www.roavs.com EISSN: 2223-0343
RESEARCH OPINIONS IN ANIMAL & VETERINARY SCIENCES
Research article DOI: 10.20490/ROAVS/16-026

The effects of *Livebios*® at different rearing stages on intestinal microflora and blood biochemical parameters in male and female broiler chickens

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Article history
Received: 20 May, 2016
Revised: 10 Jun, 2016
Accepted: 13 Jun, 2016

Abstract

This experiment was conducted to determine the effects of a probiotic (*Livebios*®) at different rearing stages on intestinal microflora and blood biochemical parameter in broiler chickens. A total of 378 one day old, male and female (Ross 308) broiler chicken were randomly allocated to one of the 21 floor pens in a completely randomized design with seven treatments and three replicate groups and eighteen chicks in each group (9 male and 9 female). Experimental treatments consisted of seven groups, including: A (control), B (1-11 days), C (1-24 days), D (1-42 days), E (11-24 days), F (11-42 days) and G (24-42 days). The results of this study indicated that probiotic at different rearing period in the diet significantly increased blood glucose and calcium during the whole period and significantly decreased blood cholesterol, low density lipoprotein (LDL) and uric acid. In addition, probiotic in diets at different rearing stage had a significant effect on *Bifidobacterium* and total aerobic bacteria counts. Gender and treatment interaction had no significant effect on the intestinal microflora population. Blood cholesterol and triglyceride levels were significantly lower in male broilers than female broilers. It is concluded that *Livebios*® at whole rearing period (1-42 days) in diets improved intestinal microflora population and blood metabolites in broiler chickens.

Keywords: Intestinal microflora; probiotic *Livebios*®; gender; *Bifidobacterium*

To cite this article: Ghasemi-Sadabadi M,Y Ebrahimnezhad and M Khaliq, 2016. The effects of Livebios® at different rearing stages on intestinal microflora and blood biochemical parameters in male and female broiler chickens. Res. Opin. Anim. Vet. Sci., 6(5): 144-150.

Introduction

Probiotic is a live microbial feed supplement that benefits the host by intestinal microbial balance. Also, the utilization of probiotics in animal nutrition can produce safe foods for human (Gaskins et al., 2002; Denli et al., 2003; Janardhana et al., 2009).

Fuller (1998) defined probiotic as an alternative to antibiotics in animal diets. Therefore, the probiotic bacteria through various mechanisms can improve the performance and health of broiler chicken (Denli et al., 2003). In recent years, scientific reports indicated that probiotic products have beneficial effects on various animals. Moreover, the probiotics are non-digestible

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food ingredients that beneficially influence the animal growth or activity of bacteria in the intestines (Gibson and Roberfroid, 1995). Wakwak et al. (2003) reported that supplementation of probiotics in the diet reduced pathogenic bacteria which can be related to the inhibitory action of probiotic bacteria. Also, Kabir et al. (2005) showed that the use of probiotic in broiler chicken's diet significantly increased the *Lactobacilli* count in the intestines. In similar studies, Mohan et al. (1996) showed that supplementation of probiotic in diet increased body weight gain compared to supplementation of antibiotic in broiler chickens. Jin et al. (1997) reported that probiotics provide microbial balance in intestinal microflora and prevention of gastrointestinal infections and improve animal performance. Poutahidis et al. (2014) indicated that the intestinal microbiota induce modulation of local gastrointestinal immunity resulting systemic effects on the immune system which activate metabolic pathways that restore tissue homeostasis and overall health. Islam et al. (2013) reported that supplementation of probiotic in the diet has significant effects on haematological and biochemical parameters in broiler chickens. Pietras (2001) showed that *L. acidophilus* and *Streptococcus faecium* reduced blood protein and total cholesterol.

Due to little information on supplementation of probiotics at different rearing period in male and female broiler, this experiment was carried out to study the effects of probiotic (*Livebios*®) at different rearing periods on intestinal microflora population and blood biochemical parameters in male and female broiler chickens.

Materials and Methods

Birds, experimental design and management

This study was conducted to determine the effects of a probiotic (*Livebios*®) at different rearing periods on intestinal microflora population and blood biochemical parameter in broiler chickens. A total of 378 one day old, male and female broiler (Ross 308) were randomly allocated to 21 floor pens in a completely randomized design with seven treatments, three replicates and 18 chicks in each replicate (nine male and nine female). On the first day, male and female chicks were separated by feather sexing method. All the chicks were kept under similar management conditions according to Ross 308 strain catalogue. The duration of the experimental period was 42 days. The feed and water were available *ad libitum* to broiler chickens. Animal handling and experimental procedures were performed according to the Guide for the Care and Use of Laboratory animals by the National Institutes of Health (USA) and the current laws of the Iranian government for animal care.

All of the chicks were divided into seven equal groups (each group was constituted by three replicates of 18 birds per replicate) according to the probiotic contents incorporated into the diets (300 g in starter, 250 g in grower and 200 g in finisher period) at different rearing periods. Therefore, experiment groups including, A (control), B (1-11 days), C (1-24 days), D (1-42 days). E (11-24 days), F (11-42 days), G (24-42 days). The treatment diets were formulated to meet the requirements of broiler as recommended by the catalogue of Ross 308 broilers (Table 1). The feed mixture of the experimental group was supplemented with probiotic (*Livebios*®) containing *Saccharomyces* cerevisiae (1×10^{11}) and Lactobacillus casei (5×10^{9}), Lactobacillus acidophilus (5×10^{9}), Streptococcus (5×10^{9}) faecium and Bacillus subtilis (1×10^{9}).

At the end of the experiment, four chicks (two male and two female) from each pens were sacrificed and collecting blood samples with EDTA from the wing vein. All the samples were properly labelled and stored for further studies at -20°C. The plasma biochemical parameters including glucose, triglyceride (TG), cholesterol, low density lipoprotein (LDL), calcium, total protein, albumin and uric acid were determined by Technicon RA 1000 Auto analyzer (Technicon Instruments Corporation, Tarrytown, New York, USA) using commercial kits (Pars Azmoon, Tehran, Iran).

At 10, 24 and 42 days, four chicks (two male and two female) per replicate were randomly selected for measurement of intestinal microflora. For this purpose, the caeca (five samples per group) were removed and opened after treatment of their surface with 70% ethanol. One gm of caeca contents was homogenized in nine ml peptone water (0.1% peptone, Wt/Vol) and serial dilutions were prepared and plated on nutrient agar, double layer hydrolyzed milk agar with China blue, Endo agar and Brilliant-green Phenol-red agar for determination of Total aerobic bacteria and *Bifidobacterium* count respectively. Results were expressed as log10 colony forming units per gram of digesta (log10 cfu/g).

Statistical analyses

The data were analyzed by ANOVA to assess the effects of *Livebios*® at different rearing periods and gender. When the ANOVA indicated significant treatment effects, means were separated using turkey's multiple range test. The general linear models procedure of the SAS system (SAS, 1996) was applied, fitting the following nonlinear equation:

$$Yij1 = \mu + Wi + Sj + (WS)\ ij + Eij1$$

Where μ is the population mean; Wi the effects of probiotic using at different rearing periods (i = 1 to 2); Sj is the effect of gender (j = 1 to 2); (WS) ij and

(WSA) ij are the interactions of the main effects; and Eij1 is the overall error term.

Results and Discussion

The Results obtained from the effects of probiotic ***Livebios***® at different rearing periods on intestinal microflora population are shown in Table 2. The results of this experiment showed that ***Livebios***® in the diet had significant (P<0.05) effect on the intestinal microbial population. Besides, the intestinal microbial population was significantly (P<0.05) improved in chickens fed with probiotic compared to the control chicken. Probiotic in diets had significantly (P<0.05) higher *Bifidobacterium* counts and lower total aerobic bacteria counts compared to the other groups. Probiotic in the diet from 1 to 10 days of age decreased total aerobic bacteria counts compared to the control.

Generally, in this study, total aerobic bacteria count had lower counts compared to control groups by using of probiotic in diets. In the same study, Watkins and Kratzer (1984) observed that using probiotic in the diet decreased harmful bacteria in the intestine in broiler chickens. These results agreed with the results of Nava et al. (2005), Line et al. (1998) and Wakwak et al. (2003) who observed that the use of probiotic in the diet reduced the pathogenic bacteria. In addition, the probiotics can be protective against *Clostridium*, *Salmonella* and *Coliforms* bacteria (Audisio et al., 2000; Toro et al., 2005). In similar case, Murry et al. (2006) found lower *Coliforms* bacteria count in birds by feeding probiotics. Therefore, the beneficial microorganisms establish in the digestive tract and prevent the pathogens (Tellez et al., 2001; La Ragione et al., 2004). Also, in our experiment, *Bifidobacterium* count was higher at whole period (1 to 42 days). Mountzouris et al. (2007) found an increase in the concentration of *Bifidobacterium* and *Lactobacillus* at 42 days of age. In this trial, gender did not significantly affect *Bifidobacterium* and total aerobic bacteria counts at each period. The interaction between gender and treatments on intestinal microflora population in broiler chicken are shown in Table 3. The results showed that interaction between treatment and gender showed no significant difference between groups.

The Results obtained from the influence of probiotic at different rearing periods on blood biochemical parameters in broiler chickens are shown in Table 4.

The results of this study showed that blood cholesterol and LDL level were also significantly decreased at 42 days in birds supplemented with probiotic compared to the control birds (P<0.05). However, the blood glucose level was significantly higher in probiotic supplemented at 1 to 42 and 11 to 42 days groups compared to the other groups (P<0.05).

Table 1: Composition of the basal diet (ingredient and nutrients) given to broiler chickens for 6 weeks

Ingredient (%)	Starter (1-10 d)	Grower (11-24 d)	Finisher (25-42 d)
Corn	56.64	57.05	61.16
Soybean meal (44% CP)	36.74	35.12	31.20
Sunflower oil	0.95	3.20	3.22
Dicalcium phosphate	1.89	1.65	1.53
Oyster shell	1.35	1.12	1.08
Sodium bicarbonate	0.26	0.26	0.26
Salt	0.23	0.23	0.23
Vitamin premix[a]	0.25	0.25	0.25
Mineral premix[b]	0.25	0.25	0.25
DL-Methionine	0.42	0.26	0.25
L-Lysinemono-HCl	0.38	0.11	0.12
L-Threonine	0.64	0.50	0.45
Nutrients (Calculated)			
ME, kcal/kg	2850	3000	3050
CP, %	22.14	20.95	19.54
Ca, %	1.05	0.90	0.85
Available P, %	0.50	0.45	0.42
Na, %	0.18	0.18	0.18
K, %	0.90	0.87	0.81
Cl, %	0.17	0.17	0.17
Met + Cys, %	1.07	0.90	0.86
Lys, %	1.43	1.18	1.09
Thr,%	0.94	0.29	0.74
Trp, %	0.94	0.80	0.26

[a]Vitamins mixture provides per 2.5 kilogram of diet: vitamin A, 12000000 IU; vitamin B_1, 4000 mg; vitamin B_2, 6000 mg; vitamin B_3, 18000 mg; vitamin B_6, 3000 mg; vitamin B_{12}, 15 mg; vitamin D_3, 5000000 IU; vitamin E, 50000 IU; vitamin K_3, 3000 mg; vitamin B_9, 1500 mg; vitamin B_5, 70000 mg; vitamin H_2, 100 mg; choline chloride, 400000 mg; [b]Mineral mixture provide per 2.5 kilogram of diet: Mn, 120000 mg; Zn, 100000 mg; Fe, 40000 mg; Cu, 20000 mg; I, 1000 mg; Se, 300 mg.

Using ***Livebios***® at different rearing periods did not show significant effect on blood TG, albumin and globulin levels (P>0.05). In addition, the blood calcium level was significantly affected between groups during the treatment period (P<0.05). The blood calcium level was significantly higher in probiotic treated at whole period and 24-42 days compared to the other treatment (P<0.05).

The use of probiotic at different rearing periods in diets showed significant effect on blood uric acid and total protein level (P<0.05). Blood uric acid level showed significantly lower concentration by using probiotic in diets compared to other groups (P<0.05). In addition, the results of blood total protein level at the end of the trial showed significantly higher level (P<0.05). The total blood protein level was significantly lower in birds of group 1-11days (P<0.05). But, the use of probiotic in diets at long time can increase blood total protein level at 1-42 days group compared to the other groups. However, TG and albumin were not markedly altered in the treated birds (P>0.05).

Table 2: Effects of probiotic *Livebios*® at different rearing periods on intestinal microflora population (log 10 CFU/g) of male and female broilers at day 10, 24 and 42

Days of probiotic treated	Gender	Total aerobic bacteria (10 day)	*Bifidobacterium* (10 day)	Total aerobic bacteria (24 day)	*Bifidobacterium* (24 day)	Total aerobic bacteria (42 day)	*Bifidobacterium* (42 day)
				Treatment			
Control		8.07^{a}	3.18	7.51	5.04	7.41^{ab}	5.60^{ab}
1- 11 days		6.54^{ab}	4.84	7.83	5.05	8.16^{a}	5.01^{b}
1- 24 days		6.30^{ab}	5.51	6.13	6.16	7.78^{ab}	6.41^{ab}
1- 42 days		5.05^{b}	6.48	6.26	6.79	7.31^{ab}	7.87^{a}
11-24 days		7.76^{a}	3.20	6.26	5.78	8.15^{a}	6.49^{ab}
11-42 days		6.53^{ab}	4.27	5.88	6.73	7.59^{ab}	8.22^{a}
24-42 days		7.86^{a}	3.74	6.32	4.85	6.25^{b}	7.98^{a}
SEM		0.97	0.84	0.57	0.47	0.44	0.62
				Gender			
	Female	7.14	4.65	6.69	6.02	7.41	6.98
	Male	6.61	4.27	6.84	5.52	7.64	6.61
SEM		0.22	0.45	0.30	0.25	0.23	0.33
				P value			
Treatment		0.0001	0.0639	0.3979	0.0603	0.0458	0.0017
Gender		0.0920	0.5557	0.3271	0.9315	0.4903	0.4398

Means in columns with same superscript do not differ significantly (P<0.05)

Table 3: Interaction between probiotic at different rearing periods and gender on intestinal microflora population (log 10 CFU/g) of male and female broilers at day 10, 24 and 42

Treatment	Gender	Total aerobic bacteria (10 days)	*Bifidobacterium* (10 days)	Total aerobic bacteria (24 days)	*Bifidobacterium* (24 days)	Total aerobic bacteria (42 days)	*Bifidobacterium* (42 days)
Control	Female	7.98	3.42	7.68	5.12	7.59	5.85
Control	Male	8.17	2.94	7.81	4.95	7.24	5.36
1- 11 days	Female	6.86	3.95	7.62	5.4	8.46	5.34
1- 11 days	Male	6.22	5.74	8.03	4.7	7.86	4.68
1- 24 days	Female	6.50	5.85	5.34	6.87	7.70	7.02
1- 24 days	Male	6.11	5.18	6.91	5.45	7.86	5.81
1- 42 days	Female	5.73	6.18	6.69	6.36	6.71	8.15
1- 42 days	Male	4.38	6.77	5.83	7.23	7.92	7.60
11-24 days	Female	7.70	3.64	6.65	5.82	8.26	6.02
11-24 days	Male	7.82	2.77	5.09	5.74	8.05	6.96
11-42 days	Female	7.93	5.68	6.48	7.45	7.37	8.18
11-42 days	Male	5.14	2.86	6.16	6.02	7.82	7.62
24-42 days	Female	7.30	3.83	6.41	5.13	5.76	7.68
24-42 days	Male	8.43	3.65	8.05	4.57	6.73	8.27
SEM		0.19	0.40	0.27	0.23	0.20	0.29
				P value			
Treatment × Gender		0.0523	0.6562	0.4325	0.1679	0.4718	0.8514

Means in columns with same superscript do not differ significantly (P<0.05)

The results of this trial indicated that gender had no significant effect on blood cholesterol and triglyceride level (P<0.05). Adding probiotic into the diet at different rearing period in female chickens showed higher blood cholesterol and TG level in probiotic-treated than male broiler chickens (P<0.05). Interaction between gender × treatments on blood biochemical parameters in broiler chicken are shown in Table 5. Generally, this experimental data regarding the interaction of treatment and gender showed no significant difference between previously mentioned treatments during the experiment. In our study, using probiotic indicated higher blood glucose level at the end of the whole period. These results were in agreement with the finding of Azza et al. (2012) who reported that the use of probiotic had enhanced the blood glucose level in broilers. In other study, Hashemzadeh et al. (2013) observed that probiotic in diets affected blood glucose level in broiler chickens at the end of the 42 days. Jouybari et al. (2009) reported that addition of yeast in broiler diet significantly increased the blood glucose level. According to these results, it seems that,

Table 4: The effects of probiotic using at different rearing periods on blood biochemical parameters on male and female broilers at 42 days

Days of probiotic treated	gender	Glucose (mg/dl)	Triglyceride (mg/dl)	Cholesterol (mg/dl)	LDL (mg/dl)	Calcium (mg/dl)	Total protein (g/dl)	Albumin (g/dl)	Uric acid (mg/dl)
					Treatment				
Control, without probiotic		197.83^{b}	69.66	126.83^{a}	39.33^{ab}	6.58^{b}	4.40^{bc}	2.52	4.60^{a}
1- 11 days		199.00^{ab}	67.66	125.16^{a}	40.16^{a}	7.11^{ab}	4.24^{c}	2.38	4.25^{a}
1- 24 days		202.00^{ab}	69.00	121.16^{ab}	39.00^{b}	7.87^{ab}	4.84^{abc}	3.09	3.88^{ab}
1- 42 days		211.00^{a}	69.33	97.50^{b}	27.66^{c}	8.05^{a}	5.44^{a}	2.93	3.36^{ab}
11-24 days		201.50^{ab}	66.50	125.16^{a}	40.33^{a}	7.35^{ab}	4.80^{b}	2.74	3.21^{ab}
11-42 days		211.00^{a}	65.50	103.50^{ab}	34.16^{abc}	7.28^{ab}	4.87^{ab}	2.74	3.20^{ab}
24-42 days		212.66^{a}	67.50	110.16^{ab}	30.00^{cb}	8.00^{a}	4.97^{ab}	3.03	3.11^{b}
SEM		5.37	1.81	5.45	1.50	0.34	0.38	0.34	1.5
					Gender				
	Female	207.70	71.28^{a}	120.71^{a}	3.8	7.35	4.83	2.76	3.8
	Male	204.28	64.47^{b}	110.57^{b}	3.7	7.58	4.76	2.79	3.7
SEM		3.33	0.96	2.91	0.81	0.18	0.15	0.10	0.81
					P value				
Treatment		0.0455	0.6296	0.0019	0.0143	0.0460	0.0197	0.1774	0.0050
Gender		0.1109	0.0001	0.0201	0.3673	0.3910	0.7317	0.8272	0.3943

^{a,b,c}. Means in columns with same superscript do not differ significantly (P<0.05)

Table 5: Interaction between probiotic at different rearing periods and gender on blood biochemical parameters of male and female broilers at days 10, 24 and 42

Treatment	Gender	Glucose (mg/dl)	Triglyceride (mg/dl)	Cholesterol (mg/dl)	LDL (mg/dl)	Calcium (mg/dl)	Total protein (g/dl)	Albumin (g/dl)	Uric acid (mg/dl)
Control	Female	201	73.62	133.56	31.24	6.74	4.41	2.41	4.8
Control	Male	194.63	65.65	120	47	6.43	4.39	2.62	4.4
1- 11 days	Female	195.31	73	135.26	48.32	6.55	4.88	2.72	4.53
1- 11 days	Male	202.56	62.23	114.13	32	7.66	3.59	2.1	3.96
1- 24 days	Female	205.66	73.33	127.22	36	7.79	5.15	3.1	3.53
1- 24 days	Male	198.33	64.51	115	42	7.96	5.71	2.86	4.23
1- 42 days	Female	219.23	71.66	103	31	97.7	4.91	3.11	3.26
1- 42 days	Male	202.60	67	99	24.31	8.13	4.78	3.8	3.46
11-24 days	Female	206	69	131.23	37.65	7.33	4.38	2.24	3.46
11-24 days	Male	197	64	119	43	7.38	5.22	3/23	3.66
11-42 days	Female	208.30	69.3	98.43	31.35	7.23	4.83	2.76	3.26
11-42 days	Male	213.46	61.5	108.29	37	7.32	4.92	2.72	3.13
24-42 days	Female	218.33	69	115.12	26	7.82	5.24	3.2	3.6
24-42 days	Male	207	66	105	34	8.18	4.70	3.3	3.16
SEM		4.05	0.85	2.56	1.36	0.16	0.13	0.09	0.71
					P value				
Treatment × Gender		0.4440	0.7686	0.6148	0.0538	0.8801	0.2239	0.1936	0.6183

Means in columns with same superscript do not differ significantly (P<0.05)

using probiotic at whole rearing period can reduce blood cholesterol level in broiler chickens. The results of blood cholesterol and LDL level in our study indicated that probiotic in the diet at whole rearing period and finisher period decreased blood cholesterol and LDL level. Hashemzadeh et al. (2013) and Tannok and Munro (2000) reported that probiotics in diets reduced the blood cholesterol level in broiler chickens at whole period. Therefore, lower blood cholesterol levels can be related to the higher *Lactobacillus* counts in the intestines. El-Rahman et al. (2012) reported that blood LDL level was significantly decreased with the use of probiotic in broiler chickens' diets. The decrease LDL might be due to anorexia or defective lipid metabolism (Garcia et al., 2010). Also, 60 to 70 percent change in plasma cholesterol had directly affected blood LDL level (Fischbach, 2004). Jouybari et al. (2009) reported that probiotic at whole rearing period showed lower blood cholesterol and LDL level in broiler chickens. Hence *Lactobacillus* and *Bifidobacteria* could contribute to the regulation of serum cholesterol concentrations by deconjunction of bile acids. Since, the excretion of deconjugated bile acids is enhanced and cholesterol is its precursor, more molecules are spent for recovery of bile acids (Pereira and Gibson, 2002). However, the blood TG level in this study did not show significant differences between groups at different period. The results of this study

showed a high calcium level in 1 to 42 days and 24 to 42 days. In a similar case, blood calcium level numerically was higher with the use of probiotic in broiler chickens (Hashemzadeh et al., 2013). The result of blood total protein level in our study was higher in 1 to 42 days groups than other groups. In contrast, Saied et al. (2011), Arslan and Saatci (2004) and Hashemzadeh et al. (2013) showed no significant changes in blood total protein using probiotic (Saied et al., 2011; Eggum et al., 1990). El-Rahman et al. (2012) reported that probiotic in diets caused increased blood total protein level in broiler chickens. The total blood protein and albumin level have been reported to be directly responsive to protein intake (Eggum et al., 1990). Paryad and Mahmoudi (2008) indicated that the use of high level of yeast (*Saccharomyces cerevisiae*) in diets increased blood total protein level in broiler chickens. The probiotic in the diet decreased uric acid level. Similar results were reported by Hashemzadeh et al. (2013). In this experiment, blood albumin was not significantly different between the groups. Hashemzadeh et al. (2013) reported that probiotic in diet did not affect the blood albumin level. Paryad and Mahmoudi (2008) indicated that supplemental yeast (*Saccharomyces cerevisiae*) in diets had a significant effect on blood albumin and globulin level. The result of this experiment is in contrast with the finding of Djouvinov et al. (2005) who reported no significant difference in the level of total proteins in a probiotic used study. In our study, gender significantly affected blood cholesterol and triglyceride level. The results showed that blood cholesterol and triglyceride level in female chicks were significantly higher than male chickens ($P<0.05$). Also, male chickens at whole rearing period had lower blood cholesterol than the other groups. This can be due to high *Lactobacilli* counts of intestine population.

Conclusions

The results of this study showed that probiotic (*Livebios*®) at whole rearing period (1 to 42 days) in the broiler chickens' diet had a positive effect on blood biochemical parameter and intestinal microflora population. Also, in this experiment results indicated that male broilers had better blood parameters and intestinal microbial population than female broilers.

Acknowledgement

This article is a part of M.Sc. thesis in Poultry Production and Management, Islamic Azad University, Shabestar Branch supervised by Dr. Y. Ebrahimnezhad. The authors would like to thank to all staff of Islamic Azad University, Shabestar Branch for providing necessary facilities for carrying out this research. Thanks are also extended to Kamran Bani-Asadian for English editing of this Manuscript.

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