J. Agr. Sci. Tech. (2011) Vol. 13: 165-172

Efficacy of *Lactobacillus acidophilus* as Probiotic to Improve Broiler Chicks Performance

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ABSTRACT

This experiment was conducted to investigate the effects of *Lactobacillus acidophilus* on performance, carcass characteristics, blood parameters, and intestinal microflora of broiler chicks. The dietary treatments were: basal diet as control 1; basal diet plus 1g. kg^{-1} of a commercial probiotic Bioplus2; basal diet plus 10 and 20 g kg^{-1} fermented milk that contained 2×10^{8} cfu g^{-1} *Lactobacillus acidophilus*. To evaluate the effect of water alone on chick performance, equal volume of water in 20 g fermented milk was added to each kg of the basal diet (control 2). A total of 280 one-day old male broiler chicks were randomly allocated to 5 experimental groups of 4 replicates of 14 chicks each. The chicks were grown to 42 d of age. The result of the experiment indicated that feed intake in chicks fed diet supplemented with commercial probiotic was significantly higher than *L. acidophilus* probiotic. Weight gain for the chicks fed with the diet that contained 20 g kg^{-1} fermented milk was higher than the control chicks, in the first 3 weeks. There was no significant difference in feed conversion and weight of organs. The number of *Lactobacilli* in ileum and colon were higher in *L. acidophilus* treated birds than the control group and also the number of *Coliforms* was lower, but the effects were not statistically significant. The levels of blood cholesterol, alanine aminotransferase, aspartate aminotrasferase and alkaline phosphatase were the highest in the control group, but the effect was statistically significant only for ALT measured at 21 d of age.

Keywords: Broiler chicken, *Lactobacillus acidophilus*, Probiotic.

INTRODUCTION

A number of health benefits have been claimed for probiotic bacteria such as *Lactobacillus acidophilus*, *Lactobacillus casei* and *Bifidobacterium spp*. It seems that probiotics have beneficial influence on: intestinal microflora balance, especially in young chicks (Nuotio *et* al., 1992), immune response (Koenen *et al*., 2004; Perdigon *et al*., 1995), blood cholesterol concentrations (De Roos and Katan, 2000) and Cancer (Matsuzaki, 1998). Some probiotics have a competitive exclusion effect on specific pathogens (Fedorka-Cray *et al*., 1999) and help to reduce duration of diarrhea (Marteau *et al*., 2001).

Lactobacilli are normal components of the healthy intestinal microflora. Various effects of lactic acid bacteria as a probiotic have been described in animal and man, which depend on strain, host, dose, timing and viability of the strain (Maassen *et al*., 2000; Isolauri *et al*., 2001). It is interesting that some probiotic strains have been selected in rodents or pigs for application in humans. This indicates that it is possible for probiotics to act over several species (Dunne *et al*., 2001; Kos *et al*., 2003; Koenen *et al*., 2004).

To provide health benefits, the suggested concentration for probiotics bacteria is 10^{6}

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cfu g^{-1} of a product. However, studies have shown low viability of probotics in market preparations. The need to monitor survival of *L. acidophilus* has often been neglected, with the result that a number of products reach the market containing a few viable bacteria (Shah, 2000).

The present study was conducted to determine the effects of *L. acidophilus* (cultured in milk) on production performance, weight of organs, intestinal microflora and blood parameters in broiler chickens.

MATERIALS AND METHODS

Preparation of L. acidophilus culture

In this experiment, *L. acidophilus* was solely cultured in milk: culture of these bacteria alone in milk is simple and also the bacteria in commercial fermented milk and commercial probiotics may not be alive and/or the number of live bacteria may be lower than the products label (Gilliland, 1981). *L. acidophilus* (KAL Dietary supplements, park city, UT 84060, USA) was cultured in sterile milk. For sterilization, low fat milk was heated to 120°C for 20 minutes, cooled to 37°C and 2 percent of the L. *acidophilus* starter was added and incubated at 37 to 40°C for 18 hours. After incubation, the product was stored at 15°c until use. On the average, the fermented milk contained 2×10^{8} cfu g^{-1} L. *acidophilus*.

Chickens diets and feeding treatments

A total of 280 one-day old male Ross chicks were assigned randomly to five treatment groups. The chicks were raised in floor pens with wood shavings litter. Four replicates of 14 chicks were considered for each treatment. The dietary treatments were: basal diet as a control; basal diet plus 1g kg^{-1} of a commercial probiotic Bioplus2 (Minimum of 1.6×10^{9} cfu/g *Bacillus licheniformis* and *Bacillus subtilis*); basal diet plus 10 and 20 g kg^{-1} fermented milk, so the number of *L. acidophilus* in these two diets were 2×10^{9} and 4×10^{9} cfu kg^{-1}, respectively. To evaluate the effect of water alone on chick performance, equal volume of water was added to another basal diet and mixed before use (control 2). The composition of the diets is shown in Table 1. The diets were isocaloric and isonitrogenous and formulated to meet the nutrient requirements of the broiler chicks during starter and grower periods according to the National Research Council (NRC, 1994). To avoid mold growth, the fermented milk and water were mixed with the diets daily. No antibiotic and anticoccidials were used in this experiment. Diets were fed *ad libitum* in mash form.

Live body weight and feed consumption were recorded weekly. One chick from each replication was selected randomly and sacrificed at 21 and 42 days of age to determine the populations of *Lactobacilli* and *coliforms* in ileum and at the end of large intestine. Weight of liver, intestines (small intestine, caeca and colon), gizzard, bursa, heart and spleen were also recorded at 21 and 42 days of age. Before weighing the intestine, intestinal digesta were removed by hand. The blood samples were also collected from the wing vein for analyses of total cholesterol, alanine aminotransferase (ALT), aspartate aminotrasferase (AST) and alkaline phosphatase (ALKPH) in serum using Pars-Azmoon kits.

Isolation and enumeration of intestinal microflora

The samples from ileum and end of colon were collected at 21 and 42 days of age. The samples were diluted to 10^{-6} and then 1 ml from 10^{-4} to 10^{-6} dilutions were added to MRS plate media for *Lactobacilli* (Oyetao *et al.*, 2003; Tae Ahn *et al.*, 2003; Vinderola *et al.*, 2000) and VRB agar for *coliforms* count. All plates were incubated at 37°C for 3 days (Vindrola *et al.*, 2000).

Table 1. Composition of the experimental diets (g kg^{-1} as fed) [1].

Feed Ingredients	Starter diets [a] (0-3 weeks)			Grower diets (3-6 weeks)		
	C	10F	20F	C	10F	20F
Corn	550	553	556	523	520	515
Wheat	100	96	92	200	200	200
Fermented milk (dry matter)	-	1.2	2.4	-	1.2	2.4
Fish meal	40	40	40	30	30	30
Soybean meal	279.6	279.4	279.2	220	211	204
Oyster shell	11	11	11	12	12	12
Dicalcium phosphate	9.6	9.6	9.6	7	7	7
Iodonized salt	3.5	3.5	3.5	2.5	2.5	2.5
DL methionin	1.3	1.3	1.3	0.5	0.5	0.5
Vitamin-mineral premix [b]	5	5	5	5	5	5
Calculated analysis						
AMEn (kcal/kg)		2890			2950	
Crude protein		208			184	
Lysin		11			9.2	
Methionine		4.8			3.6	
Methionine + cystine		8.1			6.6	
Calcium		9.1			8.3	
Phosphorus		4.1			3.3	
Sodium		1.8			1.4	
Linoleic acid		13			13.6	

[a] C=Control; 10F= Diet contained 10g kg^{-1} fermented milk; 20F= Diet contained 20g kg^{-1} fermented milk.
[b] The vitamin-mineral premix provided the following per kg of diet: retinol 3.78 mg, cholecalciferol 0.055 mg, tocopherol 30 mg, menadione 2 mg, riboflavin 6 mg, pantothenic acid 10 mg, niacin 60 mg, folic acid 0.6 mg, biotin 0.15 mg, cobalamin 0.02 mg, choline 400 mg, Zn 80 mg, Cu 10 mg, Mn 80 mg, Se 0.3 mg.

Statistical analysis

The data were analyzed as a completely randomized design, using the general linear (GLM) procedure of SAS (1998) and Duncan's Multiple Range test was used to detect (P<0.05) differences among treatment means.

RESULTS

Chick growth performance

Addition of water or probiotics to diet had no significant effect on feed intake during 0-3 week period (Table 2). Birds fed diets contained commercial probiotic from 3-6 week period, had approximately 7 and 8.5 percent higher feed intake than the diets containing 10 or 20 gkg^{-1} fermented milk, respectively (P<0.05). The same results were shown for 0-6 week period. Birds fed with the diet that contained 20 gkg^{-1} fermented milk, from 0-3 weeks, had 8 percent higher weight gain than the control (P<0.05). During the 3-6 and 0-6 week periods, birds fed with the diets containing either *L. acidophilus* or commercial probiotic grew faster than the control, but, the effect was not statistically significant. Feed conversion was improved in birds supplemented with fermented milk, but the differences were not significant. Addition of water to basal diet had no significant effect on growth performance.

Bacterial count

Addition of fermented milk containing *L. acidophilus* to the diets increased the

Table 2. Production parameters for broiler chickens fed diets with or without probiotics at different ages.

Dietary Treatments [a]	Feed intake (g/bird/d)			Weight gain (g/bird/d)			Feed conversion		
	0-21	21-42	0-42	0-21	21-42	0-42	0-21	21-42	0-42
C 1	46.6	143.3 [ab]	94.8 [ab]	32.1 [b]	73.4	52.7	1.45	1.95	1.80
C 2	46.9	140.3 [bc]	93.6 [b]	32.6 [ab]	72.1	52.3	1.44	1.94	1.78
B	46.1	148.5 [a]	97.3 [a]	32.7 [ab]	76.1	54.4	1.41	1.95	1.79
10F	48.1	135.8 [c]	91.9 [b]	33.5 [ab]	71.5	52.5	1.43	1.89	1.75
20F	47.7	138.1 [bc]	92.9 [b]	34.7 [a]	72.1	53.4	1.37	1.92	1.74
SE	0.31	1.26	0.6	0.33	0.75	0.37	0.01	0.02	0.01

[a] C1=Control 1; C2= Control 2= control 1 plus water ; B= Diet contained Bioplus2; 10F= Diet contained 10g kg^{-1} fermented milk; 20F= Diet contained 20g kg^{-1} fermented milk.
[a-d] Values within a column with no common superscript differ significantly (P<0.05).

number of *Lactobacilli* in ileum and colon (Table 3) and also the addition of either fermented milk or commercial probiotic to the basal diet decreased the number of *coliforms* in ileum and colon (Table 4), but the effects were not statistically significant.

Weight of organs

The weight of organs in broilers calculated as a percentage of the body weight is shown in Table 5. There were no significant differences in the weight of organs of broilers (except bursa weight) fed with the diets that contained 0, 10 or 20 g kg^{-1} fermented milk or commercial probiotic.

Table 3. Population of *Lactobacilli* in ileum and colon in broilers fed diets with or without probiotics at 21 and 42 days of age ($\log_{10}$).

Dietary Treatments [a]	Ileum		Colon	
	21	42	21	42
C1	8.08	8.43	8.04	8.46
B	8.05	8.62	8.30	8.59
10F	8.23	8.70	8.54	8.68
20F	8.27	8.75	8.53	8.57
SE	4.3	3.98	4.74	4.98

[a] C1=Control 1; B= Diet contained Bioplus2; 10F= Diet contained 10g kg^{-1} fermented milk; 20F= Diet contained 20g kg^{-1} fermented milk.

Blood analysis

Total cholesterol, AST, ALT and ALKPH in blood at 3 and 6 week of age is shown in Table 6. The ALKPH activity and cholesterol level of the broilers fed with either 10 or 20 g kg^{-1} fermented milk or commercial probiotic were lower than the control, at 3 and 6 week of age, but, the effects were not statistically significant. The ALT activity in the broilers fed with 10 gkg^{-1} was significantly lower than that of the broilers fed with the diets containing 0 or 20 g kg^{-1} fermented milk or commercial probiotic (P<0.05).

DISCUSSION

In this experiment, the probiotics had a

Table 4. Population of *Coliforms* in ileum and colon in broilers fed diets with or without probiotics at 21 and 42 days of age ($\log_{10}$).

Dietary Treatments [a]	Ileum		colon	
	21	42	21	42
C1	5.83	6.56	7.2	6.56
B	4.84	6.38	6.51	6.30
10F	5.27	6.48	6.81	6.44
20F	5.23	6.23	6.86	6.43
SE	3.27	3.84	3.7	2.84

[a] C1=Control 1; B= Diet contained Bioplus2; 10F= Diet contained 10g kg^{-1} fermented milk; 20F= Diet contained 20g kg^{-1} fermented milk.

[DOR: 20.1001.1.16807073.2011.13.2.9.4]

Table 5. Percentage by weight of organs from broilers fed diets with or without probiotics at 21 and 42 days of age.

Dietary Treatments [a]	Liver		Spleen		Intestines		Gizzard		Heart		Bursa	
	21	42	21	42	21	42	21	42	21	42	21	42
C1	2.8	2.04	0.13	0.12	4.9	3.94	3.0	2.48	0.65	0.49	0.29	0.13 [b]
B	2.7	2.11	0.08	0.13	6.1	4.17	3.0	2.28	0.72	0.57	0.28	0.21 [a]
10F	3.2	1.91	0.09	0.14	5.9	3.93	2.8	2.41	0.66	0.57	0.26	0.16 [b]
20F	3.1	1.89	0.10	0.12	5.3	3.43	2.9	2.07	0.72	0.55	0.25	0.15 [b]
SE	0.09	0.07	0.007	0.006	0.14	0.13	0.06	0.09	0.02	0.01	0.01	0.01

[a] C1=Control 1; B= Diet contained Bioplus2; 10F= Diet contained 10g kg^{-1} fermented milk; 20F= Diet contained 20g kg^{-1} fermented milk.
[a,b] Values within a column with no common superscript differ significantly (P<0.05).

positive effect on growth performance, but the effect was not significant. Many investigators have studied the effects of *Lactobacillus* cultures on the performance of chickens. Some results indicate that *Lactobacillus* sp. are capable of improving broilers performance (Jin *et al*., 1996a; Nahashon *et al*., 1994,1996), while other works show that *Lactobacillus* sp. does not have any positive effect on broilers performance (Buenrostro and Kratzer, 1983; Maiolino et al., 1992; Watkins and Kratzer, 1984). Factors affecting effectiveness of probiotic depends on stress condition of broilers (Jin *et al*., 1998; Lyons, 1987), capability of microorganisms to attach to the intestinal wall, their antagonism towards pathogenic bacteria, and their ability to competitively exclude some pathogenic bacteria (Jin *et al*., 1996b,c).

The result of the bacterial analysis showed improvement in intestinal microflora balance. The result on *coliform* count in the intestinal contents showed that the addition of fermented milk or the commercial probiotic depressed the *coliform* population in the ileum and colon, although the effect was not statistically significant. Jin *et al*. (1996a) and Francis *et al*. (1978) reported that chickens fed with a diet supplemented with a *Lactobacilli* culture had lower *coliform* numbers in the intestine, while, according to Jin *et al*. (1998), addition of a single strain of *L. acidophilus* or a mixture of *Lactobacillus* did not increase significantly *Lactobacilli* numbers in the ileum and cecum, except at 30 days after feeding. Also, these results agree with Watkins and Kratzer (1983, 1984) who found that chicks dosed with host specific *Lactobacillus* strains had only slightly higher numbers of *Lactobacilli* in their intestine.

Supplementing probiotics to the diets of broilers did not affect the weights of different organs. These findings are in

Table 6. Serum biochemical markers in chicks at 21 and 42 days of age.

Dietary Treatments [a]	AST (IU/l)		ALT (IU/l)		ALKPH (IU/l)		Cholesterol (mg/dl)	
	21	42	21	42	21	42	21	42
C1	188	229	10.5 [a]	14.7	13400	1640	151	125
B	176	185	10.6 [a]	12.7	7093	1482	124	125
10F	180	195	8.6 [b]	15.7	10800	808	149	118
20F	168	234	11.0 [a]	20.3	9287	900	141	119
SE	5.5	22.3	0.38	1.5	1457	314	5.2	2.3

[a] C1=Control 1; B= Diet contained Bioplus2; 10F= Diet contained 10g kg^{-1} fermented milk; 20F= Diet contained 20g kg^{-1} fermented milk.
[a,b] Values within a column with no common superscript differ significantly (P<0.05).

agreement with the results of Fethiere and Miles (1987) and Watkins and Kratzer (1984). Similarly, Perdigon *et al*. (1988) found that feeding cultured milk containing *L. acidophilus* or *L. casei* or both had no effect on the weight of the spleen or liver of mice. Pulusani and Rao (1983) also reported that liver weights were not different between the rats feeding on diets with or without skim milk fermented by *L. acidophilus*, *L. bulgaricus* or *L. thermophilus*. In one study, an implantation of *L. acidophilus* in broilers caused a reduction in the weight of the ceca (Tortuero, 1973).

AST, ALT and ALKPH activities of the serum may indicate the liver function and health. Cellular injury in liver may increase the level of these enzymes in serum. ALT is principally found in the liver and is regarded as being more specific than AST for detecting liver cell damage. According to Table 6, the probiotics showed lower level of these enzymes. There is no available report in this regard in chicks, but Oyetao *et al.* (2003) showed that adding *L. acidophilus* to the diets decreased ALT level in rats.

Only a slight anticholestrolaemic effect was also observed in chicks treated with probiotics. Oyetao *et al*. (2003) also showed similar results in rat and *Lactobacilli* had been found to have direct effect on cholesterol levels by assimilation and removal from the growth media. According to De Smet *et al*. (1994) and Ahn *et al*. (2000 and 2003), bile salt hydrolase activity of *Lactobacilli* might have some role in the reduction of serum cholesterol level.

Overall, the results indicated that addition of *acidophilus* yoghurt to broilers diet had some positive effects on growth performance, intestinal microbial balance, and liver health.

ACKNOWLEDGEMENT

The authors greatly appreciate the financial support of the present research by the Management and Budget Organization of Kerman (Project code 410/28/19181).

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بررسی امکان بهبود عملکرد جوجه های گوشتی با استفاده از پروبیوتیک لاکتوباسیلوس اسیدوفیلوس

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چکیده

این آزمایش به منظور مطالعه اثرات سطوح مختلف لاکتوباسیلوس اسیدوفیلوس (۲×۱۰^{۶} و ۴×۱۰^{۶} باکتری زنده در گرم خوراک) و یک پروبیوتیک تجاری بر عملکرد، خصوصیات لاشه، برخی فاکتورهای خونی و جمعیت میکربی روده انجام شد. در این آزمایش ۲۸۰ قطعه جوجه خروس گوشتی یک روزه به طور تصادفی به ۵ گروه آزمایشی تقسیم شدند و به هر گروه نیز ۴ تکرار ۱۴ قطعه ای جوجه اختصاص داده شد. میزان مصرف خوراک در تیمار پروبیوتیک تجاری برای کل دوره به طور معنی داری از پروبیوتیک اسیدوفیلوس بیشتر بود. میزان اضافه وزن در دوره پیشدان در تیمار حاوی سطح بالای لاکتوباسیلوس اسیدوفیلوس به طور معنی داری از تیمار شاهد بیشتر بود . در مورد ضریب تبدیل غذایی و وزن ارگانها اختلاف معنی داری مشاهده نگردید. استفاده از پروبیوتیک لاکتوباسیلوس اسیدوفیلوس سبب افزایش جمعیت لاکتوباسیلها در ایلئوم و انتهای روده بزرگ شد، همچنین انواع پروبیوتیک سبب کاهش جمعیت کلی فرم ها در این دو قسمت شدند، ولی این اختلافات از نظر آماری معنی دار نبودند. میزان کلسترول و آنزیمهای آسپارتات آمینو ترانسفراز، آلانین آمینو ترانسفراز و آلکالین فسفاتاز خون نیز در جیره های حاوی پروبیوتیک کمتر از شاهد بود، ولی این اختلاف فقط برای آلانین آمینو ترانسفراز در سن ۲۱ روزگی معنی دار گردید.