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Effects of Autochthonous Probiotic Feeding on Performances, Carcass Traits, Serum Composition and Faecal Microflora of Broiler Chickens

(Kesan Pemakanan Probiotik Autoktonus ke atas Prestasi Ciri Karkas, Komposisi Serum dan Mikroflora Tahi Ayam Daging)

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ABSTRACT

The objective of this study was to investigate the effect of autochthonous Lactobacillus plantarum *feeding on growth performance, carcass traits, serum composition and faecal microflora of broiler chickens. The results showed a significant positive effect (*$p< 0.05$*) of probiotic on body weight and feed conversion ratio. Coliform counts in the fecal matter of broiler chickens receiving probiotic were lower than the analogous population in control birds (*$p<0.05$*). In contrary, lactic acid bacteria (LAB) number increased (*$p<0.05$*) in fecal matter of experimental group. At the end of the study, the degree of serum cholesterol reduction resulted in a 20.31% compared to the control group (*$p<0.05$*). The experimental group had significantly lower serum triglycerides (*$p<0.05$*). It was concluded that autochthonous probiotic improved growth and feed efficiency in broilers chickens and considering the improvements in carcass traits. This probiotic possess the property of reducing cholesterol and triglycerides in the blood and possess a positive effect on the gut microflora.*

Keywords: Broiler chicken; carcass; microflora; performance; probiotic; serum

ABSTRAK

Objektif penyelidikan ini adalah untuk mengkaji kesan pemakanan autoktonus Lactobacillus plantarum *ke atas prestasi pertumbuhan, sifat-sifat karkas, komposisi serum dan mikroflora tahi ayam daging. Keputusan menunjukkan kesan positif (*$p<0.05$*) probiotik ke atas berat badan dan nisbah penukaran makanan. Kiraan koliform dalam bahan tahi ayam daging yang menerima probiotik adalah lebih rendah daripada populasi analog dalam burung kawalan (*$p<0.05$*). Sebaliknya, bilangan bakteria asid laktik (LAB) meningkat (*$p<0.05$*) dalam bahan tahi kumpulan eksperimen. Pada akhir kajian tahap pengurangan kolesterol serum berkurang sebanyak 20.31% berbanding dengan kumpulan kawalan (*$p<0.05$*). Kumpulan eksperimen mempunyai serum trigliserida yang lebih rendah secara signifikan (*$p<0.05$*). Disimpulkan bahawa probiotik autoktonus meningkatkan keberkesanan pertumbuhan dan makanan dalam ayam daging dengan mengambil kira peningkatan dalam sifat karkas. Probiotik mempunyai sifat mengurangkan kolesterol dan trigliserida di dalam darah dan mempunyai kesan positif ke atas usus mikroflora.*

Kata kunci: Ayam daging; kaskas; mikroflora; prestasi; probiotik; serum

INTRODUCTION

Antibiotics have been widely used in animal production for decades. Although some are used therapeutically to improve the health and well-being of animals, most were given for prophylactic purposes and to improve growth rate and feed conversion efficiency. However, due to the emergence of microbes resistant to antibiotics which were used to treat human and animal infections, the European Commission (EC) decided to phase out and ultimately ban (January 1st 2006) the marketing and use of antibiotics as growth promoters (AGP) in feed (Huyghebaert et al. 2011). With these increasing concerns about antibiotic resistance, the ban on subtherapeutic antibiotic use in Europe and the discussion of a ban in the United States, there is an increasing interest in finding alternatives to the use of AGP during poultry production (Reid & Friendship 2002). Many researchers are now focused on identifying viable alternatives to antibiotics that offer similar benefits such as increased body weight gain (BWG), increased feed and increased protection from bacterial infection. Probiotics represent potential replacements for AGP in the feed animal industry because of their reported ability to reduce enteric disease in poultry and potential food borne pathogen contamination of poultry or poultry products (Eckert et al. 2010; Reid & Friendship 2002). Probiotics have been defined as ‘live microbial feed supplements that can benefit the host by improving its intestinal balance’. As living microorganisms, they produce no drug resistance or drug residues (Scharek et al. 2005). The most common microorganisms found in the probiotic products currently available are lactic acid bacteria, especially *Lactobacillus* and *Bifidobacterium* species, which are resident microflora in the gastrointestinal tract of most animals (Simpson et al. 2004).

The beneficial effects of probiotics have been related to different modes of action. The improvement in zootechnical performances of all poultry species fed with probiotics was mostly related to the improvements that probiotics promoted in metabolic processes of digestion and utilization of nutrients. They create gut conditions that suppress harmful microorganisms and favour beneficial ones (Mead 2000). They have been largely shown to reduce disease risk, possibly through a reduction in proliferation of pathogenic species, maintaining microbiota balance in the gut (Mountzouris et al. 2007), boost immune function (Kabir et al. 2004) and increase resistance to infection (Rekiel et al. 2007). Beyond the maintenance of health, they have been shown to improve the growth performance of poultry and to have an important influence on gut morphology of broiler chickens (Idoui et al. 2009; Li et al. 2008).

The objective of this study was to examine the effects of dietary supplementation of autochthonous probiotics *Lb. plantarum* in broiler diets compared to standard broiler feed on the production performances, carcass parameters, serum composition and gut microflora of commercial broiler chickens.

MATERIALS AND METHODS

BIRDS AND TREATMENTS

As recommended by the Scientific Committee on Animal Nutrition (SCAN), the efficacy of the probiotic product was assessed according to Directive No. 87/153/EEC. The experiment was arranged and conducted in due form using animal number in groups and number of groups that are satisfactory for establishing the minimum claimed response.

The broiler chickens ISA 15 strain was assigned to two treatments with five replicates. Each replicate consisted of 11 as-hatched birds per pen. During the experimental period (42 days) all animals were fed with the commercial diet but drinking water of the experimental group was supplemented by probiotic *Lb. plantarum* and each mL of contained 65×10^{11} cfu. The composition of the commercial diet was reported in Table 1.

PRODUCTION PERFORMANCES TRAITS AND ENUMERATION OF CULTIVABLE MICROFLORA

Live body weight (LBW) and feed intake (FI) were recorded weekly and the feed conversion ratio (FCR) was calculated.

The samples of faecal matter were weekly collected. One gram of each sample was diluted and homogenised in saline buffer (0.85%) and shaken vigorously for 5 min according to the standard microbiological method, after 10-fold serial dilutions were made. The dilutions were plated in duplicate on the following media: Violet red bile lactose agar (VRBL), incubated at 37°C for 24 h for coliform bacteria; violet red bile lactose agar (VRBL), incubated at 44°C for 24 h for thermotolerant coliform bacteria and MRS agar, incubated at 37°C for 48 h to 72 h in anaerobiosis for lactic acid bacteria.

BLOOD SAMPLE COLLECTION AND CARCASS MEASUREMENTS

A blood sample was collected from the brachial vein into heparinised syringes from six (6) birds per treatment. The blood samples were centrifuged, and plasma was immediately analysed. The concentrations of plasma metabolites (cholesterol, glucose, triglycerides) were measured using standard kit (SPINREACT S.A, Spain).

At the end of the experiment, 21 birds per treatment were weighed individually and killed. Afterward, the birds were scalded, defeathered and carcasses were eviscerated. The head, neck and feet were removed and the carcass subsequently was weighed then conserved for 24 h at 4°C. The heart, liver and cloacae fat were weighed. The gizzard, crop and intestine with content were weighed too.

TABLE 1. Components and chemical composition of the commercial diet (as fed basis)

Ingredient	0-21 day	21- 42 day
Components (g kg^{-1})		
Maize	580	600
Soyameal	300	210
Cereals by-products	90	160
Premix*	15	15
Bicalcic phosphate	15	15
Chemical composition		
Metabolically energy (Kcal/ kg)	3040	3180
Crude protein	21.500	17.500
Fiber	3.066	2.556
Ash	7.50	6.00

* Provided per kg of diet: retinol, 2.64μg; cholicalciferol , 0.09μg; tocopherol , 26.6mg; phylloquinone, 3.3 mg; thiamine, 4.0 mg; riboflavin, 8.0 mg; pantothenic acid, 15 mg; niacin, 50 mg; pyridoxine, 3.3 mg; choline, 600 mg; folic acid, 1 mg; biotin, 220 mg; cobalamin, 12 mg; antioxidant, 120 mg; manganese, 70 mg; zinc, 70 mg; iron, 60 mg; copper, 10 mg; iodine, 1.0 mg; selenium, 0.3 mg

STATISTICAL ANALYSIS

For all parameters, the results were expressed as ANOVA. The results have been treated using Student test at 5%. Probability values of less than 0.05 (p<0.05) were considered significant.

RESULTS AND DISCUSSION

The results of feed intake (FI), feed conversion ratio (FCR) and body weight (BW) are presented in Table 2. These results indicate that the probiotics have a growth promoting effect on broiler chickens. FI for probiotic supplemented birds was significantly higher than the control group (p<0.05). The FI of the probiotic group was significantly higher than that of the control group in these periods: from 15 to 21 days by 6.61% (p<0.05); from 22 to 28 days by 24.48% (p<0.05) and from 29 to 35 days by 11.745 (p<0.05). The probiotic group also showed a positive effect on FCR (p<0.05).

The results showed a significant positive effect (p<0.05) of probiotic on BW of broilers chickens. The BW in the control and experimental groups at the start (seven days of age) was comparable (p>0.05). After 28 days, a positive effect on the growth produced by the probiotic became evident. The BW in the experimental group was 4.41% higher than the control group (p<0.05). At the end of the 42-day-experiment, the weight of the experimental group was by 5.03%, higher in comparison with the control group (p<0.05).

The number of coliform bacteria in faecal matter was significantly different between treatments (p<0.05) and was much lower in the experimental group and in all fecal samples (Table 3). Regarding the LAB and thermotolerant coliform populations there were significant differences (p<0.05) among treatment. Notable reduction in thermotolerant coliform was found in faecal samples of the probiotic group compared to control group. In contrary, LAB number increased (p<0.05) in fecal matter of the experimental group.

The total cholesterol in the control and experimental groups at the start was comparable (p>0.05). After 15 days, the results showed a significant positive effect of probiotic on total cholesterol concentration. In the probiotic supplemented group, cholesterol concentration was significantly reduced (p< 0.05). The degree of serum cholesterol reduction at the end of the study resulted in a 20.31% reduction of serum total cholesterol concentration from the control (Table 4). On the other hands, the triglycerides concentration of the probiotic group was lower than that of the control group in these periods: from 15 to 21 days by 6.06% (P< 0.05); from 22 to 28 days by 28% (p<0.05) and from 36 to 42 days by 1.58% (p<0.05).

The serum glucose values were elevated in experimental group during the 4th and 5th week compared to the control group (p<0.05). In contrary, this serum parameter was very high in the control group compared to the probiotic group during the 2nd and 3rd week (p<0.05).

TABLE 2. The effect of *Lb. plantarum* supplementation on the performance of broiler chickens

Parameters	day	Control group (n=5)	Experimental group (n=5)
FI (g)	07	10102.2±124.1	12401.4±108.5
	14	27085.1±150.2	26586.8±125.2
	21	29556.3±147.0	31509.0±157.5
	28	30836.3±214.2	41469.9±205.5
	35	38948.5±210.8	43524.8±254.5
	42	34051.2±232.1	32305.3±202.4
	Signification	*	*
F.C.R	07	0.68±0.02	0.78±0.03
	14	1.14±0.04	1.06±0.04
	21	0.82±0.03	0.80±0.02
	28	0.81±0.04	0.65±0.02
	35	0.69±0.01	0.61±0.01
	42	0.47±0.01	0.40±0.03
	Signification	*	*
Initial BW (g)		45.25±5.4	44.95±2.5
BW (g)	07	209.65±25.5	208.52±47.8
	14	820.74±60.5	858.67±57.5
	21	1239.32±65.8	1289.36±60.5
	28	1686.04±70.5	1760.00±65.4
	35	2001.42±75.2	2102.55±72.0
	42	2551.25±80.8	2679.70±76.7
	Signification	*	*

*: significantly difference (p<0.05), F.C.R: feed conversion ratio

TABLE 3. The effect of *Lb. plantarum* supplementation on faecal microflora of broiler chickens

Parameters	day	Control group (n=5)	Experimental group (n=5)
Mean CFU Coliform × $10^9 g^{-1}$	14	4.34	1.07
	21	23.18	2.40
	28	106	68
	35	90	76
	42	49	0.21
	Signification	*	*
Mean CFU thermotolerant Coliform × $10^9 g^{-1}$	14	2.18	0.92
	21	6.91	1.10
	28	94	80
	35	120	34
	42	96	50
	Signification	*	*
Mean CFU Lactic acid bacteria × $10^8 g^{-1}$	14	1.8	27.0
	21	2.1	34.0
	28	3.7	25.6
	35	5.9	31.6
	42	4.9	54.0
	Signification	*	*

*: significantly difference (p<0.05)

TABLE 4. The effect of *Lb. plantarum* supplementation on blood parameter of broiler chickens

Parameters ($mgdL^{-1}$)	Glucose	Cholesterol	Triglycerides
Control group (n=5)			
14	163±2.02	120±2.13	ND
21	260±2.96	160±2.03	133±0.04
28	264±2.14	134±2.09	160±0.21
35	201±2.01	113±2.20	120±0.10
42	178±2.03	128±2.12	126±0.08
Experimental group			
14	161±1.04	120±1.10	ND
21	225±1.30	118±1.30	125±0.03
28	202±1.35	108±1.30	125±0.16
35	212±1.26	107±1.09	118±0.60
42	180±1.34	102±1.17	124±0.06
Signification	*	*	*

ND : no data *: significantly difference (p<0.05)

At the end of the experiment, the birds were killed; the mean body weight was 2742.8±11.2 and 2984.0±14.5 g for control group and experimental group, respectively (Table 5). After evisceration, statistical difference (p<0.05) was found between eviscerated carcass weight groups. Finally, the birds have significantly higher weight of commercial carcass (1899.2±12.4 g) than the control (2190.3±10.3 g). As expected, the cloacae fat weight of control group (50.03±2.1 g) was higher than the experimental group (26.20±1.8 g). The results showed a significant positive effect (p<0.05) of probiotic on crop weight and Gizzard weight of broilers. On the contrary, probiotic did not affect the intestine weight and liver weight (p>0.05).

In modern poultry production, different types of growth promoters were used (probiotic, prebiotic, symbiotic and phytogenic) (Dhama et al. 2014). It has been reported recently that utilization of probiotics in animal nutrition is of economic and health benefits (A. Azza et al. 2012). The results of our study indicated that *Lb. plantarum* have a growth promoting effect on broiler chickens. These results were in agreement with a large number of studies which have shown positive effects of using different strains and combinations of probitotics (Peric et al. 2010; Safalaoh 2006). The results of this study showed that the BW in the experimental group was 4.41% higher than the control group. These results agree with the works

TABLE 5. The effect of Lb. plantarum supplementation on carcass parameters and internal organ weight of broiler chickens

Parameters	Control group (*n*=5)	Experimental group (*n*=5)	Signification
Carcass Parameters (g):			*
Mean body weight	2742.8±11.2	2984±14.5	*
Body weight after bleeding	2542.8±17.5	2774±18.1	*
Eviscerated carcass weight	1950.30±15.2	2429.98±12.7	*
Carcass weight (4°C / 24H)	1899.2±12.4	2190.3±10.3	NS
Carcass yield (%)	69.24±1.5	73.40±1.7	
Internal organ parameter (g):			
Intestine weight	141.27±17.8	147.92±15.8	NS
Liver weight	56.98±1.2	51.28±1.7	NS
Hearth weight	15.88±1.1	11.46±1.3	NS
Crop weight	90.56±2.9	67.86±2.7	*
Gizzard weight	94.54±2.7	80.90±2.3	*
Cloacae fat weight	50.03±2.1	26.20±1.8	*

NS: non significant ($p > 0.05$), *: significantly difference ($p < 0.05$)

of Afsharmanesh et al. (2010) and Sherief and Sherief (2011). Kabir et al. (2004) observed an improvement of the chickens' weights with other probiotics; however Karaoglu and Durdag (2005) did not establish any effect with *S. cerevisiae*. Over the entire trial period (0-42 d), there was difference in the FCR of broilers chickens fed on the diets with or without *Lb. plantarum*. Sherief and Sherief (2011) showed better FCR for broilers chickens fed with ration containing commercial probiotic. Endens (2003) reported that probiotics improved digestion, absorption and availability of nutrition accompanying with a positive effect on intestine activity and increasing digestive enzymes.

In this study, coliform and thermotolerant coliform counts in the faecal matter of experimental group were lower than the analogous population in control birds. Higher LAB and lower coliform counts could be expected to produce a healthier gut environment in the supplemented birds. In the study conducted by Guo et al. (2006) *Lactobacillus* counts on day 28 indicated that piglets fed with diet containing 2.2×10^5 cfu g^{-1} of feed had significantly higher lactobacilli counts than piglets fed with negative control diet. The positive effects can result from a health effect, with probiotics acting as bioreactors of the intestinal microflora by the production of antimicrobial substances, stimulation of the immune system and competition for nutrients and adhesion sites in the gastrointestinal tract which probiotics may also help to exclude or prevent pathogen colonization in the host (Mountzouris et al. 2007).

The results clearly indicated that *Lb. plantarum* has a cholesterol and triglyceride-depressing effect in the serum of broiler chickens. There were many reports that were in agreement with the presented results in the current study. It was reported that the use of 100 mg/kg of the probiotic supplement (*Lb. acidophilus*, *Bifidobacterium* and *A. oryzae*) significantly reduces the serum cholesterol level of the broiler chickens (Panda et al. 2001). Ahmadi (2011) and Jouybari et al. (2010) have observed the low levels of cholesterol synthesis in broiler chickens treated with probiotics. In reviews, Oie and Liong (2010) and Homayouni et al. (2012) conclude the same result. In the study conducted by Alloui et al. (2012), triglycerides and cholesterol were reduced in a significant manner ($p \leq 0.01$) in the group of broiler-chickens receiving *P. acidilactici* during all raising phases. It was reported that probiotics have the ability to deconjugate with bile acids, enzymatically increasing their rate of excretion and the use of cholesterol to synthesize new bile led to the reduction of serum cholesterol level (Lye et al. 2009). Furthermore, some probiotic bacteria may interfere with cholesterol absorption in the gut by de-conjugating bile salts or by directly assimilating cholesterol (Li et al. 2007).

The results showed a clear influence of the use of *Lb. plantarum* on the final quality of chickens' carcasses. These results were in agreement with those reported by Sherief and Sherief (2011). Kabir et al. (2004) reported the occurrence of a significantly ($p < 0.05$) higher carcass yield in broiler chickens fed with probiotics. Yamamoto et al. (2007) noted that when broiler chickens were fed on diets containing 0.05 and 1% of Koji-feed carcass weight was significantly increased. However, significant reduction in the cloacae fat weight of experimental group compared to the control group was obtained in this study. Our results were also in agreement with the report of Kalavathy et al. (2006).

Conclusion

The results of the present study showed that supplementation of broiler chickens drinking water with autochthonous *Lb. plantarum* induced additive benefit in growth performance and some carcass traits. In addition, this probiotic has a cholesterol and triglyceride-depressing effect in the serum and plays a positive effect on gut microflora of broiler chickens.

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