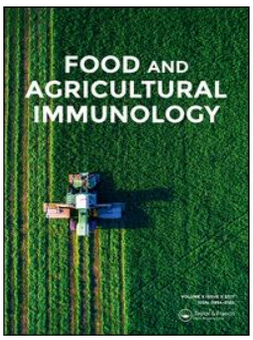

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Effects of microencapsulated probiotics and prebiotics on growth performance, antioxidative abilities, immune functions, and caecal microflora in broiler chickens

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ABSTRACT

The study was conducted to evaluate the effects of microencapsulated probiotics and prebiotics in broilers. A total of 108 one-day-old male Arbor Acres broilers were randomly divided into 3 groups (CON: basal diet; MEP: basal diet + compound microecologic products; ANT: basal diet + antibiotics), and there were 6 replicates per group and 6 birds per replicate. Compared with CON, diets supplemented with MEP or ANT significantly increased average daily gain and serum immunoglobulin M level at day 21, and serum total antioxidant capacity (T-AOC) level at day 42. Compared with CON and ANT groups, birds in MEP group had greater serum T-AOC, immunoglobulin A, interleukin-2 (IL-2) levels, and caecal *Lactobacilli* counts at day 21, and had greater serum IL-2, interleukin-6 levels, and caecal *Lactobacilli* counts at day 42. In conclusion, compound microecologic products had beneficial effects on body weight gain, serum immune function, and caecal *Lactobacillus* counts in broilers, which can be recommended as alternative to antibiotics.

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KEYWORDS
Broiler; caecal microflora; microencapsulated probiotics; immune function; prebiotic

Introduction

Antibiotic growth promoters (AGPs) are widely used to prevent poultry pathogens and disease and improve growth performance. However, the use of dietary AGPs can cause serious problems such as the antibiotic-resistant pathogens and drug residues in poultry products. So searching for alternatives to antibiotics is very urgent. Probiotics are defined as viable microorganisms used as feed additives, which could lead to beneficial effects in broilers by improving microbial balance or properties of the indigenous microflora (Fuller, 1989). However, supplementation of probiotics in diets do not always have better effects in poultry (Erdoğan, Erdoğan, Aslantaş, & Çelik, 2010; Hossain, Begum, & Kim, 2015; Jung, Houde, Baurhoo, Zhao, & Lee, 2008). The beneficial effects of probiotics were likely related to species- and strain-specific, survivability, and additive dosage

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(Abdel-Raheem, Abd-Allah, & Hassanein, 2012; Awad, Ghareeb, & Böhm, 2008), and the efficacy of probiotics can be potentiated by the selection of more efficient strains and the combination of supplementations with others strains, prebiotics, and feed enzymes (Awad et al., 2008; Awad, Ghareeb, Abdel-Raheem, & Böhm, 2009).

The prebiotics generally refer to nondigestible feed ingredients that beneficially affect the host by selectively stimulating the growth and activity of beneficial bacteria in hind intestine. The prebiotics mainly include glucose, fructose, galactose, and mannose (Hume, 2011). Although several studies have reported that combinations of probiotics (especially *Lactobacillus*) and prebiotics have the synergistic effects in promoting immune function and improving growth of beneficial indigenous bacteria and direct-fed probiotic strains in the colon (Mookiah, Sieo, Ramasamy, Abdullah, & Ho, 2014), the *Lactobacillus* is very sensitive to external environment (Corona-Hernandez et al., 2013; Prakash, Tomaro-Duchesneau, Saha, & Cantor, 2011), which may compromise the synergistic effects. Microencapsulation technology has been considered as an effective way to protect probiotics vitality, especially lactic acid bacteria (Dong et al., 2016; Song et al., 2016). Zhang et al. (2015) reported that microencapsulated *E. faecalis* group showed greater average daily gain (ADG) and lower feed conversion ratio (FCR) in the whole feeding phase than the control group in broilers. So we used the microcapsule technology to protect lactic acid bacteria liveability in our study. Therefore, the aim of the present study was to evaluate effects of microencapsulated lactic acid bacteria and other non-encapsulated probiotics and prebiotics on the growth performance, serum antioxidative abilities, serum immune functions, and caecal microflora in broilers.

Materials and methods

Source of microecologic products

The microecologic products were prepared by cereal and oil nutrition research group, Academy of State Administration of Grain (Beijing, China) (Dong et al., 2016). The blending products contained microencapsulated *Enterococcus faecium* (1×10^8 cfu/g), microencapsulated *Lactobacillus plantarum* (1×10^8 cfu/g), *Bacillius subtilis* (1×10^9 cfu/g), 250 U/g β-mannose, and 250 mg/g fructo-oligosaccharide. The count of lactic acid bacteria was analysed by GB 4789.35-2010, the count of *B. subtilis* was analysed by GB/T 26428-2010.

Experimental design and bird management

All animal procedures were approved by the Animal Ethics Committee Guidelines of Academy of State Administration of Grain (Beijing, China) following guidelines recommended in the *Guide for the care and use of agricultural animals in agricultural research and teaching* (FASS, 2010).

A total of 108 one-day-old male Arbor Acres broilers (initial body weight (BW): 42.60 ± 0.50 g) obtained from a commercial hatchery (Huadu Broiler Breeding Farms, Beijing, China) were randomly divided into three groups and there were six replicates per group and six birds per replicate. The three dietary groups were: basal diet (CON), basal diet supplemented with 2 g microecologic products/kg diets (MEP), basal diet supplemented with 0.03% aureomycin (ANT). Experimental diets (Table 1), in mash form, were formulated to

Table 1. Ingredients and nutrient composition of the basal diet (g/kg diet as fed basis)[a,b].

	Composition	
Ingredient	Starter (days 1–21)	Finisher (days 22–42)
Corn	557.50	571.10
Soybean meal	367.50	350.00
Soybean oil	29.60	41.70
Dicalcium phosphate	18.60	14.20
Limestone	12.50	14.00
Salt	3.00	3.00
Choline chloride (50%)	2.60	2.00
Minerals premix[a]	2.00	2.00
Vitamin premix[b]	0.20	0.20
L-Methionine	2.87	0.10
L-Lysine HCl	3.09	1.20
L-Threonine	0.54	0.50
Total	1000	1000
Calculated chemical composition		
ME (MJ/kg)	12.14	12.92
Crude protein	221.00	207.00
Calcium	10.90	10.10
Total phosphorus	6.50	6.50
Lysine	12.00	11.10
Methionine	5.00	3.90

[a]Vitamin premix provided the following per kilogram of diet; vitamin A, 9500 IU; vitamin D_3, 62.5 μg; vitamin K_3, 2.65 mg; vitamin B_{12}, 0.025 mg; vitamin B_2, 6 mg; vitamin E, 30 IU; biotin, 0.0325 mg; folic acid, 1.25 mg; pantothenic acid, 12 mg; nicotinic acid, 50 mg.
[b]The mineral premix provided the following per kg of diet: Cu, 8 mg; Zn, 75 mg; Fe, 80 mg; Mn, 100 mg; Se, 0.15 mg; I, 0.35 mg.

meet nutrient requirements of Chinese feeding standard of chicken (NY/T 33-2004), and all experimental diets had the same nutrient level.

Feeding trial consisted of feeding starter (days 1–21) and feeding grower (days 22–42) diets. The birds were housed in cages with wire mesh floor in an environmentally controlled room. Environmental temperature in the room was maintained at 32–35°C in the first week and then gradually reduced to 24°C until the end of the trial. Feed and water were provided *ad libitum*. The broilers were vaccinated with Newcastle disease-infectious bronchitis (ND-IB) vaccine at day 7 and infectious bursal disease (IBD) vaccine at day 14.

Growth performance

On days 21 and 42, growth performance of birds was evaluated after fasted for 12 h. Feed intake (FI), BW and FCR (FI:BW gain) were determined on a cage basis.

Serum characteristics

On days 21 and 42, one bird was randomly selected from each cage, and serum samples were taken from the wing vein. The serum samples were harvested after centrifugation (3000 g, 10 min) at 4°C and stored at −20°C. The concentrations of immunoglobulin A (IgA), immunoglobulin M (IgM), interleukin-2 (IL-2), interleukin-6 (IL-6), and interleukin-10 (IL-10) were measured using chicken-specific ELISA kits (Cusabio Biotech Co.,

Ltd, Wuhan, China). Another set of serum sample was collected for the determination total antioxidant capacity (T-AOC) activity (cat#: A005), total superoxide dismutase (T-SOD) activity (cat#: A001-3) by commercially available colorimetric diagnostic kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Caecal microflora

The caecal contents were aseptically removed and put in sterilized tubes, then stored at −20°C for subsequent enumeration of microbial population. The 0.5 g caecal contents were diluted with 4.5 mL aseptic distilled water in a flask containing glass beads and then diluted 10-fold from 10^{-2} to 10^{-9}. Caecal contents were plated on Wilkins–Chalgren agar to enumerate total anaerobes, and plated on de Man, Rogosa, and Sharpe (MRS) agar to enumerate *Lactobacillus*. Results were reported as $\log^{10}$ cfu/g of caecal digesta. All agars were obtained from Hopebiol, Bio-technology Co., Ltd (Qingdao, China).

Statistical analysis

All data were analysed by one-way ANOVA by JMP 10 (SAS Institute Inc., Cary, NC). Data were presented as the mean ± standard deviation. Differences among means of treatments were compared using Turkey's test. Caecal microflora counts were transformed to logarithms before analysis. Differences were considered statistically significant at $P \leq .05$.

Results

Growth performance

The results of growth performance were shown in Table 2. Dietary MEP or ANT supplementation significantly increased ADG at days 1–21. Dietary treatments had no significant effects on growth performance of broilers, at days 22–42 and days 1–42, but compared to CON group, diets supplemented with MEP increased ADG by 4.59% and 3.92% at days 22–42 and days 1–42, respectively. Compared to CON group, diets supplemented with ANT increased ADG by 9.32% and 10.6% at days 22–42 and days 1–42, respectively.

Table 2. Effects of microecologic products on growth performance in broilers[1].

Items	CON	MEP	ANT	*P*-value
Days 1–21				
ADG (g/d)	31.7 ± 1.24[b]	34.0 ± 1.33[a]	34.8 ± 1.97[a]	.0133
ADFI (g/d)	52.2 ± 1.06	52.3 ± 2.62	53.3 ± 2.93	.7080
FCR	1.63 ± 0.08	1.59 ± 0.02	1.56 ± 0.08	.4030
Days 22–42				
ADG (g/d)	71.9 ± 2.4	75.2 ± 4.4	78.6 ± 4.2	.0817
ADFI (g/d)	156 ± 12.7	153 ± 7.9	156 ± 12.3	.7875
FCR	2.10 ± 0.09	2.00 ± 0.09	1.90 ± 0.04	.0975
Days 1–42				
ADG (g/d)	53.5 ± 3.05	55.6 ± 2.5	59.2 ± 3.91	.0502
ADFI (g/d)	92.0 ± 5.22	94.5 ± 4.09	93.5 ± 5.85	.6742
FCR	1.68 ± 0.08	1.68 ± 0.03	1.47 ± 0.03	.8588

Note: ADFI: average daily feed intake.
[1]Within a row with no common superscripts differ significantly ($P \leq .05$).

Serum antioxidative ability

The results of serum antioxidative abilities were shown in Table 3. At day 21, birds in MEP group had greater T-AOC level than that in CON and ANT groups ($P < .05$). At day 42, birds in MEP and ANT groups had greater T-AOC level than those in CON group ($P < .05$).

Serum immunoglobulin level

The results of serum immunoglobulin levels were shown in Table 4. At day 21, birds in MEP group had greater IgA level than those in CON and ANT groups ($P < .05$). Birds in MEP and ANT groups had greater IgM level than that in CON group ($P < .05$). At day 42, compared with CON group, IgA level in CON group was increased by 34%.

Serum interleukin level

The results of serum interleukin levels were shown in Table 5. At day 21, birds in MEP group had greater IL-2 level than those in CON and ANT groups. Birds in CON and MEP groups had greater IL-6 and IL-10 levels than those in ANT group ($P < .05$). At day 42, birds in MEP group had greater IL-2 level ($P < .05$) and IL-6 level ($P < .05$) than those in CON and ANT groups.

Caecal microflora

The results of caecal microflora were shown in Table 6. At days 21 and 42, birds in MEP group had greater *Lactobacilli* counts than that in CON and ANT group. There was no significant difference in total anaerobic bacteria count among three groups.

Table 3. Effects of microecologic products on serum antioxidative ability in broilers[1].

Items	CON	MEP	ANT	*P*-value
Day 21				
T-AOC (U/mL)	6.2 ± 2.13^{b}	11.7 ± 2.33^{a}	7.9 ± 2.04^{b}	.0098
T-SOD (U/mL)	82.5 ± 14.5	99.4 ± 14.3	86.1 ± 25.2	.3992
Day 42				
T-AOC (U/mL)	5.1 ± 0.7^{b}	6.56 ± 0.8^{a}	6.34 ± 0.5^{a}	.0408
T-SOD (U/mL)	$129. \pm 15.4$	157 ± 24.4	142 ± 17.2	.1692

[1]Within a row with no common superscripts differ significantly ($P \leq .05$).

Table 4. Effects of microecologic products on serum immunoglobulin level in broilers[1].

Items	CON	MEP	ANT	*P*-value
Day 21				
IgA (mg/mL)	313 ± 78.9^{b}	418 ± 62.6^{a}	320 ± 83.9^{b}	.0382
IgM (mg/mL)	18.8 ± 3.7^{b}	29.2 ± 5.7^{a}	26.7 ± 6.0^{a}	.0093
Day 42				
IgA (mg/mL)	512 ± 122	427 ± 80	511 ± 111	.3711
IgM (mg/mL)	8.8 ± 2.4	12.9 ± 3.9	13.5 ± 4.2	.0787

[1]Within a row with no common superscripts differ significantly ($P \leq .05$).

Table 5. Effects of microecologic products on immunologic factors levels in serum of broilers[1].

Items	CON	MEP	ANT	*P*-value
Day 21				
IL-2 (mg/mL)	4.45 ± 0.69[b]	5.87 ± 1.08[a]	3.98 ± 1[b]	.009
IL-6 (mg/mL)	1.04 ± 0.2[a]	1.43 ± 0.4[a]	0.81 ± 0.3[b]	.0052
IL-10 (mg/mL)	44.38 ± 0.6[a]	44.48 ± 0.8[a]	43.52 ± 0.3[b]	.0258
Day 42				
IL-2 (mg/mL)	8.68 ± 3.6[b]	14.1 ± 4.2[a]	7.55 ± 4.6[b]	.0417
IL-6 (mg/mL)	2.04 ± 0.5[b]	2.73 ± 0.5[a]	1.42 ± 0.3[c]	.0006
IL-10 (mg/mL)	43.5 ± 19.0	33.6 ± 8.1	38.1 ± 21.5	.5959

[1]Within a row with no common superscripts differ significantly ($P \leq .05$).

Table 6. Effects of microecologic products on *Lactobacilli* counts and total anaerobic bacteria counts of caecal in broilers[1,2].

Items	CON	MEP	ANT	*P*-value
Day 21				
Lactobacilli counts	6.25 ± 0.3[b]	6.85 ± 0.2[a]	6.25 ± 0.5[b]	.0131
Total anaerobic bacteria	6.19 ± 0.3	6.0 ± 0.2	5.85 ± 0.1	.4435
Day 42				
Lactobacilli counts	6.73 ± 0.5[b]	7.66 ± 0.3[a]	6.49 ± 0.4[b]	.0007
Total anaerobic bacteria	6.87 ± 0.2	7.02 ± 0.2	6.84 ± 0.1	.2003

[1]Bacterial counts are presented as log10 CFU/g wet weight.
[2]Within a row with no common superscripts differ significantly ($P \leq .05$).

Discussion

Antibiotic use in animal feeds and as antimicrobial therapy for disease continued to grow beginning in 1951 (Dafwang, 1985), which can promote the growth performance by regulating the intestinal microorganisms in direct and indirect way. Firstly, antibiotic can directly decrease the nutriment competition of intestinal microorganism to host and inhibit the growth of intestinal pathogenic microorganism. Secondly, antibiotic can indirectly decrease the nutrient requirements for intestinal maintenance and reduce the incidence of immune response and subclinical disease (Cook, 2004). Although the use of dietary antibiotic has positive effects, it causes serious problems such as the antibiotic-resistant pathogens and drug residues in poultry products.

It is reported that combination of probiotics and prebiotics had beneficial effects on growth performance in broilers (Awad et al., 2009; Dong et al., 2016; Samli, Senkoylu, Koc, Kanter, & Agma, 2007). Abdel-Hafeez, Saleh, Tawfeek, Youssef, and Abdel-daim (2017) demonstrated that combination of *Saccharomyces cerevisiae* and mannan-oligosaccharides (MOSs) can be routinely added to broiler diets to increase BW and improve feed efficiency. Mookiah et al. (2014) reported a significant increase in BW gain and feed efficiency when birds were fed diets supplemented with isomalto-oligosaccharides and 11 strains of *Lactobacillus spp*. Probiotics can maintain the integrity of intestinal structure, inhibit the proliferation of pathogenic bacteria, produce digestive enzymes, and increase the utilization of nutrients, which all can promote the growth and development of animals (Kabir, 2009). In the present study, compared to the control group, dietary supplementation of microecologic products increased ADG, but without affecting FI and FCR, which were in agreement with Hossain et al. (2015) and Balamuralikrishnan, Lee, and Kim (2017). However, some studies reported that dietary supplementation of

synbiotics (probiotics and prebiotics) had no effects on growth performance of broilers (Jung et al., 2008; Willis, Isikhuemhen, & Ibrahim, 2007). Jung et al. (2008) reported that the oral administration of galacto-oligosaccharides alone or in combination with a *Bifidobacterium lactis*-based probiotic had no significant effects on growth, feed consumption, and FCR in broilers. Midilli et al. (2008) reported that dietary combination of probiotic (*Bacillus licheniformis*, *B. subtilis*) and a MOS derived from the cell walls of the yeast (*S. cerevisiae*) had no significant effects on BW gain and FI. The differences in growth performance may be attributed to the selection of probiotics and prebiotics, methods of preparation, administration dosage, diet composition, bird age, and hygiene condition (Mountzouris et al., 2007; Simon, Jadamus, & Vahjen, 2001; Zhang, Zhou, Ao, & Kim, 2012).

Serum T-AOC and T-SOD activities can generally be as the biomarkers to evaluate antioxidant properties (Fang et al., 2017; Wang, Xu, An, Liu, & Feng, 2008). In the present study, we found that serum T-AOC activity of birds in MEP group was significantly greater than those in CON group. Similar findings were reported by Bai et al. (2017), who demonstrated that probiotics had a better antioxidative effect in inhibiting lipid peroxidation in broilers. It is reported that intestinal bacteria could produce certain factors to capture reactive oxygen species (ROS) and prohibit the cytotoxic activity of ROS (Lin & Yen, 1999). Sohail et al. (2011) reported that MOSs and a probiotic mixture (PM) supplements can enhance antioxidant properties by reducing the total oxidants levels and improving the absorption of trace minerals. The current results indicated the dietary treatment with microecologic products can enhance positive feedback mechanism when under oxidative stress by increasing serum T-AOC and T-SOD levels of birds.

The serum immunoglobulins are the important indicators to evaluate the immune status of animal (Wang et al., 2017; Yuan et al., 2015). Many studies have shown that probiotics supplementation could enhance humoral immune response of broiler by increasing the level of immunoglobulins (Salim et al., 2013; Zhang & Kim, 2014). The current results showed the serum IgA and IgM levels of birds in MEP group were the highest among three groups at day 21. The immune response is controlled by a complex interplay among the various cytokines. T helper cell (Th) differentiates in the thymus into Th1 and Th2 cells based on differences in the cytokines they secrete. Th1 cells secrete mainly IL-2, INF-γ, and TNF-α. IL-2 is necessary for T and B cell transformation. Following antigen activation and stimulation by IL-2, the Th2 cells respond by transforming, differentiating, and dividing logarithmically, while secreting mainly IL-4, IL-5, IL-6, IL-10, and IL-13 (Johnson, 1999). So the cytokine interleukin are the most important T cell growth factors and play an important role in promoting the host immune response (Choi & Lillehoj, 2000). Hassanpour, Moghaddam, Khosravi, and Mayahi (2013) reported that compound probiotics could stimulate the immune function in an active state, and then enhance the antibody production (Hassanpour et al., 2013). The current results found dietary supplementation of MEP increased the IL-2 and IL-6 levels, and stimulate the production of IgA and IgM.

A balanced microbial population can support a healthy intestinal tract resulting in better control of intestinal pathogens (Konstantinov et al., 2006). Lactobacilli have the ability to inhibit the growth of putrefactive and pathogenic bacteria (Paton, Morona, & Paton, 2006). Some studies reported that probiotic and prebiotic had synergistic effects in maintaining caecal microbial balance in broilers (Wang et al., 2017; Zhang & Kim,

2014). The well-established growth-promoting effects of probiotic and prebiotic suggested that probiotic and prebiotic can modulate the intestinal ecosystem by increasing the numbers of lactic acid bacteria, *Bifidobacteria* and total anaerobic bacteria, and decreasing the numbers of *enteric Bacilli* and total aerobic bacteria (Schrezenmeir & de Vrese, 2001). In the current study, dietary supplementation of MEP significantly increased caecal *Lactobacilli* counts of broilers. The microecologic products could exert beneficial effects by increasing caecal *Lactobacilli* counts to balance the microbial population.

Conclusions

The compound microecologic product had beneficial effects on BW gain, the serum T-AOC activities, the serum IgA, IgM, IL-2, and IL-6 levels, and caecal *Lactobacillus* counts in broilers. Therefore, the compound microecologic product (microencapsulated probiotics and prebiotics) can be recommended as potential alternative to antibiotics in chicken diets.

Disclosure statement

No potential conflict of interest was reported by the authors.

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