

# Blood Haematological-Cholesterol Profile and Antibody Titer Response of Broilers with Added Probiotic Containing both Bacteria and Yeast or an Antibiotic in Drinking Water

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## Abstract

An experiment was conducted to investigate the haematological and cholesterol profiles and antibody titer responses to both Newcastle disease (ND) and Infectious bronchitis (IB) vaccines in broiler chickens after drinking water supplemented with either a commercial probiotic (an Active Elements®; AE) containing *Lactobacillus plantarum* and *Saccharomyces cerevisiae*, an antibiotic Chlortetracycline (CTC), or the probiotic plus the antibiotic. Four hundred one-day-old male Cobb broiler chicks were randomly allocated to 4 treatment groups designated as follow: a non-treated control (T1), a 0.05% CTC (T2), a 1:500 AE (T3), and a combination of 0.05% CTC and 1:500 AE (T4), respectively. The chicks were fed with a commercial broiler diet *ad lib.* and were reared on rice hull bedding in identical floor pens and in an environmentally controlled experimental room for 6 weeks. The result showed that the IB vaccine antibody titers of the probiotic supplemented broilers group at the 28 days of age were significantly ( $p<0.05$ ) higher than those of the other groups. At 28 days of age, the chickens originated from the probiotic and antibiotic-probiotic combination groups showed significant ( $p<0.05$ ) increases in the mean red blood cell count, the mean hemoglobin concentration, mean corpuscular haemoglobin concentration, the number of thrombocyte, monocyte, heterophil but show significant decreases in the number of lymphocyte. Finally at 42 days of age, the total serum cholesterol concentration was significantly higher ( $p<0.05$ ) in the chickens supplemented with antibiotic and with antibiotic and probiotic as compared to the control chickens. It is concluded that using this probiotic as an alternative for antibiotic in commercial broiler production may be considered.

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**Keywords :** broiler, antibody titer, cholesterol, haematology, probiotic, antibiotic

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## บทคัดย่อ

**ระดับค่าโลหิตวิทยา คอเลสเตอรอลในเลือดและการตอบสนองของระดับแอนติบอดีในไก่กระตังที่ได้รับการเติมสารเสริมชีวนะประกอบด้วยแบคทีเรียและยีสต์หรือยาปฏิชีวนะลงในน้ำดื่ม**

สฤณี กลั่นทกานนท์-ทองทรง<sup>1</sup> บุญฤทธิ์ ทองทรง<sup>2\*</sup> วิวัฒน์ ชวนะนิกุล<sup>2</sup>

การทดลองมีวัตถุประสงค์เพื่อศึกษาการระดับค่าโลหิตวิทยา คอเลสเตอรอลในเลือดและการตอบสนองของระดับแอนติบอดีต่อโรคนิวคาสเซิลและโรคหลอดลมอักเสบในไก่กระตัง ที่ได้รับการเติมผลิตภัณฑ์สารเสริมชีวนะ (แอคทีฟ อิลีเมนต์) ประกอบด้วยแบคทีเรีย *Lactobacillus plantarum* และยีสต์ *Saccharomyces cerevisiae* ในขนาด 1:500 (สารเสริมชีวนะ: น้ำดื่ม) กับการผสมยาปฏิชีวนะ (คลอร์เตตราไซคลีน; 0.05%) และการใช้ร่วมกันทั้งผลิตภัณฑ์สารเสริมชีวนะและยาปฏิชีวนะในน้ำดื่ม ถูกไก่เพศผู้อายุ 1 วัน จำนวน 400 ตัว ถูกแบ่งออกเป็น 4 กลุ่ม ได้แก่ 1) กลุ่มควบคุมไม่มีการให้สารเสริม 2) กลุ่มที่ได้รับการเสริมยาปฏิชีวนะ 3) กลุ่มที่ได้รับการเสริมสารเสริมชีวนะ และ 4) กลุ่มที่ได้รับการเสริมชีวนะร่วมกับยาปฏิชีวนะ ไก่ทดลองได้รับอาหารสำเร็จรูปและน้ำแบบไม่จำกัด และเลี้ยงในโรงเรือนที่มีการควบคุมอุณหภูมิและการระบายอากาศภายในโรงเรือนเป็นระยะเวลา 6 สัปดาห์ ผลการศึกษาพบว่า ที่อายุ 4 สัปดาห์ ไก่ที่ได้รับสารเสริมชีวนะมีผลการตอบสนองของระดับแอนติบอดีต่อโรคหลอดลมอักเสบ เพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติว่ากลุ่มทดลองอื่นๆ ส่วนกลุ่มที่ได้รับการเสริมชีวนะและหรือกลุ่มที่ได้รับการเสริมชีวนะร่วมกับยาปฏิชีวนะ มีค่าเม็ดเลือดแดง ค่าฮีโมโกลบิน ค่าเฉลี่ยความเข้มข้นของฮีโมโกลบินในเม็ดเลือดแดง แกล็ดเลือด เม็ดเลือดขาวชนิดโมโนไซต์ และเม็ดเลือดขาวชนิดเฮมาโทฟิล เพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติ ในขณะที่เม็ดเลือดขาวชนิดลิมโฟไซต์ ลดลงอย่างมีนัยสำคัญทางสถิติ เมื่อคำนึงถึงสุขภาพของผู้บริโภคที่สัมผัสกับระดับคอเลสเตอรอลโดยรวมในไก่กระตังที่อายุ 42 วัน กลุ่มที่ได้รับการเสริมยาปฏิชีวนะแสดงผลในการเพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติ ของคอเลสเตอรอลในเลือด เมื่อเปรียบเทียบกับกลุ่มควบคุม ดังนั้นการใช้สารเสริมชีวนะดังกล่าว อาจจะพิจารณาเป็นทางเลือกหนึ่งที่ทดแทนการเสริมยาปฏิชีวนะในอุตสาหกรรมผลิตไก่กระตัง

**คำสำคัญ :** ไก่กระตัง ระดับแอนติบอดี คอเลสเตอรอล ค่าโลหิตวิทยา สารเสริมชีวนะ ยาปฏิชีวนะ

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## Introduction

The control of infections and the enhancement of life performance through a non-antibiotic approach are urgently required because increases in microbial resistance to antibiotics and residues in chicken meat products can be harmful to consumers. Probiotics have been used in poultry management not only to enhance production performance (Mohan et al., 1996; Yeo and Kim, 1997; Jin et al., 1998) and to reduce mortality (Vicente et al., 2007) but also to develop and stimulate the immune response (Jin et al., 1997; Rolfe, 2000). The manipulation of gut microbial via the administration of probiotics influences the development of the immune response (Isolauri et al., 2001). In poultry, the probiotics can modulate the systemic antibody response to antigens (Huang et al.,

2004; Koenen et al., 2004). This may correlate to the physiological changes in haematological and cholesterol profiles. Probiotic microorganisms (nonpathogenic bacteria and/or yeast) are one of the alternatives for growth promotion in poultry (Abdulrahim et al., 1996; Santin et al., 2001) although their modes of action are not entirely clear and their efficacy is inconsistent.

The *Saccharomyces species* have been widely used in human medicine in combination with therapeutic antibiotic administration to avoid diarrhoea (Surawicz et al., 1989) and in growing pig via oral administration to stimulate sodium dependent glucose absorption into jejunum (Breves et al., 2000). However, the use of them as feed additives to induce physiological changes in terms of haematological-cholesterol levels and the

antibody titer responses to Newcastle disease (ND) vaccine and Infectious bronchitis (IB) vaccine of birds is still under investigation. While other common probiotic strains such as *Lactobacillus* species used in broiler nutrition have a beneficial immunomodulatory effect (Zulkifli et al., 2000a; Koenen et al., 2004), an antibiotic such as Chlortetracycline (CTC) has been suggested for administration in feed or drinking water and in medication programs to promote growth in broilers. However, various results of changes in blood haematology and biochemistry have been found (Mohan et al., 1996; Panda et al., 2006; Murwani and Bayuwardhi, 2007). Previously, Thongsong et al. (2008) reported that a combination of bacteria (*Lactobacillus plantarum*) and yeast (*Saccharomyces cerevisiae*) preparation as a probiotic supplement showed beneficially significant effects on accumulated body weight gain during the first two weeks after administration of this probiotic via the drinking water. Additionally, feed conversion ratio (FCR) and antibiotic residues of the probiotic supplemented birds were similar to those of the CTC supplemented birds. Furthermore, the percentage mortality of the probiotic supplemented birds was less than that of the non-treated control chickens. However, there are limited reports of the efficacy of this probiotic on physio-pathological changes in haematological and cholesterol profiles as well as the systemic antibody response after vaccination with some important broiler diseases vaccines.

The objective of the present study was to investigate the effect of the commercial probiotic (Active Elements®; AE) as a combination of bacteria and yeast, an antibiotic (CTC) and the probiotic plus antibiotic administration via the drinking water on the haematological and cholesterol profiles and antibody titer responses to ND and IB vaccines in broiler chickens.

## Materials and Methods

### Animals

One-day-old, male Cobb chicks were obtained from a commercial hatchery. They were vaccinated with

live vaccine against Newcastle disease (ND; VGGA strain) and infectious bronchitis (IB; H120 strain) upon hatching in the hatchery. They were vaccinated against Infectious Bursal Disease (IBD; V877 strain) on day 14 and against ND (B1 strain) and IB (Mass & Conn strain) on day 24 of broiler age. The vaccination programme was conducted according to recommendations from local veterinarians. The experiment was carried out for 42 days and other husbandries were previously published (Thongsong et al., 2008).

The experimental protocol was approved by the Animal Care and Use Committee of Chulalongkorn University.

### Probiotic and antibiotic

The probiotic (Active Elements®; AE, The Long Year Biochem. Coop. Ltd.) is a commercial liquid preparation. It is composed of both bacteria and yeast; *Lactobacillus plantarum* ( $1 \times 10^{10}$  cfu) and *Saccharomyces cerevisiae* ( $1 \times 10^9$  cfu). The antibiotic, purchased from a commercial company was chlortetracycline hydrochloride (CTC). The antibiotic and the probiotic were mixed in the drinking water individually at a final concentration of 0.05% CTC or 1:500 AE as suggested by the manufacturer.

### Experimental design

Four hundred 1-day-old broiler chicks were randomly allocated to 4 treatments, with 4 replicates per treatment and 25 chicks per replicate. The treatments were assigned either with their drinking water containing no additive (T1); 0.05% CTC (T2); 1:500 AE (T3); or a combination of CTC and AE (T4). The drinking water was freshly prepared everyday. For the T4 group, the AE or the CTC was given separately in the morning and in the evening, respectively.

### Collection of blood samples

Blood samples were collected weekly from the birds via the wing vein of 8-12 birds per treatment before

flock vaccination to assess the systemic antibody titer response to ND and IB. Blood samples were labeled, kept at room temperature for 2 hours and then at 4°C overnight for separated serums. On 28<sup>th</sup> and 42<sup>nd</sup> day of the experiment, whole blood was collected to determine the haematological and plasma cholesterol profile. All blood samples were kept on ice. The plasma and serum were kept deep frozen prior to analysis.

### Haematological and differential leukocyte examinations

The following haematological parameters such as red blood cell (RBC) and white blood cell (WBC) counts, haematocrit (Hct), haemoglobin (Hb) concentration, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were determined. Differential leukocyte counts to determine the differential percentage of white blood cells; lymphocytes, heterophils, basophils, monocytes and eosinophils, were performed manually for at least 100 leukocytes in blood smears (Ross et al., 1976). The heterophil to lymphocyte (H/L) ratios were used as an index of stress status.

### Serological and cholesterol analysis

The serum immune response was evaluated for maternal immunity and acquired immunity in response to vaccination. To determine the specific antibody production in response to ND and IB, a direct haemagglutination inhibition (HI) assay was used. The HI tests were done by standard methods (Alexander 1989) using 8 haemagglutination (HA) units of the La Sota strain of ND virus and 4 HA units of the M 41 strain of IB virus. The plasma cholesterol concentration was determined according to Allain et al. (1974) by enzymatic method.

### Statistical analysis

A general linear model procedure (SAS version 9.0; SAS Institute, Cary, NC, USA) was used to estimate the least square means of the different variables. The

effect of feed additives was evaluated within the 4x2 or 4x6 experimental design with four treatments (control and feed additives) and weeks 4 and 6 for all haematological and cholesterol profiles or weeks 1-6 for ND and IB titers:  $y_{ijk} = \mu + A_i + W_j + (A \times W)_{ij} + e_{ijk}$ , where  $y_{ijk}$  is the dependent variable;  $\mu$  is the overall mean;  $A_i$  is the fixed effect of types of feed additive ( $i = 1, 2, 3, 4$ );  $W_j$  is the fixed effect of weeks of blood sampling ( $j = 1, 2$  for haematological and cholesterol profiles;  $j = 1, 2, \dots, 6$  for ND and IB titer determination);  $(A \times W)_{ij}$  is the interaction between feed additive and weeks;  $e_{ijk}$  is the error term.

To compare the effect of feed additive on a weekly basis, each supplementation was evaluated as the only independent variable:  $y_{ij} = \mu + A_i + e_{ij}$ , where  $y_{ij}$  is the dependent variable;  $\mu$  is the overall mean;  $A_i$  is the fixed effect of types of supplementation ( $i = 1, 2, 3, 4$ );  $e_{ij}$  is the error term.

The effect of age was evaluated across types of supplementation with weekly blood sampling as the only independent variable:  $y_{ij} = \mu + W_j + e_{ij}$ , where  $y_{ij}$  is the dependent variable;  $\mu$  is the overall mean;  $W_j$  is the fixed effect of weeks of blood sampling ( $j = 1, 2, \dots, 6$  for ND and IB titer determination);  $e_{ij}$  is the error term.

The data was considered statistically significant at  $p < 0.05$ ; tendency was assigned as  $0.05 < p < 0.10$ .

## Results

### Haematological indices and differential leukocyte counts

The effects of feed additives on the haematological parameters are shown in Table 1. On day 28 of broiler age (4<sup>th</sup> week), the data revealed that the total red blood cell count (RBC), Hb and MCHC were significantly higher in broilers receiving AE and CTC+AE. The thrombocyte in AE treated groups was also significantly elevated compared to other groups. MCV was lowered in the CTC, AE and combination of CTC and AE treated groups compared to the control. Although the total white blood cell count (WBC) was unaffected by treatments, the percentages of

heterophil and lymphocyte in the combination group were affected. Consequently, the H:L ratio in this group was significantly elevated. The effects of feed additives on these parameters were lessened as the broilers became older. As shown in Table 1, at the age of 42 days (6<sup>th</sup> week), the feed additives had no effect on haematological parameters except that the MCV in the CTC treated broilers was smaller than the control group but not different from the AE or AE+CTC groups. The age of broilers had some impact on the level of complete blood count (CBC) in that the CBC was increased in older broilers; consequently, the Hb and Hct were increased as the CBC was elevated with no effect on MCV, MCH and MCHC. Moreover, the percentages of heterophil and lymphocyte were increased and decreased, respectively as the broiler aged; correspondingly the H:L ratio was then elevated. Furthermore, the statistical analysis also demonstrated that there was an interaction between age of broilers and the types of feed additive on the RBC, Hb, MCV, MCHC, thrombocyte, monocyte and basophil.

#### Total cholesterol in blood plasma

The effects of probiotic and/or antibiotic supplementation on the total cholesterol in broilers on day 28 and 42 of broiler age are shown in Figure 1. The figures show that probiotic and antibiotic supplements induced numerical decreases in total plasma cholesterol on day 28 of broiler age. Subsequently on day 42 of broiler age, there was a markedly elevated level of total cholesterol ( $p<0.05$ ) in the antibiotic supplemented groups (CTC and CTC+AE) compared to the control group.

#### Systemic antibody titer response to Newcastle disease (ND) and Infectious bronchitis (IB)

The effects of feed additives on the weekly antibody titers against Newcastle disease (ND) and infectious bronchitis (IB) are presented in Table 2 and Figures 2-4. The antibody titer response against ND was detected with no significant difference among the experimental groups ( $p>0.05$ ). However, the weekly ND

titer was changed according to the age of broilers ( $p<0.05$ ); we found that the titers dropped dramatically from week 1 to week 4 (Figure 2). After re-vaccination, the titer was increased as was evident in week 5 and then dropped to a lower level on week 6. Interestingly, at week 5 following the re-vaccination, in broilers receiving both CTC+AE, it was likely that their immunity did not respond as well although this failed to appear statistically significant as shown in Figure 2.

For the antibody titer against IB, the statistical analysis demonstrated the effects of feed additives as shown in Figure 3. At week 2, while other groups were able to maintain their antibody titer levels, the broilers receiving both CTC and AE were not; their titers were significantly lower than other groups. While the antibody titers dropped continually from week 1, it is obvious that the broilers receiving AE were able to maintain their titer levels better than others as seen at week 4, their titers were significantly higher than other groups. The age of broilers had a significant effect on the antibody titers against IB as shown in Figure 4. During the first 2 weeks, maternal immunity was maintained and then dropped continually to week 6. The re-vaccination of IB was not likely to affect broiler immunity as the titers continued to drop although were likely to elevate at week 6 (Figure 4). It should be noted that there is an interaction between the age of broiler and the types of feed additive on IB titer ( $p<0.05$ ).

### Discussion

In this study, we evaluated the effects of feed additives: antibiotic, chlortetracycline; probiotic, the combination of bacteria and yeast (*L. plantarum* and *S. cerevisiae*, respectively); and the combination of antibiotic and probiotic on haematological profiles, cholesterol level and antibody titers against ND and IB when the feed additives were supplemented for 6 weeks. It is widely accepted that haematological characteristics play an important role in the pathophysiology of chickens such as ascites in broilers (Maxwell et al., 1986; Lunger

**Table 1** The effect of feed additives (antibiotic and probiotic) on haematological profiles of broilers at 4 and 6 weeks.

Parameters	Age (Wk)	Treatments <sup>1</sup> (A)				SEM	P value <sup>2</sup>	P value <sup>3</sup>		
		control	CTC	AE	CTC+AE			A	W	AxW
RBC (x 10 <sup>6</sup> / $\mu$ l)	4	1.74 <sup>a</sup>	2.05 <sup>ab</sup>	2.50 <sup>b</sup>	2.59 <sup>b</sup>	0.11	0.0115	0.0082	0.0012	0.0271
	6	2.44	2.75	2.59	2.56	0.05	0.2047			
Hb (g/dl)	4	12.85 <sup>a</sup>	11.71 <sup>a</sup>	14.85 <sup>b</sup>	15.86 <sup>b</sup>	0.41	0.0002	0.0553	0.0071	0.0046
	6	15.58	15.44	14.70	15.10	0.38	0.8599			
Hct (%)	4	25.75	25.75	25.00	26.13	0.45	0.8517	0.8506	0.0003	0.7794
	6	28.63	27.88	27.88	27.50	0.37	0.7605			
MCV (fl)	4	135.29 <sup>a</sup>	131.40 <sup>a</sup>	103.30 <sup>b</sup>	101.94 <sup>b</sup>	5.29	0.0236	0.0098	0.0926	0.0446
	6	118.40	102.48	108.34	108.03	2.22	0.0673			
MCH (pg)	4	67.95	59.59	61.74	61.91	2.77	0.7478	0.3143	0.2902	0.9949
	6	64.51	56.68	56.81	59.24	1.71	0.3237			
MCHC (%)	4	50.00 <sup>a</sup>	45.79 <sup>a</sup>	59.50 <sup>b</sup>	61.26 <sup>b</sup>	1.72	0.0005	0.0331	0.8346	0.0092
	6	54.49	55.61	52.91	55.19	1.43	0.9206			
Thrombocyte (x10 <sup>-3</sup> / $\mu$ l)	4	39.75 <sup>a</sup>	52.88 <sup>ab</sup>	67.88 <sup>b</sup>	42.00 <sup>a</sup>	37.70	0.0207	0.4653	0.8334	0.0198
	6	56.25	57.00	42.38	51.00	36.38	0.4660			
WBC (/ $\mu$ l)	4	6694	5356	6538	5900	591	0.8539	0.9827	0.7811	0.6670
	6	5625	6450	5800	5875	265	0.7228			
Heterophil (%)	4	24.38 <sup>a</sup>	26.75 <sup>a</sup>	27.25 <sup>ab</sup>	34.75 <sup>b</sup>	1.45	0.0495	0.2210	0.0001	0.3415
	6	42.38	46.25	44.50	44.38	1.57	0.8648			
Lymphocyte (%)	4	68.13 <sup>a</sup>	61.63 <sup>a</sup>	64.75 <sup>ab</sup>	55.50 <sup>b</sup>	1.57	0.0196	0.5654	0.0001	0.1044
	6	48.38	49.75	47.75	51.50	1.88	0.9050			
Monocyte (%)	4	3.63	5.88	4.13	7.63	0.60	0.0589	0.9871	0.6857	0.0048
	6	6.88	3.88	6.25	2.88	0.69	0.1143			
Eosinophil (%)	4	1.13	2.63	1.25	0.38	0.33	0.1005	0.1365	0.0521	0.0766
	6	1.50	0.13	0.63	0.00	0.26	0.5625			
Basophil (%)	4	2.75	3.13	2.63	1.75	0.43	0.7215	0.9694	0.0009	0.3693
	6	0.88	0.00	0.88	1.25	0.28	0.4612			
H : L ratio	4	0.37 <sup>a</sup>	0.44 <sup>a</sup>	0.45 <sup>a</sup>	0.64 <sup>b</sup>	0.04	0.0237	0.7729	0.0001	0.2595
	6	0.95	1.05	1.01	0.88	0.07	0.8250			

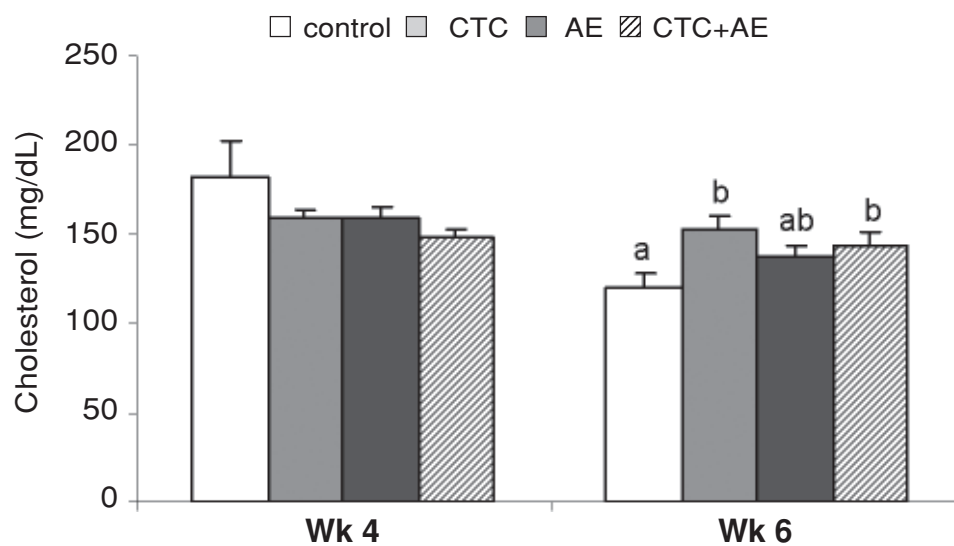
<sup>1</sup>Data represents means from each treatment at different ages; CTC: chlortetracycline; AE: Active Element®.<sup>2</sup>Compares the effect of feed additive on a weekly basis, each supplementation was evaluated as the only independent variable; different superscript denotes statistical significance at  $p < 0.05$ .<sup>3</sup>Compares the effect of feed additives (A), ages (W; weeks 4 and 6) and the interaction between feed additives and age (AxW).



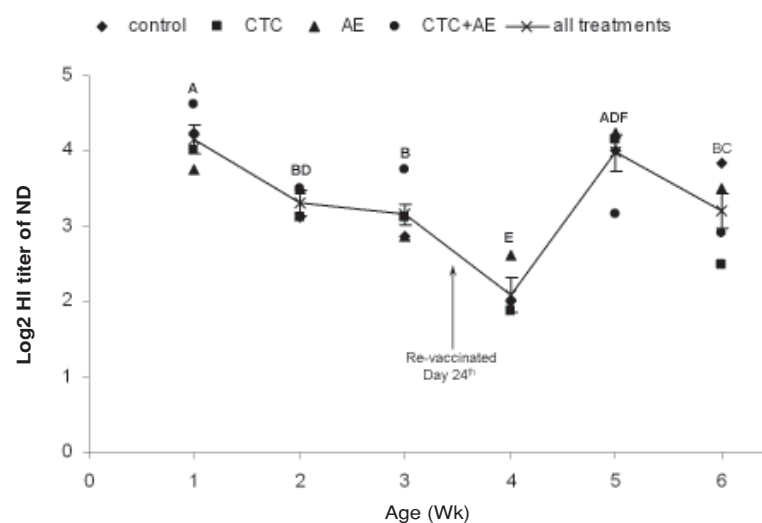
**Table 2** The effects of probiotic and/or antibiotic supplementations on the levels of antibody titer against ND as determined on a weekly basis.

	Log2 HI titer of ND				SEM	Significance
	Control	CTC	AE	CTC + AE		
Week 1	4.25	4.00	3.75	4.63	0.163	NS
Week 2	3.13	3.13	3.50	3.50	0.122	NS
Week 3	2.88	3.13	2.88	3.75	0.186	NS
Week 4	2.00	1.88	2.63	1.88	0.239	NS
Week 5	4.00	4.17	4.25	3.17	0.239	NS
Week 6	3.83	2.50	3.50	2.92	0.254	NS

Data represent means from each treatment at different age; CTC: chlortetracycline; AE: Active Element®; NS,  $p > 0.05$

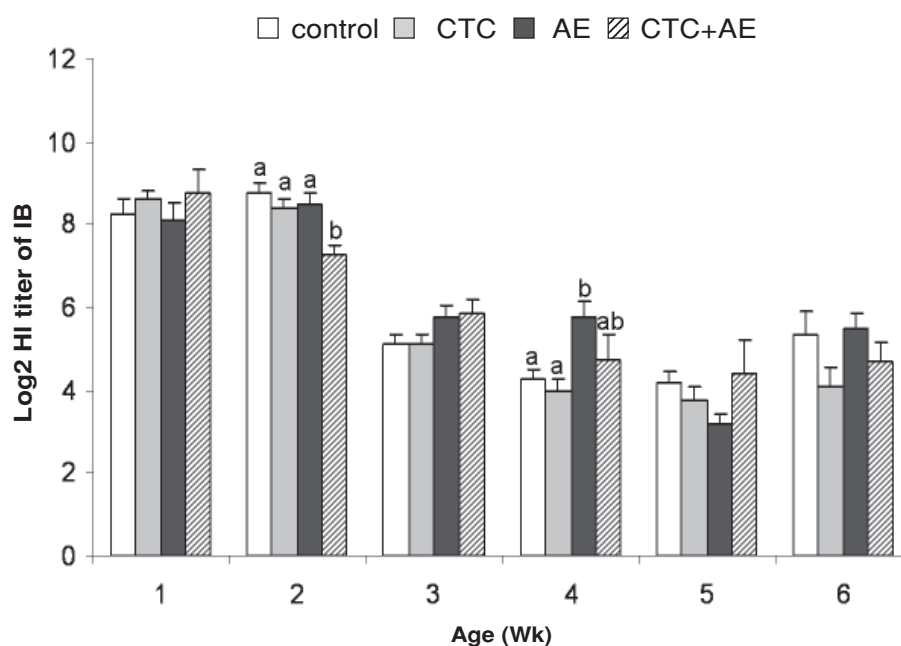
**Figure 1** The effects of probiotic and/or antibiotic supplementation on the total plasma cholesterol in broilers on day 28 (Wk 4) and 42 (Wk 6) of broiler age.

<sup>a,b</sup>Different superscripts denote significant difference at  $p < 0.05$ .

**Figure 2** The effects of age on the levels of antibody titer against ND as determined on a weekly basis. The symbols represent means from each treatment and the symbol with the connected line represents means  $\pm$  SEM of all treatments.

<sup>a,b</sup>Different superscripts denote significant difference at  $p < 0.05$ .

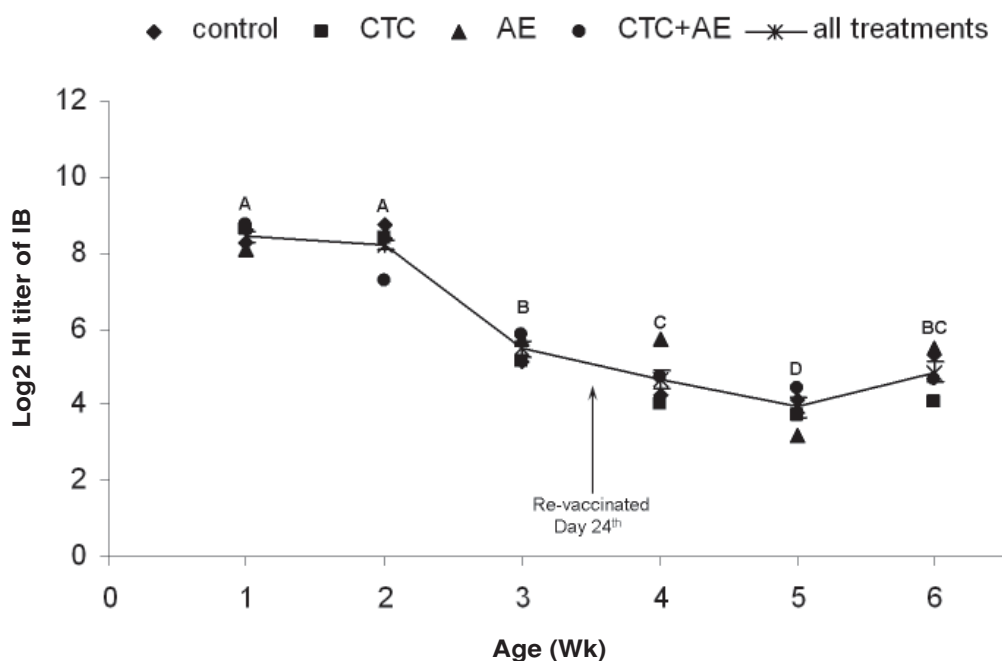
CTC: Chlortetracycline ; AE: Active Elements®



**Figure 3** The effects of probiotic and/or antibiotic supplementations on the levels of antibody titer against IB as determined on a weekly basis. The data represent means  $\pm$  SEM from each treatment.

<sup>a,b</sup>Different superscripts denote significant difference at  $p < 0.05$ .

CTC: Chlortetracycline; AE: Active Elements<sup>®</sup>



**Figure 4** The effects of age on the levels of antibody titer against IB as determined on a weekly basis. The symbols represent means from each treatments and the symbol with the connected line represents means  $\pm$  SEM of all treatments.

<sup>a,b</sup>Different superscripts denote significant difference at  $p < 0.05$ .

CTC: Chlortetracycline; AE: Active Elements<sup>®</sup>



et al., 2001; Scheele et al., 2003). The normal range values of haematological parameters and differential leukocyte counts reported by Jain (1993) are as follows: RBC = 2.5-3.5 ( $\times 10^6/\mu\text{l}$ ), Hb = 7-13 (g/dl), Hct = 22-35 (%), MCV = 90-140 (fl), MCH = 33-47 (pg), MCHC = 21-23 (%), WBC = 12000-30000 ( $/\mu\text{l}$ ), Heterophil = 15-40 (%), Lymphocyte 45-70 (%), Monocyte 5-10 (%), Eosinophil = 1.6-6.0 (%), Basophil = rare. The values retrieved in this experiment were within the normal range. However, at the 4<sup>th</sup> week, we found that probiotics had a beneficial effect on haematopoiesis as the parameters related to red blood cells were better than the control. The effect of probiotics on the blood-forming process had been reported previously in piglets (Kander, 2004). This effect was likely to be limited to the growing period of broilers. As we found at the 6<sup>th</sup> week, none of these parameters relating to haematology was different among treatments indicating that the feed additives no longer had any beneficial effect. Moreover, age was likely to be a factor affecting the blood forming process as the RBC, Hb and Hct were higher at the 6<sup>th</sup> week compared to those at the 4<sup>th</sup> week. This data suggest that as the broilers grew up, the ability to synthesize red blood cells also increased.

The differences were not limited to red blood cells, we also found that the percentages of heterophil and lymphocyte were increased and decreased, respectively in broilers receiving both probiotics and antibiotics for 4 weeks. Subsequently, the H:L ratio in this group was elevated indicating that they were stressed. It is readily accepted that the increase in heterophil and H:L ratio are indicators of stress in chickens (Siegel and Gross, 2000; Zulkifli et al., 2000b; Post et al., 2003). The stress in this group may have been caused by the management, as this group received two different types of drinking water, one contained CTC and another contained AE, switching during the day. The switching in drinking water may have led to stress and be reflected by the elevated H:L ratio in this group. However, this effect was no longer seen at the 6<sup>th</sup> week of broiler age.

Cholesterol levels in avian blood are affected by age, heredity, nutrition and various diseases. In this study, the plasma cholesterol was measured at the 4<sup>th</sup> and 6<sup>th</sup> week of broiler age and the values were in the cholesterol range (125-200 mg/dl) in chicken reported by Clarenburg (1992). The plasma cholesterol levels at the 6<sup>th</sup> week were similar to those previously reported with a value of 120-150 mg/dl (Meluzzi et al., 1992; Panda et al., 2006; Murwani and Bayuardhi, 2007). Previous reports have shown that the serum cholesterol was lower in broilers receiving diets containing probiotics (Mohan et al., 1996; Jin et al., 1998). Additionally, Kalavathy and coworkers (2006) also reported that the cholesterol in the livers and carcasses of broilers was also lower in broilers fed with diets containing *Lactobacillus* at the 6<sup>th</sup> week of age. In the current study, we did not find any lowering effect of probiotics on plasma cholesterol at the 4<sup>th</sup> or the 6<sup>th</sup> week which is inconsistent with the previous reports. However, it should be noted that the plasma cholesterol at the 4<sup>th</sup> week seemed to be lower in probiotic groups compare to the control although not significantly. The difference between the results of this study and others could be due to the difference in strain/species of probiotic microorganism used or the route of administration. Further, the age of the broilers also played a part in the level of cholesterol; the older the broilers were the lower was the plasma cholesterol. It may be that cholesterol is required during growing period for the development of muscle and tissue. In order to assess the potential health benefits to consumers of cholesterol levels in broilers, we found that at the 6<sup>th</sup> week of broiler age, birds supplemented with the antibiotic (chlortetracycline with/without probiotic) showed a significant increase in plasma cholesterol levels. This agrees with the study of Murwani and Bayuardhi (2007) who suggested that antibiotic in feed and the medication programs of broilers can affect the lipid and hepatic metabolism of broilers and be reflected by an increase in serum cholesterol. Based on the studies to date, the mechanism responsible for the cholesterol-heightening effect of antibiotics is interesting.

The effects of probiotics on the immune response of poultry are interesting and complicated. In this study, there was no significant difference in the antibody titer responses to ND among groups. In contrast, Khaksefidi and Ghoorchi (2006) reported that the antibody titers against ND in broilers fed with diets supplemented with probiotics containing *Bacillus subtilis* was significantly higher at 10 days post-immunization compared to the control birds. The difference in antibody production observed in this experiment could be attributed to the probiotic microorganism and the route of administration. The beneficial effects of probiotics seem to be not only strain-specific but have additional factors or characteristics as well. For another antibody titer against IB, likewise ND, the titer was highest and gradually decreased as the broilers got older. At the second week of age, the broilers receiving a combination of CTC and AE had the lowest antibody titer; it was likely that they were unable to maintain their titers. This may be a stress effect as shown by the H:L ratio in this group later on. The change in differential leukocyte counts could mean that the antibody formation and cell mediated immunity of broilers drinking the probiotic or probiotic plus antibiotic, were affected. At week 4, although the antibodies continually decreased, broilers supplemented with probiotic alone had higher titer levels than other groups. This may be due to two main reasons; firstly, it could be that the probiotic was somehow prolonging the antibody level. Secondly, the probiotic may have enhanced the systemic response to specific antigens which had been reported previously (Haghighi et al., 2005; 2006). From these results, it is obvious that probiotics could be important in populations where the immune response is immature or weak during the life span (Fooks and Gibson, 2002). However, *in vivo* studies are needed to further elucidate the effects of probiotics.

In conclusion, the administration of probiotics composed of bacteria (*L. plantarum*) and yeast (*S. cerevisiae*) in broiler chickens for 6 weeks via the drinking water, starting on the second day of life, had

beneficial effects. Interestingly, these effects were more pronounced at a young age during the growing period as can be seen by the higher antibody titer against IB, higher RBC and Hb implying a better blood forming process. Further, we have shown previously that the feed efficiency was also better during the first two weeks (Thongsong et al., 2008). It is then suggested that continually giving probiotics after the developmental period may have no further benefit. Further, it should be noted that at the end of the experiment there was no difference between the supplementation of chlortetracycline and probiotics in term of performance, antibody titers and haematological profiles. On the other hand, the prolonged use of antibiotics like chlortetracycline may have had some undesirable effects such as an increase in blood cholesterol. Therefore, using probiotic as an alternative for antibiotic in commercial broiler production should be considered.

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### References

- Abdulrahim, S.M., Haddadin, M.S.Y., Hashlamoun, E.A.R. and Robinson, R.K. 1996. The influence of *Lactobacillus acidophilus* and bacitracin on the layer performance of chickens and cholesterol content of plasma and egg yolk. Br. Poult. Sci. 37: 341-346.
- Alexander, D.J. 1989. Newcastle disease. In: H.G. Purchase, L.H.Arp, C.H. Domermuth, J.E. Pearson (eds.), A Laboratory Manual for the Isolation and Identification of Avian Pathogens, 3<sup>rd</sup> edn. American Association of Avian Pathologists, Kennett Square, PA., 114-120.

- Allain, C.C., Poon, L.S., Chan, C.S.G., Richmond, W. and Fu, P.C. 1974. Enzymatic determination of total serum cholesterol. *Clin. Chem.* 20: 470-475.
- Breves, G., Walter, C., Burmester, M. and Schroder, B. 2000. *In vitro* studies on the effects of *Saccharomyces boulardi* and *Bacillus cereus* var. *toyoi* on nutrient transport in pig jejunum. *J. Anim. Physiol. Anim. Nutr.* 84 : 9-20.
- Clarenburg, R. 1992. Lipid metabolism. In: Clarenburg R.; *Physiological chemistry of domestic animal*. Mosby-Year Book, Inc., St. Louis., 292-333.
- Fooks, L.J. and Gibson, G.R., 2002. Probiotics as modulators of the gut flora. *Br. J. Nutr.* 88: s39-s49.
- Haghighi, H.R., Gong, J., Gyles, C.L., Hayes, M.A., Sanei, B., Parvizi, P., Gisavi, H., Chambers, J.R. and Sharif, S. 2005. Modulation of antibody-mediated immune response by probiotics in chickens. *Clin. Diagn. Lab. Immunol.* 12: 1387-1392.
- Haghighi, H.R., Gong, J., Gyles, C.L., Hayes, M.A., Zhou, H., Sanei, B., Chambers, J.R. and Sharif, S. 2006. Probiotics stimulate production of natural antibodies in chickens. *Clin. Vaccine. Immunol.* 13: 975-980.
- Huang, M.K., Choi, Y.J., Houde, R., Lee, J.W. and Zhao, X., 2004. Effects of Lactobacilli and an acidophilus fungus on the production performance and immune responses in broiler chickens. *Poult. Sci.* 83: 788-795.
- Isolauri, E., Sutas, Y., Kankaanpää, H., Arvilommi, H. and Salminen, S. 2001. Probiotics; effects on immunity. *Am. J. Clin. Nutr.* 73: 444S- 450S.
- Jain, N.C. 1993. *Essential of Veterinary hematology*. Philadelphia ; Lea and Febiger, 222-257.
- Jin, L.Z., Ho, Y.W., Abdullah, N. and Jalaludin, S. 1997. Probiotic in poultry: modes of action. *World's Poult. Sci. J.* 53: 351-368.
- Jin, L.Z., Ho, Y.W., Abdullah, N. and Jalaludin, S. 1998. Growth performance, intestinal microbial populations and serum cholesterol of broiler diet containing *Lactobacillus* cultures. *Poult. Sci.* 77: 1259-1265.
- Kalavathy, R., Abdullah N., Jalaludin, S., Wong, M.C.V.L. and Ho, Y.W. 2006. Effects of *Lactobacillus* feed supplementation on cholesterol, fat content and fatty acid composition of the liver, muscle and carcass of broiler chickens. *Anim. Res.* 55: 77- 82.
- Kander, M. 2004. Effect of *Bifidobacterium* Sp. on the health state of piglets, determined on the basis of haematological and biochemical indices. *Electronic Journal of Polish Agricultural Universities* 7, Available Online <http://www.ejpau.media.pl/series/volume7/issue2/veterinary/art-07.html>
- Khaksefidi, A. and Ghoorchi, T. 2006. Effect of probiotic on performance and immunocompetence in broiler chicks. *Poult. Sci.* 43: 296-300.
- Koenen, M.E., Kramer, J., van der Hulst, R., Heres, L., Jeurissen, S.H.M. and Boersma, W.J.A. 2004. Immunomodulation by probiotic lactobacilli in layer- and meat-type chickens. *Br. Poult. Sci.* 45: 355-366.
- Lunger, D., Shinder, D., Rzepakovsky, V., Rusal, M. and Yahav, S. 2001. Association between weight gain, blood parameters, and thyroid hormones and the development of ascites syndrome in broiler chickens. *Poult. Sci.* 80: 965-971.
- Maxwell, M.H., Robertson, G.W. and Spence, S. 1986. Studies on an ascitic syndrome in young broilers. 1. haematology and pathology. *Avian Pathol.* 15: 511-524.
- Meluzzi, A., Primiceri, G., Giordani, R.A. and Fabris, G. 1992. Determination of blood constituents reference values in broilers. *Poult. Sci.* 71: 337-345.
- Mohan, B., Kadirvel, R., Natarajan, A. and Bhaskaran, M. 1996. Effect of probiotic supplementation on growth, nitrogen utilisation and serum cholesterol in broilers. *Br. Poult. Sci.* 37: 395-401.
- Murwani, R. and Bayuwardhi, B. 2007. Broiler serum cholesterol and glutamic oxaloacetic transaminase and their relation to antibiotic in feed and medication programs in four broiler producers in Semarang region-central Java, Indonesia. *Int. J. Poult. Sci.* 6: 266-270.

- Panda, A.K., Rama Rao, S.V., Raju, M.V.L.N. and Sharma, S.R. 2006. Dietary supplementation of *Lactobacillus Sporogenes* on performance and serum biochemico-lipid profile of broiler chickens. Poult. Sci. 43: 235-240.
- Post, J., Rebel, J.M.J. and ter Huurne, A.A.H.M. 2003. Physiological effects of elevated plasma corticosterone concentrations in broiler chickens. An alternative means by which to assess the physiological effects of stress. Poult. Sci. 82: 1313-1318.
- Rolfe, R.D. 2000. The role of probiotic cultures in the control of gastrointestinal health. J. Nutr. 130: 396s-402s.
- Ross, J.G., Christie, G., Halliday, W.G. and Jone, R.M. 1976. Determination of haematology and blood chemistry values in healthy six-week-old broiler hybrids. Avian Pathol. 5: 273-281.
- Santin, E., Maiorka, A., Macari, M., Grecco, M., Sanchez, J.C., Okada, T.M. and Myasaka, A.M. 2001. Performance and intestinal mucosa development of broiler chickens fed diets containing *Saccharomyces cerevisiae* cell wall. J. App. Poult. Res. 10: 236-244.
- Scheele, C.W., Van Der Klis, J.D., Kwakernaak, C., Buys, N. and Decuypere, E. 2003. Haematological characteristics predicting susceptibility for ascites. 1. high carbon dioxide tension in juvenile chickens. Br. Poult. Sci. 44: 477-483.
- Siegel, P.B. and Gross, W.B. 2000. General principles of stress and well-being. In: T. Grandin (ed), Livestock handling and transport. CAB International, Wailingford, UK, 27-41.
- Surawicz, C.M., Elmer, G.W., Speelman, P., McFarland, L.V., Chinn, J. and van Belle, G. 1989. Prevention of antibiotic associated diarrhoea by *Saccharomyces boulardii*: A prospective study. Gastroenterology. 96: 981-988.
- Thongsong, B., Kalandakanond-Thongsong, S. and Chavananikul, V. 2008. Effects of the addition of probiotic containing both bacteria and yeast or an antibiotic on performance parameters, mortality rate and antibiotic residue in broilers. Thai. J. Vet. Med. 38: 17-26.
- Vicente, J.L., Avina, L., Torres-Rodriguez, A., Hargis, B. and Tellez, G. 2007. Effect of a *Lactobacillus* spp-based probiotic culture product on broiler chick performance under commercial conditions. Int. J. Poult. Sci. 6: 154-156.
- Yeo, J. and Kim, K., 1997. Effect of feeding diets containing an antibiotic, a probiotic, or yucca extract on growth and intestinal urease activity in broiler chicks. Poult. Sci. 76: 381-385.
- Zulkifli, I., Abdullah, N., Azrin, N.M. and Ho, Y.W. 2000<sup>a</sup>. Growth performance and immune response to two commercial broiler strains fed diets containing *Lactobacillus* cultures and oxytetracycline under heat stress conditions. Br. Poult. Sci. 41: 593-597.
- Zulkifli, I., Che Norma, MT., Chong, C.H. and Loh, T.C. 2000<sup>b</sup> : Heterophil to lymphocyte ratio and tonic immobility reactions to preslaughter handling in broiler chickens treated with ascorbic acid. Poult. Sci. 79: 402-406.