

## INFLUENCE OF PROBIOTIC SUPPLEMENTATION ON IMMUNE RESPONSE OF BROILER CHICKS

By

A. Alkhalif<sup>1</sup>, M. Alhaj<sup>2</sup>, and I. Al-Homidan<sup>3</sup>

<sup>1</sup>Vet. Med. Dept., College of Agric and Veterinary Med, Qassim Univ.,  
Saudia Arabia

<sup>2</sup>Vet. Med. Dept., College of Agric and Veterinary Med, Tamar Univ.,  
Yemen

<sup>3</sup>Dept. of Animal Prod. and Breeding, College of Agric and Vet. Med.,  
Qassim Univ., Saudia Arabia

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**Abstract :***The objective of this study was to evaluate the effects of a commercial probiotic supplementation (Bactocell<sup>®</sup> containing live bacteria *Pediococcus acidilactici*) on immunity of broiler chickens. A total of 800 Ross one day-old broiler chicks were grown over 42 days. Chicks were wing-banded, individually weighed and randomly allocated into four equally major groups each of two replicates. Chicks of group 1 (control group) were fed the starter and finisher diets that were not supplemented with probiotic. The chicks of groups 2, 3, and 4 were fed control starter and finisher diets plus 1.6 g, 1 g and 0.8 g of probiotic per kg of feed, respectively for 42 days. The probiotic treatment groups showed significantly higher antibody levels against Newcastle Disease Virus (NDV) compared to the control group. A significant increase was recorded in the relative weight of bursa of Fabricius in all probiotic supplemented groups compared to the control one. There was a significant increase in the relative weight of spleen at 42 days of age in all probiotic treatment groups as compared to the control group. Probiotic supplementation significantly ( $p < 0.05$ ) increased the relative weight of thymus in all probiotic treatment groups at 28 and 42 days of age as compared to the control group. This indicates an enhancement effect of probiotic (*Pediococcus acidilactici*) on the immune system of broilers was detected.*

## INTRODUCTION

In most countries, poultry meat is the major animal protein produced and consumed which necessitates intensive production of broilers to meet all the needs. As a consequence, the use of antimicrobial agents became a major concern to treat and protect broiler flocks from microbial diseases. The deleterious effect of broad application of antimicrobials in poultry industry on consumers have been well documented. Therefore, there is a worldwide attempt to reduce antibiotic use in poultry production which cause increased microbial resistance to antibiotics and residues in animal products that can be harmful to consumers (van den Bogaard and Stobberingh, 2000; Caprioli *et al.*, 2000 and Pelicano *et al.*, 2004). In order to meet market and international health organization demands, the poultry industry is seeking alternatives to antibiotics that could be both economically feasible and maintain performance levels. The so called probiotics can be listed among these products.

A probiotic is a live microorganism, which when consumed in adequate amounts; confer a healthy effect on the host (Guarner and Schaafsma, 1998). According to the currently adopted definition FAO and WHO (2001), probiotics are: Live microorganisms which when administered in adequate amounts confer a healthy benefit on the host. While reports of probiotic effects on the immune response in chickens are more limited compared with mammals, similar results have been described. Panda *et al.* (2000) supplemented diet with various level of probiolac probiotic (a commercial probiotic mixture of lactic acid bacteria, *and Aspergillus oryzae*) and observed that a significant increase in antibody production at 10 days of postimmunization when sheep red blood cells (SRBCs) were injected in broiler chickens at 14 days of age and at 5 days of postimmunization when SRBC were injected at age of 21 days. However, after 28 days of age, no significant difference was found in the antibody production at 5 and 10 days of postimmunization. Haghghi *et al.* (2005) reported that probiotic-treated birds had significantly more serum antibody (predominantly immunoglobulin M (IgM)) to SRBCs than the birds that were not treated with probiotics. Rowghani *et al.* (2007) reported that broiler chickens fed diet supplemented with probiotic had a significant increase in the Newcastle antibody titers compared with those of control group. Teo and Tan (2007) observed that the birds provided feed supplemented with *Bacillus subtilis* PB6 had a significantly heavier bursa weights compared with the antibiotic supplementation and negative control groups. However, neither the studied treatments nor the control had any

effect on the relative weights of spleen. Willis *et al.* (2007) found that the bursa and spleen relative weight significantly increased in male broiler chickens receiving the probiotic supplementation. The present study was planned to investigate the effects of supplementing diets with a monospecies commercial probiotic (Bactocell<sup>®</sup>) on broiler immunity.

## MATERIALS AND METHODS

### Housing and experimental design:

A total number of 800, one day-old broiler chicks (obtained from Alwadi Company for Poultry), were grown over 42-day period. Chicks were wing-banded, individually weighed and randomly assigned to four treatment groups following completely randomized design. There were 100 birds per replicate and two replicates per treatment group. The chicks of each replicate were kept in a separate pen measuring 3 m long and 3 m wide at the Agricultural and Veterinary Experiment Station, College of Agriculture and Veterinary Medicine, Qassim University. Feed and water were provided *ad libitum*. Ventilation, air condition and temperature in each room were controlled by a DicamFSC2.2M master unit (Farm Energy and Control Services Ltd “Farmex”, Pinewood, Reading RG 303VR, United Kingdom). Chicks of group 1 (control group) were fed the starter and finisher diets that were not supplemented with probiotic. The chicks of groups 2, 3, and 4 were fed the control starter and finisher diets plus 1.6 g, 1 g and 0.8 g of a commercial probiotic (Bactocell<sup>®</sup>) per kg of ration, respectively. Diets were formulated to provide the recommended requirements for broiler (without added antibiotics, or growth promoters). The starter diet was replaced by the finisher diet at 4 weeks of age.

### Probiotic:

A probiotic commercially identified as Bactocell<sup>®</sup> was used as a test feed additive in this study. Bactocell was purchased from Lallemand Animal Health Company, France. The bacterial flora in the Bactocell probiotic has mentioned to be *Pediococcus acidilactici* in a concentration of 10<sup>9</sup> CFU/g (colony forming unit).

### Evaluation of immune response:

#### Vaccination of chickens using Newcastle disease vaccine:

At 14 days, 20 birds from each group were vaccinated with killed Newcastle disease virus vaccine (Merial Company, France). Vaccination was carried out by intramuscular inoculation using automatic syringe. Preparation and dosage of the vaccine was followed according to the

instructions of the manufacturer. Blood samples were collected prevaccination at 14 days and postvaccination at 21, 28, and 42 days of age. Detection of antibodies against Newcastle disease virus (NDV) in serum of immunized chickens was performed by enzyme linked immunosorbent assay (ELISA) using commercial kits (BioChek B.V., Holland). The assay was carried out as described by the manufacturer. Briefly, chicken serum samples were diluted and added to the microtitre wells. Then anti-chicken IgG labeled with enzyme alkaline phosphatase was then added to the wells. After another wash to remove unreacted conjugate, substrate was added to the appropriate wells in the form of pNPP (p-Nitrophenyle Phosphate) chromogen and incubated at room temperature for 30 minutes. Stop solution WAS ADDED TO STOP REACTION. FINALLY, THE ABSORBANCES OF SAMPLES WERE recorded by microtitre plate reader.

#### **Relative weights of lymphoid organs (thymus, bursa and spleen):**

At days 7, 28 and 42 of age, 10 birds from each group were sacrificed and their total body weights were recorded. Then birds were opened and the lymphoid organs (thymus, bursa and spleen) were carefully removed and individually weighed. The relative weights of the different organs were calculated as percentage of live body weight.

Data were subjected to a one-way analysis of variance using GLM procedure (SAS Institute, 1999).

## **RESULTS AND DISCUSSION**

Concerning immune response to Newcastle disease virus (NDV), the probiotic supplementation had no effect on maternal antibody levels against NDV. This was indicated by comparison between samples of probiotic-supplemented chickens and control ones prior to immunization (at 14 days of age). Findings of this study (Table1) demonstrate that probiotic had a significant enhancement effect on antibody titres against NDV at 42 days of age. This was met with the three levels of probiotic. Meanwhile, there were no significant differences between samples taken at 21 and 28 days of age (7 and 14 days postimmunization).

These findings are in agreement with those of many preceding studies. In one of those studies, Rowghani *et al.* (2007) reported that broiler chickens fed a diet supplemented with probiotic had a significant increase in the Newcastle antibody titers than control group. In another study by Lee *et al.* (2007) described that probiotic containing *Pediococcus acidilactici* enhanced serum antibody response to *Eimeria acervulina*. In a different study, Koenen *et al.* (2004) described that *Pediococcus acidilactici* had

different effects on the gastrointestinal tract and immune system of birds depending on their genetics and age, suggesting that the different types of birds (layer versus broiler) may require different doses of probiotics at different intervals.

The positive effect of feeding diet containing probiotic on the immune response indicates the enhancement of the formulating bacteria on an acquired immune response exerted by T and B lymphocytes. The direct effect might be related to stimulate the lymphatic tissue (Kabir et al., 2004), whereas the indirect effect may occur via changing the microbial population of the lumen of gastrointestinal tract. Shoeib et al. (1997) reported that the bursa of probiotic-treated chickens showed an increase in the number of follicles with high plasma cell reaction in the medulla. Christensen et al., (2002) suggested that some of these effects were mediated by cytokines secreted by immune system cells stimulated with probiotic bacteria. Commensally, bacteria presented in intestinal microbiota are in close contact with cells of the immune system. It has recently been demonstrated that resident dendritic cells (DC) in the intestinal lamina propria have the capacity to directly sample the gut lumen by projecting their dendrites through the tight junctions of epithelial cells (Rescigno et al., 2001). The recognition of commensal bacteria or their structural components by Toll-like receptors (TLR) presented on surfaces of DC could lead to the activation and maturation of these cells (Rakoff-Nahoum et al., 2004). Differential activation of DC by commensal bacteria promotes the establishment of T-helper 1 (Th1), Th2, and Th3 responses and the secretion of cytokines, such as interleukin 4 (IL-4), IL-10, and transforming growth factor  $\beta$ , that are important for antibody production and isotype switching (Christensen et al., 2002, Di Giacinto et al. 2005).

Concerning the relative weights of lymphoid organs (spleen, bursa and thymus) as indicative parameters, results are presented in Table 2. It could be concluded that there were no significant changes on the relative weight of spleen nearly at all ages except at 42 days of age, where there was a significant increase in all probiotic treatment groups compared to the control group. This may indicate the delayed response of spleen to the effect of probiotic as it is a secondary lymphoid organ that develops its proper functions with age. The increase in the relative weight of spleen at 42 days

of age is in agreement with the findings of Willis et al (2007) who found that the feeding broilers on probiotic caused increases in the relative weights of spleen of treatment group. In contrast, Teo and Tan (2007) found no significant differences in the relative weights of the spleen in broilers fed the diet containing probiotic compared with control groups. The final diversification and affinity maturation of chicken B cells in spleen germinal centers was studied by Arakawa *et al.*,(1996). Following stimulation by antigen, postbursal B cells were able to generate somatic variants in splenic germinal centers. The size of these germinal centers was maximized by day 7 of the primary response and had begun to wane by 14 day. The results obtained in this work revealed clearly that there is a significant increase in the relative weight of bursa of Fabricious in some probiotic treatment groups than the control group. Moreover, the probiotic treatment groups of levels (1.6 and 1 g/kg ration) exhibited higher increase on the relative weight of bursa than the other group receiving probiotic of 0.8 g/kg ration at 28 and 42 days of age (Table 3).Increased in the relative weight of bursa may be attributed to increase the number of immune cells. Findings encountered in this study is in agreement with that of Shoeib et al. (1997) who found that the bursa of Fabricious in probiotic- treated group showed an increase in the number of follicles with high plasma cell reaction in the medulla. Meanwhile, Teo and Tan (2007) observed that birds provided feed supplemented with *Bacillus subtilis* PB6 had a significantly heavier bursa weight compared with control groups.

The effect of probiotic on the relative weight of thymus was also investigated in this study as shown in Table (2). Probiotic supplementation significantly has increased the relative weight of thymus in all probiotic treatment groups at 42 and 28 days of age compared to control group. Increase in the weight of thymus may be due to the effect of probiotic bacteria on the functional activities of the immune system responses which led to increase in the number of lymphocytes in the primary lymphoid organs. There were no publications found to compare the current results with other work that has investigated the effect of probiotic (*pediococcus acidilactici*) on relative weight of thymus. This work is the first document in the effect of probiotic on the relative weight of thymus in broiler chickens.

In conclusion, early supplementation of the probiotic (*Pediococcus acidilactici*) to broiler diet enhances their immune response.

**Table1.** Antibody titres to Newcastle disease virus in chickens fed on rations containing different concentration of probiotic

Week of treatment	Antibodies titer against Newcastle disease by ELISA			
	Probiotic supplementation (g/kg diet)			
	Control	1.6 g/kg	1 g/kg	0.8 g/kg
2	673.6 ± 93.38 <sup>a</sup>	819.8 ± 93.38 <sup>a</sup>	685.6 ± 93.38 <sup>a</sup>	597.4 ± 93.38 <sup>a</sup>
3	2114.4 ± 87.25 <sup>a</sup>	1958.5 ± 87.25 <sup>a</sup>	1941.2 ± 87.25 <sup>a</sup>	1912.8 ± 87.25 <sup>a</sup>
4	2781.3 ± 150.19 <sup>a</sup>	3201.5 ± 150.19 <sup>a</sup>	2843.7 ± 150.19 <sup>a</sup>	3063.0 ± 150.19 <sup>a</sup>
6	2675.9 ± 247.15 <sup>b</sup>	3518.5 ± 247.15 <sup>a</sup>	3601.0 ± 247.15 <sup>a</sup>	3504.6 ± 247.15 <sup>a</sup>

Means within rows with no common letters are significantly different (P<0.05).

**Table 2.** Relative weight of lymphoid organs of chickens fed on rations containing different concentration of probiotic.

Age in week	Treatment groups	The relative weight of lymphoid organs		
		Spleen	Bursa	Thymus
1	control	0.123 ± 0.002 <sup>a</sup>	0.347 ± 0.01 <sup>a</sup>	0.360 ± 0.01 <sup>a</sup>
	1.6 g/kg	0.12 ± 0.002 <sup>a</sup>	0.341 ± 0.01 <sup>a</sup>	0.364 ± 0.01 <sup>a</sup>
	1 g/kg	0.12 ± 0.002 <sup>a</sup>	0.34 ± 0.01 <sup>a</sup>	0.360 ± 0.01 <sup>a</sup>
	0.8 g/kg	0.121 ± 0.002 <sup>a</sup>	0.351 ± 0.01 <sup>a</sup>	0.379 ± 0.01 <sup>a</sup>
4	control	0.164 ± 0.004 <sup>a</sup>	0.219 ± 0.01 <sup>b</sup>	0.379 ± 0.02 <sup>b</sup>
	1.6 g/kg	0.172 ± 0.004 <sup>a</sup>	0.243 ± 0.01 <sup>ba</sup>	0.428 ± 0.02 <sup>a</sup>
	1 g/kg	0.170 ± 0.004 <sup>a</sup>	0.243 ± 0.01 <sup>ba</sup>	0.413 ± 0.02 <sup>a</sup>
	0.8 g/kg	0.175 ± 0.004 <sup>a</sup>	0.265 ± 0.01 <sup>a</sup>	0.429 ± 0.02 <sup>a</sup>
6	control	0.146 ± 0.005 <sup>b</sup>	0.050 ± 0.002 <sup>b</sup>	0.339 ± 0.01 <sup>b</sup>
	1.6 g/kg	0.154 ± 0.005 <sup>ba</sup>	0.059 ± 0.002 <sup>a</sup>	0.412 ± 0.01 <sup>a</sup>
	1 g/kg	0.155 ± 0.005 <sup>ba</sup>	0.059 ± 0.002 <sup>a</sup>	0.43 ± 0.01 <sup>a</sup>
	0.8 g/kg	0.162 ± 0.005 <sup>a</sup>	0.056 ± 0.002 <sup>ba</sup>	0.419 ± 0.01 <sup>a</sup>

Means within rows with no common letters are significantly different (P<0.05).

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## الملخص العربي

### تأثير إضافة البروبيوتيك على الاستجابة المناعية لبداري التسمين عبد الله الخلف<sup>1</sup> - محمد الحاج<sup>2</sup> - إبراهيم الحميدان<sup>3</sup>

- 1 قسم الطب البيطري، كلية الزراعة والطب البيطري، جامعة القصيم، المملكة العربية السعودية
- 2 قسم الطب البيطري، كلية الزراعة والطب البيطري، جامعة تمار، اليمن
- 3 قسم إنتاج الحيوان وتربيته، كلية الزراعة والطب البيطري، جامعة القصيم، المملكة العربية السعودية

تهدف هذه الدراسة إلى تقييم أثر إضافة مكملات بروبيوتيك التجارية ( Bactocell ®) تحتوي على جراثيم حية (*acidilactici Pediococcus*) في علائق الدجاج اللحم علي المقدرة المناعية للطيور. تم أخذ عدد 800 كتكوت روس عمر يوم واحد حيث تم تربيتها لمدة 42 يوماً. تم ترقيم الكتاكيت في الجناح وقسمت بشكل عشوائي إلى أربع مجموعات رئيسية ( 200 في كل مجموعة). أعطيت كتاكيت المجموعة 1 (مجموعة المراقبة) عليقه بدون إضافات. بينما غذيت كتاكيت المجموعات 2 و 3 و 4 علي علف مضاف إليه 1.6 غرام ، 1 غرام و 0.8 غرام من الكائنات الحية المجهرية (البروبيوتيك) لكل كيلوجرام من الأعلاف على التوالي لمدة 42 يوماً. أوضحت النتائج المتحصل عليها أن المجموعات المغذاة علي علائق مضاف إليها بروبيوتيك كبيرة أظهرت مستويات أعلى للأجسام المناعية ضد فيروس مرض نيوكاسل ( NDV). وقد تم تسجيل زيادة كبيرة في الوزن النسبي لغدة للبرسا في جميع المجموعات. كانت هناك زيادة كبيرة في الوزن النسبي للطحال في 42 يوماً من جميع الفئات العمرية المعاملة بالبروبيوتيك مقارنة مع مجموعة التحكم. أظهرت المجموعة التي غذيت علي مستوي عالي من البروبيوتيك فرقاً معنوياً (أقل من 0.05) وزيادة الوزن النسبي لجميع الفئات في الغدة التيموثية علي عمر 28 و 42 يوماً، مقارنة مع مجموعة التحكم. وخلصت النتائج إلي وجود تأثير إيجابي لإضافة البروبيوتيك على الجهاز المناعي للدجاجة.