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Effects of dietary *Enterococcus faecium* on growth performance, carcass characteristics, faecal microbiota, and blood profile in broilers

M. Mohammadi Gheisar, A. Hosseindoust, I.H. Kim

Dankook University, Cheonan, Choongnam, Republic of Korea

ABSTRACT: This research was performed to evaluate the effect of supplementing broiler diets with a probiotic containing *Enterococcus faecium* on growth performance, carcass characteristics, faecal microbiota, and blood profile. A total of 384 one-day-old Ross 308 broiler chicks (mixed gender) with an average initial BW of 39.2 g were used in a 35 days feeding trial. The chicks were allotted to pens with 16 birds per pen and eight replications per treatment with food and water provided *ad libitum*. Treatments were: (1) basal diet, (2) 0.25% probiotic, and (3) 0.5% probiotic. Results indicated that body weight gain (BWG) on Day 7 to 21, Day 21 to 35 and overall (0 to 35) increased ($P < 0.05$) linearly but feed intake (FI) and feed conversion ratio (FCR) were not affected. A linear increase ($P < 0.05$) was observed in the relative weight of breast muscle when comparing the 0 to 0.5% concentration of probiotic, but breast meat colour was not affected by treatments. A significant impact (linear effect, $P < 0.05$) was observed on drip loss on Day 1. Inclusion of probiotic decreased ($P < 0.05$) the count of *Salmonella* linearly but the counts of *E. coli* and *Lactobacillus* were not affected. There was no remarkable influence on blood profile. Thus, it was concluded that inclusion of a probiotic containing *Enterococcus faecium* improved growth performance and altered the intestinal microbial population, without any negative effects on meat colour and blood profile in broiler chickens.

Keywords: broiler; carcass characteristics; *Enterococcus faecium*; faecal microbiota

Since antibiotics as growth promoters are being removed from poultry and swine diets worldwide, there is a pressing requirement to find alternatives. Several natural products, such as organic acids, plant extracts, probiotics, and prebiotics, have been assessed as alternatives to antibiotics as growth promoters (Patterson and Burkholder 2003; Higgins et al. 2008; Markovic et al. 2009; Mountzouris et al. 2010; Vondruskova et al. 2010; Zhang et al. 2013; Zhang and Kim 2013). Probiotics are live microbial feed additives that can beneficially influence the intestinal microflora of the host animal. Various microorganisms such as *Lactobacillus*, *Lactococcus*, *Streptococcus*, *Bacillus*, and *Enterococcus* can be used as probiotics (Fooks and Gibson 2002; Ouwehand et al. 2002; Lodemann et al. 2006; Park and Kim 2015; Zhang et al. 2014). Previous studies have confirmed the positive effects of probiotics in pigs (Meng et al. 2010; Yan and Kim 2013). Several studies have been conducted to investigate the effects of supplementing poultry diets with probiotics and it has been reported that probiotics can exert positive effects on the development and function of immune cells (Huang et al. 2004; Kabir et al. 2004). Recently, some probiotic feed additives have been produced that contain viable cells of *Enterococcus faecium*. These products are currently authorised for use in piglets and calves (Vahjen et al. 2007).

This study was conducted to investigate the effects of supplementing broiler diets with different concentrations of a probiotic containing *Enterococcus faecium* M74 on growth performance, meat quality, relative organ weights, faecal microbiota, and blood profile.

MATERIAL AND METHODS

Animals, diets, and facilities. The use and management of the broiler chickens used in this

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study were approved by the Animal Care and Use Committee of Dankook University. A total of 384 one-day-old Ross 308 broiler chicks (mixed sexes) with an average initial BW of 39.2 g were used in a 35 day experimental period. The chicks were allotted to pens with 16 birds per pen and eight pens per treatment. Treatments were: (1) basal diet, (2) 0.25% probiotic (3×10^{11} CFU/g), and (3) 0.5% probiotic (3×10^{11} CFU/g). The chicks were weighed and placed randomly in three floor battery cages in an environmentally controlled room (32 to 24 °C and 65% relative humidity). During the entire experimental period, the chickens were provided access to feed and water *ad libitum*. All diets were formulated to meet or exceed NRC recommendations for nutrient recommendations (1994). Feed ingredients and the chemical composition are presented in Table 1. Relative weight of breast meat, abdominal fat and organs were described as a percentage of live weight. The probiotic used in this experiment contained 3×10^{11} CFU of *Enterococcus faecium* M74 per gram (Lactiferm®, Chr. Hansen, Germany).

Table 1. Composition of diets (as-fed basis)

Item (%)	Phase 1 Day 0–7	Phase 2 Day 7–21	Phase 3 Day 21–35
Corn	45.48	36.69	40.80
Wheat	10.00	20.00	20.00
Soybean meal (CP 48%)	34.25	33.62	25.48
Corn gluten meal (CP 60%)	2.00	–	–
Rape seed meal	–	–	3.50
Tallow	1.90	5.54	6.01
Soybean oil	1.50	–	–
Limestone	1.06	1.12	1.17
Dicalcium phosphate	2.23	1.90	1.84
Salt	0.35	0.32	0.29
DL-Methionine	0.46	0.39	0.41
L-Lysine-HCl	0.42	0.15	0.20
Threonine	0.17	0.09	0.12
Vitamin mix[1]	0.03	0.03	0.03
Vitamin E (10%)	0.04	–	–
Mineral mix[2]	0.10	0.10	0.10
$CuSO_4 \cdot 5\ H_2O$	0.01	0.05	0.05
Total	100	100	100
Calculated nutritional content			
ME (MJ/kg)	12.62	13.03	13.31
Analysed nutritional content (%)			
CP	22.12	20.43	18.55
Lysine	1.45	1.22	1.10
Met + Cys	1.06	0.95	0.93
Ca	1.05	1.00	1.00
Available P	0.53	0.50	0.50
Crude fat	5.55	7.27	7.96
Crude fiber	3.24	3.29	3.25

[1]Provided per kg of diet: 15 000 IU of vitamin A, 3750 IU of vitamin D_3, 37.5 mg of vitamin E, 2.55 mg of vitamin K_3, 3 mg of B_1, 7.5 mg of B_2, 4.5 mg of vitamin B_6, 24 μg of vitamin B_{12}, 51 mg of niacin, 1.5 mg of folic acid, 126 mg of biotin and 13.5 mg of pantothenic acid

[2] Provided per kg of complete diet: 37.5 mg of Zn, 137.5 mg of Mn, 37.5 mg of Fe, 0.83 mg of I, and 0.23 mg of Se, and 1.408 mg of choline

Sampling and measurements. All the chickens and the remaining feed were weighed on Days 0, 7, 21, and 35 to allow calculations of body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR). On the last day of the trial period, 30 chickens were selected randomly and blood samples were taken from the wing vein (six chickens per treatment). Blood samples were collected into K_3EDTA vacuum tubes (Becton Dickinson Vacutainer Systems, Franklin Lake, NJ). The samples were centrifuged (3000 × *g*, 15 min) to recover blood plasma. Whole blood cell counts (white blood cells (WBC), red blood cells (RBC), and lymphocytes) were analysed using an automatic blood analyser (ADVIA 120, Bayer, NY). After blood sample collection, the same chickens were weighed individually and sacrificed. Breast meat, abdominal fat, gizzard, liver, spleen, bursa of Fabricius, and heart were excised, blotted to remove excess moisture, and weighed by trained personnel. Hunter *L** (lightness), *a** (redness), and *b** (yellowness) of breast meat were determined using a Minolta CR410 chromameter (Konica Minolta Sensing Inc., Osaka, Japan). Drip loss percentage was measured on Days 1, 3, 5, and 7 using approximately 2 g of breast meat sample according to the plastic bag method, described by Honikel (1998). Faecal samples from the cloacae were collected into micro-tubes and were analysed for counts of *Lactobacillus*, *E. coli*, and *Salmonella* using agar media. Viable bacteria in excreta samples were determined by plating 10-fold serial dilutions (in 1% peptone solution) onto MacConkey agar plates (Difco Laboratories, Detroit, MI) and *Lactobacilli* medium agar plates (Medium 638, DSMZ, Braunschweig, Germany) to isolate the *E. coli* and *Lactobacillus*, respectively.

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The *Lactobacilli* medium agar plates were then incubated for 48 h at 39 °C under anaerobic conditions. The MacConkey agar plates were incubated for 24 h at 37 °C. The *E. coli* and *Lactobacillus* colonies were counted immediately after removal from the incubator. For *Salmonella*, the serially diluted peptone broth tubes were incubated overnight at 37 °C, after which 1 ml was transferred to 9 ml of tetratinate broth (Neogen Corporation, Lansing, MI) followed by incubation for 48 h at 42 °C. From these tubes, 1 ml was used to inoculate 9 ml of Rappaport-Vassiliadis *Salmonella* Enrichment broth (Neogen Corporation, Lansing, MI) followed by incubation for 48 h at 42 °C. The Rappaport was used to inoculate XLT4 plates for *Salmonella* isolation, and *Salmonella* was identified using LIS (VIDAS Listeria) and TSI (Triple Sugar Iron) agar tubes (Difco Laboratories, Detroit, MI).

Statistical analysis. Data were analysed as a completely randomised design using the mixed procedures of SAS (SAS Institute 1996). Mean values and standard errors (SE) are reported. Linear and quadratic polynomial contrasts were performed to determine the effects of inclusion of 0, 0.25, and 0.5% *Enterococcus faecium* M74-containing probiotic in the diets.

RESULTS

Growth performance

The data presented in Table 2 indicate that from Day 1 to 7, there were no significant effects on body weight gain (BW), feed intake (FI), and feed conversion ratio (FCR). From Day 7 to 21, BW increased (P = 0.038) linearly in response to supplementation with 0 to 0.5% of the probiotic containing *Enterococcus faecium*, but FI and FCR were not affected. From Day 21 to 35 a linear increase (P = 0.001) was observed on BW, without any significant effect on FI and FCR. During the entire experimental period, BW increased (P = 0.005) and there was a trend for linearly increasing FI (P = 0.061), but FCR was not affected by experimental treatments.

Carcass characteristics

Supplementation with 0 to 0.5% probiotic containing 3×10^{11} CFU/g *Enterococcus faecium* resulted in a linear (P = 0.01) increase in relative weight breast muscle. Supplementing the diets with 0 to 0.5% dietary *Enterococcus faecium* had no re-

Table 2. Effect of dietary supplementation of probiotic containing *Enterococcus faecium* on growth performance in broilers

	Probiotic (%)			SE[1]	*P*-value	
	0	0.25	0.5		linear	quadratic
Day 1 to 7						
BWG (g)[2]	99.3	105.6	102.3	1.9	0.269	0.056
FI (g)[3]	129.3	130.9	132.2	3	0.704	0.582
F:G	1.30	1.25	1.28	0.03	0.734	0.352
Day 7 to 21						
BWG (g)	579.3	616.2	623.5	11.5	0.038	0.088
FI (g)	886.1	897.7	910.9	13.3	0.208	0.960
F:G	1.53	1.44	1.48	0.03	0.265	0.083
Day 21 to 35						
BWG (g)	1020.9	1075.2	1113.5	22.2	0.001	0.77
FI (g)	1753.4	1778.7	1800.9	21.8	0.144	0.957
F:G	1.72	1.66	1.62	0.04	0.105	0.753
Overall						
BWG (g)	1699.5	1804.3	1832.1	28.7	0.005	0.291
FI (g)	2768.8	2808.4	2842.7	25.8	0.061	0.933
F:G	1.63	1.56	1.55	0.03	0.071	0.302

[1]standard error, [2]gain in BW per bird, [3]feed intake per bird, number of observations per mean: 6

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Table 3. Effect of dietary supplementation of probiotic containing *Enterococcus faecium* on relative breast meat, abdominal fat and organ weights in broilers

Body part (%)	Probiotic (%)			SE[1]	*P*-value	
	0	0.25	0.5		linear	quadratic
Breast muscle	7.08	8.09	8.80	0.42	0.01	0.76
Abdominal fat	1.56	1.14	1.27	0.15	0.19	0.14
Gizzard	1.17	1.16	1.17	0.05	0.96	0.83
Heart	0.49	0.42	0.43	0.03	0.11	0.25
Liver	2.05	2.13	2.14	0.12	0.60	0.81
Spleen	0.10	0.10	0.09	0.02	0.79	0.50
Bursa of Fabricius	0.12	0.13	0.14	0.02	0.55	0.86

[1]standard error, number of observations per mean: 6

markable effect on relative weights of organs and abdominal fat (Table 3).

Faecal microbiota

The data presented in Table 4 shows that increasing concentrations (0 to 0.5%) of dietary *Enterococcus faecium* decreased (P = 0.008) the count of faecal *Salmonella* linearly. Supplementing diets with 0 to 0.5% of probiotic containing *Enterococcus faecium* resulted in a linear decrease (P = 0.008) in faecal *Salmonella* counts. The inclusion of the probiotic had no marked influence on faecal *E. coli* or *Lactobacillus* counts.

Blood characteristics

Whole blood cells and haptoglobin concentrations were not affected by dietary treatments (Table 5).

DISCUSSION

Various probiotics have been assessed in pigs and poultry, and most studies have reported that supplementing diets with probiotics represents a viable alternative to antibiotics for improved growth performance without adverse effects on mortality in poultry or pigs (Fairchild et al. 2001; Hooge

Table 4. Effect of dietary supplementation of probiotic containing *Enterococcus faecium* on faecal microbiota in broilers

Bacterial counts ($\log_{10}$ cfu/g)	Probiotic (%)			SE[1]	*P*-value	
	0	0.25	0.5		linear	quadratic
Lactobacillus	7.65	7.78	7.80	0.08	0.22	0.54
E. coli	6.56	6.43	6.46	0.06	0.25	0.35
Salmonella	2.72	2.59	2.57	0.04	0.01	0.11

[1]standard error, number of observations per mean: 6

Table 5. Effect of dietary supplementation of probiotic containing *Enterococcus faecium* on blood constituents in broilers

Constituents	Probiotic (%)			SE[1]	*P*-value	
	0	0.25	0.5		linear	quadratic
WBC (10^3/µl)	458.50	497.90	635.20	98.8	0.22	0.69
RBC (10^6/µl)	2.68	2.65	2.71	0.07	0.76	0.63
Lymphocyte (%)	81.78	71.78	71.32	11.9	0.54	0.75
Haptoglobin (mg/l)	161.6	173.3	163.3	1.4	0.93	0.54

[1]standard error, number of observations per mean: 6

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et al. 2004). The results of the current study were consistent with previous studies and demonstrated that supplementing the diet with 0.5% probiotic improved the growth performance over the whole experimental period. Other studies have also shown that supplementing diets with probiotics can improve the growth performance of broilers, and that the inclusion of probiotics may enhance the activity of digestive enzymes, such as proteases, lipases, and amylases, resulting in better nutrient utilisation and consequently improved growth performance (Fuller 2001).

Zamanzad-Ghavidel et al. (2011) reported that relative breast meat in chickens fed a diet containing *Lactobacillus*-based probiotic was higher than in those that did not receive the probiotic, consistent with the findings of the current study. Also Zheng et al. (2015) reported that feeding broiler chickens diets containing *Enterococcus faecium* led to a significant improvement in breast muscle yield. They suggested that the main effects of *Enterococcus faecium* occur in the intestine through modulation of the intestinal microbiota in favour of the host animal and through improved mucosa ultrastructure, enhanced nutrient absorption and reduced energy consumption.

Probiotics can be considered as modulators of the gut environment as they increase the population of beneficial micro-organisms and inhibit the proliferation of pathogens in the intestinal microbiota; consequently, they can improve growth performance (Patterson and Burkholder 2003; Anjum et al. 2005; Higgins et al. 2008). Several studies have confirmed the stimulatory effects of probiotics on the intestinal microbiota (Roth et al. 1992; Depta et al. 1998; Mathew et al. 1998; Jadamus et al. 2001; Scharek et al. 2005; Reiter et al. 2006; Scharek et al. 2007; Lodemann et al. 2008). Pajarillo et al. (2015) reported that supplementation of *Enterococcus faecium* NCIMB 11181 to a swine diet significantly increased faecal *Lactobacilli* counts and reduced *E. coli* counts. In agreement with our findings, Chen et al. (2005) reported that supplementing growing pig diets with a probiotic did not affect whole blood cell counts.

In conclusion, the current study demonstrates that inclusion of a probiotic containing *Enterococcus faecium* improves the growth performance and favourably alters the intestinal microbiota by increasing *Lactobacilli* and decreasing *E. coli* and *Salmonella* populations in broilers. Importantly, inclusion of the probiotic in the diet does not exert any negative effects on breast muscle colour, relative weights of organs and blood profile.

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Corresponding Author:

I.H. Kim, Dankook University, Department of Animal Resource and Science, Cheonan, Choongnam, 330-714 Republic of Korea
E-mail: inhokim@dankook.ac.kr