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Effect of enzyme preparation on egg production, nutrient retention, digestive enzyme activities and pancreatic enzyme messenger RNA expression of late-phase laying hens

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ABSTRACT

This experiment was conducted to study the effect of dietary inclusion of an enzyme preparation including phytase, xylanase, cellulase, α-amylase and acidic protease on egg production, nutrient retention, digestive enzyme activities and pancreatic enzyme mRNA expression. Three hundred and sixty ISA Brown laying hens were randomly allocated to two groups with 6 replicates of 30 birds each and fed a maize–soybean meal diet with or without enzyme supplementation (ES) from 60 to 68 wk of age. There were no differences in hen performance between the two groups. The coefficient of total tract apparent retention (CTTAR) of ether extract (EE) was increased ($P<0.05$) by ES. Birds fed the diet supplemented with enzymes tended to have higher lipase and protease activity in duodenal and jejunal digesta ($P<0.10$) than control birds. There were no significant effects of ES on pancreatic enzyme activities. The mRNA expression of amylase in pancreas tended to be downregulated by ES as compared with the control group ($P=0.097$), whereas that of pancreatic trypsinogen was not affected. It is concluded that ES did not affect productive performance of laying hens, but improved nutrient retention and tended to increase enzyme activities in the intestinal contents. The tendency for lower pancreatic amylase mRNA expression with ES, not coupled with a reduction in pancreatic enzyme activities suggests that amylase expression may be regulated not only at transcriptional level but also at post-transcriptional level.

1. Introduction

Maize and soybean meal are widely used in diets for pigs and poultry. The nutritive value of these ingredients depends partly on the amount of anti-nutritional factors such as non-starch polysaccharides (NSP) present (Kocher et al., 2003). Malathi and Devegowda (2001) reported up to 90 g/kg of NSP present in maize and 290 g/kg in soybean meal. The majority of phosphorus (P) present in plants is in the form of phytate, which is unavailable to non-ruminant animals. Carbohydrases (*i.e.* xylanase, cellulase, β-glucanase) and phytase are commonly included in the diet to degrade anti-nutritional factors, such as NSP and phytate, and to improve performance and nutrient digestibility. In addition, protease and α-amylase are often used to improve protein and starch digestion. Enzyme supplementation (ES) has been reported to improve egg production

Abbreviations: AMEn, nitrogen-corrected apparent metabolisable energy; BAEE, N-benzoyl-L-arginine ethyl ester; Ca, calcium; cDNA, complementary deoxyribonucleic acid; CTTAR, coefficient of total tract apparent retention; EE, ether extract; ES, enzyme supplementation; mRNA, messenger ribonucleic acid; NSP, non-starch polysaccharide; P, phosphorus.

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(Lázaro et al., 2003; Scheideler et al., 2005), egg weight (Um and Paik, 1999), feed conversion ratio (Mathlouthi et al., 2003; Yadav and Sah, 2006) and egg quality (Lim et al., 2003; Yadav and Sah, 2006). However, most of these studies evaluated the effects of enzyme preparation that contained a limited number of enzymes activity combinations. The use of an enzyme cocktail with several activities is likely to have greater effect on birds than when they are used separately (Olukosi et al., 2007). In addition, only a small number of such studies have determined nutrient retention and endogenous digestive enzyme activities in laying hens (Lázaro et al., 2003; Liebert et al., 2005; Kannan et al., 2008).

It has been recognised that the synthesis of pancreatic enzymes is regulated by the composition of the diet (Brannon, 1990). Some researchers have also found changes in pancreatic enzyme activities due to exogenous enzyme addition (Mahagna et al., 1995; Pinheiro et al., 2004). However, the interactions between exogenous and endogenous enzymes have not been fully elucidated. Ritz et al. (1995) showed that there was an additive effect of exogenous and endogenous amylase in male turkeys, and also suggested that exogenous amylase did not interfere with pancreatic amylase secretion because both enzymes are structurally different. However, previous research from our laboratory reported that exogenous amylase addition resulted in reduced pancreatic amylase activity as well as mRNA expression in young broilers (Jiang et al., 2008). Further research is needed to determine whether a feedback mechanism of exogenous enzyme action exists in terms of regulating pancreatic enzyme synthesis and secretion. Since there are differences between broilers and laying hens such as age, maturation of digestive organs and digestive capacity (Bedford, 2000), their physiological response to ES may differ. Therefore, the objective of this experiment was to examine the effect of exogenous enzymes on egg production, nutrient retention, digestive enzyme activities and pancreatic enzyme mRNA expression in laying hens in late-phase of production.

2. Materials and methods

2.1. Husbandry, diets and experimental design

All procedures were approved by Nanjing Agricultural University Institutional Animal Care and Use Committee.

Three hundred and sixty ISA Brown hens, 59 wk of age, were used in this experiment. After a one-week adaptation period to cages (40 cm × 40 cm × 35 cm), the birds were allocated to 2 groups with 6 replicates of 30 birds (10 adjacent cages, 3 birds per cage) each. A commercial maize–soybean meal diet (Table 1) with or without ES (1 g/kg diet) was used for an 8-wk study. The enzyme preparation was provided by VTR Bio-Tech Co., Ltd. (Zhuhai, Guangdong, China) and contained 1200 U phytase, 2000 U xylanase, 2000 U cellulase, 3000 U acidic protease and 300 U α-amylase per gram of product as determined by the manufacturer. One unit of phytase activity was defined as the amount of enzyme required to release 1 μmol of inorganic P per min from 5.0 mmol/L sodium phytate at pH 5.5 at 37 °C. One unit of xylanase activity was defined as the amount of enzyme required to release 1 μmol of xylose per min from 5.0 mg/mL xylan at pH 5.5 at 37 °C. One unit of cellulase activity was defined as the amount of enzyme required to release 1 μmol of glucose per h from 6.25 mg/mL sodium carboxymethylcellulose at pH 4.8 at 40 °C. One unit of acidic protease activity was defined as the amount of enzyme required to release 1 μg of tyrosine per min from 20 mg/mL casein at pH 3.5 at 40 °C. One unit of α-amylase activity was defined as the amount of enzyme required to dextrinise 1 mg of soluble starch per h at pH 6.0 at 60 °C. Birds were allowed *ad libitum* access to mash feed and water through the experiment and were exposed to a 16:8 light:dark cycle. Egg production, egg weight and mortality were recorded daily and feed consumption was recorded weekly per replicate. Egg mass and feed conversion ratio were calculated.

At the end of the experimental period, one hen per replicate was randomly selected and allocated into individual cages. The birds were fed their respective experimental diet for a 3-d period followed by a 72-h complete excreta collection. Excreta were collected twice per day from a plastic tray placed under the cages, stored at −20 °C, and finally pooled for each cage. The excreta samples were then dried for 48 h in an oven at 65 °C. The dried excreta were allowed to equilibrate to atmospheric conditions for 24 h before being weighed. Feed and excreta samples were then ground through a 0.45-mm screen. The samples were analysed (AOAC, 1990) for dry matter (DM, 934.01), ether extract after HCl treatment (EE, 920.39), calcium (Ca, 968.08) and P (965.17). Gross energy was determined using an adiabatic calorimeter (SXHW-III, Tianyu, Hebi, China). The coefficient of total tract apparent retention (CTTAR) of nutrients and the nitrogen-corrected apparent metabolisable energy (AMEn) were calculated according to Adeola et al. (2008).

2.2. Enzyme activity assay

At the end of the experiment, six extra hens from each group (one hen per replicate) were randomly selected and killed by cervical dislocation. Pancreas was quickly excised, frozen in liquid nitrogen and cut into two segments, with the proximal portion stored at −20 °C for enzyme activity assay and the distal stored at −70 °C for mRNA assay. The duodenal and jejunal digesta were collected separately, and stored at −20 °C until analysis.

After thawing at room temperature, the pancreas and digesta samples were homogenised (1:9, wt/vol) with ice-cold 154 mmol/L sodium chloride solution, and then centrifuged at 4550 × g for 15 min at 4 °C. Aliquots of the supernatant were collected for enzyme activity assay. All determinations were carried out in duplicate. Amylase activity was determined using the iodometric method of Somogyi (1960) and one amylase unit is defined as the amount of enzyme that hydrolyses 10 mg of starch in 30 min. Lipase activity was determined using the turbidimetric method of Verduin et al. (1973) and one lipase unit is defined as the amount of enzyme that hydrolyses 1 μmol of olive oil per min. Enterokinase was added to homogenate