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Evaluation of an intestinal *Lactobacillus reuteri* strain expressing rumen fungal xylanase as a probiotic for broiler chickens fed on a wheat-based diet

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Abstract 1. This study was conducted to evaluate the feasibility of using a transformed *Lactobacillus reuteri* Pg4 strain harbouring a rumen fungal xylanase gene as a probiotic supplement in a wheat-based poultry diet.

2. A total of 400 broiler chicks was allocated to two treatment groups with or without supplementation with 10⁶ colony forming units (cfu)/g of transformed *L. reuteri* Pg4 in a wheat-based regimen to investigate the performance, intestinal microflora populations, digesta viscosity and excreta ammonia concentrations in these broiler chickens.

3. Supplementation of the wheat-based diet with transformed *L. reuteri* Pg4 decreased intestinal viscosity, caecal coliform population, and increased body weight gain and ileal villus height and crypt depth from 0 to 21 d of age. It also decreased excreta ammonia concentrations, and increased the caecal total volatile fatty acid (VFA) and lactic acid concentrations from 0 to 21 d and 22 to 37 d of age.

4. Further, it was demonstrated that 40% of the *Lactobacillus* cells randomly isolated from the digesta of the ileum and caecum of the supplemented group possessed xylanase secretion capability.

5. It appears reasonable to assume, therefore, that the derived benefit is a result of the organism surviving, and the associated performance of some function in the intestinal tract which benefits gut health.

INTRODUCTION

For many years, bactericidal additives, including some antibiotics, have been used for treatment of diseases and for growth promotion. However, with mounting public concerns associated with antibiotic residues and increasing rates of antibiotic resistance, antibiotics have been strictly banned in some areas of the world and it appears reasonable to anticipate greater restrictions will apply in the future. Probiotics containing viable organisms might exert a beneficial effect on animal performance through modification of gastrointestinal tract (GIT) microflora, and might offer great potential both as a feed additive and as a replacement for antibiotics (Fuller, 1992). There is ample evidence to show that

probiotics enhance the immune system, increase body weight gain, reduce diarrhoea and improve feed conversion rate (Patterson and Burkholder, 2003). Currently used or researched probiotics are mostly selected from native gut microflora, with the selection of optimal strains often largely empirical. However, it has been speculated that genetic modification might be used to develop more efficacious variants. Examples of genetically modified probiotics include strains that produce antibodies, detoxification enzymes and cytokines for immune intervention (Steidler, 2003).

Wheat contains a large amount of indigestible fibre known as non-starch polysaccharide (NSP) in the endosperm layers and cell wall of grain. The NSPs in wheat are mainly

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arabinoxylans, which consist of the monosaccharides, arabinose and xylose, linked in β 1–4 linkages with arabinose side chains. These NSPs are not hydrolysed by the digestive enzymes of chicks and, as a result, the undigested arabinoxylan can often be problematic for monogastric animals on a wheat-based diet, causing intestinal disturbances, typified by sticky droppings and poor growth in young animals (Choct and Annison, 1990; Choct *et al.*, 1999). In addition, these sticky droppings also reportedly create management problems in poultry farming. Therefore, it is not surprising that addition of xylanase to wheat-based diets for non-ruminants decreases viscosity and, consequently, reduces the anti-nutritional effect of arabinoxylan, leading to better productive performance (Choct *et al.*, 1999; Chiang *et al.*, 2005). Xylanase is commonly used as an ingredient in wheat-based diets for enhancing the digestive capability of a broiler throughout its life. However, the supplementation of xylanase substantially increases the cost of feed. An alternative and less expensive strategy might be to develop probiotics with the capacity to digest plant cell wall structural carbohydrates by introduction of heterologous genes encoding polysaccharide-degrading enzymes (Cho *et al.*, 2000).

In a previous study, we isolated a strain of *L. reuteri* Pg4 from the GIT of healthy broilers and suggested that it had potential as an additive in animal feed (Yu *et al.*, 2007). In another study, the rumen fungal *Neocallimastix patriciarum* xylanase gene *xynCDBFV* was cloned and expressed into *L. reuteri* Pg4. It was further demonstrated that the transformed *L. reuteri* Pg4 not only acquired the capacity to break down xylan, but also that there was no difference from the parent strain in terms of the adhesion efficiency to mucin and mucus and its resistance to bile salts and acid (Liu *et al.*, 2005). To the best of our knowledge, use of the transformed *Lactobacillus* expressing the heterologous xylanase gene as a probiotic has not been evaluated previously.

In the present study, we investigated the effects of feeding the transformed *L. reuteri* Pg4 expressing the rumen fungal xylanase gene on body weight gain, intestinal microflora population, digesta viscosity and excreta ammonia concentrations in broiler chickens. Intestinal histological characteristics were also examined.

MATERIALS AND METHODS

Bacterial strain and media

The *L. reuteri* Pg4 strain used in this trial was isolated from the GIT of healthy broilers, and it has been successfully transformed with the ruminal fungus *N. patriciarum* xylanase gene

xynCDBFV (Liu *et al.*, 2005; Yu *et al.*, 2007). The transformed *L. reuteri* Pg4 was kept at -80°C as a stock culture. After two successive transfers in MRS broth (Difco Laboratories, Detroit, MI, USA) containing chloramphenicol ($10\text{ }\mu\text{g/ml}$), the culture solution was inoculated into the broth at 1% (vol/vol) and incubated at 37°C for 24 h. Thereafter, skim-milk powder, which was used as a cryoprotector, was added to the incubated culture at a final concentration of 50% (wt/vol). Then, the lactobacilli culture preparation was lyophilised and stored at 4°C until required. The bacterial count of the transformed *L. reuteri* Pg4 lyophilised powder was about 10^9 colony forming units (cfu)/g.

Experimental animals and growth trials

A total of 400 male and female Arbor Acres broilers (one day old; equal gender numbers) was weighed individually and assigned at random to 16 pens each containing 25 chicks of the same sex. The experiment was also randomised with respect to the two dietary treatments, and each treatment was assigned to 4 male and 4 female pens. The wheat-based regimens were with or without supplementation with 1 g transformed *L. reuteri* Pg4 powder/kg.

The feeding trial consisted of growing and finishing periods (0 to 21 d and 22 to 37 d, respectively). The composition of the experimental diet is presented in Table 1. During the experimental period, the chickens were placed in floor pens with $0.17\text{ m}^2/\text{bird}$ in an open-sided house, under natural conditions with an average temperature of 28.6°C , and 22 h of incandescence per d. Feed in a mash form and drinking water were provided *ad libitum*. The individual broiler live weights and pen feed consumption were measured at the ages of 21 and 37 d, and the feed conversion rate was also determined.

Intestinal sample and digesta collection

At the ages of 21 and 37 d, 4 chickens from each treatment group were killed and segments of duodenum, jejunum, ileum and caecum were collected for measuring the viscosity, ammonia concentration, volatile fatty acids and lactate concentration, and microbial population in the digesta. Samples of ileum were also collected for histological observation.

Determination of the viscosity of the duodenal digesta

The duodenal digesta were centrifuged at 25°C and $12000g$ for 3 min. The viscosity of the supernatants was measured according to the method of Fengler *et al.* (1987) using a

Table 1. Composition of experimental diet for broiler chickens (g/kg)

Ingredient	0 to 21 d	22 to 37 d
Wheat	569.9	597.4
Soybean meal (crude protein 445 g/kg)	185	170
Full fat soybean meal (crude protein 380 g/kg)	100	120
Soy oil	70	65
Fish meal (crude protein 650 g/kg)	35	5
Monocalcium phosphate	20	17
Limestone	11	16
L-Lysine	1.7	2.1
DL-Methionine	1.4	1.5
Sodium chloride	4.0	4.0
Mineral premix ¹	1.0	1.0
Vitamin premix ²	1.0	1.0
Calculated values, g/kg		
Crude protein (N' 6.25)	220	200
ME (MJ)	12.97	13.50
L-Lysine	12.5	11.2
DL-Methionine+cysteine	8.2	7.2
Calcium	10.0	9.0
Available phosphorus	4.7	4.0
Total phosphorus	7.1	6.5
Analysed value		
Crude protein	218	195
Ether extract	96.5	100.5
Ash	56.0	51.0

¹Contained per kg of diet: Co (CoCO₃) 0.255 mg, Cu (CuSO₄ × 5H₂O) 10.8 mg, Fe (FeSO₄ × H₂O) 90 mg, Mn (MnSO₄ × H₂O) 90 mg, Zn (ZnO) 68.4 mg, Se (Na₂SeO₃) 0.18 mg.

²Contained per kg of diet: vitamin A (as all-*trans*-retinol) 5.16 mg, cholecalciferol 75 µg, vitamin E (as *dl*- α -tocopheryl acetate) 30 mg, vitamin K₃ 4 mg, thiamine 3 mg, riboflavin 8 mg, pyridoxine 5 mg, vitamin B₁₂ 25 µg, calcium pantothenate 19 mg, niacin 50 mg, folic acid 1.5 mg, biotin 60 µg.

Brookfield digital viscometer (Model Lvidv-110P; Middleboro, MA, USA) at 25°C, with the recorded values expressed in centipoise (cP).

Determination of volatile fatty acids (VFA), lactic acid and pH level in the intestinal digesta

The concentrations of VFA and lactic acid in the ileal and caecal digesta were analysed using a high performance liquid chromatography (HPLC) system (Hitachi, Tokyo, Japan) according to the methods of Grigsby *et al.* (1992) and Marsili *et al.* (1981). The pH values of the ileal and caecal digesta were directly measured *in situ* using the glass electrode of a portable pH meter (Suntex TS-1, Taiwan).

Determination of intestinal digesta and excreta ammonia concentration

The ammonia concentrations of the intestinal digesta and excreta were assayed using the phenol-hypochlorite-nitroprusside method (Weatherburn, 1967) as follows. Intestinal digesta or excreta (1.0 g) were mixed with 4 ml

meta-phosphoric acid (25%) and then centrifuged at 3000 *g* for 10 min. The supernatant was mixed with 5.0 ml phenol-nitroprusside reagent (containing 5 g/l phenol and 25 mg/l sodium nitroprusside) and 5 ml alkaline hypochlorite solution (25 g of NaOH and 4.2 ml of sodium hypochlorite), and the absorbance of the resulting solution was measured at 625 nm.

Analysis of microbial distribution

For bacterial assays, 10-fold serial dilutions were made from 1 g aliquots of intestinal digesta using phosphate buffer saline (PBS) as a diluent. A 100 µl portion of each dilution was spread on Petri dishes containing different growth media. Reinforced clostridial agar (Oxoid, Basingstoke, UK), MRS agar (Difco) and Chromocult coliform agar (Merck, Darmstadt, Germany) were used specifically for counting anaerobic bacteria, *Lactobacillus* and coliform bacteria, respectively. Reinforced clostridial and MRS agars were incubated anaerobically at 37°C for 48 h in a GasPak system (BBL, Cockeysville, MD, USA). Chromocult coliform agars were incubated aerobically at 37°C for 24 h (Jin *et al.*, 1998). The results are expressed as logarithmic colony forming units (log cfu) per g wet weight of gastrointestinal digesta.

Detection of xylanase activity of *Lactobacillus* randomly isolated from intestinal digesta

Lactobacillus colonies randomly isolated from the intestinal digesta of the experimental birds were inoculated into MRS broth containing chloroamphenicol (10 µg/ml) and incubated at 37°C for 24 h. The xylanase activity of each *Lactobacillus* cell culture was estimated for culture supernatant (representing extra-cellular enzyme activity) and whole-cell extract (representing intra-cellular). The culture was centrifuged at 5000 *g* for 20 min at 4°C, and the supernatant was collected. The cell pellet was resuspended in 50 mM sodium citrate buffer (pH 5.0) and sonicated for 10 min with an ultrasonicator (Model XL, Misonix, Farmingdale, NY, USA). Cellular debris was subsequently removed by centrifugation at 5000 *g* for 20 min, and the clarified extract was retained for subsequent enzyme analysis. Xylanase activity was determined by measuring the amounts of reducing sugar liberated by fractions incubated with 0.5% (wt/vol) xylan in 50 mM sodium citrate buffer (pH 5.0), respectively. After incubation for 20 min at 40°C, the reaction was stopped by boiling for 10 min. The amount of reducing sugar produced was estimated using dinitrosalicylic acid (DNS) reagent (Konig *et al.*, 2002). One unit of xylanase activity was defined as that releasing 1 µmole/min of

xylose from the respective substrate under assay conditions.

Observation of intestinal histology

Intestinal histology was observed according to the method of Yu *et al.* (1999). Sample sections (3 cm in length) were taken from the middle region of the ileum, rinsed with 0.01 M PBS (pH 7.2) and placed into 10% buffered neutral formaldehyde solution (pH 7.2 to 7.4). Then, all samples were gradually dehydrated, sectioned at 6 µm and stained with hematoxylin and eosin (H&E). Finally, each sample was observed using light microscopy.

Statistical analysis

The statistical analyses were performed with the Statistical Analysis System software package (Version 6.1; SAS Institute, 1998) to analyse the significance of all results. All results were expressed as mean ± standard deviation. Student's *t*-test was used for inter-group comparison, and a *P*-value less than 0.05 was considered significant.

RESULTS

Growth performance and intestinal viscosity

The effect of supplementation of the wheat-based diet with the transformed *L. reuteri* Pg4, in terms of growth performance and digesta viscosity of the broilers, is presented in Table 2. Dietary supplementation with the transformed *L. reuteri* Pg4 significantly improved body weight gains ($P < 0.05$) and decreased duodenal viscosity ($P < 0.1$) during the growing period. Nevertheless, no significant differences were demonstrated in between-group comparison of average body weight gain, feed intake, feed conversion rate and intestinal viscosity for the two dietary variants during the finishing and whole feeding period.

Ileal and caecal VFA and lactic acid concentrations and pH levels

The effect of addition of the transformed *L. reuteri* Pg4 to the wheat-based diet in terms of VFA and lactic acid concentrations and pH in the broiler ileum and cecum are presented in Table 3. Supplementation with the transformed *L. reuteri* Pg4 significantly increased the caecal total VFA but not in the ileum during the growing phase and the finishing period. Furthermore, the experimental dietary supplementation increased the lactic acid concentrations in ileum and caecum during the growing

and the finishing period. However, the supplemented group had a trend of decreasing pH in ileal digesta only during the growing period and

Table 2. Effect of transformed *Lactobacillus reuteri* Pg 4 supplement in diet on broiler growth performance and intestinal digesta viscosity

	Control group	Supplemented group
0 to 21 d		
Body weight gain, g/bird	619 ± 8 ^b	654 ± 7 ^a
Feed intake, g/bird	1030 ± 21	1061 ± 39
Feed conversion, feed/gain	1.65 ± 0.67	1.63 ± 0.19
22 to 37 d		
Body weight gain, g/bird	1272 ± 22	1266 ± 20
Feed intake, g/bird	2281 ± 72	2279 ± 76
Feed conversion, feed/gain	1.80 ± 0.019	1.79 ± 0.021
0 to 37 d		
Body weight, g/bird	1936 ± 21	1964 ± 20
Body weight gain, g/bird	1892 ± 21	1920 ± 20
Feed intake, g/bird	3310 ± 115	3340 ± 100
Feed conversion, feed/gain	1.75 ± 0.04	1.74 ± 0.03
Viscosity, cP		
At 21 d	6.53 ± 0.52 ^x	5.79 ± 0.22 ^y
At 37 d	3.93 ± 0.33	3.79 ± 0.16

^{a, b}Means within a row with different superscripts are significantly different ($P < 0.05$).

^{x, y}Means within a row with different superscripts are different ($P < 0.1$).

Table 3. Effect of transformed *Lactobacillus reuteri* Pg 4 supplement in diet on VFA concentration, lactic acid, pH value in contents of ileum and caecum in broilers

	Control group	Supplemented group
At 21 d		
Ileum		
Total VFA (µmole/g)	63.25 ± 3.04	70.4 ± 2.26
Acetic acid (%)	69.65	85.08
Propionic acid (%)	28.57	14.91
Butyric acid (%)	1.78	0.02
Lactic acid (µmole/g)	59.62 ± 6.51 ^b	74.90 ± 1.47 ^a
pH	7.07 ± 0.56 ^x	6.38 ± 0.14 ^y
Caecum		
Total VFA (µmole/g)	140.25 ± 7.14 ^b	189.35 ± 7.00 ^a
Acetic acid (%)	65.68	77.10
Propionic acid (%)	31.08	17.67
Butyric acid (%)	3.24	7.82
Lactic acid (µmole/g)	63.42 ± 3.04 ^b	111.72 ± 15.96 ^a
pH	6.14 ± 0.35	6.83 ± 0.36
At 37 d		
Ileum		
Total VFA (µmole/g)	49.45 ± 2.6	58.45 ± 5.8
Acetic acid (%)	88.69 ^a	68.33 ^b
Propionic acid (%)	9.68 ^b	25.05 ^a
Butyric acid (%)	1.63	6.62
Lactic acid (µmole/g)	55.70 ± 9.12 ^b	73.84 ± 2.48 ^a
pH	6.37 ± 0.08	6.34 ± 0.97
Caecum		
Total VFA (µmole/g)	171.45 ± 19 ^b	198 ± 7.7 ^a
Acetic acid (%)	51.26 ^b	86.11 ^a
Propionic acid (%)	25.82	6.83
Butyric acid (%)	22.92	7.05
Lactic acid (µmole/g)	94.91 ± 24.8 ^b	136.07 ± 4.72 ^a
pH	6.43 ± 0.08	6.48 ± 0.20

^{a, b}Means within a row with different superscripts are significantly different ($P < 0.05$).

^{x, y}Means within a row with different superscripts are different ($P < 0.1$).

no effect on the pH value in the caecum during the finishing period.

Intestinal digesta and excreta ammonia concentration

As shown in Table 4, supplementation with the transformed *L. reuteri* Pg4 significantly decreased the ammonia concentrations in the excreta during the growing and finishing periods. Except for the caecal ammonia concentration at 37 d of age, however, no significant differences were demonstrated in the jejunal, ileal and caecal ammonia concentrations at any point in the feeding trial comparing the two dietary treatment groups.

Bacterial counts in the intestinal digesta

The effect of supplementation with the transformed *L. reuteri* Pg4 on the microflora population in different segments of the broiler intestinal tract is presented in Table 5. The supplementation did not affect the anaerobic bacterial and lactobacilli populations in the ileum and caecum of the treated broilers in comparison to controls at 21 and 37 d of age, whereas the count of *Lactobacillus* spp. in the supplemented group

showed a higher trend than in the control group. In addition, coliform populations were significantly lower in the caecum of the supplemented broilers compared to the controls at 21 d of age ($P < 0.05$).

Xylanase activity of bacteria isolated from digesta

A total of 150 *Lactobacillus* colonies was randomly isolated from the ileum and caecum of chicks in the treatment group; 40% of these colonies were found to exhibit xylanase secretion capability. The mean xylanase activities of the intra- and extra-cellular fractions from the *Lactobacillus* cultures randomly isolated from the GIT of animals in the treatment group were 2.78 ± 0.25 and 1.91 ± 0.37 U/ml, respectively. These isolated *Lactobacillus* colonies showed similar secretion capability to the transformed *L. reuteri* Pg4 strain in this study. In contrast, the xylanase activities of the intra- and extra-cellular fractions from *Lactobacillus* cultures randomly isolated from the GIT of animals in the control group were undetectable. This demonstrated that these *Lactobacillus* isolated from the control group did not possess the ability to produce xylanase.

Intestinal histology

The results of the intestinal histological analysis are presented in Table 6. Supplementation of the wheat-based diet with the transformed *L. reuteri* Pg4 did not affect the ileal muscle layer thickness but the associated villus height and crypt depth were significantly greater in supplemented broilers compared to the controls at 21 d of age.

DISCUSSION

The relative benefits provided by fibrolytic enzyme augmentation of cereal-based diets for monogastric animals include an overall decrease in the output of organic matter in the excreta due to better utilisation of the nutrients, reduction in

Table 4. Effect of transformed *Lactobacillus reuteri* Pg 4 supplement in diet on the ammonia concentration in broiler digesta and excreta

	mm/g	
	Control group	Supplemented group
At 21 d		
Jejunum	1.87 ± 0.48	2.72 ± 0.43
Ileum	2.55 ± 0.23	2.21 ± 0.23
Caecum	16.35 ± 2.86	14.84 ± 0.47
Excreta	36.83 ± 1.59^a	25.65 ± 1.79^b
At 37 d		
Jejunum	1.79 ± 0.11	2.63 ± 0.35
Ileum	2.30 ± 1.31	2.97 ± 0.35
Caecum	16.95 ± 0.14^a	10.95 ± 1.19^b
Excreta	34.51 ± 2.38^a	22.69 ± 1.19^b

^{a,b}Means within a row with different superscripts are significantly different ($P < 0.05$).

Table 5. Effect of transformed *Lactobacillus reuteri* Pg 4 supplement in diet on microflora population in different segments of broiler digestive tract (log cfu/g)

	Anaerobic bacteria		<i>Lactobacillus</i> spp.		Coliforms	
	Control	Supplemented	Control	Supplemented	Control	Supplemented
At 21 d						
Ileum	7.68 ± 0.03	8.06 ± 0.08	7.60 ± 0.01	7.81 ± 0.16	7.91 ± 0.29	7.54 ± 0.03
Caecum	9.42 ± 0.29	9.68 ± 0.48	8.70 ± 0.35	9.47 ± 0.34	9.09 ± 0.43^a	8.64 ± 0.19^b
At 37 d						
Ileum	9.33 ± 0.44	10.21 ± 0.61	7.10 ± 0.48	8.28 ± 0.36	8.12 ± 0.24	7.01 ± 0.08
Caecum	9.93 ± 0.21	10.02 ± 0.31	9.16 ± 0.27	9.53 ± 0.22	7.74 ± 0.10	8.28 ± 0.36

^{a,b}Means within a row with different superscripts are significantly different ($P < 0.05$).

Table 6. Effect of transformed *Lactobacillus reuteri* Pg 4 supplement in diet on ileal mucosa histology of 21-d-old broilers

	Control group	Supplemented group
Villus height, μm	862.47 $\pm$ 38 ^b	1043.29 $\pm$ 137 ^a
Crypt depth, μm	186.58 $\pm$ 28 ^b	228.82 $\pm$ 40 ^a
Muscle layer, μm	281.42 $\pm$ 52	279.44 $\pm$ 61
Villus/crypt	4.72 $\pm$ 0.83	4.75 $\pm$ 1.17

^{a,b}Means within same rows with different superscripts are significantly different ($P < 0.05$).

the moisture content of the excreta due to decreased gut viscosity and improvement in general animal health due to the prevention of those diseases associated with the proliferation of gut microflora. Therefore, fibrolytic enzymes are commonly used as ingredients in barley- or wheat-based diets for enhancing the digestive capability of the broiler throughout its life. However, the supplementation of xylanase substantially increases the cost of feed. Probiotics are now being considered as one of the alternatives to antibiotics in livestock production. Using probiotics with fibrolytic enzymes of high activities can provide additional benefits and reduce the cost of enzyme supplementation. Further, the rumen microbial population presents a rich source of novel enzymes with tremendous potential for industrial application. It is reported that cellulases and xylanases produced by ruminal fungi are among the most active fibrolytic enzymes described to date (Selinger *et al.*, 1996). To this end, we successfully cloned and expressed rumen microbial fibrolytic enzyme genes in a strain of *L. reuteri* in a previous study (Liu *et al.*, 2005). To the best of our knowledge and following a thorough review of the relevant literature, however, the evaluation of xylanase-producing *Lactobacillus* as a probiotic for broiler chickens does not appear to have been reported previously. In the present investigation, we found that the transformed *L. reuteri* Pg4 and about 40% *Lactobacillus* strains randomly isolated from the ileum and caecum of chicks in the supplemented group exhibited xylanase secretion capability, while the analogous organisms from the control birds did not. This indicates that the transformed *L. reuteri* Pg4 can survive and secrete xylanase in the chicken GIT.

When broilers are fed on wheat-based diets, the presence of NSP can give rise to highly viscous conditions in the small intestine and decrease contact between digestive enzymes and substrates, hence depressing nutrient absorption and broiler performance. It is, therefore, not surprising that addition of xylanase to wheat-based diets for broiler chickens decreases viscosity and, consequently, reduces the anti-nutritional effect of NSP, leading to better productive performance (Chiang *et al.*, 2005).

In this study, we have demonstrated that supplementation of the wheat-based diet with the transformed *L. reuteri* Pg4 harbouring the rumen fungal xylanase gene decreased intestinal viscosity during the growing phase (0 to 21 d), which suggests that the decrease in this variable may be attributable, in part, to the xylanase secreted by the transformed *L. reuteri* Pg4.

In addition to the direct effects of NSP on nutrient digestion, there appear to be secondary effects attributable to higher levels of gut microflora in the small intestine (Wang *et al.*, 2005). Choct *et al.* (1999) have indicated that excess fermentation in the chicken intestine might be detrimental to nutrient digestion and absorption. In addition, it is generally accepted that increased intestinal coliform numbers result in an imbalance in the ratio beneficial to pathogenic bacteria in the GIT, producing diarrhoea (Bazzocchi *et al.*, 2002). Inclusion of *Lactobacillus* in the diets of broiler chickens reportedly depresses coliform population in the intestinal microflora to the benefit of the host animal (Jin *et al.*, 1998). In the present study, we found that the coliform populations in the caecum of the broiler chickens supplemented with the transformed *L. reuteri* Pg4 treatment were significantly lower at 21 d than the analogous populations in the controls.

It has been demonstrated that villus height and crypt depth may be used as indicators of gut health and, hence, are associated with animal condition and growth performance. The mucosal enzyme activity per mass of intestine is closely associated with the number of enterocytes per villi in chickens. Therefore, greater digestive-enzyme activity has been noted in the higher villi (Uni *et al.*, 1995). A harsh environment may lead to abnormal changes in villus height or crypt depth. A decrease in either variable may lead to a reduction in nutrient absorption, which is associated with certain disease states. In this study, transformed *L. reuteri* Pg4 supplementation may have improved the gut health by increasing villus height and crypt depth, thus further promoting nutrient absorption.

As volatile fatty acids arise from carbohydrate fermentation and/or protein fermentation and deamination by the indigenous anaerobic microflora in the GIT, VFA concentration can be an index of the anaerobic-organism population (Savage, 1977). VFA may actually provide a number of benefits to the host animal, acting as metabolic fuel for muscle, kidney, heart, liver and brain tissue, and also affording bacteriostatic and bacteriocidal properties against organisms such as *Salmonella* and *E. coli* (Corrier *et al.*, 1990). Lactic acid, produced mainly by saccharolytic bacteria such as *Lactobacillus*, *Enterococcus* and *Bifidobacteria* during carbohydrate fermentation,

may serve to protect the animal from pathogenic bacteria by decreasing the hind gut pH, thus impeding the growth of these unfavourable bacteria (Barrow, 1992). In the present study, we found that the total VFA and lactic acid concentrations in the caecum of broilers in the experimental supplementation group were significantly greater than in controls. Thus, we suggest that oral administration of transformed *L. reuteri* Pg4 powder may benefit the animal by enhancing the health of the GIT.

Ammonia emission and subsequent deposition can be a major source of pollution in poultry production, causing nitrogen enrichment, acidification of soils and surface waters, and nitrate elevation in ground and surface waters. In the housing environment, ammonia emissions can also adversely affect the health, performance and welfare of both the farmed animals and their human attendants (McCrory and Hobbs, 2001). Uric acid is the major nitrogenous component in poultry manure. Conversion of uric acid to ammonia requires urease, an enzyme which is excreted in animal faeces (McCrory and Hobbs, 2001). Yeo and Kim (1997) reported that a diet containing *L. casei* decreased urease activity in the small intestinal contents of young chicks. In our study, we demonstrated that supplementation of the wheat-based diet with the transformed *L. reuteri* Pg4 significantly decreased the ammonia concentrations in the excreta of broiler chickens, suggesting that this decrease may be attributable, in part, to the reduction of fecal urease activity produced by the transformed *L. reuteri* Pg4.

In conclusion, supplementation of the wheat-based diet with the transformed *L. reuteri* Pg4 harbouring the rumen fungal xylanase gene decreased intestinal viscosity, caecal coliform populations and excreta ammonia concentrations, while increasing the caecal total VFA and lactic acid concentrations, ileal villus height and crypt depth, and body weight gains during the growing phase of the broiler chickens. Further, it was demonstrated that *Lactobacillus* isolated from the digesta of ileum and caecum of the supplemented group possessed xylanase secretion capability, while the analogous organisms from the control animals did not. It appears reasonable to assume, therefore, that the benefit derived is a result of the organism surviving and performing some function in the intestinal tract, enhancing gut health.

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