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Original research article

Novel probiotics: Their effects on growth performance, gut development, microbial community and activity of broiler chickens

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ABSTRACT

A total of 294 one-day-old Cobb broiler chickens were used to investigate the effects of four *Lactobacillus* strains on gut microbial profile and production performance. The six dietary treatments, each with 7 replicates were: 1) basal diet (negative control), 2) one of four strains of *Lactobacillus* (tentatively identified as *Lactobacillus johnsonii, Lactobacillus crispatus*, *Lactobacillus salivarius* and an unidentified *Lactobacillus* sp.) and 3) basal diet with added zinc-bacitracin (ZnB, 50 mg/kg). Results showed that the addition of probiotic *Lactobacillus* spp. to the feed did not significantly improve weight gain, feed intake and feed conversion rate (FCR) of broiler chickens raised in cages during the 6-week experimental period, but tended to increase the number of total anaerobic bacteria in the ileum and caeca, and the number of lactic acid bacteria and lactobacilli in the caeca; and to significantly increase the small intestinal weight (jejunum and ileum). Furthermore, all 4 probiotics tended to reduce the number of *Enterobacteria* in the ileum, compared with the control treatments. The probiotics did not affect the pH and the concentrations of short chain fatty acids (SCFA) and lactic acid in both the ileum and caeca.

1. Introduction

The use of probiotics has become a field of science, medicine and business that is growing rapidly. In agricultural science, probiotic, prebiotics, feed enzymes and organic acids, have been seen as potential alternatives to in-feed antibiotics (IFA) (Choct, 2002).

The addition of either pure *Lactobacillus* cultures or mixtures of lactobacilli and other bacteria to broiler diets has produced variable results. Kalavathy et al. (2003) found an improvement in body weight gain (BWG) and feed conversion ratio (FCR) of broilers fed a mixture of different *Lactobacillus* strains from 1 to 42 days of age. A consistent improvement in BWG of chickens fed a culture of *Lactobacillus* has also been reported (Awad et al., 2009). Feeding broiler chickens up to 6 weeks of age with a diet containing a single strain of *Lactobacillus acidophilus* or a mixture of lactobacilli significantly improved BWG and FCR (Jin et al., 1998a). Cao et al. (2013) found that supplementation the broiler diets with a single strain of *Lactobacillus* (*Enterococcus faecium*) significantly improved the BW and BWG compared to the control. However, Ashayerizadeh et al. (2011) did not find any significant difference in the performance of chickens fed on diets containing a mixture of *Lactobacillus* cultures and other bacteria, compared with a non-supplemented diet. Variation in the effects of probiotics on growth performance of broiler chickens may be attributed to the differences in the strains of bacteria used as the dietary supplements.

In the present study, the effects of four strains of *Lactobacillus* spp. on pH, the concentrations of short chain fatty acids (SCFA) and lactic acid, and growth performance of broiler chickens were investigated; the populations of total anaerobic bacteria, lactic acid bacteria, *Lactobacilli*, *Enterobacteria* and *Clostridium perfringens* in gut environment were detected.

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2. Materials and methods

2.1. Probiotic strains

A total of 235 *Lactobacillus* isolates were tested using an antagonistic activity assay as described by Schillinger and Lucke (1989), Teo and Tan (2005), and the four strains of *Lactobacillus* isolates were selected as probiotic candidates by largest inhibition zone appearance with indicator pathogenic strains of *C. perfringens* and *Escherichia coli*. These four strains of *Lactobacillus* were tentatively identified as *Lactobacillus johnsonii, Lactobacillus crispatus*, *Lactobacillus salivarius* and one unidentified *Lactobacillus* sp.

All the strains were kept at −20°C in de Man, Rogosa, Sharpe (MRS) broth (Oxoid, CM0359) with 40% glycerol. The culture medium used for growth was MRS agar (Oxoid, CM0361). The overnight culture of each *Lactobacillus* isolate was used as a feed additive probiotic candidate after anaerobic incubation at 39°C for 24 h.

2.2. Experimental design and bird management

A total of 294 one-day-old male Cobb broiler chickens vaccinated against Marek's disease, infectious bronchitis, and Newcastle disease were randomly assigned to 6 diets each with 7 replicates with 7 birds per replicate. Chickens were reared in multi-tiered brooder cages placed in a climate-controlled room up to 21 d, and then the birds were transferred to a metabolic cage room to 35 d. Feed and water were provided ad libitum. The room temperature was gradually decreased from 33°C on d 1 to 24°C on d 35. Eighteen hours of light was provided per day throughout the trial, excluding d 1 to 7 during which 23 h of light was provided. Each cage was equipped with a feeding and water trough placed outside and also an excreta collection tray. The commercial starter and finisher diets was formulated by Ridley AgriProducts (Tamworth, NSW, Australia) as shown in (Table 1) and fed as a one-phase mash feed to avoid inactivation of the probiotics. Four strains of *Lactobacillus* (No. 1286 tentatively identified as *L. johnsonii*, No. 709 tentatively identified as *L. crispatus*, No. 697 tentatively identified as *L. salivarius* and No. 461 unidentified *Lactobacillus* sp.) were selected as probiotic candidates and added to the feed to make up four different treatments. Two control treatments were also included, a negative control, with no additives and a positive control treatment with the antibiotic, zinc-bacitracin (ZnB, 50 mg/kg), added. The experimental diets with the probiotic candidates were mixed weekly. The individual strains were grown in MRS broth contained 5 g/L of yeast extract (powder, Oxoid, LP0021) and 20 g/L of glucose, for overnight (at 39°C) and harvested by centrifugation at 4,420 × *g* for 15 min (Induction Drive Centrifugation, Beckman Model J2-21M, Beckman Instruments Inc., Palo Alto, California, USA), resuspended in phosphate buffered saline (PBS, pH 7.4) and mixed into a premix with the basal diet for 10 min using a miniature mixer. This pre-mixture of product with feed (1 kg) was then transferred into a larger mixer (total capacity 300 kg) where the final volume of the weekly feed batch was prepared. The mixer equipment was thoroughly cleaned between the mixing of different treatments by using a vacuum cleaner and a wash diet (basal feed).

2.3. Probiotic bacterial concentrations in feed samples

Representative feed samples of each feed batch were tested for bacterial concentrations on d 1, 3, and 7 of each week during the experimental period. Ten grams of sample feed were dissolved in 90 mL of peptone water (Oxoid, CM0009) and 10-fold dilutions were performed in Hungate tubes with 9 mL of peptone water. The numbers of lactic acid bacteria in the feed samples were determined on de MRS agar inoculated with 0.1 mL of diluted sample and after anaerobic incubation at 39°C for 48 h.

Table 1
Ingredient composition and calculated chemical composition of basal diets (as-fed basis).

Item	1 to 3 weeks (Starter)	4 to 6 weeks (Finisher)
Ingredient, g/kg		
Wheat	262.0	214.0
Sorghum	350.25	400.2
Mung beans	100.0	100.0
Tallow in mixer	32.5	34.0
Sunflower meal		25.0
Canola meal	60.0	60.0
Cottonseed meal		50.0
Soybean meal	157.0	81.5
Limestone B10	15.5	16.0
Kynofos/Biofos MDCP	11.5	11.0
Salt	1.75	1.5
Sodium bicarbonate	2.0	2.0
Choline Chloride (75%)	0.6	0.6
DL-Methionine	2.1	1.3
L-Lysine scale 3	2.1	0.4
L-Threonine	0.2	
Vitamin and mineral premix[1]	2.5	2.5
Calculated chemical composition, g/kg		
ME, MJ/Kg	12.26	12.39
Crude protein	200.02	190.00
Crude fibre	35.17	43.14
Crude fat	52.16	54.47
Lys	11.49	8.98
Met + Cys	8.32	7.37
Ca	9.73	9.79
Available phosphorous	6.50	6.71
Na	1.62	1.65
Cl	2.19	1.75

[1] Vitamin and mineral premix contained the following: vitamin A (as *all-trans* retinol), 12,000 IU; cholecalciferol, 3,500 IU; vitamin E (as D-α-tocopherol), 44.7 IU; vitamin B_{12}, 0.2 mg; biotin, 0.1 mg; niacin, 50 mg; vitamin K_3, 2 mg; pantothenic acid, 12 mg; folic acid, 2 mg; thiamine, 2 mg; riboflavin, 6 mg; pyridoxine hydrochloride, 5 mg; D-calcium pantothenate, 12 mg; Mn, 80 mg; Fe, 60 mg; Cu, 8 mg; I, 1 mg; Co, 0.3 mg; and Mo, 1 mg.

Representative samples from all experimental feeds were tested as above for bacterial concentrations before being added to the probiotic candidates to make up six different treatments.

2.4. Sample collection and processing

Feed leftovers and birds were weighed on a weekly basis for calculation of average feed intake and body weight. Mortality was recorded when it occurred and FCR (feed intake/weight gain) was corrected for mortality. On d 21 and 35, two birds from each cage were randomly selected and killed by cervical dislocation. The abdominal cavity was opened and visceral organs were weighed. The weight and the length of the full small intestine and then the empty weight of each intestinal segment were recorded.

The contents of the gizzard were collected into plastic containers. An approximately 2 cm piece of the proximal ileum was flushed with ice-cold PBS at pH 7.4 and fixed in 10% formalin for morphological measurements. The contents of the ileum and caeca were collected, and then stored at −20°C until volatile fatty acids (VFA) analysis was performed.

2.5. Enumeration of intestinal bacteria

About 1 g of fresh digesta samples from the ileum and caeca were transferred into 15 mL MacCartney bottles containing 10 mL of anaerobic broth. The suspension was homogenized for 2 min in CO_2-flushed plastic bags using a bag mixer (Interscience, St. Norm, France) and serially diluted in 10-fold increments in anaerobic broth according to the technique of Miller and Wolin (1974). One millilitre of the homogenized suspension was then transferred into 9 mL of