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Short communication

Effect of dietary supplementation of bacteriophage on growth performance and cecal bacterial populations in broiler chickens raised in different housing systems

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ABSTRACT

This experiment was conducted to investigate the effect of dietary bacteriophage (BP) on growth performance and cecal bacterial populations in broiler chickens raised in different housing systems. A total of 1170 1-d-old broiler chickens were housed in either battery cages (120 birds) or conventional floor pens (1050 birds). Within each housing system, birds were randomly allotted to 1 of 3 dietary treatments with 5 replicates. Dietary treatments included basal diets (negative control; NC), basal diets with 0.025 g/kg avilamycin (positive control; PC), and basal diets with 0.5 g/kg BP mixture (BP5). The mixture of the individual BP targeting at *Salmonella gallinarum*, *Salmonella typhimurium*, *Salmonella enteritidis*, *Salmonella derby*, *Staphylococcus aureus*, and *Clostridium perfringens* was used in this experiment. Diets were fed to birds for d 35. The effects of housing systems, dietary treatments, and their interactions were analyzed. No interactions for all measurements were observed, and thus, the main effects were presented. During overall experiment, birds raised in battery cages had greater ($P < 0.01$) BW gain (BWG), feed intake, and less ($P < 0.01$) feed conversion ratio (FCR) than those raised in floor pens. Greater BWG was observed ($P < 0.05$) for PC treatment than for NC treatment, but those for BP5 treatment had intermediate values between other treatment groups. The FCR was less ($P < 0.05$) for PC and BP5 treatment groups than for NC treatment, but there was no difference between PC treatment and BP5 treatment. For cecal bacterial populations, birds raised in battery cages had less ($P < 0.05$) DNA copy numbers for *C. perfringens*, but greater ($P < 0.05$) DNA copy numbers for *Escherichia coli* than those raised in floor pens. The BP5 treatment had less ($P < 0.05$) DNA copy numbers for *C. perfringens* compared with NC treatment. In conclusion, dietary BP improves growth performance of broiler chickens and decreases targeted pathogenic bacteria populations, especially for *C. perfringens* in the gastrointestinal tract. This positive effect is likely independent of housing systems.

1. Introduction

Dietary antibiotic growth promoters (AGPs) have been widely used in the poultry industry as an effective means to improve growth performance and treat bacterial diseases (Baurhoo et al., 2009). However, recently several side-effects of AGPs such as the presence of antibiotic-resistant pathogens and antibiotic residues in poultry products have been reported (Nakphaichit et al., 2011; Nisha, 2008). Consequently, there has been a worldwide increase in the regulation or ban of the use of AGPs in poultry diets. This phenomenon currently forces poultry

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nutritionists to search for new alternatives to AGPs. Several potential alternatives such as probiotics, prebiotics, and acidifiers have been developed and used in poultry diets (Fuller, 1989; Ganguly, 2013; Patterson and Burkholder, 2003), but their efficacy has been inconsistent as compared to dietary AGPs.

The bacteriophage (BP) or phage is an infectious virus that kills bacteria by multiplying within their cells and subsequently destroying the host bacteria (Monk et al., 2010). Implication of BP has been based on its specificity targeting at particular species or strains of pathogenic bacteria (Monk et al., 2010). There has been increasing evidence to suggest that the applications of a single or mixture of specific BP by aerosol spray, muscle injection, or oral gavage to chickens challenged with specific pathogens ameliorate clinical symptoms of infection and decrease mortality (Johnson et al., 2008). However, limited data pertaining to the effects of dietary supplementation of BP on growth performance and health of broiler chickens have been available although dietary application may be one of the most practical methods.

Previous experiments investigating the efficacy of possible alternatives to AGPs for broiler chickens have been conducted in either battery cages or floor pens. It can be speculated that the different housing systems may influence the efficacy of the alternatives to AGPs because of the differences in the extent of bacterial load, pathogenic challenge, and environmental stress between housing systems. Therefore, it can be hypothesized that the efficacy of dietary BP for broiler chickens may differ between 2 different housing systems (i.e., battery cages vs. conventional floor pens), but this hypothesis has not been tested previously.

The objective of the current experiment, therefore, was to investigate the effect of dietary supplementation of BP on growth performance and cecal bacterial populations in broiler chickens raised in different housing systems.

2. Materials and methods

2.1. Birds, diets, and experimental design

The experiment was performed using a completely randomized design with 2 × 3 factorial arrangements of 2 housing systems and 3 dietary treatments. A total of 1170 1-d-old Ross 308 broiler chickens (initial BW = 42.7 ± 0.41 g) were obtained from a local hatchery (Yangji hatchery, Pyeongtaek, Republic of Korea) and were housed in either conventional floor pens (1050 birds; 200 cm × 230 cm × 100 cm = width × length × height for each pen) or battery cages (120 birds; 76 cm × 78 cm × 45 cm = width × length × height for each cage) in an environmentally controlled room. Higher stock density for birds raised in floor pens (0.066 m^2/bird) than for those raised in battery cages (0.074 m^2/bird) was set to more closely simulate a commercial situation of raising broiler chickens.

Within each housing system, all chicks were randomly allotted to 1 of 3 dietary treatments with 5 replicates. Each replicated pen consisted of 70 and 8 chicks for conventional floor pens and battery cages, respectively. A 2-phase feeding program with a starter diet from d 0 to 21 and a grower diet from d 22 to 35 was used in this experiment. Within each phage, a basal diet as a negative control (NC) was prepared mainly with corn, soybean meal, wheat, and corn gluten meal. The energy and nutrient concentrations of the basal diets were formulated to meet or exceed the current recommendations of NRC (1994) for broiler chickens of each phase. Two additional diets were prepared by adding 0.025 g/kg avilamycin as a positive control (PC) or 0.5 g/kg BP mixture to the basal diet (BP5) at the expense of corn. The BP (CTC Bio Inc., Seoul, Republic of Korea) used in this experiment was a mixture of individual BP targeting specifically at *Salmonella gallinarum*, *Salmonella typhimurium*, *Salmonella enteritidis*, *Salmonella derby*, *Staphylococcus aureus*, and *Clostridium perfringens*. The concentrations of individual BP in the mixture were 10^8 pfu per g for *S. gallinarum*, *S. typhimurium*, *S. enteritidis*, *S. derby*, and *S. aureus*, whereas those of BP targeting at *Clostridium perfringens* were 10^6 pfu per g. The experimental diets were in mash form. The diets and water were provided ad libitum for d 35. The room temperature for both conventional floor pens and battery cages was maintained at 30 °C during the first wk of the experiment and then gradually decreased to 24 °C at the end of the experiment as recommended by Ross manual. A 24-h lighting schedule was used throughout the experiment. The BW gain (BWG) and feed intake (FI) were recorded at d 21 and 35 of the experiment. Feed conversion ratio (FCR) was calculated by dividing BWG (g) by FI (g). The protocol for this experiment was reviewed and approved by the Institutional Animal Care and Use Committee at Chung-Ang University.

2.2. Sample collection and bacterial population analyses

At the conclusion of the experiment, 2 birds per replicate with a BW close to the replicate mean BW (i.e., 10 birds per treatment) were euthanized by cervical dislocation. The cecal contents were collected from the euthanized chickens and used for analyzing bacterial populations. The cecum was ligated at both sides and removed from the gastrointestinal tract, and the contents were aseptically collected into a 2-mL Eppendorf tube. The cecal contents were immediately frozen at −80 °C before analysis. The bacterial populations were analyzed with individual chicken by the quantitative PCR (qPCR) method as demonstrated by Castillo et al. (2006). The data were expressed as $\log_{10}$ DNA copy numbers for analyzed bacteria per g of cecal samples.

2.3. Statistical analysis

All data were analyzed by 2-way ANOVA according to a completely randomized design using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC) with the replicate as the experimental unit. Outlier data were checked using the UNIVARIATE procedure of SAS (Steel et al., 1997), but no outliers were identified. The statistical model used was

$$Y_{ijk} = \mu + H_i + B_j + \mathrm{HB}_{ij} + e_{ijk}$$

where Y_{ijk} is the individual observation, μ is the overall mean, H_i is the effect of housing system, B_j is the effect of dietary BP, HB_{ij} is the effect of interaction, and e_{ijk} is the random error. An alpha level of 0.05 was used to determine statistical significance. When the model was significant, the Tukey's test was performed to make pairwise