861               | 225              | 269            | 3.20          |
|      | Fungus   | 955               | 224              | 298            | 3.20          |
|      | Bacteria | 872               | 214              | 271            | 3.22          |
| Probability |          |                   |                  |                |               |
| Diet | Corn     | 0.021             | 0.139            | 0.730          | 0.046         |
|      | Xylanase | 0.410             | 0.787            | 0.196          | 0.927         |
| Diet × xylanase |          |                   |                  |                |               |
| Corn | Bacillus | 0.733             | 0.686            | 0.924          | 0.958         |
|      | Fungus   | 32.58             | 6.737            | 6.924          | 0.0167        |

SEM: standard error of means.

a,bMeans assigned different letters within a factor of analysis (diet, xylanase and their interactions) are significantly different (p < .05).

| Item | Xylanase | Liver, g/kg | Gizzard, g/kg | Pancreas, g/kg | Duodenum, g/kg | Jejunum, g/kg | Ileum, g/kg | Small intestine, g/kg | Ceca, g/kg | Ceca, cm/kg |
|------|----------|-------------|---------------|---------------|----------------|---------------|-------------|------------------------|------------|------------|
| Diet |          |             |               |               |                |               |             |                        |            |            |
| Corn | Bacillus | 30.1bc      | 24.0          | 3.96          | 10.5           | 16.1          | 11.9c       | 38.5bc                 | 5.34       | 32.8       |
|      | Fungus   | 29.0bc      | 23.8          | 3.78          | 10.7           | 16.5          | 12.2bc      | 39.4bc                 | 5.28       | 32.8       |
|      | –        | 28.0c       | 22.4          | 3.72          | 11.3           | 19.5          | 13.5a       | 44.3a                  | 4.68       | 32.2       |
| Wheat| Bacillus | 31.3abc     | 21.3          | 3.54          | 9.7            | 16.8          | 12.2bc      | 38.7bc                 | 5.73       | 33.6       |
|      | Fungus   | 33.3ab      | 21.4          | 3.78          | 10.1           | 16.2          | 12.9abc     | 39.2ab                 | 5.5        | 33.8       |
|      | –        | 29.8bc      | 20.2          | 3.62          | 10.7           | 16.4          | 13.1abc     | 40.2abc                | 5.64       | 32.6       |
| Main effect | |             |               |               |                |               |             |                        |            |            |
| Diet | Corn     | 29.0b       | 23.4a         | 3.82          | 10.9           | 17.4          | 12.5        | 40.8                   | 5.1b       | 32.6       |
|      | Wheat    | 31.5a       | 21.0b         | 3.65          | 10.1           | 16.5          | 12.7        | 39.4                   | 5.62a      | 33.3       |
| Xylanase |          |             |               |               |                |               |             |                        |            |            |
| Corn | Bacillus | 28.9b       | 21.3          | 3.67          | 11.0           | 17.9          | 13.3a       | 42.3                   | 5.17       | 32.4ab     |
|      | Fungus   | 31.2a       | 22.6          | 3.78          | 10.4           | 16.4          | 12.6ab      | 39.3                   | 5.39       | 33.3a      |
|      | Bacteria | 30.7abc     | 22.7          | 3.75          | 10.1           | 16.5          | 12.0b       | 38.6                   | 5.54       | 33.2a      |
| Probability |          |             |               |               |                |               |             |                        |            |            |
| Diet |          | 0.0068      | 0.0007        | 0.2637        | 0.1260         | 0.3680        | 0.5609      | 0.3524                 | 0.0372     | 0.8945     |
| Xylanase |          | 0.0835      | 0.1387        | 0.8338        | 0.2816         | 0.3429        | 0.0153      | 0.1173                 | 0.6315     | 0.0221     |
| Diet × xylanase |          | 0.0289      | 0.9398        | 0.4965        | 0.9912         | 0.2609        | 0.0372      | 0.0465                 | 0.6020     | 0.5185     |
| SEM |          | 0.048       | 0.038         | 0.007         | 0.225          | 0.486         | 0.191       | 0.761                  | 0.067      | 0.523      |

a,bMeans with different letters within a factor of analysis (diet, xylanase and their interactions) are significantly different (p < .05); SEM, standard error of means.

Table 4. Effects of supplementing corn and wheat-based diets with xylanase of microbial or fungal origin on jejunal morphology of broiler chickens (21 d).

Table 5. Effects of supplementing corn and wheat-based diets with xylanase of microbial or fungal origin on relative weight (g/kg body weight) of selected digestive organs and caeca length (cm/kg BW) in broiler chickens (21 d).
three fungal (Aspergillus niger, Aspergillus aculeatus and Trichoderma viride) and one microbial source (Bacillus subtilis) increased weight gain and AMEs in broiler chicks regardless of its origin. In the study of Hew et al. (1998) on the effects of two types of enzyme preparations on digestibility of nutrients in broiler chicks, responses in amino acid digestibility and AME were similar for both enzymes.

In the current study, observations concerning lack of enzyme supplementation effect, regardless of its origin, on performance of birds could be justified by no consistent and pertained changes in gut morphological measurements. However, certain promising effects of enzyme such as increased caeca length were observed in birds fed with enzyme-supplemented diets. Unlike to our results, Mathlouthi et al. (2002) in broilers found significant changes in gut morphological measurements accompanied with improved nutrients digestion and absorption in birds fed with xylanase supplemented diets. Wang et al. (2005) reported that supplementing a wheat-based diet with xylanase reduced the relative length and weight of certain gut segments in broiler chicken. They attributed the results to the contracting viscosogenic effects of wheat AXs resulting in reduced digesta mixing and endogenous enzymes secretion. Our observation on lack of enzyme inclusion on evaluated parameters could be explained by the fact that diets, irrespective of basal ingredient, were low in soluble NSPs. Many studies have revealed that promising effects of xylanase supplementation on gut morphometric parameters were only realised in birds fed with diets containing high concentrations of soluble NSPs (Wang et al. 2005; Masey-O’Neill et al. 2014).

Feeding birds with wheat-based diets, irrespective of xylanase inclusion, resulted in improved BW gain and feed intake when compared with birds grown on corn-based diets. Even though, there are few supporting reports (Chiang et al. 2005; Kiarie et al. 2014), these findings disagree with several previous reports confirming the detrimental effects of NSPs fraction in wheat-based diets (Choct & Annison 1990; Annison 1991) due to their potential ability to increase viscosity of digesta in gut causing suppressed nutrients digestibility. The preceding reports have attributed such unexpected results to composition of diet and chemical composition of ingredients, in particular the NSPs content (Wiseman 2000), amino acid profile of wheat-based diets (Abadi et al. 2014) and physical characteristics of wheat used in the diets (Gutierrez Del Alamo et al. 2008). The wheat cultivar used in this study (Pishtaz) contains a very low soluble NSP concentration. The soluble NSP content of this cultivar is lower than the most wheat cultivars used in the other studies (Pirgozliev et al. 2003; Parsaie et al. 2006).

Moreover, AMEn of Pishtaz cultivar is higher than the most Iranian wheat cultivars (Parsaie et al. 2006). Since this cultivar contains very low level of soluble NSP, it should be highly digestible, a fact realised by the greater ileal dry matter and crude protein digestibility of wheat-based diets compared with corn-based diets. It has also to be mentioned that the improved body weight gain in the birds fed with wheat-based diets was accompanied by the increased feed intake.

| Table 6. Effects of supplementing corn and wheat-based diets with xylanase of microbial or fungal origin on relative weight (g/kg body weight BW) of selected digestive organs and caeca length (cm/kg BW) of broiler chickens (42 d). |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Item                           | Xylanase        | Liver, g/kg     | Gizzard, g/kg   | Pancreas, g/kg  | Duodenum, g/kg  | Jejunum, g/kg   | Ileum, g/kg     | small intestine, g/kg | Ceca, cm/kg |
|--------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Diet                           |                 |                 |                 |                 |                 |                 |                 |                 |                 |
| Corn                           | Bacterial       | 21.6bc          | 13.9            | 2.14            | 4.80            | 9.03            | 9.47            | 23.3            | 3.68            | 13.8            |
|                                | Fungus          | 23.4bc          | 13.7            | 2.44            | 5.30            | 9.81            | 9.31            | 24.4            | 4.23            | 13.8            |
| Wheat                          | Bacterial       | 23.9ab          | 10.1            | 2.36            | 5.27            | 8.96            | 9.52            | 23.8            | 4.60            | 14.7            |
|                                | Fungus          | 25.6a           | 10.9            | 2.50            | 5.07            | 9.72            | 9.60            | 24.4            | 4.66            | 14.7            |
|                                | –               | 22.5bc          | 10.6            | 2.16            | 5.56            | 7.50            | 9.45            | 22.5            | 4.34            | 13.2            |
| Main effect, diet              |                 |                 |                 |                 |                 |                 |                 |                 |                 |
| Diet                           |                 |                 |                 |                 |                 |                 |                 |                 |                 |
| Corn                           |                 |                 |                 |                 |                 |                 |                 |                 |                 |
|                                | 21.6b           | 13.9b           | 2.32            | 5.11            | 9.70            | 8.75            | 23.6            | 4.01b           | 13.6            |
| Wheat                          | 24.0a           | 10.5a           | 2.34            | 5.30            | 8.72            | 9.52            | 23.6            | 4.54a           | 14.2            |
| Xylanase                       |                 |                 |                 |                 |                 |                 |                 |                 |                 |
| –                              | 21.1c           | 12.3            | 2.27            | 5.39            | 8.89            | 8.46            | 22.8            | 4.23            | 13.2b           |
| Fungus                         | 24.5a           | 12.3            | 2.47            | 5.19            | 9.76            | 9.45            | 24.4            | 4.44            | 14.3a           |
| Bacteria                       | 22.8b           | 12.0            | 2.25            | 5.04            | 8.99            | 9.50            | 23.5            | 4.14            | 14.3a           |
| Probability                    |                 |                 |                 |                 |                 |                 |                 |                 |                 |
| Diet                           | 0.003           | 0.001           | 0.8624          | 0.3800          | 0.0654          | 0.1444          | 0.9897          | 0.0186          | 0.1629          |
| Xylanase                       | 0.0002          | 0.7565          | 0.3253          | 0.3965          | 0.3237          | 0.1972          | 0.2807          | 0.4926          | 0.0476          |
| Diet x xylanase                | 0.0214          | 0.6586          | 0.3809          | 0.3717          | 0.0619          | 0.2664          | 0.9104          | 0.4025          | 0.6618          |
| SEM                            | 0.042           | 0.036           | 0.006           | 0.010           | 0.028           | 0.027           | 0.040           | 0.011           | 0.022           |

a–dMeans with different letters within a factor of analysis (diet, xylanase and their interactions) are significantly different (p < .05); SEM, standard error of means.
These observations revealed that improved BWG in the birds grown on wheat-based diets may in part be attributed to their enhanced feed utilisation.

In the current study, experimental diets were iso-caloric and iso-nitrogenous, thus the mechanism through which wheat-based diets improved performance compared with corn-based diets on Day 21 of age cannot be fully justified by the collected data. Moreover, increased villi height and villi height to crypt depth ratio on Day 21 in birds fed on wheat-based diets suggest that digestive and absorptive capacity of the same birds could have been increased and, in part, encouraged a greater flow of nutrients into the small intestine. It is speculated that diets effect in birds could in some extent be exerted through their influence on gut microflora responses. Rodriguez et al. (2012) reported that digesta count of Escherichia coli and Lactobacilli in the birds fed with wheat-based diets was greater than those received corn-based diets. These bacteria mainly produce acetic and butyric acid, respectively (Guilloteau et al. 2010). Butyric acid improves gut health and function by reducing pH and inhibiting proliferation of putrefactive organisms in the intestinal lumen. In addition, butyric acid promotes the proliferation of enterocytes and stimulates secretion of digestive enzymes (Guilloteau et al. 2010).

In this study, wheat or corn as the main ingredient of diets induced many alterations in size (weight or length) of gut segments and digestive organs. Increased caeca weight was prominent in the birds fed wheat-based diets which could partly explain their superior growth performance compared to the birds fed with corn-based diets. In broiler chickens, caeca are the main sites of volatile fatty acids (VFA) production as the major end-products of microbial fermentation (Jozefiak et al. 2004). The wheat-based diets may have provided more resistant carbohydrates to enzymatic digestion or slowly digested carbohydrates in the proximal part of the gut (Masey-O’Neill et al. 2014) resulting in an increased quantity of fermentable substrates in caeca, where they were fermented to VFA leading to hypertrophy of caeca (Jozefiak et al. 2004). In agreements with our results, Masey-O’Neill et al. (2014) reported that caeca in birds grown on wheat-based diets were greater in size than those fed corn-based diets. It is worthy to note that increased caeca size in the birds received wheat-based diets was accompanied with increased liver weight giving the impression that wheat effects on caeca and liver weight was mediated through an increased VFAs flow form caeca to liver causing enhanced liver activity for secreting lipoproteins. This interpretation seems sensible because caeca size as well as liver weight was increased in the birds fed with enzyme-supplemented diets (either from fungal or bacterial source), irrespective of cereal type in the diet. Brenes et al. (1993) reported that increased microorganisms count in distal part of the GIT and their fermentation end-products may alter liver size because liver is the major site of short chain fatty acids metabolism, where butyric and propionic acids are almost entirely taken up.

Conclusions

The results of this study showed that dietary supplementation with xylanase from either a bacterial or fungi source had no effect on growth performance and nutrient utilisation in broiler chickens. However, reduced ileum and small intestine weight, increased caeca length and liver weight in birds received xylanase-supplemented diets suggest promising effects of xylanase on gut health and function. Furthermore, improved body weight gain and feed intake in birds grown on wheat-based diets were reflected as improvement of gut health through increased caeca size, and improvements in certain morphological measurements.

Disclosure statement

No potential conflict of interest was reported by the authors.

Geolocation information

The research was performed in Iran, Islamic Republic of, Esfahan (Latitude: 32.65722, Longitude: 51.67761), Coordinates: 32°39’26”N 51°40’39”E.

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