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Growth performance, nutrient digestibility and immune response of broiler chicks fed diets supplemented with a culture of *Lactobacillus bulgaricus*

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Abstract

BACKGROUND: Probiotics are being developed for use in animal feed to enhance production performance and prevention of gastrointestinal infections. The ban on using antibiotics as growth promoters, antibiotic resistance and the inherent problems of developing new vaccines make a compelling case for developing alternatives for in-feed antibiotics. The alternatives of choice have to be considered under the environmental conditions of the animal. Among the probiotics in use today, *Lactobacillus* has been shown to play a vital role in disease prevention, immune enhancement, improved growth and carcass yield in poultry. The present study investigates the effect of *Lactobacillus bulgaricus* (LB)-based probiotic on the growth performance, nutrient digestibility and immune response of broilers under tropical environmental conditions.

RESULTS: Broilers fed LB diets consumed more feed ($P < 0.05$) and had greater body weight gain than the control group. Feed/gain ratio improved significantly ($P < 0.05$) with the 20, 40 and 60 mg kg^{-1} LB diets compared with the control or 80 mg kg^{-1} LB diet. The apparent digestibilities of nitrogen and fat increased with LB supplementation. However, there was no significant difference ($P > 0.05$) in fibre digestibility. White blood cell count increased significantly in broilers fed higher levels (>40 mg kg^{-1}) of LB compared with the control group. Antibody production measured as antibody titre against Newcastle disease vaccine showed a curvilinear response over the range of LB concentrations examined.

CONCLUSION: The results indicate that LB addition to broiler chick diets significantly improved growth performance, increased nutrient digestibility and stimulated humoral immune response.
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Keywords: broiler chick; *Lactobacillus bulgaricus*; performance; nutrient digestibility; immune response

INTRODUCTION

Many feed additives have been used to promote animal health and improve growth performance and utilisation of nutrients in poultry production.[1] The use of antibiotics as growth promoters and for prevention and treatment of diseases has become commonplace as poultry producers have adopted intensive farming systems. However, there is growing concern regarding the possible build-up of antibiotic residues in animal products, which can be harmful to consumers, and the development of drug-resistant bacteria, attributed to continuous feeding of antibiotics at sub-therapeutic levels. These have resulted in increased interest being paid to valuable alternatives for in-feed antibiotics in the poultry industry. The modern alternative feed additives that are being used or studied include (i) feed acidifiers, which contain blends of organic and inorganic acids that can enhance growth of broiler chickens by reducing the pH value of both the feed and the forepart of the intestinal tract, thus controlling harmful bacteria and improving the digestion of nutrients,[2,3] (ii) prebiotics, which are non-digestible substrates that beneficially affect the host by selectively stimulating growth and/or activity of one or a limited number of bacteria in the colon,[4] and (iii) probiotics (direct fed microbials) such as *Lactobacillus* culture in poultry production.[5]

Probiotics are characterised as 'live microbial feed supplements that can benefit the host by improving its intestinal balance'.[6] Probiotic micro-organisms have to be viable to accomplish their putative beneficial effects such as producing antimicrobial factors, inhibiting undesirable organisms and competing for receptor sites on the gut epithelial tissue.[7] Feeding chickens on diets containing *Lactobacillus* cultures has been shown to improve growth, feed efficiency, carcass yield and cut-up meat parts.[8–10] These investigators ascribed such improvements in productive and quantitative

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traits to the ability of *Lactobacillus* cultures to improve microbial balance in the gastrointestinal tract. However, the responses to performance and microbial balance have not always been consistent.[11] According to Huang *et al*.,[12] such inconsistency could be due to the strain of selected micro-organisms, dose level and frequency of feeding the strain, method of preparation, drug interactions and condition of the animal. Because of the conflicting findings, more studies are needed to establish consistent modes of action by which probiotic bacteria exert their beneficial functions. Nevertheless, several modes of action of probiotics involving direct bacterial antagonism, competitive exclusion of harmful pathogens and immune stimulation have been demonstrated in poultry.[13,14]

Although *Lactobacillus* species have been widely studied as probiotics, there is a scarcity of information on the possible effect of *Lactobacillus bulgaricus* on poultry productivity under hot and humid stresses naturally prevailing in the tropics. In the present study, several levels of a skim milk *Lb. bulgaricus* culture were included in the diet of broiler chicks. Their effects on growth performance, nutrient digestibility and immune response were evaluated in a humid tropical environment.

MATERIALS AND METHODS

Lactobacillus bulgaricus culture preparation

The strain of *Lb. bulgaricus* (LB) used in this study was isolated from a commercial yoghurt starter (Yogourmet, Lyo-san, Canada) containing three bacteria, *Lactobacillus acidophilus*, *Lb. bulgaricus* and *Streptococcus thermophilus*. A selective medium of de Man–Rogosa–Sharpe (MRS) agar was used for isolation of *Lactobacillus* species, after which identification of LB spores was confirmed biochemically and enumerated following the procedures outlined by Cowan and Steel.[15] A pure colony of the bacterial culture was inoculated into skim milk before addition to the diets described below; this protocol has been confirmed as suitable in chicken feeding trials.[16] Skim milk was used as the suspension medium because of its strong effect on the stability of probiotic bacteria.[17]

Chicks, diets and management

Two hundred and ten 1-day-old unsexed Shaver broiler chicks were obtained from a local hatchery. The birds were divided into five groups of 42 chicks each, which were allocated randomly to five dietary treatments using a completely randomised design. Each treatment had three replicate cage units of 14 chicks. The experimental diets had skim milk culture of LB at 0, 20, 40, 60 and 80 mg kg^{-1} levels added to separate batches of the basal diet (Table 1). An inoculation of 10 mg kg^{-1} gave a mean cell count of 1×10^6 colony-forming units (cfu) g^{-1}, so the increasing concentrations used for inoculation gave determined mean cell counts of 0, 2×10^6, 4×10^6, 6×10^6 and 8×10^6 cfu g^{-1} of the bacterium respectively. The basal diet without LB (0 mg kg^{-1}) culture served as control. The bacterial culture was stored at 4 °C and its viability was retained over the experimental period. Viability was checked periodically by plating a small portion of the culture on agar plates.

In preparing the diets, batches of the basal diet were weighed and mixed with fresh skim milk culture of LB containing the different levels described above every other day. The diets, in mash form, and water were provided *ad libitum*. All chicks were reared under natural tropical conditions (29–33 °C, 80–100% relative humidity) throughout the 35 day period of study. Routine management was followed. Over the entire period, all chicks were weighed in each cage weekly and the values were divided by the total number of chicks. Mean weight gains were obtained by calculation. The cumulative mean value for feed intake was calculated from weekly records obtained by subtracting the weight of feed remaining at the end of each week from the total weight introduced during the week. Feed/gain ratio was calculated by dividing the average amount of feed intake per chick by the average weight gain over the entire period of the trial. Protein intake/gain ratio was also determined. Newcastle disease vaccine (ND Lasota, Cuxhaven,

Table 1. Composition and nutrient content of basal diet[a]

Component	Amount (g kg^{-1})
Ingredients	
Yellow maize	453
Soybean meal	210
Fish meal	30
Groundnut cake	150
Wheat bran	120
Bone meal	20
Oyster shell	10
Vitamin/mineral premix[b]	2.5
Salt	2.5
DL-Methionine	2.0
Nutrients[c]	
Crude protein	230
Crude fibre	40.7
Fat	52.8
Metabolisable energy (MJ kg^{-1})	11.76
Lysine	11.9
Methionine	3.5
Calcium	13.6
Phosphorus	6.0

[a] Five dietary treatments were derived by adding skim milk culture of *Lactobacillus bulgaricus* at 0, 20, 40, 60 and 80 mg kg^{-1} levels to separate batches of the basal diet.

[b] Provided 1.5 mg retinol, 25 mg cholecalciferol, 16 mg α-tocopherol, 1 mg menadione, 0.8 mg thiamine, 2.4 mg riboflavin, 14 mg nicotinic acid, 4 mg calcium D-pantothenate, 1.4 mg pyridoxine, 10 mg cyanocobalamin, 0.4 mg folic acid, 0.02 mg biotin, 120 mg choline chloride, 0.62 mg zinc bacitracin, 36 mg avaten, 40 mg Mn, 18 mg Zn, 0.8 mg Cu, 0.09 mg Co, 20 mg Fe and 0.04 mg Se kg^{-1} diet.

[c] Determined values except for metabolisable energy, lysine, methionine, calcium and phosphorus, which were calculated from the published[34] compositions of the ingredients used.

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Germany) was administered to the chicks in their drinking water on day 14 of the feeding trial.

Digestibility trial

On day 33 a digestibility trial was carried out with the chicks. Weighed quantities of the diets were supplied and excreta were collected over 72 h in plastic sheeting placed under the wire mesh floor of the cage using the total collection method. Excreta samples were oven dried (70 °C for 20 h), weighed, ground and stored in airtight Kilner jars. Duplicate samples of diets and dried excreta were analysed for proximate components by AOAC[18] methods and the values were used to compute apparent nutrient digestibility.

Blood sampling, white blood cell count and agglutination antibody titre assay

At the end of the experimental period, two chicks were randomly selected from each replicate and whole blood samples were collected from the wing vein into (i) bottles containing ethylene diamine tetraacetic acid (EDTA) anticoagulant for white blood cell (WBC) measurement and (ii) clean dry test tubes for serum antibody levels against Newcastle disease antigen. Serum was isolated by centrifugation at $1916 \times g$ for 20 min and stored at −20 °C until used for analysis. Total WBC count was enumerated in a (USA) Spencer Bright line haemacytometer. Anti-Newcastle disease antibody titres were determined using a haemagglutination inhibition test[19] with 20 g L^{-1} saline-washed erythrocytes from other chicks. Briefly, serial twofold dilutions from the serum were made in well round-bottom microtitre plates. To each well were then added aliquots of Newcastle disease vaccine suspension and washed erythrocytes. The contents of the plates were incubated at room temperature for 2 h. Agglutination end point was established as the last serum dilution where cell agglutination was visible after incubation. Agglutination titres were logarithmically transformed to $\log_{10}$ antibody titres as described by Yildirim *et al*.[20]

Statistical analysis

Data were subjected to one-way analysis of variance and Duncan's multiple range test to determine significant differences at the 0.05 level.[21] Regression analysis was run using the GenStat software package.[22]

RESULTS AND DISCUSSION

Growth performance of broiler chicks was affected by dietary LB supplementation at the end of the feeding trial (Table 2). Feed intake and weight gain with the LB-supplemented diets were significantly higher than with the control diet. The highest weight gain was achieved by chicks fed 60 mg kg^{-1} LB, with a value 11% greater than the control. Feed/gain ratio was significantly improved at 20, 40 and 60 mg kg^{-1} LB compared with the control, but at higher LB concentration it was similar to the control. Feed intake and crude protein intake followed a fairly similar trend. Among dietary treatments, 20 and 60 mg kg^{-1} LB elicited the best protein intake/gain ratio. There was no mortality at all levels during the feeding trial period. Broilers consumed more feed with the LB-supplemented diets, probably because of the use of skim milk growth medium which encouraged appetite.[23] The data on feed conversion indicate that those chicks fed 80 mg kg^{-1} LB consumed more feed but failed to show any extra weight gain. The reason for the relatively lower feed conversion of the group fed this level of LB is unclear. In general, the growth response showed that LB produced significant improvements over the control. This observation confirms previous findings[9,24,25] that dietary supplementation with *Lactobacillus* cultures improved the performance of chickens. The enhanced growth with LB may be partly attributed to the colonisation of the gastrointestinal tract of the chicks, which improved the digestion of essential nutrients. However, it is known that nutrients, especially protein, metabolised by microflora are not utilised by the host. Thus it possible that skim milk usage as a medium to ensure stability of the probiotic might have stimulated proteolytic or protein digestion activity, thereby contributing to the improvement in nutrient digestion.

Dietary LB significantly increased the apparent digestibilities of nitrogen and fat but had no significant effect on the apparent digestibility of fibre (Table 3). Digestion of nitrogen and fat was highest with

Table 2. Growth performance of broilers fed basal or *Lactobacillus bulgaricus* (LB)-supplemented diets for 35 days[a]

Dietary LB (mg kg^{-1})	Feed intake (g per chick)	Weight gain (g per chick)	Feed/gain ratio (g g^{-1})	Protein intake (g per chick)	Protein intake/gain ratio (g kg^{-1})
0	1998a	746c	2.70a	460a	617b
20	2036b	805ab	2.53b	468a	581a
40	2141b	811ab	2.64b	492b	607b
60	2091b	833a	2.51b	481b	577a
80	2230c	814ab	2.74a	513c	630c
Pooled SEM	26.71	10.45	0.28	6.32	3.75
Regression equation	$Y = 2.56X + 1997.2$	$Y = 0.728X - 774.5$	$Y = 0.0005X + 2.60$	$Y = 0.595X + 459$	$Y = 0.11X + 598$
r	0.89	0.74	0.14	0.90	0.15

[a] Values are mean of three replicates per treatment. Means in the same column with different letters are significantly different at $P < 0.05$. SEM, standard error of means.

Table 3. Apparent nutrient digestibility of broilers fed basal or *Lactobacillus bulgaricus* (LB)-supplemented diets[a]

	Digestibility (%)		
Dietary LB (mg kg^{-1})	Nitrogen	Fat	Fibre
0	68.9a	63.4a	41.7
20	70.5a	64.1a	42.8
40	73.7b	71.9c	43.0
60	76.9c	67.5b	41.8
80	74.1b	68.0b	42.5
Pooled SEM	3.1	2.7	0.96
Regression equation	$Y = 0.08X + 69.78$	$Y = 0.06X + 64.63$	$Y = 0.0033X + 42.23$
r	0.80	0.53	0.11

[a] Values are mean of three replicates per treatment. Means in the same column with different letters are significantly different at $P < 0.05$. SEM, standard error of means.

60 and 40 mg kg^{-1} LB respectively. This finding suggests that LB increased the chicks' ability to digest some nutrients. Gopal[26] demonstrated that *Lactobacillus* strains can inhibit the adhesion of a range of pathogenic bacteria that compete for available nutrients. This action could produce an improvement in the intestinal environment for the processes of absorption and retention of nutrients, as evidenced in the increase in nitrogen and fat digestion for all LB treatment groups.

The antibody titre and WBC count are presented in Table 4. Chicks fed LB exhibited greater antibody production against Newcastle disease vaccine antigen than their control counterparts. WBC count increased significantly with higher levels (>40 mg kg^{-1}) of LB compared with the control group. The response of WBC count did not correspond to that of antibody production over the same increments. A curvilinear antibody response was obtained over the range of LB levels (Fig. 1). The monotonic curve showed that the optimum was at 60 mg kg^{-1} LB ($Y = 0.0175X + 7.83$, $r = 0.66$). The presence of a stimulatory effect on systemic immunity upon ingestion of LB as evidenced in this study could provide a mechanism for LB probiosis. According to Perdigon *et al*.[27] and Tannock,[28] the antigenic load resulting from other species of *Lactobacillus* can induce stimulation of the immune system. Maassen *et al*.[29] reported that a strain-dependent cytokine production profile was induced after oral administration of *Lactobacillus* and that such a cytokine profile determined the direction and efficiency of the humoral response. Furthermore, Zulkifli *et al*.[9] showed that feeding *Lactobacillus* cultures to broiler chickens under heat stress conditions increased antibody response. The present results extend information on immune enhancement of chickens fed LB diets, especially with respect to tropical conditions. However, the metabolic cost of immune response may be considerable[30] and, from the viewpoint of sufficient dietary nutrients from increased feed intake of the birds, these may have replaced nutrients diverted for the formation of a wide range of α-amino acids and organic phosphates which provide essential support function for the immune system. As such, growth performance was not compromised among the birds.

Table 4. Antibody titre against Newcastle disease vaccine and white blood cell (WBC) count of broilers fed basal or *Lactobacillus bulgaricus* (LB)-supplemented diets[a]

Dietary LB (mg kg^{-1})	Antibody titre ($\log_{10}$)	WBC count (10^9 L^{-1})
0	7.25c	9.34a
20	8.33b	10.27a
40	8.67ab	9.95a
60	9.67a	11.78ab
80	8.53b	13.49b
Pooled SEM	1.24	1.06
Regression equation	$Y = 0.0175X + 7.83$	$Y = 0.0506X + 8.91$
r	0.66	0.92

[a] Values are mean of six determinations per treatment. Means in the same column with different letters are significantly different at $P < 0.05$. SEM, standard error of means.

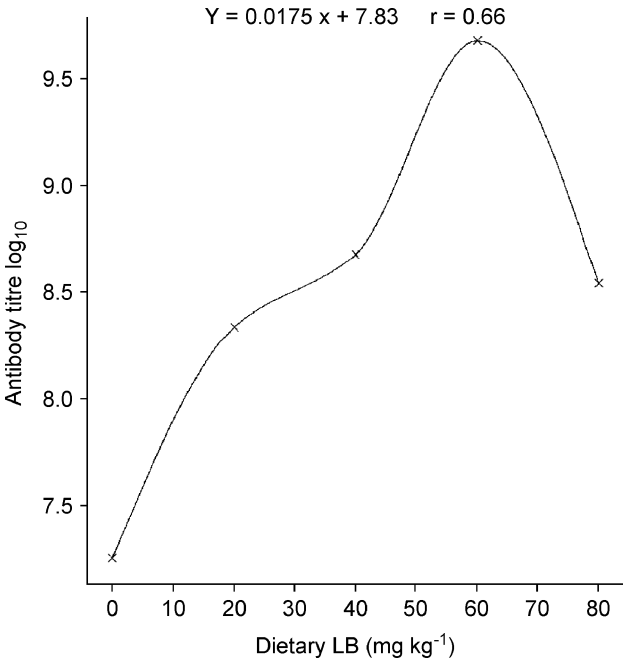


Figure 1. Antibody production in broiler chicks fed different *Lactobacillus bulgaricus* (LB) levels for 35 days.

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WBCs function primarily as a defence system and contain lymphocytes (not analysed in the current study) which play a central role in the immunological defence mechanism of the body.[31] The increase in the population of WBCs obtained may be attributed to the presence of LB in the diet stimulating the production of lymphocytes, particularly the B type which are responsible for forming antibodies that provide humoral immunity.[32] Previously, Pollmann *et al*.[33] showed elevated WBC counts following inoculation of *Lb. acidophilus* in pigs. It seems that the number of WBCs present is a good indicator of the exposure of the chicks to pathogens and the ability of the chicks to eliminate pathogenic bacteria, and, reasonably, a degree of nutritional enhancement can follow.

In conclusion, the present study showed that broiler chicks can be fed an LB strain as a growth promoter to improve performance, nutrient digestibility and antibody production under hot and humid conditions which prevail in the tropics.

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REFERENCES

1 Babalola TOO, Apata DF and Atteh JO, Effect of β-xylanase supplementation of boiled castor seed meal-based diets on the performance, nutrient absorbability and some blood constituents of pullet chicks. *Trop Sci* **46**:216–223 (2006).
2 Skinner JT, Izat AL and Waldroup PW, Fumaric acid enhances performance of broiler chickens. *Poultry Sci* **70**:1444–1447 (1991).
3 Luckstadt C, Senkoylu N, Akyurek H and Agma A, Acidifier – a modern alternative for anti-biotic free feeding in livestock production, with special focus on broiler production. *Veterinarija ir Zootechnika* **49**:91–93 (2004).
4 Gibson GR and Roberfroid MB, Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *J Nutr* **125**:1401–1412 (1995).
5 Hong HA, Duc LH and Cutting SM, The use of bacterial spore formers as probiotics. *FEMS Microbiol Rev* **29**:813–835 (2005).
6 Fuller R, Probiotics in man and animals. *J Appl Bacteriol* **66**:365–378 (1989).
7 Fuller R, Probiotics for farm animals, in *Probiotics. A Critical Review*, ed. by Tannock GW. Horizon Scientific Press, Wymondham, pp. 15–22 (1998).
8 Abdulrahim SM, Haddadin MSY, Odetallah NHM and Robinson RK, Effect of *Lactobacillus acidophilus* and zinc bacitracin as dietary additives for broiler chickens. *Br Poultry Sci* **40**:91–94 (1999).
9 Zulkifli I, Abdullah N, Azrin MN and Ho YW, Growth performance and immune response of two commercial broiler strains containing *Lactobacillus* cultures and oxytetracycline under heat stress conditions. *Br Poultry Sci* **41**:593–597 (2000).
10 Kabir SML, Rahman MM, Rahman MB and Ahmed SU, The dynamics of probiotics on growth performance and immune response in broilers. *Int J Poultry Sci* **5**:361–364 (2004).
11 Radecki SY and Yokoyama MT, Intestinal bacteria and their influence on swine nutrition, in *Swine Nutrition*, ed. by Miller ER, Duane EU and Lewis AJ. Butterworth-Heinemann, Boston, MA, pp. 439–447 (1991).
12 Huang M-K, Choi YJ, Houde J-W, Lee B and Zhao X, Effects of lactobacilli and an acidophilic fungus on the production performance and immune responses in broiler chickens. *Poultry Sci* **83**:788–795 (2004).
13 Patterson JA and Burkholder KM, Application of prebiotics and probiotics in poultry production. *Poultry Sci* **82**:627–631 (2003).
14 Jin LZ, Ho YW, Abdullah N and Jalaludin S, Probiotics in poultry: modes of action. *World Poultry Sci J* **53**:351–368 (1997).
15 Cowan ST and Steel S, *Manual for the Identification of Medical Bacteria* (2nd edn). Cambridge University Press, London, pp. 237–238 (1997).
16 Haddadin MSY, Abdulrahim SM, Odetallah NHM and Robinson RK, A proposed protocol for checking the suitability of *Lactobacillus acidophilus* cultures for use during feeding trials with chickens. *Trop Sci* **37**:16–20 (1997).
17 Sodini I, Lucas A, Oliveira MN, Remeuf F and Corrieu G, Effect of milk base and starter culture on acidification, texture, and probiotic cell counts in fermented milk processing. *J Dairy Sci* **85**:2479–2488 (2002).
18 AOAC, *Official Methods of Analysis* (15th edn). Association of Official Analytical Chemists, Washington, DC (1990).
19 Brown J, Resurrection RS and Dickson TG, The relationship between the hemagglutination-inhibition test and the enzyme-linked immunosorbent assay for the detection of antibody to Newcastle disease. *Avian Dis* **34**:585–587 (1990).
20 Yildirim M, Lim C, Wan PJ and Klesius PH, Growth performance and immune response of channel catfish (*Ictalurus punctatus*) fed diets containing graded levels of gossypol-acetic acid. *Aquaculture* **219**:751–768 (2003).
21 Steel RGD and Torrie JH, *Principles and Procedures of Statistics* (2nd edn). McGraw-Hill, New York, NY (1980).
22 *GenStat – Release 4.2 Discovery Edition 2*. VSN International, Hemel Hempstead (2005).
23 Shah NP, Probiotic bacteria: selective enumeration and survival in dairy foods. *J Dairy Sci* **83**:894–907 (2000).
24 Tortuero F, Influence of implantation of *Lactobacillus acidophilus* in chicks on the growth, feed conversion, malabsorption of fat syndrome and intestinal flora. *Poultry Sci* **52**:197–203 (1993).
25 Jin LZ, Ho YW, Abdullah N and Jalaludin S, Growth performance, intestinal microbial populations, and serum cholesterol of broilers fed diets containing *Lactobacillus* cultures. *Poultry Sci* **77**:1259–1265 (1998).
26 Gopal PK, *In vitro* adherence properties of *Lactobacillus rhamnosus* DR20 and *Bifidobacterium lactis* DR10 strains and their antagonistic activity against an enterotoxigenic *Escherichia coli*. *Int Food Microbiol* **97**:207–216 (2001).
27 Perdigon G, Alvarez S, Medici M and Holgado AAPR, Influence of the use of *Lactobacillus casei* as an oral adjuvant on the levels of secretory immunoglobulin A during an infection with *Salmonella typhimurium*. *Food Agric Immunol* **5**:27–37 (1993).
28 Tannock GW, Studies of the intestinal microflora: a prerequisite for the development of probiotics. *Int Dairy J* **8**:527–533 (1998).
29 Maassen CB, Holten JC, Balk F, Heiine Den Bak-Glashouwer MJ, Leer R, Laman JD, *et al*, Orally administered *Lactobacillus* strains differentially affect the direction and the efficacy of the immune response. *Vet Q* **20**:81–83 (1998).
30 Glick B, The immune system of poultry, in *Poultry Production*, ed. by Hunton P. Elsevier Science, Amsterdam, (1995).

31 Eroschenko VP, *Di Fiore's Atlas of Histology with Functional Correlations* (9th edn). Lippincott Williams and Wilkins, Philadelphia, PA (2000).
32 Guyton AC and Hall JE, *Textbook of Medical Physiology* (11th edn). Saunders, Philadelphia, PA (2006).
33 Pollmann DS, Danielson DM and Peo ER, Effects of microbial additives on performance of starter and growing finishing pigs. *J Anim Sci* **51**:577–581 (1980).
34 NRC, *Nutrient Requirements of Poultry* (13th edn). National Academy of Science, Washington, DC (1984).

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DOI: 10.1002/jsfa