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Original research article

Delivery routes for probiotics: Effects on broiler performance, intestinal morphology and gut microflora

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ABSTRACT

Four delivery routes, via, feed, water, litter and oral gavage, were examined for their efficacy in delivering a novel probiotic of poultry origin, *Lactobacillus johnsonii*, to broilers. Seven treatments of 6 replicates each were allocated using 336 one-day-old Cobb broiler chicks. The treatments consisted of a basal diet with the probiotic candidate, *L. johnsonii*, added to the feed, and three treatments with *L. johnsonii* added to the drinking water, sprayed on the litter, or gavaged orally. In addition, a positive control treatment received the basal diet supplemented with zinc-bacitracin (ZnB, 50 mg/kg). The probiotic strain of *L. johnsonii* was detected in the ileum of the chicks for all four delivery routes. However, the addition of *L. johnsonii* as a probiotic candidate did not improve body weight gain, feed intake and feed conversion ratio of broiler chickens raised on litter during the 5-week experimental period regardless of the route of administration. The probiotic treatments, regardless of the routes of delivery, affected ($P < 0.05$) the pH of the caecal digesta and tended ($P = 0.06$) to affect the pH of the ileal digesta on d 7, but the effect disappeared as the birds grew older. All probiotic treatments reduced the number of *Enterobacteria* in the caeca on d 21, and tended ($P < 0.054$) to reduce it in the ileum and caeca on d 7 and in the ileum on d 21 compared with the controls. The probiotic also tended to increase the number of lactic acid bacteria and lactobacilli in the ileum and caeca on d 7, but this trend was not evident on d 21. The trend appeared most pronounced when the probiotic was delivered orally or via litter. The probiotic also decreased ($P < 0.05$) the population of *Clostridium perfringens* rapidly from an early age to d 21 in the caeca, leading to a 3-fold decrease in the number of *C. perfringens* between d 7 and 21. It also showed that the probiotic treatment presented the lowest number of *C. perfringens* in the caeca. Delivery of the probiotic through feed, water and litter increased ($P < 0.01$) the weight of the pancreas on d 21, but the probiotic did not affect other morphometric parameters of the gut. Furthermore, the probiotic did not affect the pH and the concentrations of short chain fatty acids and lactic acid in either the ileum or caeca.

1. Introduction

Probiotics display numerous health benefits beyond providing basic nutritional advantages. Probiotic products consisting of beneficial microflora can help to establish and maintain the balance of the intestinal microflora in commercial broilers. However, selecting a probiotic microorganism that has beneficial effects in broiler chickens requires an extensive search for the optimum candidate, and one which will perform under practical conditions. Inoculating one-day-old chicks with competitive exclusion (CE) cultures or more classical probiotics serves as an effective model for determining the modes of action and efficacy of these microorganisms. Because of the susceptibility of one-day-old chicks to infection, this practice is also of commercial importance. By using this model, a number of probiotics have been shown to reduce colonization and shedding of *Salmonella* and *Campylobacter* (Netherwood et al., 1999; Fritts et al., 2000). However, one of the

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key factors determining their efficacy in practical use is stability during storage, delivery and feed processing.

There are many different methods for administering probiotic preparations to broiler chickens: through feed, water, gavage (including droplet or inoculations), spray or litter, but adding to feed is the most commonly used method in poultry production.

Introducing probiotics through drinking water, into the crop by tube and syringe, with crumbles, or by spraying on bird environment and litter had no effects on the survival rate of bacteria (Gardiner et al., 2000; Morelli, 2000; Corcoran et al., 2004). The feed-type probiotic products rarely produce optimum results in pelletized diets usually fed to broilers (Nguyen et al., 1988; Scheuerman, 1993). Kozasa (1986) found that two probiotic bacteria incorporated into crumbles, successfully survived the duration of the experiment. Also, Gould and Hurst (1969) reported that spores of bacillus are well known for being able to survive high temperatures. Thus, the best natural solution to the challenge of stability in direct-fed microbial products is to use spore-forming beneficial strains of microbes or fed as crumbles (Crawford, 1979). However, Seuna et al. (1978) showed that the viability of the organisms rapidly declined, especially in chlorinated water when bacteria via the drinking water rather than gavage compared.

The literature suggests that spray application of probiotic cultures, either on the environment of the birds or on the litter material seems to be an effective way of administering probiotic cultures (Blankenship, 1992), whilst according to Nurmi and Rantala (1973) intubation into the crop is perhaps the most satisfactory method for delivering a precise dose of probiotics to the animal.

The aim of this study was to determine the efficacy of administering a probiotic strain of *Lactobacillus johnsonii* which chosen by antimicrobial activities showed the best resistant in promoting growth performance, intestinal morphology and gut microflora in broiler chickens.

2. Materials and methods

2.1. Probiotic strains

The bacterial strain used in this experiment was selected using the antagonistic activity assay described by Teo and Tan (2005).

A pure *L. Johnsonii* isolate was grown in MRS broth 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). It was re-suspended in phosphate-buffered solution (PBS, pH 7.4) and mixed by constant mechanical stirring (Heidolph MR 3001K stirrer, Heidolph Instruments GmbH & Co., Schwabach, Germany) for 10 min. This premixture of PBS probiotic solution was added to feed, drinking water, or was gavaged orally. The quantities of MRS broth and premix phosphate-buffered solution (PBS, solution used were calculated by determining the bacterial concentration needed for the experiment. In this study, the concentration of the probiotic candidate, *L. johnsonii*, supplied via different routes was: feed delivery $> 10^6$ cfu/gram of feed samples; oral delivery $> 10^8$ cfu/mL of BPS solution; litter delivery $> 10^8$ cfu/mL of PBS spray solution and water delivery $> 10^6$ cfu/mL of water sample.

Representative feed, water, and litter samples of each treatment batch were tested for bacterial concentrations weekly on d 1 and 7. Ten grams (or millilitres) of samples were dissolved in 90 mL of peptone water (Oxoid, CM0009) and 10-fold dilutions were performed in Hungate tubes with 9mL of peptone water. The numbers of lactic acid bacteria in the samples were determined on MRS agar (Oxoid, CM0361) inoculated with 0.1 mL of diluted sample and after anaerobic incubation at 39°C for 48 h.

2.2. Bird husbandry

A total of 336 one-day-old male Cobb broiler chicks, which were vaccinated against Marek's disease, infectious bronchitis, and Newcastle disease, were obtained from a local hatchery (Baiada hatchery, Kootingal, NSW, Australia) and randomly allocated to 42 cages in four-tier floor pens (600 × 600 × 300 mm dimension, with a floor space of 0.36 m^2/cage) sit on sawdust litter in climate-controlled rooms. Each of the 7 dietary treatments was randomly assigned to 6 cages with 8 birds per cage (except for the water treatment group which needed to be in line in order to be serviced by the same water pipe that supplied the water containing the probiotics). At d 21, birds were transferred to slide-in cages (800 × 740 × 460 mm) in an environmentally controlled room.

The room temperature was gradually decreased from 33°C on d 1 to 24°C on d 21. Eighteen hours of light was provided per day throughout the trial, excluding d 1 to 7 during which 23 h of light was provided. Relative humidity was between 65 and 70%. Each cage was equipped with a feeding trough placed outside and had water pipes providing drinking nipples inside. Feed and water were provided ad libitum.

2.3. Experimental treatments

2.3.1. The diet and treatments

The basal diets (starter and finisher) were based on corn, wheat and soybean meal as shown in (Table 1), and fed as a one-phase mash feed to avoid inactivation of the probiotic. Seven treatments

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 (Ridley Agriproducts Pty Ltd., Tamworth, NSW) contained the following minerals in milligrams per kilogram of diet: 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.