

The effect of probiotic *Bacillus amyloliquefaciens* on growth performance, immune organ index and immune function of weaned meat rabbits

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ABSTRACT

Bacillus has been reported to improve the production performance and immunity of chickens and pigs, so we hypothesized that *Bacillus amyloliquefaciens* (BA) could effectively increase the growth performance and immunological ability of weaned meat rabbits. To verify this hypothesis, this study was designed to examine the effect of BA on the growth performance, immune organ index, and immune function of meat rabbits. A total of 160 weaned New Zealand meat rabbits (35 ± 3 days) were randomly divided into 4 groups with 4 replicates per group (n = 10 per replicate, 5 males and 5 females). The rabbits of the control group were fed with a basal diet and the rabbits of treatment groups I, II and III were fed with the basal diet supplemented with 150, 300 and 450 mg/kg BA, respectively, for a total of 8 weeks. Our results showed that BA supplementation increased daily gain and reduced feed to gain ratio. Furthermore, BA supplementation significantly increased splenic and thymic indexes and the levels of immunoglobulin M (IgM), complement 3 (C3), and C4 in the treatment groups II and III (P<0.05). Therefore, BA supplementation in the diet could effectively improve the growth performance, immune organ index and immune function of weaned meat rabbits.

Key words: *Bacillus amyloliquefaciens*; meat rabbits; growth performance; immune organ index; immune function

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Introduction

The wide use of antibiotics in livestock farming may bring about many positive outcomes, including improved animal production performance and reduced disease (DOWARAH et al, 2017; GADDE et al., 2017). Irrational or abusive use of antibiotics, however, has also led to several

problems, such as occurrence of antibiotic-resistant bacteria, a weakened immune system, antibiotic residual in animal-origin foods, and environmental contamination (BARTON, 2000; GAO et al., 2017; LI et al., 2017; ZOU et al., 2016). To tackle these problems, many countries have developed regulations and restrictions on antibiotics as feed

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supplements (COGLIANI et al., 2011). Therefore, the search for a highly effective, non-toxic, residual-free and safe feed product, as a substitute for antibiotics, has become the major focus of animal nutrition studies (YANG and MCHOCT, 2009).

A probiotic is a new type of functional feed additive that is safe, non-toxic, and residual-free, and has many healthy functions, such as disease prevention and growth promotion, and is hence a promising effective substitute for feed antibiotics (LUTFUL KABIR, 2009). *Bacillus* is an acid-tolerant, salt-tolerant, heat-tolerant, and stable probiotic, and its biological function is mainly derived from its ability to generate numerous bio-active substances, such as proteases, lipases, and amylases, as well as antimicrobial substances that inhibit the growth of pathogenic bacteria and viruses (PARK et al., 2016; GIBSON et al., 2017).

At present, *Bacillus* has been reported to improve the production performance and immunity of chickens and pigs (GUO et al., 2018; SAMOLINSKA et al., 2018). In addition, the rabbit digestive system and immune system are different from those of pigs and chickens. However, information about the effects of *Bacillus* on the growth and immune functions of meat rabbit is scant. Therefore, in this study, the daily diet of weaned New Zealand meat rabbits was supplemented with *Bacillus amyloliquefaciens* (BA) to examine its effect on the growth performance and immune functions of these animals, in order to provide a theoretical and scientific basis for wide application of BA.

Materials and methods

Ethics statement. Animal experiments in this study were carried out under the supervision of the Animal Care and Use Committee of Shandong Province, China (Permit No. 20181126).

Experimental design. A total of 160 weaned New Zealand meat rabbits (35 ± 3 days; 782 ± 31 g) were randomly divided into a control group and treatment groups I, II, and III, with 4 replicates per group, and 10 rabbits (5 males and 5 females) per replicate. The basal feed used in this study was produced on the basis of the standard Nutrient Requirements of Rabbits (NRC, 1977) (Table 1).

Table 1. Composition and nutrient levels of the basal diet (air-dry)

Items	Content
Ingredients (%)	
Alfalfa meal	30.0
Corn	21.0
Soybean meal	10.7
Wheat bran	21.0
Wheat	7.0
Silkworm chrysalis	3.0
Rapeseed meal	4.0
CaHPO ₄	0.9
Limestone	0.7
NaCl	0.5
Lysine	0.2
Premix [#]	1.0
Total	100.0
Nutrient levels	
Crude protein	16.02
Crude fiber	13.10
Ca	0.90
P	0.62
Digestible energy (DE) (MJ/kg) ^{**}	10.71

[#]Premix provided the following per kg of diet: Cu, 120 mg; Fe, 100 mg; Zn, 90 mg; Mn, 30 mg; Mg, 150 mg; vitamin A, 4,000 IU; vitamin D₃, 1,000 IU; vitamin E, 50 mg; choline, 1 mg; ^{**}DE was calculated (de Blas and Mateos, 1998), and the other nutrient levels were measured values.

The control group was fed with the basal diet, while treatment groups I, II, and III were fed with the basal diet supplemented with 150, 300 and 450 mg/kg BA (viable bacteria $\geq 1.5 \times 10^{10}$ CFU/g), respectively, for a total of 8 weeks.

All experimental rabbits were housed in a closed rabbit shed, with two rabbits per cage (60 cm × 60 cm × 60 cm). The rabbit shed and its ancillary facilities were strictly disinfected prior to the experiment. The experimental rabbits had access to water and food *ad libitum*, and the rabbit shed was regularly cleaned. At the end of the experiment, the rabbits were anesthetized with 10% chloral hydrate and then sacrificed for further study.

Determination of growth performance. During the experimental period, the food consumption and fasting weight of the experimental rabbits were measured weekly. Average daily gain (ADG), average daily feed intake (ADFI), and feed to gain ratio (F/G) were calculated as follows:

ADG = Total gain / (Number of rabbits per group × experimental days); ADFI = (Total feed provided - total feed remaining) / (Number of rabbits per group × experimental days); F/G = Daily feed intake/daily gain.

Determination of immune organ index. At the end of the experiment, 5 experimental rabbits were randomly selected from each group, and the thymus and spleen of each rabbit were weighed to calculate the thymic and splenic index, respectively. Thymic index = (thymus weight /weight before euthanasia) ×100%; Splenic index = (spleen weight /weight before euthanasia) ×100%.

Results

The effect of BA on growth performance. In terms of ADFI, no significant difference was found between the four groups ($P>0.05$). During weeks 0-4, ADG was significantly higher in the three treatment groups than in the control group ($P<0.05$), and ADG was significantly higher in treatment

Determination of serum immune markers. At the end of the experiment, 4 experimental rabbits were randomly selected from each group, and blood was collected via cardiac puncture into 5 mL tubes. Blood samples were centrifuged at 3,000 r/min for 5 min, and supernatants were transferred to new tubes and stored at -20 °C. Serum concentrations of immunoglobulin (Ig) G, IgM, complement 3 (C3) and C4 were determined by the immunoturbidimetric assay (ZHAO and JIANG, 2006).

Statistical analysis. Statistical analysis was performed using the SPSS18.0 software (SPSS Inc., Chicago, Illinois, USA). Single and multiple comparisons were performed using one-way analysis of variance (ANOVA) and Duncan's test, respectively. Data are expressed as mean ± SD and $P<0.05$ was considered statistically significant.

groups II and III than in treatment group I ($P<0.05$). During weeks 5-8, ADG was significantly higher in the three treatment groups than in the control group ($P<0.05$). During the entire experimental period (weeks 0-8), ADG was significantly higher in treatment groups II and III than in treatment

Table 2. The effect of BA on the growth performance of meat rabbits.

Items	Treatment groups			Control group
	I	II	III	
ADFI (g/d)				
0-4 week	96.98 ± 3.96	97.71 ± 4.03	98.12 ± 4.62	97.16 ± 4.28
5-8 week	134.23 ± 3.28	135.61 ± 4.88	134.91 ± 4.78	134.29 ± 5.22
0-8 week	115.87 ± 4.68	116.66 ± 4.56	116.51 ± 5.12	115.22 ± 6.48
ADG (g/d)				
0-4 week	32.02 ± 0.21 ^b	33.26 ± 0.37 ^c	33.68 ± 0.49 ^c	30.29 ± 0.19 ^a
5-8 week	30.18 ± 0.31 ^b	31.20 ± 0.42 ^b	31.27 ± 0.21 ^b	28.58 ± 0.49 ^a
0-8 week	31.18 ± 0.15 ^b	32.29 ± 0.28 ^c	32.42 ± 0.23 ^c	29.36 ± 0.52 ^a
F/G (g/g)				
0-4 week	3.01 ± 0.03 ^b	2.92 ± 0.03 ^c	2.91 ± 0.02 ^c	3.18 ± 0.05 ^a
5-8 week	4.43 ± 0.08 ^b	4.38 ± 0.06 ^b	4.31 ± 0.04 ^b	4.62 ± 0.13 ^a
0-8 week	3.70 ± 0.06 ^b	3.60 ± 0.05 ^c	3.58 ± 0.07 ^c	3.91 ± 0.08 ^a

^{a-c}: Means in the same row with no common superscripts differ significantly ($P<0.05$). ADG, average daily gain; ADFI, average daily feed intake; F/G, feed to gain ratio.

Table 3. The effect of BA on the immune organ index of meat rabbits

Items	Treatment groups			Control group
	I	II	III	
Thymus weight(g)	5.02 ± 0.61 ^a	6.18 ± 0.59 ^b	6.56 ± 0.67 ^b	4.58 ± 0.39 ^a
Spleen weight(g)	1.51 ± 0.37 ^a	1.93 ± 0.38 ^b	2.01 ± 0.26 ^b	1.45 ± 0.42 ^a
Thymic index(g/kg)	2.03 ± 0.38 ^a	2.36 ± 0.26 ^b	2.45 ± 0.21 ^b	1.81 ± 0.22 ^a
Splenic index(g/kg)	0.59 ± 0.21 ^{ab}	0.72 ± 0.16 ^{ab}	0.76 ± 0.15 ^b	0.57 ± 0.12 ^a

^{a-b}:Means in the same row with no common superscripts differ significantly(P<0.05).

Table 4. The effect of BA on the immune function of meat rabbits

Items	Treatment groups			Control group
	I	II	III	
IgG(mg/dL)	4.71 ± 0.36	4.89 ± 0.46	4.87 ± 0.51	4.66 ± 1.02
IgM(mg/dL)	0.38 ± 0.06 ^{ab}	0.46 ± 0.05 ^b	0.46 ± 0.02 ^b	0.28 ± 0.05 ^a
C3(mg/dL)	0.15 ± 0.01 ^a	0.21 ± 0.02 ^b	0.22 ± 0.01 ^b	0.14 ± 0.03 ^a
C4 (mg/dL)	0.03 ± 0.01 ^a	0.05 ± 0.01 ^b	0.05 ± 0.01 ^b	0.02 ± 0.01 ^a

^{a-b}:Means in the same row with no common superscripts differ significantly(P<0.05).

group I and the control group (P<0.05), and ADG was significantly higher in treatment group I than in the control group (P<0.05) (Table 2).

During weeks 0-4, F/G was significantly higher in the control group than in the three treatment groups (P<0.05), and significantly higher in treatment group I than in treatment groups II and III (P<0.05). During weeks 5-8, F/G was significantly higher in the control group than in the three treatment groups (P<0.05) but similar between the three treatment groups (P>0.05). During the entire experimental period (weeks 0-8), F/G was significantly higher in the control group than in the three treatment groups (P<0.05), and was significantly higher in treatment group I than in treatment groups II and III (P<0.05) (Table 2).

The effect of BA on immune organ index and immune function. Compared with the control group, the weights of the thymus and spleen were significantly higher in treatment groups II and III (P<0.05), but no significant difference was found in treatment group I (P>0.05). Similarly, compared with the control group, the thymic index was significantly higher in treatment groups II and III (P<0.05), but not significantly in treatment group I (P>0.05) (Table 3).

Compared with the control group, the splenic index was significantly higher in treatment group III (P<0.05), but no significant difference was observed in treatment groups I and II (P>0.05) (Table 3). In addition, no significant difference was found in serum IgG level between the four groups (P>0.05). However, serum IgM, C3, and C4 levels were significantly higher in treatment groups II and III than in the control group (P<0.05) (Table 4).

Discussion

In this study, our results showed that BA had little effect on the ADFI but it increased the growth performance of weaned meat rabbits by improving ADG and reducing F/G. In addition, these effects were significantly higher as the dose of BA supplementation increased, demonstrating that the positive effect of BA on the growth performance of weaned rabbits was dependent on a certain quantity, which was consistent with the findings in broilers (AHMED et al., 2014; WANG et al., 2018). Upon entrance into the digestive tract of weaned rabbits, BA creates an anaerobic environment by microbial oxygen consumption, and provides a niche for the substantial proliferation of microbial species that produce xylan-degrading enzymes, pectinolytic

enzymes and mucolytic enzymes (TROCINO et al., 2005; CAO et al., 2018; TSUKAHARA et al., 2018). This effect enhances the absorption of nutrients, and thereby improves the growth performance of weaned rabbits. Furthermore, since animals are under stress before and after weaning, and undergo major changes in their intestinal enzyme system, the benefit of BA on the growth performance of pre-weaning and weaned rabbits may be attributable to the rapid establishment of an enzyme system in weaned rabbits that is able to reduce the effect of weaning stress (ALEXOPOULOS et al., 2004; AYYAT et al., 2018).

Our findings demonstrated that BA supplementation in the daily diet increased the weight of the immune organs in weaned rabbits, and the thymic and splenic indexes were significantly higher in treatment group III than in the control group. This result is consistent with previous studies, where *Bacillus* significantly increased the thymic and splenic indexes of broiler chicks and weaned piglets, and actively promoted the growth and development of immune organs (ZHANG et al., 2005; XIN et al., 2011). These effects may be related to the antigenic or adjuvant properties of various components of the *Bacillus*, including cell wall sugar, peptidoglycan, peptides and proteins, that constantly stimulate the intestinal mucosa or act on immune organs to promote their growth and development (VITETTA et al., 2017). In addition, *Bacillus* may produce or synthesize various nutrients in the animal's gastrointestinal tract that are required for the growth and development of the immune organs (TROCINO et al., 2005).

Immunoglobulins are the major immune mediator of the humoral immune response, and play an important role in the defense system of the animal. IgG is a key indicator for the overall humoral immune status of the body (SEBINA and PEPPER, 2018). *Bacillus subtilis* supplementation significantly increases serum IgG levels in broiler chicks, but has no effect on IgM levels (YU et al., 2010). Moreover, another study demonstrated that *Bacillus licheniformis* significantly increases local IgG and IgM levels in the respiratory and digestive tract of broiler chicks (YANG et al., 2005). Our findings are somewhat similar but slightly different

from the above results, and this might be attributable to the different animal species or probiotic products. Nonetheless, our study confirmed that BA had a certain humoral immunity-promoting effect in meat rabbits.

The complement system is a key component of the immune system and is involved in defense response and immune regulation. The biological functions of the complement are dependent on its activation. C3 plays a critical role in the process of complement activation, and C4 is important for the activation phase of the complement (FU et al., 2018). As a result, serum C3 and C4 levels are biomarkers for assessing immune status. Our results showed that BA at high dose significantly increased levels of C3 and C4, which is consistent with the findings of a previous study, which showed that *Bacillus* can significantly increase serum levels of C3 and C4 in grass carp (SHEN et al., 2011).

Conclusions

Supplementation with an appropriate amount of BA in the diet could effectively improve the growth performance, immune organ index, and immune function of weaned New Zealand rabbits.

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SAŽETAK

Pokazalo se da bakterije roda *Bacillus* mogu poboljšati proizvodna svojstva i imunost pilića i svinja, pa je pretpostavka ovog rada da bi *Bacillus amyloliquefaciens* (BA) mogao učinkovito povećati prirast i imunološku sposobnost kunića za meso nakon odbića. S tom svrhom, istražen je učinak BA-a na prirast, imunološki indeks organa i imunološki status kunića mesne pasmine. Ukupno 160 kunića novozelandske pasmine nakon odbića (35 ± 3 dana), slučajnim je odabirom, podijeljeno u 4 skupine s 4 ponavljanja po skupini ($n = 10$ po ponavljanju, 5 mužjaka i 5 ženki). Kunići iz kontrolne skupine dobivali su osnovni obrok, dok je kunićima u pokusnim skupinama I, II i III u osnovni obrok dodavano 150, 300 i 450 mg/kg BA-a, ukupno osam tjedana. Rezultati su pokazali da je dodatak BA-a povećao dnevni prirast i smanjio konverziju hrane. Osim toga dodatak BA-a znakovito je povećao imunološke indekse slezene i timusa te razinu imunoglobulina M (IgM), komplementa 3 (C3) i 4 (C4) u pokusnim skupinama II i III ($P < 0,05$). Zaključeno je da bi dodavanje BA-a u obrok kunića za meso nakon odbića moglo doprinijeti povećanju njihova prirasta, imunološkog indeksa organa i imunološkog statusa.

Ključne riječi: *Bacillus amyloliquefaciens*; meso kunića; prirast; imunološki indeks organa; imunološki status
