Original Research

Effect of *In Ovo* Injection of *Lactobacillus acidophilus* on the Serum Bio-Chemistry of Commercial Broiler Chicken

P. Kanagaraju[1*], S. Ravichandran[1], K. Kumanan[2], P. Muthusamy[3], S. Rathnapraba[4] and G. Srinivasan[1]

[1]Department of Poultry Science, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), Chennai-600 007, Tamil Nadu, INDIA
[2]Centre for Animal Health Studies, TANUVAS, MMC, Chennai-600 051, Tamil Nadu, INDIA
[3]PGRIAS, TANUVAS, Kattupakkam-603 203, Tamil Nadu, INDIA
[4]Department of Animal Biotechnology, Madras Veterinary College, TANUVAS, Chennai-600 007, Tamil Nadu, INDIA

Part of M.V.Sc thesis work submitted to Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu, India 600 007.

***Corresponding author:** kanraj2007@gmail.com

Rec. Date:	Apr 06, 2018 10:35
Accept Date:	Jun 02, 2018 18:07
DOI	10.5455/ijlr.20180406103558

Abstract

A biological trial was conducted to study the effect of in ovo injection of Lactobacillus acidophilus to 18 days old chicken embryo on the serum bio-chemistry of commercial broiler chicken. Broiler hatching eggs (Cobb-400) with uniform weight were randomly selected, divided in to five treatments each with 6 replicates of 24 eggs each and were incubated in forced draft incubator under standard conditions. In ovo injection was carried out on 18th day of incubation, out of total 720 eggs, 144 eggs served as non-injected control (T_1), 144 eggs served as sham control and the remaining 432 eggs (144 for each treatment group) were injected with 0.2 ml of 1x10^6 Lactobacillus acidophilus (T3), 0.2 ml of 1x10^9 Lactobacillus acidophilus (T4) and 0.2ml of 1x10^{12} Lactobacillus acidophilus (T5). The positive control group was injected with 0.2 ml of 0.9% normal saline solution. At hatch, 480 chicks were randomly selected (96 birds from each treatment) with six replicates of 16 birds each as per treatment wise. Data on total cholesterol, high-density lipoproteins cholesterol (HDLC), low-density lipoprotein cholesterol (LDLC) and triglycerides were estimated and subjected to statistical analysis. Sixth week serum total cholesterol, low-density lipoprotein cholesterol, triglycerides were significantly (P<0.01) decreased in L. acidophilus injected broilers. Whereas, serum high-density lipoprotein cholesterol levels were significantly (P<0.01) increased compared to control birds. Based on the results of the present study it can be concluded that the in ovo injection of Lactobacillus acidophilus reduced serum bad cholesterol and increased good cholesterol content of commercial broilers which may help in the production of designer meat.

Key words: Cobb-400, *In ovo* Injection, *Lactobacillus acidophilus*, Serum bio-chemistry

How to cite: Kanagaraju, P., Ravichandran, S., Kumanan, K., Muthusamy, P., Rathnapraba, S., & Srinivasan, G. (2019). Effect of In Ovo Injection of *Lactobacillus acidophilus* on the Serum Bio-Chemistry of Commercial Broiler Chicken. International Journal of Livestock Research, 9(12), 106-111. doi: 10.5455/ijlr.20180406103558

Introduction

The early stage of post-hatch period is critical for the establishment of gut microflora. Probiotics are considered as alternative to antibiotics to reduce the intensity and severity of enteric infections in poultry due to competitive inhibition, colonization, changes in pH and production of antibiotic like substances (Hajati and Rezaei, 2010). In artificial hatching, the settlement of the intestinal beneficial bacteria is delayed, even if this process is induced by feed additives. *In ovo* technology, an emerging technique which helps in promoting early colonization of beneficial bacteria in intestine and maintenance of gut health (Cox, 2013). Many studies have been carried in broilers by feeding probiotics through feed but *in ovo* injection of probiotics in broilers are found to be meager. The objective of the present study was to investigate the effect of *in ovo* injection of *Lactobacillus acidophilus* (LA) on the serum biochemical profile of commercial broilers.

Materials and Methods

Seven hundred and twenty fertile eggs with uniform weight were randomly selected from 38 weeks old commercial broiler breeder flock (Cobb 400). *In ovo* injection of *Lactobacillus acidophilus* was done as per the modified Noor *et al*. (1995) method. On 18th day of incubation, out of total 720 eggs, 144 eggs served as non-injected control (T_1), 144 eggs served as injected control (T_2- positive control) and the remaining 432 eggs (144 for each treatment group with six replicates of 24 eggs each) were injected with 0.2 ml of 1x10^6 *Lactobacillus acidophilus* (T3), 0.2 ml of 1x10^9 *Lactobacillus acidophilus* (T4) and 0.2ml of 1x10^{12} *Lactobacillus acidophilus* (T5). The positive control group was injected with 0.2 ml of sterile water (Sham). The *Lactobacillus acidophilus* (MTCC NO.10307) culture was obtained from Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh, India-160 030. *In ovo* injection was carried out in an empty incubation cabinet where the temperature and humidity was maintained at 37.5°C and 60 per cent, respectively. Each egg was candled and earmarked to identify the site of the injection (Aminion). After disinfection of egg shell surface with 99.90 % ethyl alcohol, a pin head size hole was made using a sharp egg puncture and 0.2 ml of respected treatment solution was injected into the amnion using an insulin syringe with 31g needle (0.25 mm x 8 mm) to a depth of about 8 mm without disturbing the air cell. The hole was sealed and incubated up to 21 days.

After completion of *in ovo* injection, all eggs were transferred to hatcher trays and incubation was continued till hatching of the chicks. Number of chicks hatched in each replicate within each treatment was noted and recorded. The hatch was taken on day 21 and the chicks were wing banded. The hatch weight of each chick was individually recorded on a balance

of 0.01 g accuracy. The hatched-out chicks were allotted in to five treatments with six replicates of 16 chicks each. Experimental birds were provided with standard broiler ration (BIS, 2007). Birds were provided with *ad libitum* feed and water. Standard managemental practices were followed throughout the experiment. At the end of the experiment (day 42), one bird was randomly selected from each replicate (6 birds for each treatment group) and blood samples were collected from the wing vein. The collected blood samples were centrifuged at 3000 rpm for 10 min and the sera were decanted into aseptically treated vials and stored at –20˚C until further analysis.

The data on total cholesterol, high-density lipoproteins cholesterol (HDLC), low-density lipoprotein cholesterol (LDLC) and triglycerides were estimated based on CHOD-PAP methodology (Allain *et al.*, 1974) and GPO-TAPS method (Buccolo and David, 1973), respectively in A15 Biosystem Auto Analyser by using commercial diagnostic kits from AGAPPE Diagnostic Ltd. The LDL cholesterol estimated based on selective solubilization method (Crouse, 1985) in A15 Biosystem Auto Analyser by using commercial diagnostic kits from M/s. AGAPPE Diagnostic Ltd and expressed in mg/dl. The data were analyzed by one way ANOVA using V.17 SPSS (1999) software. Differences between treatment means were detected by using Tukey post-hoc test.

Results and Discussion

The data on mean (±SE) serum total cholesterol, High-density lipoprotein cholesterol (HDLC), Low-density lipoprotein cholesterol (LDLC) and Triglycerides of broiler chicken estimated at the end of trial (42nd day) as influenced by *in ovo* injection of *Lactobacillus acidophilus* are presented in Table 1 along with statistical interpretation. Serum total cholesterol was significantly (P<0.01) low in all the three *Lactobacillus acidophilus* (109.49 to 113.85 mg/dl) treatments when compared to sham (143.51 mg/dl) and negative control (142.33 mg/dl) indicating that *in ovo* supplementation of *Lactobacillus acidophilus* is an efficient suppressor of serum cholesterol level.

Table 1: Mean (±SE) total cholesterol, HDLC, LDLC and triglyceride of broilers chicken as influenced by *in ovo* injection of *Lactobacillus acidophilus*

Treatments		Serum lipid profile (mg/dl)			
		Cholesterol	HDLC	LDLC	Triglycerides
	Non injected control	142.33^{a} ± 1.57	78.06^{b} ± 0.41	80.52^{a} ± 0.23	90.83^{a} ± 0.36
In ovo injection of 0.2 ml of	Sterile water (Sham)	143.51^{a} ± 1.59	77.57^{b} ± 0.40	80.18^{a} ± 0.15	91.03^{a} ± 0.30
	L.acidophilus 1x10^{6}cfu	109.49^{c} ± 1.34	81.63^{a} ± 0.30	75.99^{b} ± 0.21	85.34^{b} ± 0.24
	L.acidophilus 1x10^{9}cfu	122.53^{b} ± 1.19	81.76^{a} ± 0.51	73.89^{c} ± 0.36	84.31^{b} ± 0.16
	L.acidophilus 1x10^{12}cfu	113.85^{c} ± 1.94	83.00^{a}± 0.37	75.48^{b} ± 0.16	80.90^{c} ± 0.28
F- value		104.979	36.296	160.433	255.707
Significance		**	**	**	**

*No. of observations (n) = 6; Means within column bearing different superscripts differ significantly (P<0.01); ** Highly significant (P<0.01)*

 DOI 10.5455/ijlr.20180406103558

Serum HDLC levels differed significantly (P<0.01) and the value improved in the *in ovo* treated groups with proportionate decrease of bad LDLC level in the serum. Hence, probiotics bacteria LA *in ovo* injection was found to be an ideal method to reduce the bad LDLC and improve the good HDLC; which is indication of better health expressed as higher livability of broilers. Triglyceride values were also followed the similar trend as that of total cholesterol. All the three *in ovo* supplemented *Lactobacillus acidophilus* probiotics groups showed significantly (P<0.01) lower serum triglycerides levels with the values ranged from 80.90 (LA $1x10^{12}$) to 85.34 mg/dl (LA $1x10^{6}$) when compared to control (90.83 mg/dl) and sham birds (91.03 mg/dl). Serum total cholesterol, low-density lipoprotein cholesterol (LDLC) and serum triglycerides estimated in six weeks broiler chicken received *in ovo* treatment with three level of *Lactobacillus acidophilus* were significantly lower than sham and non-injected control. However, high-density lipoprotein cholesterol (HDLC), the good cholesterol was significantly increased in the serum of broilers injected with *L. acidophilus* on 18^{th} day of incubation through intra amnion. By throwing light on literatures we could not able to find the literatures to support the present results of influence of *in ovo* delivery of probiotics on serum lipid profile of broilers, hence the present study was planned. However, the results obtained in this study were discussed with the available results of dietary supplementation. These results are in accordance with Mohan *et al.* (1995) and Arun *et al.* (2006) who reported significantly lowered total cholesterol, very low-density lipoprotein (VLDL) cholesterol and triglyceride concentrations in the serum of broiler chickens fed with *Lactobacillus sporogenes* supplemented diet.

In agreement with the present findings, Panda *et al.* (2001) who found that the dietary supplementation of probiotic (*Lactobacillus acidophilus*, *Bifidobacterium* and *Aspergillus oryzae*) significantly reduced the serum cholesterol level of broiler chickens. Similar findings were also reported by Kalavathy *et al.* (2003) who observed that dietary supplementation of a mix culture of 12 strains of *Lactobacillus* at 1% level resulted in lowered serum cholesterol concentration of broilers. Mayahi *et al.* (2009) fed 0.1% of two commercial probiotics, one consisting of *E. faecium* and the other consisting of *Bifidobacterium* to broilers and found significant reduction in their serum total cholesterol and triglyceride concentrations. Mansoub, (2010) findings also observed that the dietary supplementation of *Lactobacillus acidophilus* and *Lactobacillus casei* either alone or in combination with water significantly (P<0.01) declined total cholesterol and triglycerides in the serum of broilers compared to control. The findings of the present *in ovo* study concurred with the findings of Shokryazdan *et al.* (2017) who found that supplementation of the three *L. salivarius* strains to broilers significantly reduced their serum LDL-cholesterol, but not their HDL-cholesterol levels at 21 and 42 d of age. Similar results of reduction in LDL-cholesterol but not HDL-cholesterol were also reported by Kalavathy *et al.* (2003) who fed 1 g kg^{-1} of a multistrain probiotic comprising 12 *Lactobacillus* strains to broilers. Panda *et al.* (2006) also found a decrease in serum LDL-cholesterol but not HDL-cholesterol in broilers supplemented with *L. sporogenes* at 100 or 200 mg kg^{-1} diet

 DOI 10.5455/ijlr.20180406103558

is also in line with the present findings. Conversely, Ashayerizadeh *et al*. (2009) did not find any significant differences in the serum HDL- and LDL-cholesterol concentrations of chickens fed with commercial probiotics (PrimaLac) when compared to control birds.

The decreased level of serum total cholesterol, low-density lipoprotein cholesterol (LDLC) and serum triglyceride observed in the *in ovo* treated broilers in this study could be explained by the fact that *L. acidophilus* is capable to deconjucate glycocholic and taurocholic acids (gallbladder acids) by the action of bile salt hydrolase an enzyme, while deconjucated acids are not capable to dissolve and absorb fatty acids as conjucated acids. As a consequence, they prevent absorption of cholesterol. Further, free gallbladder acids attach to bacteria and fibres and this can increase the excretion of them (Gilliland and Speck, 1977). Since, cholesterol is the precursor for the synthesis of new bile acids, the use of cholesterol to synthesize new bile would lead to a decreased concentration of cholesterol in blood (Lye *et al.*, 2009). In addition, Ooi and Liong (2010) suggested that the hypocholesterolemic effect of probiotics and synbioticare mediated via altering the pathways of cholesteryl esters and lipoprotein transporters. Hence, the result of the present study indicated that *Lactobacillus acidophilus* can be exploited as an efficient probiotic in the production of low cholesterol meat from broiler chicken.

Conclusion

In ovo injection of *Lactobacillus acidophilus* at the dose of $1x10^6$ and $1x10^9$ significantly reduced total cholesterol, low-density lipoprotein cholesterol (LDLC) and triglyceride but high-density lipoprotein cholesterol (HDLC) was significantly increased in commercial broilers. Based on the results of the present study it can be concluded that the *in ovo* injection of *Lactobacillus acidophilus* reduced serum bad cholesterol and increased good cholesterol content of commercial broilers which may help in the production of designer meat.

Acknowledgement

The author greatly acknowledges the Tamil Nadu Veterinary and Animal Sciences University, Chennai-51 for providing fund and all infra structural facilities to carry out this work.

References

1. 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.
2. Arun, A., Murrugappan, R., Ravindran, A.D.D., Veeramanikandan, V. and Balaji, S. (2006). Utilization of various industrial wastes for the production of poly-β-hydroxy butyrate (PHB) by *Alcaligenes eutrophus*. *Afr. J. Biotechnol*. 5 : 1524-1527.
3. Ashayerizadeh, O., Dastar, B., Shargh, M.S., Ashayerizadeh, A. and Mamooee, M. (2009). Influence of antibiotic, prebiotic and probiotic supplementation to diets on carcass characteristics, hematological Indices and internal organ size of young broiler chickens. *J Anim Vet Adv*. 8 : 1772–1776.

DOI 10.5455/ijlr.20180406103558

4. BIS. (2007). Bureau of Indian Standards of poultry feed. Manak Bhawan, 9, Bahadur Shah Zafar Marg, New Delhi, India.
5. Buccolo, G. and David, H. (1973). Quantative determination of triglyceride by the enzyme. *Clin. Chem*., 19 : 476-482.
6. Cox, CM. (2013). *In Ovo* Supplementation of Primalac and the Effects on Performance and Immune Response of Broilers. Ph.D thesis submitted to Animal and Poultry Sciences, the faculty of the Virginia Polytechnic Institute and State University, Blacksburg, VA.
7. Crouse, J.R. (1985). Studies of low density lipoprotein molecular weight in human being with coronary artery disease. *J. lipid Res*. 26 : 56-66.
8. Gilliland, S.E. and Speck, M.L. (1977). Enumeration andidentity of lactobacilli in dietary products. *J. Food Prot*. 40 : 760-762.
9. Hajati, H. and Rezaei, M. (2010). The application of probiotics in poultry production. *Inter. J. Poult. Sci.*, 9:298-304.
10. Kalavathy, R., Abdullah, N., Jalaludin, S. and Ho, Y. W. (2003). Effects of *Lactobacillus* cultures on growth performance, abdominal fat deposition, serum lipids and weight of organs of broiler chickens. *Br. Poult. Sci*., 44 : 139–144.
11. Lye, H.S., Kuan, C.Y., Ewe, J.A., Fung, W.Y. and Liong, M.T. (2009). The improvement of hypertension by probiotics: Effects on cholesterol, diabetes, Renin and phytoestrogens. *Int. J. Mol. Sci*., 10 : 3755-3775.
12. Mayahi, M., R.Jalali, M. and Kiani, R. (2009). Effects of dietary probiotic supplementation on cholesterol and triglyceride levels in broiler chicks sera. *Antalya*. pp. 589–591.
13. Mohan, B., Kadirvel, M., Bhaskaranand, M. and Natarajan, A. (1995). Effect of probiotic supplementation onserum/yolk cholesterol and on egg shell thickness in layers. *Br. Poult. Sci*., 36 : 799–803.
14. Mansoub, N.H. (2010). Effect of probiotic bacteria utilization on serum cholesterol and triglycrides contents and performance of broiler chickens. *Global Veterinaria*. 5 : 184–186.
15. Ooi, L.G. and Liong, M.T. (2010). Cholesterol-Lowering Effects of Probiotics and Prebiotics: A Review of *in Vivo* and *in Vitro* Findings. *Intl. J. Mol. Sci.* 11(6) : 2499–2522.
16. Panda, A.K., Reddy, M.R. and Praharaj, N.K. (2001). Dietary supplementation of probiotic on growth, serum cholesterol and gut microflora of broilers. *Ind. J. Anim. Sci*. 71 : 488-490.
17. Panda, A.K., Rao, S.V.R., 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.
18. Shokryazdan, P., FaselehJahromi, M., Liang, J.B., Ramasamy, K., Sieo, C.C. and Ho, Y.W. (2017). Effects of a *Lactobacillus salivarius* mixture on performance, intestinal health and serum lipids of broiler chickens. *PLoSone.*,12(5) : 0175959.
19. SPSS (1999). Statistical software package for the social sciences SPSS, version 17. Int., USA.

DOI 10.5455/ijlr.20180406103558