Effects of probiotic and whey powder supplementation on growth performance, microflora population, and ileum morphology in broilers

Abolfazl Zarei, Abolghasem Lavvaf and Mohammad Motamedi Motlagh

Department of Animal Science, Karaj Branch, Islamic Azad University, Karaj, Iran

ABSTRACT
A study was conducted to examine how supplementation using probiotics and whey powder affects microflora population, ileum morphology, and growth performance in broilers. In this experiment, 400 one-day-old male chickens were randomly assigned into four groups: the control group (CON), the probiotics group (0.2%) (PRO), the whey powder group (4%) (WHP), and a group that received a combination of probiotics (0.2%) and whey powder (4%) (PRO-WHP). Microflora populations as well as ileum morphology were assessed on day 42 and growth performance parameters were measured at the end of the rearing period (day 42). The findings indicated that the PRO-WHP group had the largest Lactobacillus count (P < .05) while the lowest Escherichia coli count was found in the WHP. No significant difference was found between the PRO group and the WHP (P > .05) for villus:cyt ratio. The largest feed intake and body weight gain were observed in the PRO-WHP group. In addition, the PRO-WHP group had the best feed conversion ratio, but was not significantly different from the PRO and the WHP groups. Our experiment indicated that adding whey powder alone or in combination with probiotics can improve broiler performance by modifying intestinal microflora and changing intestinal morphology.

Introduction
Growth-promoting antibiotics are among feed additives used to prevent growth of intestinal pathogens and improve growth performance in broilers. Recent concerns over human resistance to antibiotics together with consumers’ increasing demand for healthy food have led to prohibition of use of growth-promoting antibiotics in poultry diets (Dibner and Richards 2005; Castanon 2007). Different studies attempting to find alternatives to growth-promoting antibiotics suggest that these antibiotics can be replaced by probiotics, prebiotics, organic acids, or herbs (Dibner and Richards 2005; Windisch et al. 2007; Darabighane and Nahashon 2014; Zhang et al. 2015; Abudabos et al. 2017). Among these animal and poultry feed additives, probiotics and prebiotics have drawn considerable attention.

Probiotics are live microorganisms that can replace growth-promoting antibiotics and beneficially affect the host animal by improving its intestinal microbial balance (Yang et al. 2009; Khan et al. 2011; Khan and Naz 2013). Though competitive elimination and creating a balanced population in the digestive system, they can enhance health conditions in poultry (Majidi-Mosleh et al. 2017). Numerous studies have been carried out to examine effects of probiotics on growth performance of broilers, and Blajman et al. (2014), in a study using meta-analytical methods, reported body weight gain and improved feed efficiency in broilers as a result of using probiotics, leading to increased body weight gain and enhanced feed efficiency in chickens that received probiotics through water compared to those receiving it in their feed. In addition, results of experiments on chickens with coccidiosis indicated the effectiveness of probiotics in improving growth performance and mitigating unfavourable effects of Eimeria (Ritzi et al. 2014).

Another feed additive with prebiotic-like effects is whey powder. As a byproduct of cheese processing, whey contains 65–80% lactose (Samli et al. 2007). Because their bodies lack lactase, birds cannot digest lactose which is eventually fermented into lactic acid and volatile fatty acids that may stimulate intestinal bacteria. Mehri et al. (2004) reported improved FCR by adding 4% whey powder to broiler diet. In addition, Rastad et al. (2008) added probiotics and whey powder to broiler feed and found that diets containing 500 and 750 g probiotics per ton with 2% whey led to weight gain in chickens on days 0–21. Gülşen et al. (2002) found that adding 3.85% whey powder to broiler feed increased their body weight, although it did not affect FI and FCR.

Supplementing diets with probiotics and prebiotics is a significant factor contributing to modified microflora intestinal microflora which, in turn, may effectively influence birds’ growth performance and health (Yang et al. 2009). Therefore, application of feed additives, such as probiotics or prebiotics and prebiotics (symbiotic), which can potentially modify intestinal microflora population, may help achieving maximum growth in poultry. To date, only a limited number of studies have examined effects of whey powder, and therefore the present study aims to investigate effects of supplementing diets with probiotics, whey powder, and mixture combination.
of the two (probiotic + whey) on micro flora, ileum morphology, and growth performance in broilers.

Materials and methods

Dietary treatments and animal management

The experiment has been approved by the Karaj Islamic Azad University Committee of Animal Ethics and complied with Iranian guidelines for animal welfare. In this experiment, 400 one-day-old Ross 308 male chickens were randomly distributed in 4 groups with 5 replicates, each containing 20 chickens: the control group (basal diet, no additive) (CON), the probiotic group (0.2%) (PRO), the whey power group (4%) (WHP), and the whey + probiotic group (4%+0.2%) (PRO-WHP). The broiler diet was formulated according to the Ross 308 production manual using UFFDA (Using Friendly Feed Formulating Done Again) for three rearing periods: starter (days 0–10), grower (days 11–24), and finisher (days 25–42). Table 1 presents the feed ingredients and chemical composition of the basal diet. During the rearing period, the chickens had constant access to water and feed. The lighting programme was 23 h of light and 1 h of darkness. Automatic heaters were used to raise the temperature in the house. The initial temperature was 32°C which was gradually reduced according to breeder standards. Control parameters, such as temperature, humidity, light, and ventilation, were the same for all treatments. Vaccination was performed according to advice provided by a veterinarian and was the same for all experimental groups.

Preparing probiotic and whey powder

LactoFeed, the probiotic used in this study, was obtained from Tech Gen Zist Company in Iran. The product is free from genetically modified organisms. Chemical composition of LactoFeed consists of Lactobacillus acidophilus at 2.5 × 10^10 CFU/kg, Lactobacillus casei at 2.5 × 10^10 CFU/kg, Bifidobacterium thermophilum at 2.5 × 10^10 CFU/kg, and Enterococcus faecium at 2.5 × 10^10 CFU/kg. Whey powder was purchased from NasrDalya, an Iranian company. This E. coli-free powder contained 60% lactose.

Measurement of ileum microflora

On day 42, two chickens were randomly selected from each replicate and slaughtered by cervical dislocation. To determine Lactobacillus and Escherichia coli counts, 1-g samples were taken from the ileum. Samples were serially diluted from 10^−1 to 10^−7. Lactobacillus count was performed in MRS (de Man, Rogosa, and Sharpe) agar after incubation in anaerobic chamber at 37°C for 48 h. The E. coli count was performed in MacConkey agar after aerobic incubation at 37°C for 24 h. The numbers of colony-forming units (CFUs) were expressed as log10 CFU per gram.

Measurement of ileum morphological parameters

On day 42, two birds were randomly selected from each replicate and slaughtered by cervical dislocation. To measure ileum morphology, 1.5 cm of the middle part of the ileum was excised and then rinsed using NaCl 9.0% solution. Clean ileum samples were fixed in a buffered 10% formal solution for 8 h, washed three times in a 70% ethanol solution and stored until use. A 0.5 cm² subsample of each ileum sample was then stained by the Feulgen method using Schiff reagent. At least 10 villi and 10 crypts from each subsample were dissected under a stereo microscope and then

Table 1. Ingredients and chemical composition of the experimental basal diets.

| Composition of basal diet | Composition of basal diet | Composition of basal diet |
|---------------------------|---------------------------|---------------------------|
|                          | Starter (1–10 days)       | Grower (11–24 days)       | Finisher (25–42 days)  |
|                          | CON  PRO  WHP  PRO-WHP    | CON  PRO  WHP  PRO-WHP    | CON  PRO  WHP  PRO-WHP  |
| Corn (g/kg)               | 578  576  551  549        | 601  599  579  577        | 635  633  609  608     |
| Soybean meal (g/kg)       | 266  266  252.2  252.2    | 228  228  183  183        | 226  226  184  183     |
| Corn Gluten (g/kg)        | 84.1  84.1  84.1  84.1     | 93.1  93.1  120  120      | 57   57   87   87      |
| Soybean oil (g/kg)        | 20   20   20   20         | 30   30   30   30        | 40   40   40   40      |
| Dicalcium phosphate (g/kg)| 20.6  20.6  20.6  20.6     | 18   18   18   18        | 16.5  16.5  14.5  14.5   |
| Sodium bicarbonate (g/kg) | 1.4   1.4   1.4   1.4      | 1.4   1.4   1.4   1.4    | 2.3   2.3   2.3   2.3    |
| Salt (g/kg)               | 2.2   2.2   2.2   2.2      | 2.2   2.2   2.2   2.2    | 1.9   1.9   1.9   1.9    |
| Vitamin Premix (g/kg)    | 2.5   2.5   2.5   2.5      | 2.5   2.5   2.5   2.5    | 2.5   2.5   2.5   2.5    |
| Mineral Premix (g/kg)     | 2.5   2.5   2.5   2.5      | 2.5   2.5   2.5   2.5    | 2.5   2.5   2.5   2.5    |
| L-Ly HCL (g/kg)           | 63    63    71    71       | 5.9   5.9   6.2   6.2     | 3.4   3.4   3.4   3.4    |
| DL-Met (g/kg)             | 3.1   3.1   3.1   3.1      | 2.1   2.1   1.9   1.9     | 2.3   2.3   2.3   2.3    |
| Probiotic                 | 0.2   0.2   0.2   0.2      | 0     0     0     0       | 0     0     0     0       |
| whey powder               | 0     0     40    40       | 0     0     40    40      | 0     0     40    40      |

Calculated composition

Metabolizable energy (kcal/kg) 3025 3025 3025 3025 3015 3015 3150 3150 3150 3150 3150 3150 3200 3200 3200 3200 3200 3200 3200 3200 3200 3200

Crude protein (%) 22 22 22 22 22 21 21 21 21 19 19 19 19 19 19 19 19 19 19 19 19 19 19 19 Ca (%) 0.15 0.15 0.15 0.15 0.15 0.08 0.08 0.08 0.08 0.04 0.04 0.04 0.04 0.04 0.04 0.04 0.04 0.04 0.04 0.04 0.04 0.04 Available Phosphorous (%) 0.5 0.5 0.5 0.5 0.5 0.47 0.47 0.47 0.47 0.43 0.43 0.43 0.43 0.43 0.43 0.43 0.43 0.43 0.43 0.43 0.43 0.43 Met + Cys (%) 1.08 1.08 1.08 1.08 0.9 0.9 0.9 0.9 0.8 0.8 0.8 0.8 0.8 0.8 0.8 0.8 0.8 0.8 0.8 0.8 0.8 Lysine (%) 1.40 1.40 1.40 1.40 1.21 1.21 1.21 1.21 1.21 1.19 1.19 1.19 1.19 1.19 1.19 1.19 1.19 1.19 1.19 1.19 1.19 1.19

α-Vitamin premix provided per kilogram of diet: vitamin A (transretinyl acetate), 10,000 IU; vitamin D3 (cholecalciferol), 5000 IU; vitamin E (d-l-tocopherol acetate), 50 IU; vitamin K3 (bisulphate menadione complex), 3 mg; thiamine (thiamine mononitrate), 3 mg; riboflavin, 9 mg; nicotinic acid, 50 mg; pantothenic acid (D-calcium panthenolate), 15 mg; vitamin B6, 4 mg; d-biotin, 0.1 mg; folic acid, 2 mg; vitamin B12 (cyanocobalamin), 0.02 mg and choline (choline chloride), 1000 mg.

β-Mineral premix provided per kilogram of diet: iron (FeSO4·7H2O), 55 mg; iodine (I2), 1.3 mg; manganese (MnSO4·H2O), 120 mg; zinc (ZnO), 100 mg; copper (CuSO4·5H2O), 16 mg; selenium (Na2SeO3), 0.3 mg.
photographed. Villus height and crypt depth were measured using the image analysis software Visilog 6.3 Viewer Lite (Noesis, Saint Aubin, France) (Catalá-Gregori et al. 2008).

**Measurement of performance parameters**

Growth performance was measured by calculating BWG, FI, and FCR for the whole rearing period (42 days) in broilers. We corrected the parameters by recording the number and weights of chickens that died during experimental periods.

**Statistical analysis**

The experiment was conducted using a completely randomized design with 4 treatments and 5 replicates, each containing 20 broilers. We used SAS (2002) to analyse ANOVA results and compare mean values through Duncan’s multiple range test at the level of $P < .05$.

**Results and discussion**

As a part of the digestive ecosystem, microflora in the digestive system have significant impacts on poultry health and performance (Wu et al. 2016). Therefore, creating a healthy intestinal environment with increased counts of healthful bacteria using probiotics and prebiotics can enhance growth performance (Huyghebaert et al. 2011). Table 2 presents the results of using probiotics, whey, and whey plus probiotics on micro flora populations of the ileum in 42-day-old broilers. Our findings indicate the largest *Lactobacillus* count in the PRO-WHP group, showing a significant difference from other groups ($P < .05$), while the lowest *Lactobacillus* count was observed in the CON group ($P < .05$). The largest *E. coli* count was found in the CON group, which was significantly higher compared to the other groups ($P < .05$). The lowest *E. coli* count was found in the WHP group, although it was not significantly different from the values found for the PRO and the PRO-WHP group ($P > .05$). Our findings are consistent with the results of Samli et al. (2007) who reported largest lactic acid bacteria count in the ileum of the group receiving a combination of probiotics (*E. faecium*, 0.2%) and whey powder (3.5%) with no significant difference between these two treatments. Because their bodies lack lactase, birds cannot digest lactose which is eventually fermented into lactic acid and volatile fatty acids that may stimulate intestinal bacteria. Gülşen et al. (2002) proposed that whey powder may activate pH-improving microorganisms like *Lactobacillus* in the intestine and reduce digestion which, in turn, can decrease the growth of pathogenic bacteria.

Table 3. Effect of probiotic, whey powder, and combination of probiotic and whey powder on ileum morphology of broilers.

| Dietary treatment | CON | PRO | WHP | PRO-WHP | SEM |
|-------------------|-----|-----|-----|---------|-----|
| Villus height (µm) | 706.5$^a$ | 858.6$^b$ | 834$^b$ | 835.6$^b$ | 2.85 |
| Crypt depth (µm) | 121.3$^a$ | 114.3$^b$ | 115.1$^b$ | 110.4$^b$ | 1.08 |
| Villus height: crypt depth | 5.83$^a$ | 7.51$^b$ | 7.24$^a$ | 7.56$^a$ | 0.07 |

Note: The means within the same row with at least one common letter do not have significant difference ($P > .05$).

An important impact reported in some studies in relation to using probiotics and prebiotics is the changes in intestinal morphology as a result of modified microflora (Yang et al. 2009). Table 3 shows the results of the ileum morphology on day 42. The results of our experiment indicated that the PRO group had the greatest villus height ($P < .05$) while no significant difference was found between the PRO-WHP group and the WHP group in terms of villus height ($P > .05$). Broilers in the CON group had the smallest villus height. Broilers in the PRO-WHP group had the smallest crypt depth with no significant difference from other groups. No significant difference was found between the WHP group and the PRO group in terms of crypt depth ($P > .05$). The deepest crypts were observed in the CON group. Regarding villus:crypt ratio, the lowest ratio was found in the CON group ($P < .05$). No significant difference was observed between the PRO group and the WHP group. Broilers in the PRO-WHP group showed significant increase in their villus: crypt ratios compared to the control group. Samli et al. (2007) reported the greatest villus height in broilers receiving *E. faecium* and the lowest height in the control group, which is consistent with our findings. In addition, in an experiment by Samli et al. (2007), no significant difference was found between the whey powder plus probiotics group and the whey powder group in terms of villus height. However, structure of the intestinal mucosa indicates intestinal health in chickens (Xu et al. 2003). Short-chain fatty acids, as the final product of fermentation by lactobacilli, seem to be capable of reducing pH and making the intestinal environment unfavourable for pathogen growth, and this in turn can enhance intestinal health and reduce level of damage to intestine mucosa (Farthing 2004). As noted above in relation to villus height, the lowest height was found in the CON group and this can lower absorption of nutrients. In our experiment, the CON group had the smallest villus: crypt ratio which can be attributed to presence of toxin, reduced absorption of nutrients, and consequently lowered growth performance in broilers (Xu et al. 2003).

Table 4. Effect of probiotic, whey powder, and combination of probiotic and whey powder on growth performance of broilers.

| Parameters | CON | PRO | WHP | PRO-WHP | SEM |
|------------|-----|-----|-----|---------|-----|
| FI | 4268.4$^b$ | 4350.7$^a$ | 4546.8$^a$ | 4610.8$^a$ | 50.18 |
| BWG | 2100.2$^b$ | 2276.2$^a$ | 2362.5$^a$ | 2384.6$^a$ | 28.08 |
| FCR | 2.03$^b$ | 1.91$^a$ | 1.90$^a$ | 1.89$^a$ | 0.09 |

Note: The means within the same row with at least one common letter do not have significant difference ($P > .05$).

CON: control; PRO: probiotic; WHP: whey powder; PRO-WHP: combination of probiotic and whey powder; FI: feed intake; BWG: body weight gain; FCR: feed conversion ratio; SEM: standard error of the means.
Table 4 presents the effects of supplementation with probiotics, whey powder, and probiotic mixed with whey on growth performance (FI, BWG, and FCR) for broilers on day 42. The results indicated the smallest FI in the CON group with no significant difference from the PRO group \( (P > .05) \). In addition, the largest BWG was found in the PRO-WHP group with no significant difference from other groups except for the CON group. These findings are in line with those of Rastad et al. \( (2008) \) who reported greater FI in broilers receiving 500 g and 750 g probiotics per ton with 2% whey powder, in comparison to the control group. On the other hand, however, Gülşen et al. \( (2002) \) and Kermanshahi and Rostami \( (2006) \) who supplemented the feed with respectively 3.85% whey powder and 2%, 4%, and 8% whey powder found no significant difference from the control group and the broilers receiving whey powder in terms of feed intake. This increased feed intake is attributable to provision of a favourable environment for growth and multiplication of probiotics and stimulation of growth which leads to increased intake of nutrients.

Regarding broilers BWG, the smallest BWG was observed in the CON group, showing a significant difference from other groups \( (P < .05) \). The PRO-WHP group had the largest BWG with no significant difference from the WHP group and the PRO group \( (P > .05) \). Similarly, Samli et al. \( (2007) \) reported improved weight gain in broilers receiving probiotics \( (E. \ faecium, 0.2\%) \) plus whey powder \( (3.5\%) \) compared to the control group while Rastad et al. \( (2008) \) reported greater weight gain in broilers receiving 500 and 700 g per ton probiotics plus 2% whey powder when compared to the control group. Consistent with these findings are the results of Gülşen et al. \( (2002) \) who added 3.85% whey powder to broilers feed and found a significant increase in weight gain in comparison to the control group.

Our findings on FCR on day 42 showed that the highest FCR was that of the CON group \( (P < .05) \). The best FCR was found in the PRO-WHP group which showed no significant difference from the PRO group and the WHP group \( (P > .05) \). Samli et al. \( (2007) \) noted improved FCR in broilers that received probiotic plus whey powder. However, Rastad et al. \( (2008) \) reported no significance difference from the control group in broilers that received 2% whey powder mixed with probiotics at 500 and 750 g per ton. Kermanshahi and Rostami \( (2006) \) found no improvement in FCR after adding 2%, 4%, and 8% whey powder, which is inconsistent with the findings of Samli et al. \( (2007) \).

It can be concluded however that improved intestinal micro flora \( (increased \text{ Lactobacillus} \text{ and reduced } \text{ E. coli} \text{ counts}) \) with modified intestine morphology which, in turn, can boost growth performance in probiotic and whey treatments. The CON group had a lower \text{ Lactobacillus} \text{ count than other groups and also a smaller villus:crypt ratio which may account for reduced growth performance in the CON group. Although the PRO group and the WHP group were almost the same in terms of villus:crypt ratio, they showed no significant difference from the PRO-WHP group, and the modified ileum morphology \( (changes \text{ in villus:crypt ratio}) \) can be another factor explaining positive effect of supplementation with probiotics plus whey powder.

Conclusions

One can conclude from this experiment that supplementing broiler feed with a mixture of probiotics and whey powder improves intestinal micro flora \( (increased \text{ Lactobacillus} \text{ and reduced } \text{ E. coli} \text{ count}) \) and enhanced growth performance in broilers. It is recommended that future studies focus on identifying the optimum level for probiotics and whey powder supplementation as well as examining the effects of whey and probiotics on humoral and cellular immune systems in broilers.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by Islamic Azad University, Karaj Branch.

References

Abudabos AM, Alyemni AH, Dafalla YM, Khan RU. \( 2017 \). Effect of organic acid blend and \text{ Bacillus subtilis} \text{ alone or in combination on growth traits, blood biochemical and antioxidant status in broilers exposed to \text{ Salmonella typhimurium} \text{ challenge during the starter phase. } \text{ J Appl Anim Res. 45:538–542.} \)
Blajman J, Frizzo L, Zbrun M, Astesana D, Fusari M, Soto L, Rosmini M, Signorini M. \( 2014 \). Probiotics and broiler growth performance: a meta-analysis of randomised controlled trials. Br Poult Sci. \( 55:483–494. \)
Castanon J. \( 2007 \). History of the use of antibiotic as growth promoters in European poultry feeds. Poult Sci. \( 86:2466–2471. \)
Catalá-Gregori P, Mallet S, Travel A, Orengo J, Lessire M. \( 2008 \). Efficiency of a prebiotic and a plant extract alone or in combination on broiler performance and intestinal physiology. Can J Anim Sci. \( 88:623–629. \)
Darabighane B, Nahashon SN. \( 2014 \). A review on effects of \text{ Aloe vera} \text{ as a feed additive in broiler chicken diets. }\text{ Ann Anim Sci. 14:491–500.} \)
Dibner J, Richards J. \( 2005 \). Antimicrobial growth promoters in agriculture: history and mode of action. Poult Sci. \( 84:634–643. \)
Farthing MJ. \( 2004 \). Bugs and the gut: an unstable marriage. Best Pract Res Clin Gastroenterol. \( 18:233–239. \)
Gülşen N, Coşkun B, Umucalilar H, Inal F, Boydak M. \( 2002 \). Effect of lactose and dried whey supplementation on growth performance and histology of the immune system in broilers. Arch Anim Nutr. \( 56:131–139. \)
Huyphebaert G, Ducatelte R, Van Immerseel F. \( 2011 \). An update on alternatives to antimicrobial growth promoters for broilers. Vet J. \( 187:182–188. \)
Kermanshahi H, Rostami H. \( 2006 \). Influence of supplemental dried whey on broiler performance and cecal flora. Int J Poult Sci. \( 5:538–543. \)
Khan R, Naz S. \( 2013 \). The applications of probiotics in poultry production. World Poult Sci J. \( 69:621–632. \)
Khan Sh, Yousaf B, Miani AA, Rehman A, Faroq MS. \( 2011 \). Assessing the effect of administrating different probiotics in drinking water supplement on broiler performance, blood biochemistry and immune response. J Appl Anim Res. \( 39:418–428. \)
Majidi-Mosleh A, Sadeghi A, Mousavi S, Chamani M, Zarei A. \( 2017 \). Ileal MUC2 gene expression and microbial population, but not growth performance and immune response, are influenced by in ovo injection of probiotics in broiler chickens. Br Poult Sci. \( 58:40–45. \)
Mehr M, Zare Sa, Samie A. \( 2004 \). The effects of supplementation of whey powder on broiler performance. Iran J Agr Sci. \( 35:1007–1013. \)
Rastad A, Samie A, Daneshvar F. \( 2008 \). Effect of bactocell and dry whey on performance and carcass characteristics of broiler chickens. JWSS – Isfahan Univ Technol. \( 12:473–480. \)
Ritzi MM, Abdelrahman W, Moheln M, Dalloul RA. \( 2014 \). Effects of probiotics and application methods on performance and response of broiler chickens to an \text{ Eimeria} \text{ challenge. }\text{ Poult Sci. 93:2772–2778.} \)
Samli HE, Senkoylu N, Koc F, Kanter M, Agma A. 2007. Effects of Enterococcus faecium and dried whey on broiler performance, gut histomorphology and intestinal microbiota. Arch Anim Nutr. 61:42–49.

SAS Institute. 2002. SAS/STAT Software, Release 9.2. NC, USA: SAS Institute, Inc.

Windisch W, Schedle K, Piltzner C, Kroismayr A. 2007. Use of phytogenic products as feed additives for swine and poultry. J Anim Sci. 86:E140–E148.

Wu Y, Zhou Y, Lu C, Ahmad H, Zhang H, He J, Zhang L, Wang T. 2016. Influence of butyrate loaded clinoptilolite dietary supplementation on growth performance, development of intestine and antioxidant capacity in broiler chickens. PloS one. 11:e0154410.

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 Sci. 82:1030–1036.

Yang Y, Iji P, Choct M. 2009. Dietary modulation of gut microflora in broiler chickens: a review of the role of six kinds of alternatives to in-feed antibiotics. World Poultry Sci J. 65:97–114.

Zhang X, Sun Z, Cao F, Ahmad H, Yang X, Zhao L, Wang T. 2015. Effects of dietary supplementation with fermented ginkgo leaves on antioxidant capacity, intestinal morphology and microbial ecology in broiler chicks. Br Poultry Sci. 56:370–380.