Effects of Probiotics on Early Growth, Dietary Nutrient Utilization, and Intestinal Microbiota in Laying Hens (Postprint)

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Abstract

This experiment was conducted to investigate the effects of dietary probiotic supplementation on early growth, nutrient utilization, and gut microbiota in layer chickens. A total of 540 healthy 1-day-old Lohmann Brown male chicks with similar body weight were randomly allocated to 3 groups, each consisting of 6 replicates of 30 birds. The control group received a basal diet, the antibiotic group received the basal diet supplemented with 0.1 g/kg zinc bacitracin premix, and the probiotic group received the basal diet supplemented with 1 g/kg probiotic preparation. The experimental period lasted 70 days. The results showed: 1) Compared with the control group, the 70-day body weight was significantly increased in both the probiotic and antibiotic groups ($P<0.05$), with no significant difference between these two groups ($P>0.05$). 2) Compared with the control group, the apparent utilization rate of dietary crude protein in the probiotic group increased by 8.7% ($P=0.054$), and the apparent utilization rate of calcium increased by 22.96% ($P<0.05$); the apparent utilization rate of dietary energy in the antibiotic group was significantly increased ($P<0.05$). 3) Compared with the control group, the number of microbial species and the abundance of beneficial bacteria in the cecum of the probiotic group were increased, while the abundance of harmful bacteria was decreased; in the antibiotic group, the abundance of harmful bacteria in the cecum was decreased, but the microbial richness and abundance of beneficial bacteria in the cecum were also decreased. It can be concluded that dietary probiotic supplementation can promote early growth, improve nutrient utilization, optimize gut microbiota structure, and enhance physiological health status in layer chickens, and that probiotics are more beneficial to gut health than antibiotics.

Full Text

Effects of Probiotics on Growth, Diet Nutrient Utilization and Intestinal Microflora in Early Life of Layer Chickens

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Abstract

This experiment was conducted to investigate the effects of dietary probiotics on growth performance, nutrient utilization, and intestinal microflora in early life of layer chickens. Five hundred and forty 1-day-old healthy male Roman Brown layer chicks with similar body weight were randomly allocated into 3 groups with 6 replicates per group and 30 chicks per replicate. The control group was fed a basal diet, the antibiotic group received the basal diet supplemented with 0.1 g/kg zinc bacitracin premix, and the probiotic group received the basal diet supplemented with 1 g/kg probiotic preparation. The experiment lasted for 70 days. The results showed that: 1) Compared with the control group, the body weight at 70 days of age was significantly increased in both probiotic and antibiotic groups ($P<0.05$), with no significant difference between these two groups ($P>0.05$). 2) The probiotic group exhibited an 8.7% improvement in apparent crude protein utilization ($P=0.054$) and a 22.96% increase in apparent calcium utilization ($P<0.05$) compared to the control group, while the antibiotic group showed significantly improved apparent energy utilization ($P<0.05$). 3) Compared with the control group, the probiotic group demonstrated increased microbial diversity and beneficial bacterial abundance in the cecum, along with reduced pathogen abundance; the antibiotic group showed decreased pathogen abundance but also reduced microbial richness and beneficial bacterial abundance. These findings indicate that dietary probiotic supplementation can promote early growth, improve nutrient utilization, enhance intestinal microflora structure, and improve physiological health status in layer chickens, with probiotics being more beneficial for intestinal health than antibiotics.

Keywords: probiotic; layer chicken; early life; nutrient utilization; intestinal microflora

Introduction

With growing concerns over antibiotic-induced bacterial resistance and drug residues in animal-derived food products, probiotics have gained widespread application and recognition in practical production [1]. Previous studies have reported that dietary probiotics can improve growth performance [2-4], enhance nutrient utilization [5], promote intestinal development [6-8], and reduce harmful bacteria populations in the gut [9-10] in broiler chickens. However, most probiotic research has focused on broilers, with limited studies on early-stage layer chickens. The brooding and early rearing periods represent critically important foundational stages in layer chicken development, during which nutrient utilization capacity, intestinal development, and gut health status directly affect normal growth during rearing and subsequent laying performance. Maintaining optimal physiological health from the chick stage would facilitate long-term healthy growth and support high production levels during the laying period. Therefore, this study aimed to investigate the effects of dietary probiotics on early growth, nutrient utilization, and intestinal microflora in layer chickens, thereby clarifying the role of probiotics in early-stage layer diets and providing a reference for probiotic application in layer chicken nutrition.

Materials and Methods

1.1 Experimental Materials The probiotic preparation used in this experiment consisted of *Bacillus subtilis* and *Pediococcus acidilactici*, each with viable counts of 1×10 CFU/g, purchased from Shandong Baolai-LaiLai Bioengineering Co., Ltd. The zinc bacitracin premix contained 10% zinc bacitracin, purchased from Tianjin Xinxing Veterinary Pharmaceutical Factory. Experimental animals were 1-day-old Roman Brown layer chicks provided by Jinhu County Xingda Poultry Co., Ltd. The dietary premix (1% addition rate) was provided by Yangzhou Yangda Feed Factory.

1.2 Experimental Design Five hundred and forty 1-day-old Roman Brown layer chicks from the same hatch with similar body weight were randomly divided into 3 groups: a control group fed the basal diet without any probiotics or antibiotics, an antibiotic group fed the basal diet supplemented with 0.1 g/kg zinc bacitracin premix, and a probiotic group fed the basal diet supplemented with 1 g/kg probiotic preparation. Each treatment group consisted of 6 replicates with 30 chicks per replicate. The basal diet was formulated as a powder according to NRC (1994) [11] nutrient requirements, and the premix contained no antibiotics. The composition and nutrient levels of the basal diet are presented in Table 1 .

The 70-day experiment was conducted in indoor cages with ad libitum access to feed and water. The health status of the chickens was observed and recorded daily.

1.3 Sample Collection and Analysis

1.3.1 Metabolism Trial At 70 days of age, one chicken with body weight close to the replicate average was selected from each replicate in all groups for a metabolism trial. The total collection method was used to collect feces continuously for 4 days. Crude protein in feces was determined according to the national standard method [12], while dry matter and energy were analyzed using methods described in "Feed Analysis and Quality Detection Technology" edited by Zhang Liying [13].

1.3.2 Cecal Microflora Determination Total DNA was extracted from aseptically collected cecal contents. After optimization, high-throughput sequencing was performed on the NGS Illumina MiSeq 2×300 bp platform to determine cecal microflora composition and structure.

1.4 Data Analysis Data were compiled using Excel 2007 software and analyzed using one-way ANOVA and Duncan' s multiple comparison tests in SPSS 17.0 software. Differences were considered significant at $P<0.05$. Results are expressed as "mean ± standard deviation" .

Results

2.1 Effects of Dietary Probiotics on Early Growth Performance The effects of dietary probiotics on early growth performance are shown in Table 2 . At 70 days of age, body weight in both probiotic and antibiotic groups was significantly higher than in the control group ($P<0.05$), with no significant difference between the probiotic and antibiotic groups ($P>0.05$).

2.2 Effects of Dietary Probiotics on Nutrient Apparent Utilization As shown in Table 3 , the probiotic group exhibited a trend toward improved apparent crude protein utilization ($P=0.054$), with an 8.7% increase compared to the control group. The antibiotic group showed significantly higher apparent energy utilization than both the control and probiotic groups ($P<0.05$), while no significant difference was observed between the control and probiotic groups for energy utilization ($P>0.05$). Apparent calcium utilization in the probiotic group was significantly higher than in both antibiotic and control groups ($P<0.05$), with no significant difference between the latter two groups ($P>0.05$). No significant differences were detected among groups for apparent utilization of dry matter or phosphorus ($P>0.05$).

2.3 Effects of Dietary Probiotics on Cecal Microflora

2.3.1 Effects on Cecal Microbial Community Abundance Operational Taxonomic Units (OTUs) are uniform markers artificially assigned to specific taxonomic units (strain, genus, or species) in population genetics research to facilitate information analysis. Through clustering operations, sequences are grouped based on similarity, with each group representing one OTU. All sequences can be classified into OTUs at different similarity levels, with bioinformatic statistical analysis typically performed at the 97% similarity level. Venn diagrams intuitively display OTU composition and similarity among samples.

Since Venn diagrams can only analyze 2-5 samples or groups, inter-group comparisons were conducted accordingly. The Venn diagram (Figure 1 [Figure 1: see original paper]) revealed that the probiotic group had a total of 466 OTUs, greater than the antibiotic group (453 OTUs) and control group (450 OTUs).

2.3.2 Effects on Cecal Microflora Structure The species composition of each sample at phylum and genus levels is illustrated in histograms (Figure 2 [Figure 2: see original paper] and Figure 3 [Figure 3: see original paper]). At the phylum level, 13 different bacterial phyla were detected in the cecum, with Bacteroidetes and Firmicutes being the dominant phyla. These two dominant phyla accounted for averages of 49.3% and 41.8% in the probiotic group, 49.5% and 40.9% in the antibiotic group, and 49.2% and 41.7% in the control group, respectively. Although Fusobacteria were not dominant, their abundance was lower in the probiotic group than in the other two groups. Additionally, Actinobacteria abundance averaged 1.5% in the probiotic group, lower than 1.9% in the antibiotic group and 1.7% in the control group. In the antibiotic group, B2 and B4 Proteobacteria were higher than in other samples.

Among the 13 bacterial phyla, 168 different genera were identified. As shown in Figure 3, Barnesiella was the dominant genus, accounting for 24.00%, 27.49%, and 27.54% of the microflora in the control, antibiotic, and probiotic groups, respectively. *Bacteroides* was also a dominant genus in the cecum, with 11.1% abundance in the probiotic group, higher than 5.0% in the antibiotic group and 7.3% in the control group. Among *Bacteroides* species, *Bacteroides uniformis*, *Bacteroides fragilis*, and *Bacteroides eggerthii* were detected through high-throughput sequencing. *Bacteroides uniformis* abundance was 0.98% in the antibiotic group, lower than 2.96% in the probiotic group and 2.45% in the control group. *Bacteroides fragilis* abundance was 1.8% in the probiotic group, higher than 1.1% in the antibiotic group and 0.7% in the control group. *Bacteroides eggerthii* abundance was 0.56% in the antibiotic group, lower than 0.85% in the probiotic group and 0.78% in the control group.

Furthermore, dietary antibiotics reduced cecal *Bacillus* content to less than 0.001%, substantially lower than 0.036% in the probiotic group and 0.012% in the control group. Enterobacteriaceae abundance was 0.029% in the probiotic group and 0.01% in the antibiotic group, both lower than 0.044% in the control group.

Discussion

3.1 Effects of Dietary Probiotics on Early Growth Performance Probiotics can produce various vitamins, amino acids, and digestive enzymes in the intestine to promote nutrient digestion and utilize excess oxygen in the gut, thereby promoting the growth of anaerobic bacteria such as Bacteroidetes that dominate intestinal digestion and absorption, ultimately enhancing nutrient metabolism and absorption and improving growth performance. Research on probiotics in layer chickens during early development is limited compared to extensive studies in broilers. Lu et al. [14] reported that a composite probiotic preparation containing *Bacillus cereus*, *Bacillus subtilis*, and *Enterococcus faecalis* significantly improved growth performance in broilers aged 22-35 days. Salim et al. [15] also demonstrated that dietary probiotic supplementation promoted broiler growth and significantly increased body weight. Zhang et al. [16] found that *Bacillus subtilis* supplementation significantly increased broiler body weight after 35 days of feeding. The present results align with these findings, showing that dietary probiotic supplementation significantly promoted growth in layer chickens. Xie et al. [17] compared various probiotic preparations with antibiotic feed additives and concluded that probiotics exhibited equal or even superior growth-promoting effects. The specific growth-promoting mechanisms of probiotics, which involve secretion of nutrients and digestive enzymes during proliferation and metabolic activities, warrant further investigation.

3.2 Effects of Dietary Probiotics on Nutrient Utilization in Young Layers Previous studies have shown that *Bacillus subtilis* supplementation in yellow-feathered broilers and layer chickens can significantly improve crude protein, calcium, and phosphorus utilization [18] without affecting dry matter utilization [19]. However, Li et al. [5] and Apata [20] reported that probiotics significantly improved ileal apparent utilization of dry matter, crude protein, and energy in broilers. In this study, although probiotic supplementation did not significantly affect apparent crude protein utilization, it showed a trend toward improvement compared to the control group. Probiotics significantly improved calcium apparent utilization, consistent with findings by Zhou et al. [18] and Li et al. [19]. Calcium is closely related to bone development and eggshell formation in layers. Wan [21] proposed that *Bacillus subtilis* can synthesize B vitamins and vitamins E and K during growth and reproduction, participating in animal metabolism and promoting calcium and phosphorus absorption. Nahashon et al. [22] also demonstrated that microbial agents can improve calcium absorption and utilization in chickens, suggesting they facilitate adequate calcium supply during egg production. Therefore, the significant improvement in calcium utilization by probiotics compared to the basal diet and antibiotics indicates that probiotic supplementation in early-stage layer diets can promote growth by enhancing calcium absorption, with continued use potentially improving laying performance. No significant differences in dry matter apparent utilization among groups in this study align with Li et al. [19]. Probiotics produce and secrete various enzymes during intestinal proliferation, particularly bacilli, which can enhance activities of amylase, protease, and lipase [23]. Wang et al. [24] reported that probiotic supplementation significantly improved pancreatic amylase and trypsin activities and stimulated secretion of these enzymes, thereby promoting nutrient absorption. Other studies have indicated that active substances such as digestive enzymes and growth-promoting factors produced by probiotics can synergistically enhance host digestion and absorption of nutrients [25]. The significantly improved energy apparent utilization in the antibiotic group suggests that both probiotics and antibiotics enhanced nutrient utilization in different aspects, which may be related to the improved growth performance observed at 70 days of age.

3.3 Effects of Dietary Probiotics on Cecal Microflora in Early-Stage Layers This experiment identified a total of 477 OTUs, including 437 OTUs common to all three groups, 4 unique OTUs in each group, 16 unique OTUs shared between probiotic and control groups, 9 unique OTUs shared between probiotic and antibiotic groups, and only 4 unique OTUs shared between antibiotic and control groups. The greater total OTU number in the probiotic group indicates that probiotics increased cecal microbial diversity, whereas antibiotics reduced OTU numbers. Cao [26] reported that dietary supplementation with bacitracin zinc and colistin sulfate significantly reduced cecal OTU numbers in broilers, consistent with our findings.

At the phylum level, mammalian gut microbiota are dominated by Bacteroidetes, Firmicutes, Actinobacteria, and Proteobacteria, with Firmicutes and Bacteroidetes as the predominant phyla. Cao [26] similarly found that broiler ceca primarily contained Firmicutes, Bacteroidetes, Actinobacteria, and small amounts of Tenericutes, with Firmicutes and Bacteroidetes as the dominant phyla. The present results showed that layer chicken ceca mainly contained Bacteroidetes, Firmicutes, Actinobacteria, Proteobacteria, and Tenericutes, with the first two being the most dominant phyla, indicating that layer chickens, broilers, and mammals share similar dominant microflora structures. Choi et al. [27] demonstrated that chicken gastrointestinal microbiota primarily consisted of Bacteroidetes, Firmicutes, Actinobacteria, Proteobacteria, and Acidobacteria, with structural differences likely related to species, breed, dietary composition, and environmental conditions [28].

At the genus level, *Barnesiella* and *Bacteroides* were the dominant genera. *Bacteroides* is crucial for maintaining host health [29]. The probiotic group showed significantly higher *Bacteroides* abundance than other groups, particularly *Bacteroides fragilis*. As a normal intestinal commensal bacterium, *B. fragilis* is well understood and acts as an opportunistic pathogen only when tissue damage or pathological changes occur. Reduced *B. fragilis* populations are associated with abnormalities; Sha [30] found that patients with ulcerative colitis and Crohn' s disease had lower intestinal *B. fragilis* abundance than healthy individuals. Other studies have linked *B. fragilis* to glucose metabolism, with Zhang [31] reporting that it can indirectly promote glucose metabolism and utilize polysaccharides effectively. Zhu et al. [32] demonstrated that *B. fragilis* BF-839 supplementation improved immune function in broilers. Since no lesions were observed in this study, the increased *B. fragilis* in the probiotic group may have contributed to enhanced digestive and immune functions, though specific mechanisms require further investigation. The significantly reduced *Bacteroides uniformis* in the antibiotic group suggests antibiotics may affect immune function by altering this species.

Both antibiotics and probiotics reduced Enterobacteriaceae abundance, which includes pathogenic genera such as *Escherichia*, *Salmonella*, *Shigella*, *Enterobacter*, and *Proteus*, indicating that both can inhibit harmful bacteria. However, probiotics significantly increased *Bacillus* abundance, whereas antibiotics substantially reduced it. *Bacillus* not only promotes nutrient digestion and absorption but also inhibits pathogen growth, providing important probiotic benefits. Consistent with these results, Wang [33] reported that probiotics increased beneficial bacteria such as *Lactobacillus* and *Bifidobacterium* while reducing pathogen numbers. Combined with the improved body weight and nutrient utilization observed in this study, probiotics exert positive effects by increasing beneficial bacteria like *Bacillus*, whereas antibiotics, despite inhibiting pathogens, also suppress beneficial bacteria, compromising intestinal health.

Conclusion

Dietary probiotic supplementation can improve early growth performance and nutrient utilization while enhancing intestinal microflora structure in layer chickens. Although antibiotic supplementation also promotes early growth and nutrient utilization, it is detrimental to cecal microbial colonization and stability of microflora structure.

References

[1] Tang Zhigang, Qian Qiaoling, Hou Xiaoying, et al. Mechanism of probiotics and their application in broilers and layers [J]. Journal of Domestic Animal Ecology, 2010, 31(2): 5-8.
[2] Yang C M, Cao G T, Ferket P R, et al. Effects of probiotic, *Clostridium butyricum*, on growth performance, immune function, and cecal microflora in broiler chickens [J]. Poultry Science, 2012, 91(9): 2121-2129.
[3] Tang Zhigang, Wang Junfeng, Wen Chao, et al. Effects of probiotics on production performance, immune organ indices and serum parameters in broilers [J]. Jiangsu Agricultural Sciences, 2010(4): 208-210.
[4] Hossain M M, Begum M, Kim I H. 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 [J]. Veterinarni Medicina, 2015, 60(2): 77-86.
[5] Li L L, Hou Z P, Li T J, et al. Effects of dietary probiotic supplementation on ileal digestibility of nutrients and growth performance in 1- to 42-day-old broilers [J]. Journal of the Science of Food and Agriculture, 2008, 88(1): 35-42.
[6] Sen S, Ingale S L, Kim Y W, et al. Effect of supplementation of *Bacillus subtilis* LS 1-2 to broiler diets on growth performance, nutrient retention, caecal microbiology and small intestinal morphology [J]. Research in Veterinary Science, 2012, 93(1): 264-268.
[7] Samanya M, Yamauchi K. Histological alterations of intestinal villi in chickens fed dried *Bacillus subtilis* var. natto [J]. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 2002, 133(1): 95-104.

[8] Wolfenden R E, Pumford N R, Morgan M J, et al. Evaluation of selected direct-fed microbial candidates on live performance and *Salmonella* reduction in commercial turkey brooding houses [J]. Poultry Science, 2011, 90(11): 2627-2631.
[9] Knap I, Kehlet A B, Bennedsen M, et al. *Bacillus subtilis* (DSM17299) significantly reduces *Salmonella* in broilers [J]. Poultry Science, 2011, 90(8): 1690-1694.
[10] Liu Yongjie, Zhao Yanbing, Cui Bintao, et al. Effect of feeding *Lactobacillus* to chicks on colonization of major normal microflora in cecum [J]. Chinese Veterinary Science, 1999, 29(5): 29-30.
[11] NRC. Nutrient requirements of poultry [S]. Washington, D.C.: National Academy Press, 1994.
[12] National Feed Industry Standardization Technical Committee. GB 6432–1994 Method for determination of crude protein in feed [S]. Beijing: China Standards Press, 1995.
[13] Zhang Liying. Feed analysis and feed quality detection technology [M]. 2nd ed. Beijing: China Agricultural University Press, 2003: 49-53.
[14] Lu Yin, Wu Xufeng, Fei Yongjun, et al. Effects of compound probiotics on growth performance and carcass quality of broilers [J]. Chinese Journal of Animal Science, 2013, 49(1): 50-53.
[15] Salim H M, Kang H K, Akter N, et al. Supplementation of direct-fed microbials as an alternative to antibiotic on growth performance, immune response, cecal microbial population, and ileal morphology of broiler chickens [J]. Poultry Science, 2013, 92(8): 2084-2090.
[16] Zhang Z F, Cho J H, Kim I H. Effects of *Bacillus subtilis* UBT-MO2 on growth performance, relative immune organ weight, gas concentration in excreta, and intestinal microbial shedding in broiler chickens [J]. Livestock Science, 2013, 155(2/3): 343-347.
[17] Xie Quanxi, Cui Shifa, Xu Haiyan, et al. Effects of compound microbial preparation and feed antibiotics on growth performance, immune performance and antioxidant indices in broilers [J]. Chinese Journal of Animal Nutrition, 2012, 24(7): 1336-1344.
[18] Zhou Liqiang, Lan Liyan, Xu Chunsheng, et al. Effects of *Bacillus subtilis* on growth performance and nutrient metabolic rate in yellow-feathered broilers [J]. China Animal Husbandry & Veterinary Medicine, 2012, 39(2): 71-74.
[19] Li Junbo, Cheng Tingshui, Lü Wuxing, et al. Effects of *Bacillus subtilis* preparation on performance, egg quality and nutrient digestibility in laying hens [J]. China Poultry, 2009, 31(4): 15-17.
[20] Apata D F. Growth performance, nutrient digestibility and immune response of broiler chicks fed diets supplemented with a culture of *Lactobacillus bulgaricus* [J]. Journal of the Science of Food and Agriculture, 2008, 88(7): 1253-1258.
[21] Wan Fuchang. Research and application of microecological drugs [M]. Beijing: Chemical Industry Press, 2009: 90-220.
[22] Nahashon S N, Nakaue H S, Mirosh L W. Production variables and nutrient retention in Single Comb White Leghorn laying pullets fed diets supplemented

with direct-fed microbials [J]. Poultry Science, 1994, 73(11): 1699-1711.
[23] Zhou Bing, Zhang Weicai. Advances in protein secretion mechanisms of *Bacillus subtilis* [J]. Letters in Biotechnology, 2004, 15(3): 281-284.
[24] Wang Y B, Xu Z R. Effect of probiotics for common carp (*Cyprinus carpio*) based on growth performance and digestive enzyme activities [J]. Animal Science Technology, 2006, 127(3/4): 283-292.
[25] Sandusky C L, Heath J L. Effect of dietary sacchariterpenin to rabbit production performance [J]. Nutrition Research, 2003, 159(4): 880-884.
[26] Cao Guangtian. Antimicrobial mechanism of *Bacillus amyloliquefaciens* and its effects on growth metabolism and cecal microflora in broilers [D]. Ph.D. thesis. Hangzhou: Zhejiang University, 2015: 98-131.
[27] Choi J H, Kim G B, Cha C J. Spatial heterogeneity and stability of bacterial community in the gastrointestinal tracts of broiler chickens [J]. Poultry Science, 2014, 93(8): 1942-1950.
[28] Mueller S, Saunier K, Hanisch C, et al. Differences in fecal microbiota in different European study populations in relation to age, gender, and country: a cross-sectional study [J]. Applied and Environmental Microbiology, 2006, 72(2): 1027-1033.
[29] Rocha E R, Smith C J. Ferritin-like family proteins in the anaerobe *Bacteroides fragilis*: when oxygen storm coming, take shelter [J]. Biometals, 2013, 26(4): 577-591.
[30] Sha Sumei. Study on fecal microflora biodiversity and main composition in IBD patients [D]. M.S. thesis. Xi' an: The Fourth Military Medical University, 2011: 38-59.
[31] Zhang Yong. Effects of probiotic *Lactobacillus casei* Zhang on improvement of impaired glucose tolerance and prevention of type II diabetes in rats [D]. Ph.D. thesis. Hohhot: Inner Mongolia Agricultural University, 2013: 1-66.
[32] Zhu Yanxu, Shang Erbin, Wang Zhanhong. Effects of *Bacteroides fragilis* BF-839 on production performance of broilers [J]. Modern Journal of Animal Husbandry and Veterinary Medicine, 2012(3): 40-42.
[33] Wang Lifeng. Study on effects of probiotic *L. plantarum* P-8 on intestinal microflora, intestinal immunity and growth performance in broilers [D]. Ph.D. thesis. Hohhot: Inner Mongolia Agricultural University, 2014: 1-65.

Note: Figure translations are in progress. See original paper for figures.