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Effect of probiotic on growth performance and digestive enzyme activity of Arbor Acres broilers

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ABSTRACT

The effect of probiotic, *Bacillus coagulans* NJ0516, on growth performance and digestive enzyme activity of Arbor Acres (AA) broilers was investigated. Strains NJ0516 were added to commercial basal diets as probiotic at three final concentrations: T-1, 1×10^6 cfu g^{-1}; T-2, 2×10^6 cfu g^{-1} and T-3, 4×10^6 cfu g^{-1}, respectively. Twelve groups, of 30 broilers, with three replicates for each treatment group (T-1, T-2 and T-3) and the control group treated without probiotic were used. After 49 days, broilers receiving the diets supplemented with probiotic showed significantly better growth performances including final weight and daily weight gain (DWG) than those fed the basal diet (control). As for feed conversion ratio (FCR), T-2 and T-3 showed lower value ($P < 0.05$) than the control. However, there was no significant different in final weight, DWG and FCR between T-1, T-2 and T-3 and the survival rate was not affected ($P > 0.05$) by the dietary treatments. The higher protease activities were observed in T-2 and T-3 ($P < 0.05$) compared with the control and T-1. However, there was not significantly different ($P > 0.05$) between T-2 and T-3 in protease activity. Amylase activity in T-1, T-2 and T-3 was remarkably higher ($P < 0.05$) than that in the control. Significantly higher amylase activity was observed in T-2 compared with that of T-1. There was no remarkable difference ($P > 0.05$) in amylase activity of T-2 compared with that of T-3, even though there was a tendency for increased activity. As for lipase activity of duodenum in broilers, assays showed no difference in all treatment groups. It showed that probiotic, *B. coagulans* NJ0516 administration in feed with a certain concentration displayed a growth promoting effect and increased the protease and amylase activities.

1. Introduction

There is a worldwide attempt to reduce antibiotic use in animal production because increased microbial resistance to antibiotics and residues in animal products can be harmful to consumers (Jin et al., 1998). Therefore, the need for alternative techniques for poultry production is increasing and the contribution of probiotics may be considerable (Patterson and Burkholder, 2003). Probiotic, which means "for life" in Greek (Gibson and Fuller, 2000), has been defined as "a live microbial feed supplement which beneficially affects the host animal by improving its intestinal balance" (Fuller, 1989). Therefore, several terms such as "friendly", "beneficial", or "healthy" bacteria are also commonly used to describe probiotics. In the poultry industry, probiotic supplementation has been shown to improve daily weight gain, feed conversion ratio and mortality rate in broiler chickens (Mohan et al., 1996; Huang et al., 2004; Schocken-Iturrino et al., 2004; Mountzouris et al., 2007). Nevertheless, contradictory results have been reported by other researchers (Senanl et al., 1997; Panda et al., 1999; Willis and Reid, 2008). The strain of selected microorganism, the dosage, method of preparation, and condition of animals could be partially responsible for such discrepancies.

Probiotics used in animals such as broilers include *Lactobacillus*, *Bifidobacterium*, *Bacillus*, *Streptococcus*, *Pediococcus*, *Enterococcus* and yeast such as *Saccharomyces cerevisiae* and *Saccharomyces boulardii* (Fuller, 1992; Patterson and Burkholder, 2003; Kabir et al., 2004; Mountzouris et al., 2007). Recently, emphasis has been placed on the selection, preparation and application of probiotic strains especially lactic acid bacteria (LAB). The natural adaptation of LAB to the gut environment and the lactic acid produced by them has provided these organisms with an advantage over other microorganisms to be used as probiotics (Guerra et al., 2007). Most works have also focused on *Bacillus* (Kumprecht and Zobac, 1996; Casula and Cutting, 2002). The spores of *Bacillus* spp. are especially easy to introduce in dry feed, and this is an additional advantage of these promising candidate probiotics (Ragione et al., 2001). Few studies have been carried out on spore forming lactic acid producing bacteria such as *Bacillus coagulans* (*B. coagulans*) as probiotics in broilers. The strains of *B. coagulans* share characteristics common

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to the genera *Bacillus* (spore forming, motile) and *Lactobacillus* (micro-aerophilic, lactic acid production) (Hyronimus et al., 2000). Many factors make *B. coagulans* good candidates for a probiotic use: (1) they are easily cultured in "bulk", (2) they produce organic acids and (3) they possess the capacity to sporulate (Hyronimus et al., 2000). Therefore, this study attempted to investigate the effect of probiotic, *B. coagulans*, on growth performance and digestive enzyme activity of the Arbor Acres (AA) broiler, which was one of the most valuable chicken species farmed in the world. For the digestive enzyme parameter assays, the protease activity, amylase activity and lipase activity were used as indicators.

2. Materials and methods

2.1. Bacteria

The microorganism employed in this study was *B. coagulans* NJ0516 obtained from the floors soil of buildings housing organically reared AA broilers in Poultry Field Hatchery of Huzhou, Zhejiang province. Care was taken to avoid gross contamination with environmental material. The probiotic strain, *B. coagulans* NJ0516 were cultured and counted on normal nutrient agar by spore staining with the spread plate technique (Marshall and Beers, 1967). For taxonomic identification, the Gram staining test, catalase tests and other physiological and biochemical tests were made. Cell size, motility and morphology were determined microscopically (Leica Standard DMR, 1000×). Identification of the isolate was achieved by comparing the results of taxonomic tests with the properties in the manual of Dong and Cai (2001). The strains were cultured in the laboratory and checked routinely for purity based on their morphological and biochemical characteristics during this investigation. Stock culture of the probiotics was stored at −70 °C (Forma 702, Thermo, USA) in powdered skimmed milk suspension with 25% glycerol prior to use.

2.2. Experimental design

Healthy 1 day-old AA broiler chickens were obtained from Poultry Field Hatchery of Huzhou and were reared in an environmentally controlled isolation facility for 49 days from October to December. Ambient temperature was gradually decreased from 35 °C at first day to 24 °C after 3 week of age (rate of decrease: 3 °C during the first week, 4 °C during the second and the third weeks). Relative humidity was maintained at 50 ± 5%. Three hundred and sixty male and female feather sexed (180 male and 180 female) broilers were randomly segregated into 12 groups of 30 broilers so that three replicates were available for each of the three treatments and the control groups. All broiler chickens had similar initial weights (39.3 ± 0.8 g). The commercial basal diets used in this experiment were according to the National Research Council (1994) and obtained from Yixing Co. in Haiyan, China. A complete starter diet with 21.8% crude protein (CP) and 3034 kcal/kg of metabolic energy (ME) from 0 to 3 week, and a grower diet with 19.6% CP and 3105 kcal/kg of ME from 4 to 7 week were used.

The strains of *B. coagulans* NJ0516 were grown in culture medium (normal nutrient broth, Difco, Shanghai, China) in a shaking incubator at 37 °C for 24 h. After incubation, the cells were harvested by centrifugation (2000*g*), washed three times with PBS (Phosphate Buffered Saline, pH 7.2, Sangon, China), and re-suspended in the same buffer. The above commercial basal diet was used for the supplementation of probiotic strains. The control groups were fed unsupplemented commercial basal diets during the entire trial period. Treatment 1 (T-1), treatment 2 (T-2) and treatment 3 (T-3) were fed with diets containing different concentration viable *B. coagulans* NJ0516 (with a final concentration 1×10^6 cfu g^{-1}, 2×10^6 cfu g^{-1} and 4×10^6 cfu g^{-1}, respectively). Every day, the probiotic strains with a certain concentration were added to the diets according to the above final concentration, respectively. In order to reach these final concentrations, strains were slowly applied into the diets, mixing part by part in a drum mixer. The amount of probiotic strains (cfu g^{-1} diet) in each diet was determined and modified by plate counting on medium agar. Finally, the diets supplemented with different concentrations of probiotic strains were distributed to the corresponding broilers groups.

This feeding trial process was acceptable to the commercial producer. The experimental broilers were provided continuous lighting from incandescent lamps in the ceilings of each room, but each brood-grow battery provided an area of subdued light for sleeping and resting. Fresh water and diets were provided *ad libitum*. The feeding trial was conducted under the supervision of the Animal Care and Use Committee of the University. Records of feed consumed in each pen were kept and birds were weighted individually at 49 days of age so that feed conversion ratio (FCR) and average body weights could be calculated. Mortality was recorded on a per-pen basis as it occurred, and the mortality rate was calculated in each group as the number of dead divided by the total number of birds at the beginning of the experiment.

2.3. Sampling and analytical methods

Weight of all collected broilers determined at 1 day-old and 49 day-old were treated as initial weight and final weight, respectively. The daily weight gain (g d^{-1}) (DWG) was calculated as: (final weight − initial weight)/49 (g d^{-1}). The FCR used the following formula: total feed consumption/(total final weight − total initial weight + total mortality weight).

For enzymatic analysis, 10 randomly selected broilers (5 male and 5 female) were collected from each group at the end of the experiment and slaughtered by severing the jugular vein in order to study the effect of *B. coagulans* NJ0516 with different concentrations on digestive enzyme activities of AA broilers. The birds were then immediately eviscerated for collection of duodenum samples. Dissection produced a crude mixture of whole duodenum by operating on ice following the method of Jin et al. (2000). A homogenous duodenum digesta sample was collected by massaging the tract from both ends. The digesta samples were stored immediately at −70 °C (Forma 702, Thermo, USA) until used. The broilers duodenum digesta samples were diluted 10×, based on the sample weight, with icecold phosphate buffered saline (PBS, pH 7.0), homogenized using a hand held glass homogenizer, respectively. The homogenate was then centrifuged at 18,000*g* for 20 min at 4 °C. The supernatants were divided into small portions and stored at 4 °C prior to analysis. All enzymatic assays were conducted within 24 h after extraction.

Protein concentration was evaluated according to Bradford (1976) using bovine serum albumin as a standard protein. Protease activity was evaluated according to Lowry et al. (1951) using Folin-phenol reagent and amylase activity was measured by dinitrosalicylic acid according to Bernfeld (1951). Lipase activity was determined based on measurement of fatty acids release due to enzymatic hydrolysis of triglycerides in stabilized emulsion of olive oil using commercial kits (Nanjing Jiancheng Bioengineering Institute, China) (Jin, 1995). Enzyme activities including protease and amylase were both expressed as specific activity (U g^{-1} protein) and lipase activity was expressed as U mg^{-1} protein.

2.4. Statistic analysis

Analysis of variance (ANOVA) and Student's *t*-test were used to determine the significant ($P < 0.05$) difference between the tested