

Effect of *Bacillus subtilis*, *Clostridium butyricum* and *Lactobacillus acidophilus* endospores on growth performance, nutrient digestibility, meat quality, relative organ weight, microbial shedding and excreta noxious gas emission in broilers

M.M. HOSSAIN, M. BEGUM, I.H. KIM

Department of Animal Resource and Science, Dankook University, Cheonan, Choongnam, Republic of Korea

ABSTRACT: This study was conducted to evaluate the effects of *Bacillus subtilis*, *Clostridium butyricum* and *Lactobacillus acidophilus* (tri-strain probiotics, TSP) endospores in broilers. TSP can benefit the host animal by increasing nutrient absorption from the gastrointestinal tract and altering the intestinal ecosystem in poultry. A total of 500 day-old ROSS 308 mixed sex broiler chickens with an average initial body weight (IBW) of $45 \text{ g} \pm 0.6 \text{ g}$ were used in this 35-day feeding study. Broiler chickens were randomly allotted to one of five dietary treatments: (1) CON (antibiotic free diet), (2) ANT1 (CON + enramycin 5 ppm), (3) ANT2 (CON + avilamycin 5 ppm), (4) TSP1 (CON + 0.1% TSP), and (5) TSP2 (CON + 0.2% TSP) with five replicates per treatment and 20 chicks per pen. Broiler chickens fed on TSP diets exhibited linearly increasing body weight gain (BWG) and decreased feed conversion ratio (FCR) compared to those on the CON diet ($P < 0.05$; Day 21 to Day 35 and Day 1 to Day 35, respectively). Further, dry matter (DM) and nitrogen (N) digestibility were improved ($P < 0.05$) in the TSP treatment at the end of study. The inclusion of TSP reduced (linear, $P = 0.02$) meat lightness (L^*) compared with CON and ANT treatments. Broiler chickens fed with TSP diets had relatively higher (linear, $P < 0.05$) bursa weight than those fed with ANT and CON diets. The supplementation of TSP increased ($P < 0.05$) the ileal and caecal *Lactobacillus* count compared with CON and ANT diets. The ileal and caecal *Escherichia coli* and caecal *Clostridium perfringens* counts were reduced ($P < 0.05$) in the TSP2 group compared with the CON group. Broiler chickens fed with TSP diets exhibited increased ($P < 0.05$) caecal *Bifidobacteria* counts compared with CON and ANT diets. Excreta ammonia (NH_3) gas emission was lower ($P < 0.05$) with the TSP treatment compared with the CON treatment. In conclusion, the supplementation of TSP improved growth performance, nutrient digestibility, meat quality, gut health and reduced noxious gas emission in broilers.

Keywords: ammonia gas emission; body weight gain; bursa weight; *Escherichia coli*; *Lactobacillus*; meat lightness

List of abbreviations

BWG = body weight gain, **FCR** = feed conversion ratio, **DM** = dry matter, **L^*** = meat lightness, **N** = nitrogen, **NH_3** = ammonia, **TSP** = tri-strain probiotics

In the poultry business, antibiotics are used worldwide with the goal of enhancing growth performance and preventing illnesses. However, repeated use of antibiotics in poultry diets results in undesirable outcomes such as the resistance of microorganisms to antibiotics, accumulation

of antibiotic residues in animal products and the environment, imbalance of normal microflora and reduction in beneficial intestinal microflora (Hinton et al. 1986; Sinol et al. 2012). This has resulted in severe restrictions or total bans on the employment of antibiotics in animal and poultry

production in several countries. As a result, the poultry industry must develop alternatives to antibiotics to maintain health and performance under commercial conditions. Probiotics have been increasingly adopted as an alternative to antibiotic growth promoters in poultry diets (Mountzouris et al. 2010; Zhang and Kim 2014). Probiotics, which are live cultures of harmless bacteria or yeast species that equilibrate intestinal microflora to benefit the host (Ferencik et al. 2000; Chen et al. 2013), have been demonstrated to be helpful in maintaining the intestinal ecosystem and enhancing animal health. Probiotics have been reported to improve growth performance and nutrient digestibility, balance intestinal microflora, promote immune function and benefit the intestinal morphology (Li et al. 2009; Sinol et al. 2012; Zhang and Kim 2013). Although, the efficiency of probiotics depends upon various factors such as, selection of strain, administration level, application method, ability of the selected strain to survive at environmental temperatures, long-term storage and viability.

Among a number of bacterial species used as probiotics, spore-forming *Bacillus* spp. has been identified as a suitable probiotic because of the resistance of its spores to harsh conditions and long-term storage at ambient temperature (Sinol et al. 2012; Chen et al. 2013). *Clostridium butyricum* is a butyric acid bacterium that was isolated from soil and healthy animal and human faecal matter (Finegold et al. 1983). *Clostridium butyricum* can produce endospores, which is critical for its capacity to survive at lower pH and relatively greater bile concentrations compared with *Lactobacillus* and *Bifidobacterium* (Kong et al. 2011). In an earlier study the impact of a probiotic complex (*Bacillus subtilis* endospores and *Clostridium butyricum* endospores) in increasing growth performance and meat quality in growing-finishing pigs was confirmed (Meng et al. 2010). However, to the best of our knowledge, the effects of *Bacillus subtilis* DSM 17299, *Clostridium butyricum* and *Lactobacillus acidophilus* endospores in broiler chickens have not been investigated in detail. Thus, this study was designed to assess the effect of *Bacillus subtilis* DSM 17299, *Clostridium butyricum* and *Lactobacillus acidophilus* endospores on growth performance, nutrient digestibility, meat quality, relative organ weight, microbial shedding and excreta noxious gas emission in broiler chickens.

MATERIAL AND METHODS

Source of TSP. The probiotic preparation used in the current experiments was manufactured by a commercial company (Probiion, Woogene B & G Co. Ltd., Seoul, Republic of Korea). The product is composed of a mixture of spray-dried spore-forming bacteria, which was guaranteed to contain 2×10^8 viable spores/kg of *Bacillus subtilis* DSM 17299, *Clostridium butyricum* and *Lactobacillus acidophilus*.

Table 1. Diet composition (as-fed basis)

| Ingredients (%) | Starter (1–21 day) | Finisher (21–35 day) |
|---|--------------------|----------------------|
| Corn grain | 57.84 | 63.49 |
| Corn gluten meal | 9.30 | 5.20 |
| Soybean meal | 21.60 | 21.60 |
| Fish meal (60%) | 4.18 | 2.70 |
| Wheat bran | 0.40 | 0.40 |
| Tallow | 3.67 | 3.73 |
| Limestone | 1.60 | 1.60 |
| TCP | 0.50 | 0.45 |
| L-lysine | 0.18 | 0.10 |
| Salt | 0.25 | 0.25 |
| Vit.-Min. mixture ¹ | 0.25 | 0.25 |
| Choline | 0.22 | 0.22 |
| Selenium | 0.01 | 0.01 |
| Calculated composition² | | |
| ME (kcal/kg) | 3,200 | 3,200 |
| CP (%) | 23.09 | 20.08 |
| Lys (%) | 1.21 | 1.05 |
| Met + Cys (%) | 0.50 | 0.41 |
| Ca (%) | 1.02 | 0.93 |
| Total P (%) | 0.57 | 0.51 |
| Analysed composition | | |
| CP (%) | 23.05 | 20.05 |
| Lys (%) | 1.20 | 1.04 |
| Met + Cys (%) | 0.49 | 0.41 |
| Ca (%) | 1.02 | 0.92 |
| Total P (%) | 0.55 | 0.50 |

¹Vit.-Min. = mixture contains the following amounts of micronutrients per kg: Vitamin A, 1 600 000 IU; Vitamin D3, 300 000 IU; Vitamin K3, 130 mg; Vitamin B2, 1000 mg; Niacin, 2000 mg; Ca-Pantothenate, 800 mg; folic acid, 60 mg; DL-methionine, 6000 mg; Mn, 12 000 mg; Zn, 9000 mg; Fe, 4000 mg; Cu, 500 mg; I, 250 mg; Co, 100 mg; Ca, 7140 mg; BHT, 6000 mg

²values for ingredients used in diet formulation were based on broilers requirements in NRC (1994)

doi: 10.17221/7981-VETMED

Experimental design, animals and diets. A total of 500 day-old ROSS 308 mixed sex broiler chickens with an average initial body weight (IBW) of $45 \text{ g} \pm 0.6 \text{ g}$ were randomly allotted to five treatments with five replicates per treatment and 20 chicks per pen. Broiler chickens were fed with starter (one to 21 days) and finisher (21 to 35 days) diets in the form of mash. All nutrients in diets were formulated to meet or exceed the recommendations of the NRC (1994) for broiler chickens (Table 1). Dietary treatments were (1) CON (antibiotic free diet), (2) ANT1 (CON + enramycin 5 ppm), (3) ANT2 (CON + avilamycin 5 ppm), (4) TSP1 (CON + 0.1% TSP), and (5) TSP2 (CON + 0.2% TSP). It is well accepted that enramycin and avilamycin can be used as antibiotic growth promoters (AGPs) to compare the efficacy of probiotics (Mountzouris et al. 2010; Abudabos 2012). Broiler chickens were raised in a temperature-controlled room with three floors of stainless steel pens of identical size ($1.75 \times 1.55 \text{ m}$). Room temperature began at $33 \text{ }^\circ\text{C}$ from Day 1 to Day 3 and was reduced gradually to $24 \text{ }^\circ\text{C}$ until the end of the experiment; the relative humidity was around 60%. The broiler chickens had free access to diet and water. The Animal Care and Use Committee of Dankook University approved all of the experimental protocols used in the current study.

Chemical analysis. Feed samples were ground to pass through a 1-mm screen, after which they were analysed for DM (method 934.01; AOAC 2000), N (method 968.06; AOAC 2000), calcium (Ca, method 984.01; AOAC 1995), and phosphorus (P, method 965.17; AOAC 1995). Individual AA composition was measured using an AA analyser (Beckman 6300; Beckman Coulter Inc., Fullerton, CA) after 24-h hydrolysis in HCl. For the determination of cysteine (Cys) and methionine (Met), the samples were oxidized with performic acid overnight at $0 \text{ }^\circ\text{C}$. Performic acid is an oxidizing reagent that converts Cys quantitatively to cysteic acid and Met to Met sulfone (Moore 1963). Nitrogen was determined (Kjectec 2300 Nitrogen Analyser; Foss Tecator AB, Hoeganaes Sweden) and crude protein (CP) was calculated as $\text{N} \times 6.25$. Gross energy (GE) was analysed using an oxygen bomb calorimeter (Parr 1600 Instrument Co., Moline, IL, USA).

Sampling and measurements. Birds were weighed on Day 1, Day 21 and Day 35, with birds in each pen being weighed as a group. Mean BW for each treatment was calculated from the pen replicates for each weighing day. The BWG was calculated individu-

ally for each period and cumulatively based on pen weights. For the same period, feed intake (FI) of each pen as a group was measured as BW (Day 21 and Day 35) with cumulative averages calculated. The FCR was calculated as $(\text{feed intake})/(\text{body weight gain})$.

At the end of the experiment, apparent total tract digestibility (ATTD) of DM, N and energy (E) were determined using chromic oxide as an indicator (Fenton and Fenton 1979). All broiler chicks were fed diets mixed with 2% Cr_2O_3 for seven days before excreta collection at Week 5. All excreta were pooled by pen and mixed, after which a representative sample was stored in a freezer at $-20 \text{ }^\circ\text{C}$ until analysis. Before chemical analysis, the excreta samples were thawed and dried for 72 h at $50 \text{ }^\circ\text{C}$ in a forced-air oven (model FC-610, Advantec, Toyo Seisakusho Co. Ltd., Tokyo, Japan), after which they were finely ground to a size that could pass through a 1-mm screen. All feed and excreta samples were then analysed for DM, N and E as described above. Chromium was analysed using UV absorption spectrophotometry (UV-1201, Shimadzu, Kyoto, Japan). The ATTD was then calculated using the following formula:

$$\text{digestibility (\%)} = \{1 - [(\text{Ne} \times \text{Cd})/(\text{Nd} \times \text{Ce})]\} \times 100$$

where:

Ne = nutrient concentration in excreta (% DM)

Nd = nutrient concentration in diet (% DM)

Cd = chromium concentration in diet (% DM)

Ce = chromium concentration in excreta (% DM)

At the end of experiment, five broiler chickens were randomly selected from each treatment (one bird per pen), euthanised by cervical dislocation and weighed individually. The liver, spleen, bursa, breast, gizzard and abdominal fat were removed by trained personnel and weighed. Relative organ weight (percent of live BW) was calculated. The breast meat Hunter lightness (L^*), redness (a^*) and yellowness (b^*) values were measured using a Minolta CR410 Chroma meter (Konica Minolta Sensing Inc., Osaka, Japan). Yan et al. (2011) described a similar method for calculating meat colour. Cook loss was determined as described previously by Sullivan et al. (2007). Briefly, 5 g of breast meat were heat-treated in plastic bags separately in a water bath ($100 \text{ }^\circ\text{C}$) for 5 min. Samples were cooled at room temperature. Cooking loss was calculated as $(\text{sample weight before cooking} - \text{sample weight after cooking})/\text{sample weight before cooking} \times 100$. Drip loss was measured as de-

scribed by Honikel et al. (1986). Two (2.5 × 2.5 cm) chops were weighed, placed in a drip loss tube (C. Christensen Laboratory, Hillerod, Denmark) and held at 2 °C for 24 h. Then, meat samples were removed, blotted dry on paper towels and reweighed. Differences between sample weights were used to calculate drip loss percent. Duplicate pH values for each sample were measured using a pH meter (Fisher Scientific, Pittsburgh, PA). To determine TBARS, 5 g of breast meat with 20% trichloroacetic acid (TCA), 12.5 ml 2M phosphoric acid were homogenised in one minute at a speed of 14 000 rpm using a homogenizer (AM-8, Nissci Co., Japan) and the extract was titrated by methanol and then homogenised with 5 ml distilled water. Finally, 25 ml of the mixture solution were centrifuged for 15 min at a speed of 1500 rpm at 4 °C. Two ml of the solution were mixed with 2ml 0.005M thiobarbituric acid (TBA) and incubated in a 95 °C water bath for 30 min and then spectrophotometric analysis was performed under 530 nm.

The same slaughtered broiler chickens were used for microbial counts. Ileal and caecal contents were collected into Qorpak glass containers (118 ml) under CO₂, sealed and placed on ice until transported to the laboratory for enumeration of microbial populations. Caecal and ileal samples were assessed for populations of *Lactobacillus*, *Escherichia coli*, *Clostridium perfringens*, and *Bifidobacteria*. One gram of the composite excreta sample from each cage was diluted with 9 ml of 1% peptone broth (Becton, Dickinson and Co., Franklin Lakes, NJ) and then homogenised. Viable counts of bacteria in the caecal and ileal samples were then conducted by plating serial 10-fold dilutions (in 1% peptone solution) onto lactobacilli medium III agar plates (Medium 638; DSMZ, Braunschweig, Germany), MacConkey agar plates (Difco Laboratories, Detroit, MI), Perfringens agar base (Perfringens TSC Agar; Oxoid, Basingstoke, UK) and Wilkins-Chalgren agar (Oxoid, Nepean, Ontario, Canada) supplemented with glacial acetic acid (1 ml/l) and mupirocin (100 mg/l) extracted from antimicrobial discs (Oxoid; Rada et al. 1999) to isolate the *Lactobacillus*, *Escherichia coli*, *Clostridium perfringens* and *Bifidobacteria*, respectively. The lactobacilli medium III agar plates were then incubated for 48 h at 37 °C under anaerobic conditions. The MacConkey and Perfringens agar plates were incubated for 24 h at 37 °C and the Wilkins-Chalgren agar plates were incubated for 72 h at 37 °C. The

microflora colonies were counted immediately after removal from the incubator. The concentration of microflora was expressed as log₁₀ colony-forming units per gram of intestinal content.

Excreta NH₃ and hydrogen sulphide (H₂S) contents were measured at day 35 according to the method described by Zhang and Kim (2013). A total of 300 g excreta samples from each pen were collected in 2.6-l plastic boxes, with adhesive tape-sealed holes for pumps. The samples were allowed to ferment for one day at room temperature (25 °C), after which 100 ml of the headspace air were sampled from approximately 2.0 cm above the excreta sample. The concentrations of NH₃ and H₂S were measured within the scope of 5.0–100.0 ppm (No. 3La, detector tube; Gastec Corp. Kanagawa, Japan) and 2.0–20.0 ppm (4LK, detector tube; Gastec Corp.). After collection, the boxes were re-sealed with adhesive plaster to measure the excreta noxious content at Day 3 and Day 5 as aforementioned. Prior to measurement, the excreta samples were manually shaken for approximately 30 s to disrupt any crust formation on the surface of the excreta sample and to homogenise the samples. The excreta volatile fatty acid (VFA) concentration was determined by the method of Erwin et al. (1961). The VFA concentration in the supernatant liquid was determined using a gas chromatograph (VARIAN, CP-3800, CA, USA).

Statistical analysis. Data were statistically analysed by ANOVA using the GLM procedure of SAS/STAT[®]9.2 (SAS 2008), for a randomised complete block design. Differences among all treatments were separated by Tukey's range test. Before conducting statistical analysis of the *Lactobacillus*, *Escherichia coli*, *Clostridium perfringens* and *Bifidobacteria* counts, the value was transformed logarithmically. The linear and quadratic effects of TSP among treatments were analysed using a contrast statement. Orthogonal contrasts were used for the effects of ANT vs. TSP treatments. Variability in the data was expressed as the pooled SE and probability values of less than 0.05 were considered significant.

RESULTS

Growth performance and ATTD of nutrients

In this study, the inclusion of TSP linearly improved BWG and FCR in the grower and overall experimental period compared with the CON diet

doi: 10.17221/7981-VETMED

Table 2. Effect of *Bacillus subtilis*, *Clostridium butyricum* and *Lactobacillus acidophilus* endospores on the growth performance of broilers

| | Treatment | | | | | SE ¹ | P-values | | ANT vs. TSP |
|----------------------|--------------------|--------------------|---------------------|--------------------|--------------------|-----------------|----------|-----------|-------------|
| | CON | ANT1 | ANT2 | TSP1 | TSP2 | | linear | quadratic | |
| BWG (g/chick) | | | | | | | | | |
| Day 1 to 21 | 880 | 910 | 907 | 990 | 1001 | 58 | 0.39 | 0.17 | 0.31 |
| Day 21 to 35 | 886 ^b | 882 ^b | 919 ^{ab} | 955 ^a | 964 ^a | 17 | 0.04 | 0.24 | 0.12 |
| Day 1 to 35 | 1.766 ^b | 1.792 ^b | 1.816 ^b | 1.945 ^a | 1.965 ^a | 18 | 0.04 | 0.31 | 0.66 |
| FI (g/chick) | | | | | | | | | |
| Day 1 to 21 | 1.095 | 1.734 | 1.149 | 1.221 | 1.207 | 65 | 0.53 | 0.14 | 0.84 |
| Day 21 to 35 | 1.716 | 1.927 | 1.709 | 1.668 | 1.756 | 44 | 0.32 | 0.78 | 0.54 |
| Day 1 to 35 | 2.923 | 2.996 | 2.940 | 3.104 | 3.144 | 95 | 0.33 | 0.38 | 0.32 |
| FCR | | | | | | | | | |
| Day 1 to 21 | 1.244 | 1.253 | 1.266 | 1.234 | 1.206 | 0.08 | 0.42 | 0.61 | 0.56 |
| Day 21 to 35 | 1.937 ^a | 1.965 ^a | 1.859 ^{ab} | 1.828 ^b | 1.822 ^b | 0.04 | 0.01 | 0.31 | 0.27 |
| Day 1 to 35 | 1.655 ^a | 1.672 ^a | 1.619 ^b | 1.596 ^b | 1.600 ^b | 0.02 | 0.02 | 0.34 | 0.48 |

CON = antibiotic free diet; ANT1 = CON + enramycin 5 ppm; ANT2 = CON + avilamycin 5 ppm; TSP1 = CON + 0.1% TSP; TSP2 = CON + 0.2% TSP

¹pooled SE

^{a,b}means in the same row with different superscripts differ ($P < 0.05$)

($P < 0.05$, Table 2). No significant differences were observed in FI throughout the whole experimental period. TSP2 treatment increased DM and N digestibility ($P = 0.03$ and $P = 0.02$, respectively) compared with the CON treatment (Table 3). No differences were observed in E digestibility among the treatments.

ANT1 and ANT2 treatments (51.2, 51.8 vs. 56.2, 53.2 and 55.4; $P = 0.02$; Table 4). Moreover, broilers fed TSP diets showed a higher relative bursa weight compared with other treatments ($P = 0.02$). No significant differences were observed in other parameters ($P > 0.05$).

Meat quality and relative organ weight

The supplementation of TSP1 and TSP2 linearly increased relative bursa weight compared with CON,

Microbial shedding and excreta noxious gas emission

The impact of TSP on ileal and caecal microbial shedding is shown in Table 5. In this study, broil-

Table 3. Effect of *Bacillus subtilis*, *Clostridium butyricum* and *Lactobacillus acidophilus* endospores on the nutrient digestibility of broilers

| Item (%) | Treatment | | | | | SE ¹ | P-values | | ANT vs. TSP |
|----------|--------------------|---------------------|--------------------|---------------------|--------------------|-----------------|----------|-----------|-------------|
| | CON | ANT1 | ANT2 | TSP1 | TSP2 | | linear | quadratic | |
| DM | 70.48 ^b | 72.36 ^b | 71.89 ^b | 77.43 ^a | 77.22 ^a | 1.53 | 0.03 | 0.18 | 0.04 |
| N | 68.43 ^b | 69.21 ^{ab} | 68.03 ^b | 69.49 ^{ab} | 71.93 ^a | 1.23 | 0.02 | 0.47 | 0.41 |
| E | 72.84 | 71.94 | 74.33 | 72.69 | 73.21 | 1.48 | 0.36 | 0.44 | 0.21 |

CON = antibiotic free diet; ANT1 = CON + enramycin 5 ppm; ANT2 = CON + avilamycin 5 ppm; TSP1 = CON + 0.1% TSP; TSP2 = CON + 0.2% TSP

¹pooled SE

^{a,b}means in the same row with different superscripts differ ($P < 0.05$)

Table 4. Effects of *Bacillus subtilis*, *Clostridium butyricum* and *Lactobacillus acidophilus* endospores on meat quality and relative organ weight of broilers

| Item | Treatment | | | | | SE ¹ | P-values | | ANT vs. TSP |
|----------------------------------|--------------------|--------------------|---------------------|--------------------|--------------------|-----------------|----------|-----------|-------------|
| | CON | ANT1 | ANT2 | TSP1 | TSP2 | | linear | quadratic | |
| Meat colour | | | | | | | | | |
| <i>L</i> * | 56.2 ^b | 53.2 ^b | 55.4 ^b | 51.2 ^a | 51.8 ^a | 2.01 | 0.02 | 0.32 | 0.04 |
| <i>a</i> * | 14.9 | 15.6 | 15.9 | 14.2 | 15.2 | 1.20 | 0.19 | 0.55 | 0.55 |
| <i>b</i> * | 8.54 | 8.33 | 8.92 | 8.66 | 7.99 | 0.78 | 0.65 | 0.34 | 0.29 |
| pH | 6.2 | 6.65 | 6.21 | 6.29 | 6.37 | 0.18 | 0.33 | 0.29 | 0.17 |
| Drip loss (%) | 2.43 | 2.62 | 2.21 | 2.09 | 1.98 | 0.33 | 0.28 | 0.35 | 0.25 |
| Cook loss (%) | 16.7 | 17.2 | 15.7 | 15.1 | 15.9 | 0.51 | 0.81 | 0.47 | 0.96 |
| TBARS (mg/kg) | 0.024 | 0.027 | 0.025 | 0.021 | 0.018 | < 0.01 | 0.06 | 0.21 | 0.41 |
| Relative organ weight (%) | | | | | | | | | |
| Liver | 2.52 | 2.49 | 2.66 | 2.60 | 2.57 | 0.13 | 0.57 | 0.19 | 0.42 |
| Spleen | 0.058 | 0.077 | 0.078 | 0.065 | 0.071 | < 0.01 | 0.61 | 0.29 | 0.71 |
| Bursa | 0.115 ^b | 0.166 ^b | 0.143 ^{ab} | 0.171 ^a | 0.182 ^a | 0.01 | 0.02 | 0.31 | 0.09 |
| Breast | 8.34 | 8.45 | 8.72 | 8.64 | 8.51 | 0.22 | 0.33 | 0.59 | 0.24 |
| Gizzard | 1.67 | 1.64 | 1.72 | 1.59 | 1.57 | 0.04 | 0.54 | 0.60 | 0.42 |
| Abdominal fat | 1.62 | 1.54 | 1.51 | 1.55 | 1.59 | 0.03 | 0.19 | 0.77 | 0.17 |

CON = antibiotic free diet; ANT1 = CON + enramycin 5 ppm; ANT2 = CON + avilamycin 5 ppm; TSP1 = CON + 0.1% TSP; TSP2 = CON + 0.2% TSP; *L** = indicates lightness; *a** = indicates redness; *b** = indicates yellowness

¹pooled SE

^{a,b}means in the same row with different superscripts differ ($P < 0.05$)

ers fed TSP2 exhibited increased ($P < 0.05$) ileal *Lactobacillus* but reduced *Escherichia coli* counts compared with CON and ANT diets. Moreover, TSP1 and TSP2 diets exhibited increased caecal

Lactobacillus and *Bifidobacteria* but diminished *Escherichia coli* and *Clostridium perfringens* counts compared with CON diets ($P < 0.05$). The excreta NH_3 was reduced ($P < 0.05$) by TSP2 supplement-

Table 5. Effects of *Bacillus subtilis*, *Clostridium butyricum* and *Lactobacillus acidophilus* endospores on *Clostridium perfringens* on ileal and caecal microbial shedding in broilers

| Item (log ₁₀ CFU/g) | Treatment | | | | | SE ¹ | P-values | | ANT vs. TSP |
|--------------------------------|-------------------|--------------------|--------------------|-------------------|-------------------|-----------------|----------|-----------|-------------|
| | CON | ANT1 | ANT2 | TSP1 | TSP2 | | linear | quadratic | |
| Ileum | | | | | | | | | |
| <i>Lactobacillus</i> | 8.55 ^b | 8.65 ^b | 8.62 ^b | 8.83 ^a | 8.91 ^a | 0.09 | < 0.01 | < 0.01 | 0.03 |
| <i>Escherichia coli</i> | 7.79 ^a | 7.51 ^b | 7.55 ^b | 7.41 ^b | 7.17 ^c | 0.07 | < 0.01 | 0.03 | 0.31 |
| <i>Clostridium perfringens</i> | 7.64 | 7.55 | 7.52 | 7.41 | 7.35 | 0.39 | 0.44 | 0.75 | 0.25 |
| <i>Bifidobacteria</i> | 8.37 | 8.39 | 8.59 | 8.71 | 8.81 | 0.30 | 0.11 | 0.56 | 0.51 |
| Caecum | | | | | | | | | |
| <i>Lactobacillus</i> | 9.03 ^c | 9.47 ^{bc} | 9.25 ^c | 9.71 ^a | 9.76 ^a | 0.15 | < 0.01 | 0.03 | 0.04 |
| <i>Escherichia coli</i> | 8.11 ^a | 7.87 ^{ab} | 7.89 ^{ab} | 7.67 ^b | 7.55 ^b | 0.11 | 0.01 | 0.01 | 0.41 |
| <i>Clostridium perfringens</i> | 8.15 ^a | 7.98 ^a | 7.95 ^a | 7.07 ^b | 7.15 ^b | 0.25 | 0.72 | 0.04 | 0.61 |
| <i>Bifidobacteria</i> | 8.45 ^b | 8.50 ^b | 8.61 ^b | 8.82 ^a | 8.97 ^a | 0.09 | 0.02 | 0.25 | 0.55 |

CON = antibiotic free diet; ANT1 = CON + enramycin 5 ppm; ANT2 = CON + avilamycin 5 ppm; TSP1 = CON + 0.1% TSP; TSP2 = CON + 0.2% TSP

¹pooled SE

^{a,b,c}means in the same row with different superscripts differ ($P < 0.05$)

doi: 10.17221/7981-VETMED

Table 6. Effects of *Bacillus subtilis*, *Clostridium butyricum* and *Lactobacillus acidophilus* endospores on excreta noxious gas emission in broilers

| Item | Treatment | | | | | SE ¹ | P-values | | ANT vs. TSP |
|-----------------------------|-----------------|------------------|------------------|-----------------|-----------------|-----------------|----------|-----------|-------------|
| | CON | ANT1 | ANT2 | TSP1 | TSP2 | | linear | quadratic | |
| NH₃ (ppm) | | | | | | | | | |
| Day 1 | 36 ^a | 34 ^b | 32 ^{ab} | 29 ^b | 30 ^b | 0.89 | < 0.01 | 0.01 | 0.41 |
| Day 3 | 55 ^a | 56 ^a | 54 ^a | 47 ^b | 49 ^b | 0.95 | 0.03 | 0.07 | 0.01 |
| Day 5 | 80 ^a | 76 ^{ab} | 77 ^{ab} | 74 ^b | 70 ^c | 1.39 | 0.03 | 0.26 | 0.21 |
| H₂S (ppm) | | | | | | | | | |
| Day 1 | 1.7 | 1.6 | 1.8 | 1.5 | 1.7 | 0.31 | 0.60 | 0.86 | 0.41 |
| Day 3 | 2.2 | 2.4 | 2.3 | 2.0 | 2.1 | 0.20 | 0.31 | 0.63 | 0.32 |
| Day 5 | 3.7 | 3.6 | 4.0 | 3.8 | 3.5 | 0.42 | 0.67 | 0.33 | 0.68 |
| VFA (μmol/N) | | | | | | | | | |
| Propionic acid | 45.9 | 40.1 | 43.8 | 45.9 | 45.2 | 2.51 | 0.43 | 0.66 | 0.45 |
| Butyrate | 24.5 | 23.1 | 21.8 | 23.2 | 22.0 | 1.60 | 0.31 | 0.74 | 0.97 |

CON = antibiotic free diet; ANT1 = CON + enramycin 5 ppm; ANT2 = CON + avilamycin 5 ppm; TSP1 = CON + 0.1% TSP; TSP2 = CON + 0.2% TSP

¹pooled SE

^{a,b,c}means in the same row with different superscripts differ ($P < 0.05$)

tation compared with the CON (Table 6). No significant differences were observed in H₂S and VFA concentrations among the treatments.

DISCUSSION

In the experiments described here, BWG, DM and N digestibility were increased and FCR was reduced; however, dietary supplementation of TSP had no effect on the FI. In agreement with our results, Zhang and Kim (2014) reported that dietary supplementation with 1×10^5 CFU or 2×10^5 CFU/kg of multistrain probiotic significantly improved BWG and reduced FCR in broiler chickens compared with chickens from the control treatment. Likewise, Sinol et al. (2012) reported that inclusion of *Bacillus subtilis* C-3102 in broiler chicken diets resulted in improved FCR (21–42 days) and weight gain (42 days). Zhang et al. (2013) found that BWG was increased by the administration of 10^5 and 10^8 CFU/kg of *Bacillus*-based probiotic. Yang et al. (2012) found that adding 2×10^7 CFU or 3×10^7 CFU/kg of *Clostridium butyricum* to the diet improved the ADG in broiler chickens. Similar positive effects were also reported by other researchers (Talebi et al. 2008; Zhou et al. 2010). However, it has also been reported that probiotics exerted only a minimal effect on growth performance in broiler chickens (Lee et

al. 2010; Sinol et al. 2012). The inconsistency might be attributed to the strains of probiotic, technique of preparation, administration dosage, diet composition, bird age and hygienic status (Sinol et al. 2012; Zhang et al. 2012).

Improvement in growth performance and feed efficiency of broiler chickens supplemented with different strains of probiotics (Sinol et al. 2012; Zhang and Kim 2014) are thought to be induced by the cumulative effect of probiotic action including the improvement of feed intake and digestion (Shim et al. 2010), increased digestive enzyme activity and decreased NH₃ production (Chen et al. 2013), maintenance of beneficial microbial populations (Chen et al. 2013) and alteration of bacterial metabolism (Sinol et al. 2012). In this study, DM and N digestibility were increased by dietary supplementation of TSP. The underlying mechanism is related to the fact that increased villus height and villus height-to-crypt depth ratio are directly correlated with an increased epithelial turnover (Sinol et al. 2012), and longer villi are linked with activation of cell mitosis (Samanya and Yamauchi 2002). Shortening of villi and deeper crypts lead to poor nutrient absorption, increased secretion in gastrointestinal tract and reduced performance (Xu et al. 2003). Intestinal morphology including duodenal and ileal villus height and crypt depth and villus height-to-crypt depth ratio are indicative

of gut health in broiler chickens. Sen et al. (2012) reported that supplementation of *Bacillus subtilis* LS 1-2 in broiler chicken diets resulted in increased villus height and villus height-to-crypt depth ratio in duodenum and ileum at Day 35. Thus, in the present study, improved growth performance in birds fed *Bacillus subtilis* DSM 17299, *Clostridium butyricum* and *Lactobacillus acidophilus* endospores might be due to greater nutrient retention and improved gut health.

In the current study, the inclusion of tri-strain probiotics increased relative weight bursa to the BW compared to the control group. Chen et al. (2013) suggested measuring immune organ weight as a method for evaluating immune status in chickens. Willis et al. (2007) also suggested that the bursa was the primary lymphoid organ in broiler chickens and concluded that probiotics could increase the relative weight of bursa to BW. The colour of broiler chicken meat is important because consumers regard it as a sign of a fresh and high quality product. Froning (1995) had recommended that lower L^* value, showing the level of paleness, could reduce the broiler chicken consumption. Chen et al. (2013) also suggested that broiler chicken size could affect the sensory attributes of breast meat. In this study, broiler chickens that were given probiotics had reduced L^* values compared to those that were given the non-probiotic treatment. Chen et al. (2013) also found similar results after supplementation of probiotics in broiler chickens. In the present study, we hypothesise that the difference in L^* values may be due to the growth rate in broiler chickens. Collectively, our results suggest that the inclusion of probiotics could reduce the lightness value of the breast muscle in broiler chickens and subsequently increase their market price.

Probiotics beneficially affect the host animal by improving intestinal balance (Chen et al. 2013), creating gut micro-ecological conditions that suppress harmful microorganisms like *Clostridium* and *Coliforms* (Shim et al. 2010; Sinol et al. 2012) and by favouring beneficial microorganisms like *Lactobacillus* and *Bifidobacterium*. In our study, the dietary supplementation of *Bacillus subtilis* DSM 17299, *Clostridium butyricum* and *Lactobacillus acidophilus* endospores increased gut *Lactobacillus* and *Bifidobacteria* counts and decreased *Escherichia coli* and *Clostridium perfringens* counts compared with those of other treatments. Suppression of harmful organisms resulted

in higher growth and metabolism of beneficial microorganism which might have improved the performance and nutrient retention in the present study. A number of previous studies have demonstrated the potential of probiotics to enhance the growth of beneficial bacteria and suppress potentially pathogenic bacteria in the intestine (Sinol et al. 2012; Zhang and Kim 2013). Ng et al. (2009) suggested that probiotics may influence the intestinal microflora by facilitating antibody production, promoting epithelial barrier integrity, augmenting toll-like receptor signalling and through other mechanisms. Thus, we can confirm the effect of TSP on gut microflora in broiler chickens.

Ammonia is a major aerial pollutant originating from farm animal operations and poultry is one of the principal contributors among domestic animals (Zhang et al. 2013). High concentrations of NH_3 or H_2S can cause hazardous effects to humans and animals (Drummond et al. 1980; Zhang and Kim 2014). It has been suggested that excreta ammonia gas emission is related to nutrient utilisation and the intestinal microbial ecosystem (Ferket et al. 2002). In our study, NH_3 gas emission was reduced by addition of *Bacillus subtilis* DSM 17299, *Clostridium butyricum* and *Lactobacillus acidophilus* to the diet. In agreement with our results, Zhang et al. (2013) found that dietary supplementation with *Bacillus subtilis* UBT-MO2 resulted in 26.5% and 37.9% lower excreta NH_3 concentrations compared with no supplementation. Moreover, Zhang and Kim (2013) also reported that the dietary application of probiotics could reduce the NH_3 contents in the excreta. Accordingly, the reduction of excreta NH_3 may be considered as a manifestation of improved nutrient digestibility and an enhanced gut micro-ecological condition in the current study.

In conclusion, the inclusion of TSP might improve growth performance and nutrient digestibility. Broiler chickens fed with TSP may exhibit increased relative bursa weight and reduced meat lightness. Under the conditions of our study, TSP supplementation could enhance gut health and reduce the noxious toxic gas emission in broiler chickens.

REFERENCES

- Abudabos AM (2012): Effect of Primalac® or Enramycin supplementation on performance, intestinal morphology

doi: 10.17221/7981-VETMED

- and microbiology of broilers under *Clostridium perfringens* challenge. *Journal of Food, Agriculture and Environment* 10, 595–599.
- AOAC (1995): Official method of analysis. 16th ed. Association of Official Analytical Chemists, Washington, DC.
- AOAC (2000): Official method of analysis. 17th ed. Association of Official Analytical Chemists, Gaithersburg, MD.
- Chen W, Wang JP, Yan L, Huang YQ (2013): Evaluation of probiotics in diets with different nutrient densities on growth performance, blood characteristics, relative organ weight and breast meat characteristics in broilers. *British Poultry Science* 54, 635–641.
- Drummond JG, Curtis SE, Simon J, Norton HW (1980): Effects of aerial ammonia on growth and health of young pigs. *Journal of Animal Science* 50, 1085–1091.
- Erwin ES, Marco GT, Emery EM (1961): Volatile fatty acid analysis of blood and rumen fluid by gas chromatography. *Journal of Dairy Science* 44, 1768–1771.
- Fenton TW, Fenton M (1979): An improved method for chromic oxide determination in feed and feces. *Canadian Journal of Animal Science* 59, 631–634.
- Ferencik M, Mikes Z, Seman M, Ebringer L (2000): Beneficial modification of the human intestinal microflora using orally administered enterococci. *High Tatras Slovak Republic* 46, 11–14.
- Ferket PR, van Heugten E, van Kempen TATG, Angel R (2002): Nutritional strategies to reduce environmental emissions from nonruminants. *Journal of Animal Science* 80, E168–E182.
- Finegold SM, Sutter VL, Mathisen GE (1983): Normal indigenous intestinal flora. In: Hentiges DJ (ed.): *Human Intestinal Microflora in Health and Disease*. Academic Press, New York, NY. 3–31.
- Froning GW (1995): Color of poultry meat. *Poultry and Avian Biology Reviews* 6, 83–93.
- Hinton M, Kaukas A, Linton AH (1986): The ecology of drug resistance in enteric bacteria. *Journal of Applied Bacteriology Symposium Supplement* 15, 77–92.
- Honikel KO, Kim CJ, Hamm R, Roncales P (1986): Sarcomere shortening of prerigor muscle and its influence on drip loss. *Meat Science* 16, 267–282.
- Kong Q, He GQ, Jia JL, Zhu QL, Ruan H (2011): Oral administration of *Clostridium butyricum* for modulating gastrointestinal microflora in mice. *Current Microbiology* 62, 512–517.
- Lee KW, Lee SH, Lillehoj HS, Li GX, Jang SI, Babu US, Park MS, Kim DK, Lillehoj EP, Neumann AP, Rehberger TG, Siragusa GR (2010): Effects of direct-fed microbials on growth performance, gut morphometry, and immune characteristics in broiler chickens. *Poultry Science* 89, 203–216.
- Li SP, Zhao XJ, Wang JY (2009): Synergy of astragalus polysaccharides and probiotics (*Lactobacillus* and *Bacillus cereus*) on immunity and intestinal microbiota in chicks. *Poultry Science* 88, 519–525.
- Meng QW, Yan L, Ao X, Zhou TX, Wang JP, Lee JH, Kim IH (2010): Influence of probiotic in different energy and nutrient density diets on growth performance, nutrient digestibility, meat quality, and blood characteristics in growing finishing pig. *Journal of Animal Science* 88, 3320–3326.
- Moore S (1963): On the determination of cystine as a cysteic acid. *Journal of Biological Science* 238, 235–237.
- Mountzouris KC, Tsirtsikos P, Palamidi I, Arvaniti A, Mohnl M, Schatzmayr G, Fegeros K (2010): Effects of probiotic inclusion levels in broiler nutrition on growth performance, nutrient digestibility, plasma immunoglobulins, and caecal microflora composition. *Poultry Science* 89, 58–67.
- Ng SC, Hart AL, Kamm MA, Stagg AJ, Knight SC (2009): Mechanisms of action of probiotics: Recent advances. *Inflammatory Bowel Disease* 15, 300–310.
- NRC (1994): *Nutrient requirements of poultry*. 9th revised ed. National Academy Press, Washington, DC.
- Rada V, Sirotek K, Petr J (1999): Evaluation of selective media for bifidobacteria in poultry and rabbit caecal samples. *Journal of Veterinary Medicine Series B* 46, 369–373.
- Samanya M, Yamauchi K (2002): Histological alterations of intestinal villi in chickens fed dried *Bacillus subtilis* var. natto. *Comparative Biochemistry and Physiology* 133, 95–104.
- SAS (2008): *SAS User's Guide*. SAS Institute Inc., Cary, NC, USA.
- Sen S, Ingale SL, Kim YW, Kim JS, Kim KH, Lohakarea JD, Kim EK, Kim HS, Ryu MH, Kwon IK, Chae BJ (2012): Effect of supplementation of *Bacillus subtilis* LS 1-2 to broiler diets on growth performance, nutrient retention, caecal microbiology and small intestinal morphology. *Research in Veterinary Science* 93, 264–268.
- Shim YH, Shinde PL, Choi JY, Kim JS, Seo DK, Pak JI, Chae BJ, Kwon IK (2010): Evaluation of multi-microbial probiotics produced by submerged liquid and solid substrate fermentation methods in broilers. *Asian-Australasian Journal of Animal Science* 23, 521–529.
- Sinol S, Ingale SL, Kim YW, Kim JS, Kima KH, Lohakarea JD, Kim EK, Kim HS, Ryu MH, Kwon IK, Chae BJ (2012): Effect of supplementation of *Bacillus subtilis* LS 1-2 to broiler diets on growth performance, nutrient retention, caecal microbiology and small intestinal morphology. *Research in Veterinary Science* 93, 264–268.
- Sullivan ZM, Honeyman MS, Gibson LR, Prusa KJ (2007): Effects of triticale-based diets on finishing pig perfor-

- mance and pork quality in deep-bedded hoop barns. *Meat Science* 76, 428–437.
- Talebi A, Amirzadeh B, Mokhtari B, Gahri H (2008): Effects of a multi-strain probiotic (PrimaLac) on performance and antibody responses to Newcastle disease virus and infectious bursal disease virus vaccination in broiler chickens. *Avian Pathology* 37, 509–512.
- Willis WL, Isikhuemhen OS, Ibrahim SA (2007): Performance assessment of broiler chickens given mushroom extract alone or in combination with probiotic. *Poultry Science* 86, 1856–1860.
- Xu ZR, Hu CH, Xia MS, Zhan XA, Wang MQ (2003): Effects of dietary fructooligosaccharide on digestive enzyme activities, intestinal microflora and morphology of male broilers. *Poultry Science* 82, 1030–1036.
- Yan L, Meng QW, Ao X, Zhou TX, Yoo JS, Kim HJ, Kim IH (2011): Effect of fermented garlic powder supplementation on growth performance, blood characteristics and meat quality in finishing pigs fed low-nutrient density diets. *Livestock Science* 137, 255–259.
- Yang CM, Cao GT, Ferket PR, Liu TT, Zhou L, Zhang L, Xiao YP, Chen AG (2012): Effects of probiotic, *Clostridium butyricum*, on growth performance, immune function, and cecal microflora in broiler chickens. *Poultry Science* 91, 2121–2129.
- Zhang ZF, Kim IH (2013): Effects of probiotic supplementation in different energy and nutrient density diets on performance, egg quality, excreta microflora, excreta noxious gas emission, and serum cholesterol concentrations in laying hens. *Journal of Animal Science* 91, 4781–4787.
- Zhang ZF, Kim IH (2014): Effects of multistrain probiotics on growth performance, apparent ileal nutrient digestibility, blood characteristics, cecal microbial shedding, and excreta odor contents in broilers. *Poultry Science* 93, 364–370.
- Zhang ZF, Zhou TX, Ao X, Kim IH (2012): Effects of β -glucan and *Bacillus subtilis* on growth performance, blood profiles, relative organ weight and meat quality in broilers fed maize–soybean meal based diets. *Livestock Science* 150, 419–424.
- Zhang ZF, Cho JH, Kim IH (2013): Effects of *Bacillus subtilis* UBT-MO2 on growth performance, relative immune organ weight, gas concentration in excreta, and intestinal microbial shedding in broiler chickens. *Livestock Science* 155, 343–347.
- Zhou X, Wang Y, Gu Q, Li W (2010): Effect of dietary probiotic, *Bacillus coagulans* on growth performance, chemical composition, and meat quality of Guangxi Yellow chicken. *Poultry Science* 89, 588–593.

Received: 2014–08–12

Accepted after corrections: 2015–01–06

Corresponding Author:

I.H. Kim, Dankook University, Department of Animal Resource and Science, No.29 Anseodong, Cheonan, Choongnam, 330-714, Republic of Korea
E-mail: inhokim@dankook.ac.kr



Hossain MM, Begum M, Kim IH (2015)
 Effect of *Bacillus subtilis*, *Clostridium butyricum* and *Lactobacillus acidophilus* endospores on growth performance, nutrient digestibility, meat quality, relative organ weight, microbial shedding and excreta noxious gas emission in broilers
 Veterinarni Medicina, 60, 77-86
[Additional material](#)

References (available DOI included):

- Abudabos AM (2012): Effect of Primalac® or Enramycin supplementation on performance, intestinal morphology and microbiology of broilers under *Clostridium perfringens* challenge. *Journal of Food, Agriculture and Environment* 10, 595–599.
- AOAC (1995): Official method of analysis. 16th ed. Association of Official Analytical Chemists, Washington, DC.
- AOAC (2000): Official method of analysis. 17th ed. Association of Official Analytical Chemists, Gaithersburg, MD.
- Chen W, Wang JP, Yan L, Huang YQ (2013): Evaluation of probiotics in diets with different nutrient densities on growth performance, blood characteristics, relative organ weight and breast meat characteristics in broilers. *British Poultry Science* 54, 635-641
[doi:10.1080/00071668.2013.825369](https://doi.org/10.1080/00071668.2013.825369)
- Drummond JG, Curtis SE, Simon J, Norton HW (1980): Effects of aerial ammonia on growth and health of young pigs. *Journal of Animal Science* 50, 1085–1091.
- Erwin E.S., Marco GJ, Emery EM (1961): Volatile Fatty Acid Analyses of Blood and Rumen Fluid by Gas Chromatography. *Journal of Dairy Science* 44, 1768-1771 [doi:10.3168/jds.S0022-0302\(61\)89956-6](https://doi.org/10.3168/jds.S0022-0302(61)89956-6)
- FENTON TW, Fenton M (1979): An improved procedure for the determination of chromic oxide in feed and feces. *Canadian Journal of Animal Science* 59, 631-634 [doi:10.4141/cjas79-081](https://doi.org/10.4141/cjas79-081)
- Ferencik M, Mikes Z, Seman M, Ebringer L (2000): Beneficial modification of the human intestinal microflora using orally administered enterococci. *High Tatras Slovak Republic* 46, 11–14.
- Ferket PR, van Heugten E, van Kempen TATG, Angel R (2002): Nutritional strategies to reduce environmental emissions from nonruminants. *Journal of Animal Science* 80, E168–E182.
- Finegold SM, Sutter VL, Mathisen GE (1983): Normal indigenous intestinal flora. In: Hentiges DJ (ed.): *Human Intestinal Microflora in Health and Disease*. Academic Press, New York, NY. 3–31.
- Froning GW (1995): Color of poultry meat. *Poultry and Avian Biology Reviews* 6, 83–93.
- Hinton M, Kaukas A, Linton AH (1986): The ecology of drug resistance in enteric bacteria. *Journal of Applied Bacteriology Symposium Supplement* 15, 77–92.
- Honikel KO, Kim CJ, Hamm R, Roncales P (1986): Sarcomere shortening of prerigor muscles and its influence on drip loss. *Meat Science* 16, 267-282 [doi:10.1016/0309-1740\(86\)90038-0](https://doi.org/10.1016/0309-1740(86)90038-0)
- Kong Q, He G-Q, Jia J-L, Zhu Q-L, Ruan H (2011): Oral Administration of *Clostridium butyricum* for Modulating Gastrointestinal Microflora in Mice. *Current Microbiology* 62, 512-517
[doi:10.1007/s00284-010-9737-8](https://doi.org/10.1007/s00284-010-9737-8)
- Lee K W, Lee S H, Lillehoj HS, Li GX, Jang SI, Babu US, Park MS, Kim DK, Lillehoj EP, Neumann AP, Rehberger TG, Siragusa GR (2009): Effects of direct-fed microbials on growth performance, gut morphometry, and immune characteristics in broiler chickens. *Poultry Science*, 89, 203-216
[doi:10.3382/ps.2009-00418](https://doi.org/10.3382/ps.2009-00418)
- Li SP, Zhao XJ, Wang JY (2009): Synergy of Astragalus polysaccharides and probiotics (*Lactobacillus* and *Bacillus cereus*) on immunity and intestinal microbiota in chicks. *Poultry Science* 88, 519-525
[doi:10.3382/ps.2008-00365](https://doi.org/10.3382/ps.2008-00365)
- Meng QW, Yan L, Ao X, Zhou TX, Wang JP, Lee JH, Kim IH (2009): Influence of probiotics in different energy and nutrient density diets on growth performance, nutrient digestibility, meat quality, and blood characteristics in growing-finishing pigs. *Journal of Animal Science*, 88, 3320-3326
[doi:10.2527/jas.2009-2308](https://doi.org/10.2527/jas.2009-2308)
- Moore S (1963): On the determination of cystine as a cysteic acid. *Journal of Biological Science* 238, 235–237.
- Mountzouris KC, Tsitsrikos P, Palamidi I, Arvaniti A, Mohnl M, Schatzmayr G, Fegeros K (2009): Effects of probiotic inclusion levels in broiler nutrition on growth performance, nutrient digestibility, plasma immunoglobulins, and cecal microflora composition. *Poultry Science*, 89, 58-67
[doi:10.3382/ps.2009-00308](https://doi.org/10.3382/ps.2009-00308)



- Ng SC, Hart A. L., Kamm M A, Stagg AJ, Knight SC (2009): Mechanisms of action of probiotics: Recent advances. *Inflammatory Bowel Diseases* 15, 300-310 <[doi:10.1002/ibd.20602](https://doi.org/10.1002/ibd.20602)>
- NRC (1994): Nutrient requirements of poultry. 9th revised ed. National Academy Press, Washington, DC.
- Rada V, Sirotek K, Petr J (1999): Evaluation of Selective Media for Bifidobacteria in Poultry and Rabbit Caecal Samples. *Journal of Veterinary Medicine Series B*, 46, 369-373 <[doi:10.1046/j.1439-0450.1999.00241.x](https://doi.org/10.1046/j.1439-0450.1999.00241.x)>
- Samanya M, Yamauchi K (2002): Histological alterations of intestinal villi in chickens fed dried *Bacillus subtilis* var. natto. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* 133, 95-104 <[doi:10.1016/S1095-6433\(02\)00121-6](https://doi.org/10.1016/S1095-6433(02)00121-6)>
- SAS (2008): SAS User's Guide. SAS Institute Inc., Cary, NC, USA.
- Sen S, Ingale SL, Kim YW, Kim JS, Kim KH, Lohakare JD, Kim EK, Kim HS, Ryu MH, Kwon IK, Chae BJ (2012): Effect of supplementation of *Bacillus subtilis* LS 1-2 to broiler diets on growth performance, nutrient retention, caecal microbiology and small intestinal morphology. *Research in Veterinary Science* 93, 264-268 <[doi:10.1016/j.rvsc.2011.05.021](https://doi.org/10.1016/j.rvsc.2011.05.021)>
- Shim YH, Shinde PL, Choi JY, Kim JS, Seo DK, Pak JI, Chae BJ, Kwon IK (2010): Evaluation of multi-microbial probiotics produced by submerged liquid and solid substrate fermentation methods in broilers. *Asian-Australasian Journal of Animal Sciences* 23, 521-529 <[doi:10.5713/ajas.2010.90446](https://doi.org/10.5713/ajas.2010.90446)>
- Sen Sinol, Ingale S.L., Kim Y.W., Kim J.S., Kim K.H., Lohakare J.D., Kim E.K., Kim H.S., Ryu M.H., Kwon I.K., Chae B.J. (2012): Effect of supplementation of *Bacillus subtilis* LS 1-2 to broiler diets on growth performance, nutrient retention, caecal microbiology and small intestinal morphology. *Research in Veterinary Science*, 93, 264-268 <[doi:10.1016/j.rvsc.2011.05.021](https://doi.org/10.1016/j.rvsc.2011.05.021)>
- Sullivan Zebblin M, Honeyman Mark S, Gibson Lance R, Prusa Ken J (2007): Effects of triticales-based diets on finishing pig performance and pork quality in deep-bedded hoop barns. *Meat Science* 76, 428-437 <[doi:10.1016/j.meatsci.2006.12.002](https://doi.org/10.1016/j.meatsci.2006.12.002)>
- Talebi A, Amirzadeh B, Mokhtari B, Gahri H (2008): Effects of a multi-strain probiotic (PrimaLac) on performance and antibody responses to Newcastle disease virus and infectious bursal disease virus vaccination in broiler chickens. *Avian Pathology* 37, 509-512 <[doi:10.1080/03079450802356995](https://doi.org/10.1080/03079450802356995)>
- Willis WL, Isikhuemhen OS, Ibrahim SA (2007): Performance assessment of broiler chickens given mushroom extract alone or in combination with probiotics. *Poultry Science* 86, 1856-1860 <[doi:10.1093/ps/86.9.1856](https://doi.org/10.1093/ps/86.9.1856)>
- Xu Z, Hu C, Xia M, Zhan X, Wang M (2003): Effects of dietary fructooligosaccharide on digestive enzyme activities, intestinal microflora and morphology of male broilers. *Poultry Science* 82, 1030-1036 <[doi:10.1093/ps/82.6.1030](https://doi.org/10.1093/ps/82.6.1030)>
- Yan L, Meng QW, Ao X, Zhou TX, Yoo JS, Kim HJ, Kim IH (2011): Effects of fermented garlic powder supplementation on growth performance, blood characteristics and meat quality in finishing pigs fed low-nutrient-density diets. *Livestock Science* 137, 255-259 <[doi:10.1016/j.livsci.2010.09.024](https://doi.org/10.1016/j.livsci.2010.09.024)>
- Yang CM., Cao GT, Ferket PR, Liu TT, Zhou L, Zhang L, Xiao YP, Chen AG (2011): Effects of probiotic, *Clostridium butyricum*, on growth performance, immune function, and cecal microflora in broiler chickens. *Poultry Science*, 91, 2121-2129 <[doi:10.3382/ps.2011-02131](https://doi.org/10.3382/ps.2011-02131)>
- Zhang ZF, Kim IH (2013): Effects of probiotic supplementation in different energy and nutrient density diets on performance, egg quality, excreta microflora, excreta noxious gas emission, and serum cholesterol concentrations in laying hens. *Journal of Animal Science*, 91, 4781-4787 <[doi:10.2527/jas.2013-6484](https://doi.org/10.2527/jas.2013-6484)>
- Zhang ZF, Kim IH (2013): Effects of multistrain probiotics on growth performance, apparent ileal nutrient digestibility, blood characteristics, cecal microbial shedding, and excreta odor contents in broilers. *Poultry Science*, 93, 364-370 <[doi:10.3382/ps.2013-03314](https://doi.org/10.3382/ps.2013-03314)>
- Zhang ZF, Zhou TX, Ao X, Kim IH (2012): Effects of β -glucan and *Bacillus subtilis* on growth performance, blood profiles, relative organ weight and meat quality in broilers fed maize-soybean meal based diets. *Livestock Science* 150, 419-424 <[doi:10.1016/j.livsci.2012.10.003](https://doi.org/10.1016/j.livsci.2012.10.003)>
- Zhang ZF, Cho JH, Kim IH (2013): Effects of *Bacillus subtilis* UBT-MO2 on growth performance, relative immune organ weight, gas concentration in excreta, and intestinal microbial shedding in broiler chickens. *Livestock Science* 155, 343-347 <[doi:10.1016/j.livsci.2013.05.021](https://doi.org/10.1016/j.livsci.2013.05.021)>
- Zhou X, Wang Y, Gu Q, Li W (2009): Effect of dietary probiotic, *Bacillus coagulans*, on growth performance, chemical composition, and meat quality of Guangxi Yellow chicken. *Poultry Science*, 89, 588-593 <[doi:10.3382/ps.2009-00319](https://doi.org/10.3382/ps.2009-00319)>