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Interaction between protease and xylanase in broiler chicken diets containing sorghum distillers' dried grains with solubles

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ABSTRACT

Individual or combined effects of xylanase and protease on the nutritive value of diets containing sorghum distillers' dried grains with solubles (sDDGS) in broiler chickens were investigated. A total of 480 day-old male broiler chickens were assessed in a 3 × 2 × 2 factorial arrangement of treatments (0, 150 or 300 g sDDGS/kg diet, with or without xylanase, and with or without protease) in a 21 d study. Each of the 12 treatments was replicated 5 times, accommodating 8 birds per replicate. Feed intake and body weight gain (BWG) of the birds were increased ($P < 0.001$) by inclusion of sDDGS to the diets independent of enzyme supplementation. Feed conversion ratio (FCR) deteriorated ($P < 0.001$) with the incorporation of sDDGS into the diets at both levels. Protease improved feed consumption ($P < 0.05$) and BWG ($P < 0.01$) of the birds when assessed for the 21 d study with an interaction with inclusion of sDDGS ($P < 0.05$). Regardless of sDDGS and protease, xylanase significantly improved ($P < 0.01$) FCR at any period of the trial. Digestibility of protein and most amino acids was adversely affected ($P < 0.001$) by the inclusion of 150 and 300 g sDDGS/kg diet. While protease individually improved ($P < 0.01$) methionine digestibility, independent of sDDGS inclusion, an admixture of xylanase and protease did not result in further improvement in amino acid digestibility. Addition of xylanase reduced ($P < 0.05$) the concentration of insoluble non-starch polysaccharides (NSP) in the ileum. Noticeably, the response of birds to xylanase supplementation on the concentrations of arabinose, xylose and total insoluble NSP was compromised when xylanase and protease were added to the diet simultaneously. To conclude, despite lack of significant synergy, supplementation of xylanase and protease to the diets containing sDDGS was beneficial for the FCR and BWG of the birds, respectively.

1. Introduction

Due to increased demand for biofuels, the use of grain resources for ethanol production has led to increased supply of distillers' dried grains with solubles (DDGS). As a result, this by-product of the ethanol production industry has drawn the attention of feed producers to be incorporated into poultry diets. So far, research into the use of DDGS has focused largely

Abbreviations: AA, amino acid(s); AID, apparent ileal digestibility; Ala, alanine; Arg, arginine; Asp, aspartic acid; BWG, body weight gain; CP, Crude protein; DDGS, distillers' dried grains with solubles; DM, dry matter; FCR, feed conversion ratio; FI, feed intake; GIT, gastrointestinal tract; Glu, glutamine; Gly, glycine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; NSP, non-starch polysaccharides; Phe, phenylalanine; Pro, proline; sDDGS, sorghum distillers' dried grains with solubles; Ser, serine; Thr, threonine; Ti, titanium; Tyr, tyrosine; TME, true metabolisable energy; Val, valine.

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on maize- or wheat-DDGS and less work has been done on DDGS of sorghum origin. The results of our previous experiment (Barekatain et al., 2013) revealed a lower protein digestibility when diets contained sorghum DDGS, which also contributed to the observed poor feed conversion in broilers receiving diets containing 300 g DDGS/kg. Similar observations were reported by Olukosi et al. (2010) when maize DDGS was incorporated in the diets of broiler chickens at 100 g/kg. It has also been widely documented that DDGS has a lower amino acid (AA) digestibility in poultry compared to the parent grain. The reason for this variation in AA digestibility is most likely due to the effect of the various processing and drying techniques involved in the production of DDGS, and inherent chemical composition of the material. Bandegan et al. (2009) found that all AA digestibility estimates for standardized and apparent coefficients of wheat DDGS were lower in broiler chickens compared to figures for wheat as a main grain with lysine (Lys) the least digestible amino acid. In another study, Kim et al. (2010) showed higher AA digestibility for maize germ than for both conventional and high-protein DDGS samples in broilers. This poor AA availability is further exacerbated when sorghum is used as the main grain owing to its low protein digestibility. A substantial portion of sorghum protein is composed of kafirin, which is known to negatively impact digestibility of AA due to resistance to proteolysis (Selle et al., 2010). Kafirin resistance to pepsin hydrolysis is further compounded by the lack of improvement in protein digestibility when sorghum is treated with moist heat or cooked (Oria et al., 1995). This considered, application of exogenous protease appears to be a logical approach to enhancing protein, and hence AA digestibility of DDGS.

In general, protease has been primarily used in combination with other enzymes to improve performance of broiler chickens (Marsman et al., 1995; Cowieson et al., 2006; Cowieson and Ravindran, 2008) although there are few reports on the application of protease in broiler diets containing DDGS. In the study conducted by Olukosi et al. (2010) a mixture of carbohydrases and protease was not effective in improving the performance of birds fed DDGS-containing diets although it improved the performance of the control birds, which were fed a low-density diet. Irrespective of the inclusion of DDGS, there are some contradictory reports with regard to the influence of protease on broiler performance. Recently, Kalmendal and Tauson (2012) observed an improvement in FCR in broilers fed diets supplemented with xylanase and protease individually or in combination with no sub-additive effect of the two enzymes. Using a protease as a sole treatment, live performance and crude protein (CP) digestibility in broilers were also restored (Angel et al., 2011). Ghazi et al. (2003) reported an improvement in nutritive value of soybean meal when protease was added to broiler diets. However, when protease and α-glucosidase were used simultaneously, the responses in terms of true metabolisable energy and nitrogen digestibility of the birds were compromised compared to individual application of either enzyme.

The high content of non-starch polysaccharides (NSP) remains the primary concern for using DDGS in broiler diets, regardless of the source grain. In this regard, arabinoxylans make up a substantial proportion of NSP content of DDGS (Choct and Petersen, 2009). In addition, for sorghum DDGS (sDDGS), there is clear evidence of poor protein digestion. Therefore, the present study was conducted to test the effect of protease and xylanase supplementation and the synergistic effect of both enzymes in diets containing different levels of DDGS for broiler chickens.

2. Materials and methods

2.1. Experimental design and diets

A $3 \times 2 \times 2$ factorial arrangement of treatments was employed in a completely randomized design to test the effect of varying levels of sDDGS, xylanase, protease and their interactions. All diets were formulated to meet the minimum requirements of Cobb 500 broiler chickens (Cobb, 2008). Three levels of dietary sDDGS were formulated at 0, 150 and 300 g/kg, partly replacing maize, soybean meal and soy-protein concentrate in the basal diet. The composition of the experimental diets is shown in Table 1.

The sorghum DDGS sample was analyzed prior to feed formulation and was found to contain (g/kg) 286.6 CP, 103 ether extract, 51.6 ash, 260 total NSP, 52.8 free sugars, 62 starch, 1.7 P, 7.2 Ca, 4.6 Lys and 4 methionine (Met). Protein and energy contents of the experimental diets were maintained at the same level. Two levels of xylanase supplementation, with or without xylanase, were created by adding Ronozyme WX (DSM Nutritional Products, Pty Ltd, Wagga Wagga, Australia) at 0 and 0.25 g/kg of diets. The Ronozyme WX is derived from *Thermomyces lanuginosus spp*. as an endoxylanase, hydrolyzing arabinoxylans and xylans in the feed with a minimum 1000 fungal xylanase unit (FXU)/g. Protease (Ronozyme ProAct, DSM Nutritional Products, Pty Ltd., Wagga Wagga, Australia) was also supplemented at 0 and 0.2 g/kg of experimental diets. The protease used was a commercial enzyme produced from fermentation of *Bacillus licheniformis* containing transcribed genes from *Nocardiopsis prasina* (Angel et al., 2011). The protease activity is defined in PROT units, with 1 unit as the amount of enzyme that releases 1 μmol of *p*-nitroaniline from 1 μM of substrate/min at pH 9.0 and 37 °C (Angel et al., 2011). To measure digestibility of nutrients, all experimental diets were supplemented with 5 g/kg of titanium dioxide (TiO_2) as an indigestible marker.

2.2. Housing and general management

A total of 480 male day-old Cobb 500 broiler chickens, vaccinated for Marek's disease and infectious bronchitis, were collected from a commercial hatchery. Sixty multi-tiered brooder cages (42 cm × 75 cm × 25 cm) with wire floor were used to house the birds in a climate-controlled system. The cages were randomly assigned to each of twelve treatments, each replicated five times, with 8 birds per replicate.