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Effects of the Addition of Probiotic Containing both Bacteria and Yeast or an Antibiotic on Performance Parameters, Mortality Rate and Antibiotic Residue in Broilers

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Original Articles

Effects of the Addition of Probiotic Containing both Bacteria and Yeast or an Antibiotic on Performance Parameters, Mortality Rate and Antibiotic Residue in Broilers

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Abstract

The experiment was conducted to investigate the effects of supplementing a commercially available probiotic (an Active Elements®; AE) containing *Lactobacillus plantarum* and *Saccharomyces cerevisiae*, an antibiotic; Chlortetracycline (CTC), and the probiotic plus antibiotic, via the drinking water on broiler production performance, mortality rate and the antibiotic residue in meat. Four hundred one-day-old male Cobb broiler chicks were randomly allocated to 4 treatments; a non-treated control, a 0.05% CTC, a 1:500 AE, and a combination of CTC and AE. Chicks were fed with a commercial broiler diet *ad libitum*. They were reared under an environmentally controlled house for 6 weeks. The data revealed that supplementation with probiotic (group 3-4) resulted in significantly higher accumulated body weight gain during the first two weeks. The final body weight, final body weight gain, feed intake and FCR were not significantly different among treatments. The mortality rate was highest in the combination group, followed by control and the AE-treated group. The antibiotic residues in the breast tissue sampling at the 6th week of age were less than 50 μg/kg tissue. The gross and histological examinations revealed no obvious pathological lesions in any treatment. In conclusion, this study indicated that administration of this probiotic to broiler chickens early in life had beneficial effects on weight gain. The FCR and antibiotic residues of the probiotic-supplemented birds were similar to antibiotic-supplemented birds. The percentage mortality was less than the non-treated group. Therefore, this probiotic may be used as an alternative to replace the adverse effect of antibiotic.

Keywords : antibiotic, broiler, growth performance, mortality rate, probiotic

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บทคัดย่อ

ผลการเติมสารเสริมชีวนะประกอบด้วยแบคทีเรียและยีสต์หรือยาปฏิชีวนะต่อตัวบ่งชี้ด้านสมรรถนะ อัตราการตาย และการตกค้างของยาปฏิชีวนะในไก่กระทง

บุญฤทธิ์ ทองทรง[1*] สฤณี กลันทกานนท์-ทองทรง[2] วิวัฒน์ ชวนะนิกุล[1]

การทดลองครั้งนี้มีวัตถุประสงค์เพื่อเปรียบเทียบผลการใช้ผลิตภัณฑ์สารเสริมชีวนะ (แอคทีฟ อิลีเมนต์) ประกอบด้วยแบคทีเรีย *Lactobacillus plantarum* และยีสต์ *Saccharomyces cerevisiae* ในขนาด 1:500 (สารเสริมชีวนะ:น้ำดื่ม) กับการผสมยาปฏิชีวนะ (คลอร์เตตร้าไซคลีน; 0.05%) และการใช้ร่วมกันทั้งผลิตภัณฑ์สารเสริมชีวนะและยาปฏิชีวนะ ในน้ำดื่ม ที่มีต่อสมรรถนะผลผลิต อัตราการตายและการตกค้างของยาในเนื้ออกของไก่กระทง ลูกไก่เพศผู้อายุ 1 วัน จำนวน 400 ตัว ถูกแบ่งออกเป็น 4 กลุ่ม ได้แก่ 1) กลุ่มควบคุมไม่มีการให้สารเสริม 2) กลุ่มที่ได้รับการเสริมยาปฏิชีวนะ 3) กลุ่มที่ได้รับการเสริมสารเสริมชีวนะ และ 4) กลุ่มที่ได้รับสารเสริมชีวนะร่วมกับยาปฏิชีวนะ ไก่ทดลองได้รับอาหารสำเร็จรูปและน้ำแบบไม่จำกัด และเลี้ยงในโรงเรือนที่มีการควบคุมอุณหภูมิและการระบายอากาศภายในโรงเรือนเป็นระยะเวลา 6 สัปดาห์ ผลการศึกษาพบว่า ไก่ที่ได้รับสารเสริมชีวนะ (กลุ่มที่ 3-4) มีน้ำหนักตัวเพิ่มสะสมในช่วง 2 สัปดาห์แรก เพิ่มสูงกว่ากลุ่มควบคุมและกลุ่มที่ได้ยาปฏิชีวนะเพียงอย่างเดียว อย่างมีนัยสำคัญทางสถิติ แม้ว่าน้ำหนักตัวเฉลี่ย น้ำหนักตัวที่เพิ่มขึ้นและปริมาณอาหารที่กินได้รวมทั้งอัตราการแลกเนื้อในช่วงสุดท้ายไม่มีความแตกต่างอย่างมีนัยสำคัญทางสถิติระหว่างกลุ่มทดลอง สำหรับอัตราการตายของไก่กระทงเรียงลำดับจากมากไปน้อย คือ กลุ่มที่ 4 > 1 > 3 การตกค้างของยาในเนื้ออกของกลุ่มที่ได้รับยาปฏิชีวนะ (กลุ่ม 2 และ 4) พบว่ามีสารตกค้างในระดับต่ำกว่า 50 ไมโครกรัมต่อกิโลกรัมเนื้อเยื่อ เมื่อเก็บตัวอย่างที่อายุ 6 สัปดาห์ นอกจากนี้ ผลการผ่าซากและเก็บตัวอย่างเนื้อเยื่อเพื่อตรวจทางจุลพยาธิ ไม่พบความผิดปกติในทุกกลุ่มการทดลอง ดังนั้นการศึกษาครั้งนี้ ชี้ให้เห็นว่าการให้สารเสริมชีวนะดังกล่าวผสมในน้ำดื่มในระยะแรก ส่งผลให้น้ำหนักตัวเพิ่มสะสมในช่วงอายุสองสัปดาห์ โดยอัตราการแลกเนื้อและปัญหายาปฏิชีวนะตกค้างมีผลไม่ต่างจากการได้รับยาปฏิชีวนะ และกลุ่มที่ได้รับสารเสริมชีวนะมีร้อยละการตายต่ำเมื่อเทียบกับกลุ่มควบคุม ดังนั้นสารเสริมชีวนะดังกล่าวนี้ อาจเป็นทางเลือกหนึ่งในการพิจารณานำมาใช้ในการเลี้ยงไก่กระทง เพื่อทดแทนผลที่ไม่พึงประสงค์ของยาปฏิชีวนะ

คำสำคัญ : ยาปฏิชีวนะ ไก่กระทง สมรรถนะการเจริญ อัตราการตาย สารเสริมชีวนะ

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Introduction

It is well recognized that gastrointestinal normal flora plays an important role in the health and performance of poultry. Probiotics are defined as live microbial supplements which beneficially affect the host animal by improving some beneficial functions in its intestinal microbial balance (Fuller, 1989). They have been used in poultry management to enhance production performances (Mohan et al., 1996; Yeo and Kim, 1997; Jin et al., 1998), to develop and stimulate the immune response (Jin et al., 1997[a]; Rolfe, 2000) and to reduce mortality (Vicente et al., 2007). In recent years, antibiotics have not been a major player in most poultry company programs. The use of antibiotics, including chlortetracycline as growth promoters to increase production performance and to decrease mortality, was completely banned by the European Union (EU) because increases in microbial resistance to antibiotics and residues in chicken meat products can be harmful to consumers. The control of infections and enhancement of live performance through a non-antibiotic approach is thus urgently required. Consequently, several alternatives have been investigated to reduce or replace antibiotics. Probiotic microorganisms (nonpathogenic bacteria and/or yeast) are one of the alternatives for growth promotion in poultry although modes of action are not entirely clear. *Lactobacillus* species such as *Lactobacillus plantarum, Lactobacillus acidophilus* and *Lactobacillus bulgaricus* have been widely studied as probiotics (Abdulrahim et al., 1996). The *Lactobacillus* has shown beneficial effects on resistance to infectious agents such as *Escherichia coli, Salmonella* spp. and *Campylobacter* spp. (Jin et al., 1996[b]; Pascual et al., 1999; Stern et al., 2001). A nonpathogenic yeast; *Saccharomyces cerevisiae* has also been added to broiler diets to improve body weight gain (Santin et al., 2001). However, there are limited reports on the improvement of broiler performance and the reduction in the proliferation of enteropathogenic bacteria (Stanley et al., 1993). In the broiler industry, probiotic supplementation has been shown to improve body weight gain, feed conversion ratio and mortality rate (Jin et al., 1996[a], 1997[b]; Mohan et al., 1996; Vicente et al., 2007). However, contradictory results of probiotic supplementation have also been reported by other investigators (Senanl et al., 1997; Panda et al., 1999), and depend on many factors such as strain of selected microorganism, method of preparation and the use and condition of the birds. For application in broiler production, a combination of bacteria and yeast preparations may be more effective than bacteria or yeast alone.

The objective of the present study was to compare the potentially beneficial effects of this commercial probiotic (the bacterial and yeast preparative) and antibiotic supplementation on growth performance, feed efficiency, mortality and drug residues in commercial broilers.

Materials and Method

Animals and housing:

1-day-old, male Cobb chicks were obtained from a commercial hatchery. They were vaccinated against Newcastle Disease (ND) and Infectious Bronchitis (IB) upon hatching in the hatchery. They were vaccinated against Infectious Bursal Disease (IBD) at day 14 and against ND and IB at day 24. The experiment was carried out for 42 days in an environmentally controlled broiler house with evaporative cooling system. The average temperature and the relative humidity ranged between 28.31±0.14^{o}C and of 82.16±0.49%, respectively. The average airflow in the house was 0.84±0.05 m/sec as measured from eight different locations. The housing was layered with rice hulls as bedding and with a stocking density of 8 chicks/m^2. They were provided with a commercial diet. A starter diet and a finisher diet were used during the first 2 weeks and the last 4 weeks according to their nutrient requirements. Feed and water were provided *ad libitum* in each pen via a hanging waterer and feeder.

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

Feed additives:

The feed additives used in this experiment were antibiotic and probiotic. Antibiotic, purchased from a commercial company was chlortetracycline hydrochloride (CTC). Probiotic (Active Elements®; AE, supplied from Long Year Biochem. Cooperative Ltd.) was a commercial liquid preparation. It was composed of both bacteria and yeast; *Lactobacillus plantarum* (1x10^{10} cfu) and *Saccharomyces cerevisiae* (1x10^9 cfu). The antibiotic and the probiotic were mixed in drinking water individually or in combination at a final concentration of 0.05% CTC or 1:500 AE as suggested by the manufacturer.

Experimental designs

Four hundred 1-day-old broiler chicks were randomly allocated to 4 treatments, 4 replicates per treatment and 25 chicks per replicate. The treatments were assigned according to their drinking water containing no additive (T1); 0.05% CTC (T2); 1:500 AE (T3); or the 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. For T2 and T4, antibiotic containing groups, CTC was withdrawn 2 days before the end of experiment to minimize antibiotic residual in meat.

Performance parameters

Chicks in each replicate were weighed on a weekly basis (batch-weighing of each replicate for the first four weeks, thereafter individually). The feed intake (FI) in each replicate was recorded daily. The calculated parameters were an average body weight(BW), body weight gain (BWG), average daily feed intake (FI) and feed conversion ratio (FCR). Further, the mortality rate was also determined weekly and at the end of the experiment.

Postmortem examination and tissue sampling

On the last day of the experiment, four chicks from each treatment were randomly selected and humanely killed by cervical dislocation. The carcasses were immediately necropsied and examined for gross pathological lesions by a veterinarian. Trimmed sections were taken and fixed in 10% buffered formalin solution and embedded in paraffin wax. The intestinal organs of each group were processed for histopathological examinations by slide stained with haematoxylin and eosin and examined by light microscopy. To evaluate the antibiotic residual in chicken meat, breast tissues from T2 and T4, were sampled and frozen at -20^{o}C before evaluation.

Antibiotic residue analysis

To determine the chlortetracycline residue in chicken meat, the breast meat (approximately 500 g) from antibiotic-treated groups (T2 and T4) was submitted for analysis by a standard laboratory utilizing a liquid chromatography/mass spectrometry (LC/MS) technique.

Statistical analysis

Data on performance parameters; BW, BWG, FI and FCR were calculated based on a replicate basis. Experimental data were analyzed by one-way analysis of variance (ANOVA) and followed by the Duncan's multiple range tests. Statistical significance was determined at $p < 0.05$.

Results

Performance parameters

Body weight (BW) and body weight gain (BWG)

There was no difference in body weight recorded weekly among the treatments (Table 1). Interestingly, probiotic supplementation via drinking water during the first 4-wk period slightly improved body weight and body weight gain as compared to the control and the antibiotic-supplemented group (Table 1) but not significantly. However, the accumulated body weight gain was increased ($p < 0.05$) by the probiotic supplementation (T3 and T4) during the first 2 weeks as compared to the control (T1) and antibiotic supplemented group (T2); Figure 1 (inset). The final body weight gain was not significantly different among groups although it is likely that the control group gained less weight than the others (2382.67±37.02 vs. 2441.82±37.62, 2411.26±55.54 and 2408.71±43.33; values for T1-T4, consequently).

Feed intake(FI)

Broiler FI on a weekly basis during the experiment is shown in Figure 2. There was no significant difference among groups but it is likely that the overall feed intake in the antibiotic treated group (T2) tended to be lower than the others (3950.81±84.54, 3850.79±67.53, 3963.66±59.62 and 3910.58±59.08; values for T1-T4, consequently).

Feed conversion ratio (FCR)

Broiler FCR on a weekly basis during the experiment is shown in Figure 3. The weekly FCR for the experiment did not differ among the treatments. However probiotic supplementation via drinking water during the first 3-wk period showed slightly better FCR as compared to the others. Interestingly, although not significantly different ($p > 0.05$), the FCR of the antibiotic treated groups (T2 and T4) tended to be lower in the last week of experiment, noticeably T2 as shown in Figure 3. The overall FCR for T1-T4 were 1.66±0.04, 1.58±0.03, 1.65±0.03 and 1.62±0.02, respectively.

Broiler mortality and postmortem examination

The percentage mortality is shown in Table 2. The overall mortality rate was highest in the antibiotic and probiotic combination group, followed by the non-treated group and probiotic treated group, respectively. There was no loss in birds treated with antibiotic alone (T2). The mortality rate in the combination group (T4) was evident during the last 2 weeks while the others were distributed evenly throughout the experiment period. Moreover, it is worth noting that some of the broilers had leg deformities in the last 2 weeks because of their heavy body weight. These broilers were then reluctant to move and as the clinical signs progressed this resulted in paralysis and eventually death (observation data). There was no obvious sign of abnormality in any organs observed grossly or histologically in any groups.

Antibiotic residue in breast meat

The concentrations of chlortetracycline in the breast meat of broilers receiving antibiotic (T2 and T4) were below the detectable limit (< 50 μg/kg) as measured by LC/MS. In Thailand, safety considerations regarding drug residues in livestock products are the task of the committees of livestock development department and ministry of public health who publish the recommended announcements. The maximum residue limit (MRL) of chlortetracycline in poultry meat is 100-200 μg/kg (personal communication).

Table 1 Effects of probiotic and/or antibiotic supplementation on body weight (BW) as shown on a weekly basis.

Age	Treatments			
	T1	T2	T3	T4
Start	54.11 ± 0.44	52.57 ± 0.75	53.32 ± 0.58	53.35±0.89
Wk 1	100.58 ± 0.87	100.35 ± 1.65	104.62 ± 3.00	103.01 ± 1.25
Wk 2	237.44 ± 0.47	235.53 ± 1.88	244.98 ± 1.59	245.50 ± 3.97
Wk 3	558.28 ± 5.26	555.55 ± 8.19	572.36 ± 6.02	562.11 ± 10.43
Wk 4	971.26 ± 9.51	960.79 ± 14.73	985.78 ± 8.98	978.68 ± 19.71
Wk 5	1683.95 ± 7.58	1679.34 ± 20.29	1697.26 ± 25.35	1688.58 ± 34.45
Wk 6	2436.78 ± 36.62	2494.38 ± 37.30	2464.57 ± 55.67	2462.06 ± 43.91

Remark: T1 : control, T2 : 0.05% CTC, T3 : 1:500 AE, T4 : 0.05% CTC + 1:500 AE

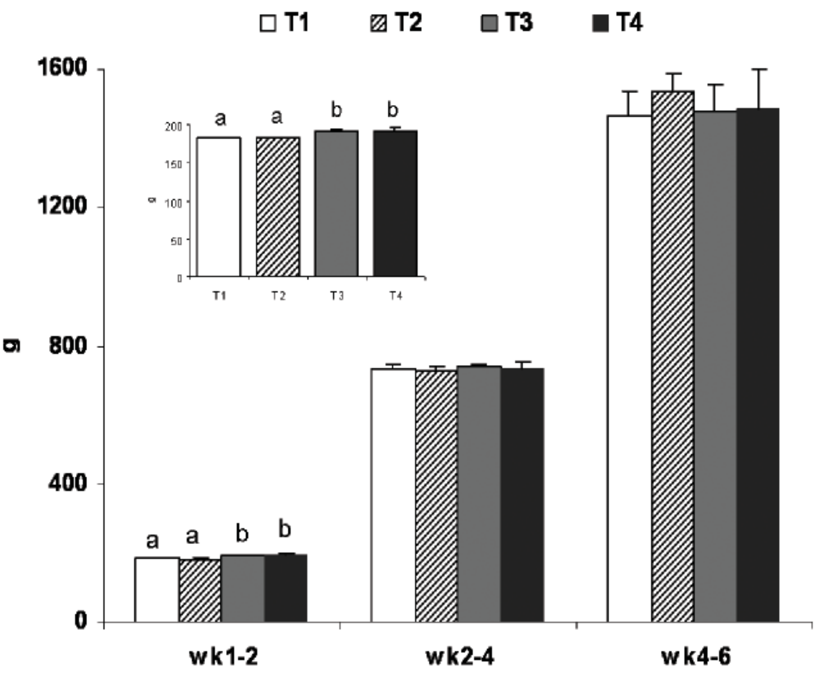

Figure 1 Effect of treatments on the accumulated body weight gain in control (T1), antibiotic T2; 0.05% CTC), probiotic (T3; 1:500 AE) and antibiotic-probiotic combination (T4) treated-birds during the experiment. Bars represent means±SE, n=4 per treatment. Inset represents the accumulated body weight gain for the first 2-weeks of the experiment. a,b denote significantly differences among treatments ($p < 0.05$), ANOVA followed by Duncan's multiple range tests.

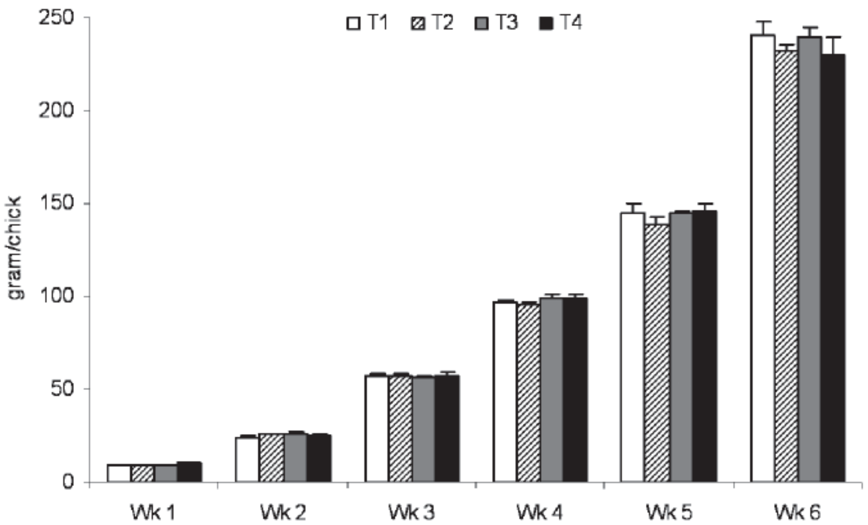

Figure 2 Effect of treatments on the weekly daily feed intake in control (T1), antibiotic (T2; 0.05% CTC), probiotic (T3; 1:500 AE) and antibiotic-probiotic combination (T4) treated- birds during the experiment. Bars represent means±SE, n = 4 per treatment.

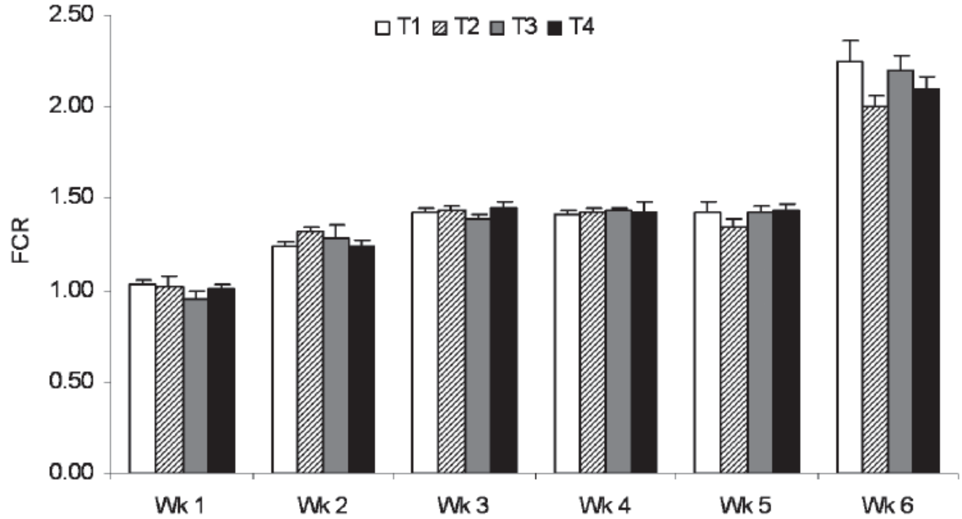

Figure 3 Effect of treatments on the feed conversion rate (FCR) in control (T1), antibiotic (T2; 0.05% CTC), probiotic (T3; 1:500 AE) and antibiotic-probiotic combination (T4) treated- birds during the experiment. Bars represent means±SE, n = 4 per treatment.

Table 2 Effect of probiotic and/or antibiotic supplementation on mortality in broilers as shown on a weekly basis and overall throughout the 6-week period.

Age	Treatments			
	T1	T2	T3	T4
Wk 1	0	0	0.98 (1)	0.98 (1)
Wk 2	0.98 (1)	0	0	0
Wk 3	0	0	0.99 (1)	0
Wk 4	1.01 (1)	0	0	0
Wk 5	1.02 (1)	0	0	2.97 (3)
Wk 6	1.03 (1)	0	1.02 (1)	1.02 (1)
Total	3.92	0	2.94	4.90

Remark: T1 : control, T2 : 0.05% CTC, T3 : 1:500 AE, T4 : 0.05% CTC + 1:500 AE
Numbers in parentheses represent the numbers of deaths in each treatment.

Discussion

In this study, the using of probiotic composed of bacteria *(Lactobacillus plantarum)* and yeast *(Saccharomyces cerevisiae)* in broiler for 6 weeks revealed that most of the performances (i.e. body weight, feed intake, FCR) were not significantly different in either the antibiotic (chlortetracycline) or control group. However, the accumulated body weight gain was increased significantly ($p < 0.05$) by probiotic supplementation during the first 2 week period as compared to the control and antibiotic supplemented group. Healthy birds generally maintain a balanced microbial population that plays an important role in the growth performance and health status. Previously, Amit-Romach et al. (2004) reported that colonization of the chicken intestine by commensal bacteria belonging to the *Lactobacillus* spp. is present predominantly in the small intestines of young chickens(2 weeks of age). During early life, colonization patterns are unstable and chicks are then susceptible to environmental pathogens. Initial colonization is of great importance to the host because the bacteria can modulate the expression of genes in epithelial cells (Hooper et al., 2001), thus this microorganism will probably reside permanently in the intestine and determine the colonization pattern of bacteria introduced later in life (Ducluzeau, 1993). Probiotic compounds supplied early in life may then be beneficial in aiding permanent colonization of the administered probiotic strains, indigenous microbes, or both or them (Santosa et al., 2006; Timmerman et al., 2006).

It is not known, from this result, why the addition of the probiotic failed to increase significantly the over all BWG, and FCR for the whole duration of the experiment although the probiotic supplementation via drinking water during the first 3-wk period slightly showed better BWG and FCR as compared to the others. Results of experiments using probiotics in broilers have been inconsistent because the efficacy of probiotic application depends on many factors such as the administration level, application methods (spraying, feed or water), viability, the frequency of application (intermittent or continuous), environmental stress factors (stocking density, temperature and humidity) and broiler age (Patterson and Burkholder, 2003). The most widely used probiotic strains are of the genus Lactobacillus, which is also the dominant genus in the proximal intestine of chickens early in life (Barnes et al., 1972). It is general accepted that efficacy for most probiotic microorganisms is demonstrated with a daily consumption of 10^8 to 10^9 cfu in animals (Ewing and Cole, 1994). In consideration of commercial application, the optimal dose for probiotics varies from one strain to another, and a higher dose does not always lead to a better performance (Huang et al., 2004). For application, the administration of probiotics in the drinking water resulted in a lower increase of average daily gain when compared with probiotic administration via the feed (Jin et al., 2000; Zulkifli et al., 2000; Kalavathy et al., 2003). Different modes of administering probiotics might be a factor affecting efficacy and are currently being investigated which may ultimately lead to the widespread use of probiotics in animals. The results of this work show that Active Elements® (AE) administration in water display a growth-promoting effect similar to an antibiotic (chlortetracycline). Thus, there is the benefit such as food safety to using AE as an alternative way to replace antibiotic in poultry industry.

The performance improvements resulting from use of a growth promoting feed antibiotic is due to factors such as reduced competition for nutrients in the intestine and reduced local inflammation due to the control of pathogens (Mohan et al., 1996). The effect of administering probiotic with, or subsequent to, antibiotic treatment on the performance parameter has been investigated since it has been of concern that antibiotic treatment can disrupt homeostasis of microorganism in the gastrointestinal system or have an impact in terms of the safety of antibiotic resistant probiotics (Mohan et al., 1996; Courvalin, 2006). Simultaneously antibiotic and probiotic administration can be suggested to reduce the side effects of antibiotic (Santosa et al., 2006; Miles, 2007) but not at the same time of day. In order for probiotics to be the most efficient, they should be taken at least two hours after the antibiotic application and in large doses. Additionally, antibiotic susceptibility testing should be advocated as an essential selection criterion for potentially probiotic microorganisms, singly and in combination with antibiotic prophylaxis. This study has shown that the simultaneous administration of probiotic plus antibiotic resulted in an increasing mortality rate even higher than the control, while no mortality was found in the antibiotic-supplemented group. This could be due to the fact that the management of switching between probiotic and antibiotic containing-drinking water may be stressful. The effect of probiotics (i.e. *Lactobacillus* preparations) on mortality in broilers was inconsistent (Jin et al., 2000; Zulkifle et al., 2000). However, in this study, the mortality was lowered in the probiotic group as compared to the control and similar to the study reported by Timmerman et al. (2006). Concerning the clinical signs and the organ gross as well as intestinal histology findings, there were no differences observed following this probiotic application compared with the control group. The broiler leg abnormalities and reluctance to move were more prevalent in high performance flocks of fast-growing birds.

This study provides evidence that the administration of this probiotic, the combination of yeast and bacteria, via the drinking water to broiler chickens, starting on the second day of life, had beneficial effects on production performance, mortality rate and no antibiotic residue in the birds similar to the responses obtained with antibiotic administration was detected. A use of this probiotic is thus an alternative in field conditions to replace the adverse effect of antibiotic and economic losses.

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