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In ovo delivery of *Lactobacillus acidophilus* on the growth and immune response of commercial broiler chicken

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Abstract
A nutritional trial was conducted to investigate the effect of *in ovo* injection of *Lactobacillus acidophilus* to 18 days old broiler chicken embryo on the growth performance and humoral immune response of commercial broilers. *In ovo* injection was carried out on 18th day of incubation, out of total 720 broiler hatching 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 in each treatment) with six replicates of 16 birds each as per treatment wise. Data on hatchability, sixth week weight gain, cumulative FCR, livability and antibody titres against sheep RBC antigen were recorded and statistically analysed. There was no significant difference in percent hatchability among treatment and control groups. The 6th week body weight and weight gain were significantly (P<0.01) affected by *in ovo* injection of *Lactobacillus acidophilus* irrespective of concentration. The feed consumption was significantly (P<0.01) more in probiotic injected groups compared to other groups only on second week. The 6th week cumulative FCR was not significantly affected by *in ovo* infusion of probiotics. The livability was significantly (P<0.05) higher in probiotic infused groups. Antibody titre against SBRC antigen was significantly (P<0.01) increased by *in ovo* treatments compared to sham and control. The TLR-2 gene was significantly (P<0.01) up regulated in the spleen and caecal tonsil's of *L. acidophilus* infused broilers. The results of this trial indicated that the *in ovo* injection of *Lactobacillus acidophilus* has got beneficial effect on the growth performance, survivability and immune status of commercial broilers.

Keywords: *In ovo* delivery, *Lactobacillus acidophilus*, growth performance, immune status, broilers

Introduction
Probiotics are nowadays widely used as growth promoter to produce antibiotic residue free broiler chicken meat due to increased awareness about development of antibiotic resistance. Probiotics have the ability to reduce the intensity and severity of enteric infections in broilers due to competitive inhibition, colonization, changes in pH and production of antibiotic like substances (Hajati and Rezaei, 2010) [1]. In artificial hatching, the colonization of the intestinal beneficial bacteria is delayed, even if this process is induced by feed and water additives. Hence, there is necessity for early colonization of beneficial microbiota in the gastro intestinal tract of poultry. On 18th day of embryonic development, the embryo will have its first meal when it consumes the amniotic fluid before internal pipping (Ferket and Uni, 2006) [2]. *In ovo* techniques take the advantage of this crucial time and help to promote early colonization of probiotic bacteria in order to improve the gut efficiency and health which is utmost importance in case of fast growing commercial broilers. Though numerous studies have been carried in broiler chickens by feeding probiotics through feed and water, *in ovo* supplementation of probiotics in broiler chickens found to be meagre. Hence, the present study was under taken to investigate the effect of *in ovo* injection of *Lactobacillus acidophilus* at different concentrations on the growth performance and immune status of commercial broilers.

Materials and method
One thousand hatching eggs with uniform weight were collected from 38 weeks old commercial broiler breeder flock (Cobb 400). Out of which 720 eggs with live embryos were

selected based on 18th candling for in ovo study. *In ovo* injection of nutrient solutions was done as per the modified Noor *et al.* (1995) [3] 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 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 percent, respectively.

After completion of *in ovo* injection, all eggs were transferred to hatcher trays and incubation was continued till hatching of the chicks. The hatch was taken on day 21, the number of chicks hatched in each replicate within each treatment was recorded and wing band applied for identification. The hatch weight of each chick was individually recorded on a balance of 0.01 g accuracy treatment wise. 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) [4]. Birds were provided with *ad libitum* feed and water. Standard management practices were followed throughout the experiment. The data on hatchability, biweekly body weight, body weight gain, feed consumption, feed conversion ratio (FCR), livability and antibody titres against sheep RBC antigen were recorded. Immune status of the experimental birds was assessed indirectly by haemagglutination (HA) titres against sheep red blood cell (SRBC) as a specific antigen. For immunization one bird is randomly selected from each replicate (six birds per each treatment group) were sensitized with one percent SRBC suspension through intravenous route in jugular vein by using tuberculin syringe at 21day of age for humoral immune response study. One ml of blood was collected on 7th, 14th and 21st day of post immunization. Later, serum was separated from the blood and then stored at -20 °C till further assay. Haemagglutination assay for SRBC was carried out as per procedure outlined by Van der Zijpp and Leenstra (1980) [5].The data were analyzed by one way ANOVA using V.17 SPSS (1999) [6] software. Differences between treatment means were detected by the Tukey test.

Results and discussion

The effect of *in ovo* injection of probiotics at different levels on hatchability, bi weekly body weight, weight gain, feed consumption, FCR, livability and antibody titre against SBRC antigen were given in Table 1 and 2. *In ovo* treatment did not significantly affect hatchability in this study and the value ranged from 92.36% (Negative Control) to 95.14% in T3 (*Lactobacillus acidophilus* of $1x10^6$). Similar findings were reported by Cox (2013) with commercial probiotic (Primalac) in broilers. Whereas De Olivera *et al.,* (2014) [7] reduced hatchability by about 10% in probiotics injected group compared with non injected control (98%), with exception of *B. subtilis* group (95%). These findings suggest that the probiotic bacteria can be safely supplemented through *in ovo* method without affecting hatchability.

The sixth week body weight was significantly (P<0.01) increased in all *in ovo* groups injected with *Lactobacillus acidophilus* irrespective of concentration ranged from 1969.45 ± 24.93 (LA $1x10^{12}$ cfu) to 2067.29 ± 24.20 (LA $1x10^6$ cfu) when compared to control groups. However there was no significant difference among treatment groups. In agreement with this finding, Edens *et al.* (1997) [8] reported that *in ovo* injection of *L. reuteri* increased body weight of broiler chickens. On the other hand, several researchers reported no significant improvement due to probiotic supplementation (Wolfenden *et al.*, 2011) [9].Similar trend was observed in body weight gain also, at six weeks significantly (P< 0.01) higher body weight gain (1927.19 to 2025.00 g) was recorded in groups injected with L. acidophilus than control group (1818.44 g). Similar findings were reported by Chasity *et al.* (2017) [10] who reported that *in ovo* injection of probiotc had significantly (P<0.01) improved body weight gain.

The cumulative 6th week feed consumption was not affected significantly. However, there was numerical increase in the feed consumption in the *in ovo* probiotic injected groups. This finding was in agreement with Cox CM (2013) [11] who reported that *in ovo* supplementation of Primalac did not significantly alter feed consumption in broilers. Similar findings were also reported by Majidi-Mosleh *et al.*, 2017 [12] who found that *in ovo* infusion of different probiotics strain did not affect the daily feed intake significantly (P>0.05) among treatments at different periods or in the whole period.

The sixth week cumulative FCR was not significantly affected by the different *in ovo* treatments. This finding of the present study concurred with the findings of Zulkifli *et al.* (2000) [13] who observed better feed conversion ratio in broilers fed a diet containing *Lactobacillus* cultures during the growing period (1 to 21d), but did not find good feed efficiency during finishing period (22 to 42 d). On contrary, Majidi-Mosleh *et al.,* 2017 [12] reported that *in ovo* infusion of different probiotics strain did not have effect on feed conversion ratio between the treatments during the experimental period. The findings of the present study indicated that the *in ovo* injection of *L. acidophilus* at a dose level of $1x10^6$ cfu or $1x10^9$cfu had numerical beneficial effects on the cumulative feed efficiency at 6 weeks of age.

Significantly (P<0.01) higher antibody titre against SBRC was noted in all *in ovo* injected *L. acidophilus* groups compared to sham and control on 7th day post inoculation. On 14th day post inoculation, the antibody titre increased more significantly (P<0.01) in *L. acidophilus* injected groups as 3.12, 3.00 and 3.24 log_2 values in LA $1x10^6$, LA $1x10^9$ and LA 10^{12} injected groups respectively when compared to sham and negative control (2.08 log_2 values). Similar trend was observed on 21st day post inoculation of SRBC but the antibody titre values were higher than that of 14th day titre values and ranged from 4.51 (LA $1x10^{12}$ cfu) to 2.08 (Negative control) log_2. The antibody titre against SBRC antigen in broilers injected with *L. acidophilus* on 18th day of incubation increased more significantly compared to sham and control on 7th, 14th, 21st and 28th day of post immunization. On contrary to this findings Hosseini *et al.* (2013) [14] have reported that supplementation of probiotic did not have any effects on antibody production against SRBC antigen in broilers. Increased antibody titre against SBRC was obtained by Hosein Nikpiran *et al.* (2013) [15], Afsharmanesh and Sadaghi (2014) [16] in broilers fed with probiotics supplemented diet. On contrary to the present result, Majidi-Mosleh *et al.* (2017) [12] reported non significant antibody response to SRBC antigen in broilers injected with *B. subtilis*, *Pediococcus acidilactici* solution and *Enterococcus faecium* probiotics on 18^{th} day of incubation through amniotic route.

However, Haghighi *et al.* (2005) [17] reported that the immune modulatory activities of probiotics in enhancing the antibody response are highly dependent on the antigen, immunization regimen, type and number of species of bacteria present in probiotics and genetic background of the host. The antibody titre values obtained in this study clearly indicated that *in ovo* supplementation of *L. acidophilus* to 18 days old embryos had more significant (P<0.01) effect on humoral immune status; which was reflected by better livability during the experimental period compared to non injected birds.

The livability of the broilers were significantly (P<0.05) influenced by the *in ovo* supplementation of *L. acidophilus*. The livability was significantly (P<0.05) higher in *in ovo* treated groups ranged from 93.75 to 97.92 percent compared to negative (87.50%) and sham (92.71%) controls. Necropsy of dead birds did not reveal any pathological lesions that were attributable to treatment effects. The overall mortality in this study was within the standards prescribed for commercial broilers with the exception of control. The percentage mortality was high in the control chicks due to exposure to *E. coli* organisms might have been occurred through orofaecal contamination and lack of immune status which was confirmed by high coliform counts in the intestinal contents. Similar reports were observed by Anjum *et al.* (2005) [18]. The present findings were on contrary to Sohail Hassan Khan *et al.*, 2011 [19] who reported that the dietary supplementation of probiotics did not have influence on the mortality of the birds and was in the expected range.

Table 1: Effect of *in ovo* supplementation of *Lactobacilus acidophilus* at different concentrations on hatchability, chick weight, body weight (g) and body weight gain (g) of broiler

Treatments	Hatchability (%) (n=6)	Day-old chick weight (g)	6^{th} week Body weight gain (g)	6^{th} week Cumulative FCR	6^{th} week Cumulative livability (%)
Non injected control	92.36 ± 1.23		1818.44^{c} ± 25.15	1.84 ± 0.13	87.50^{b} ± 3.61
Injected control (0.2 ml of sterile water)	94.44 ± 0.88		1887.63^{bc} ± 24.74	1.87 ± 0.17	92.71^{ab} ± 1.92
Lactobacillus acidophilus of $1x10^{6}$ cfu	95.14 ± 0.27		2025.00^{a} ± 24.17	1.74 ± 0.08	93.75^{ab} ± 1.61
Lactobacillus acidophilus of $1x10^{9}$ cfu	93.07 ± 0.27		1969.53^{ab} ± 23.37	1.75 ± 0.03	97.92^{a} ± 1.32
Lactobacillus acidophilus of $1x10^{12}$ cfu	94.44 ± 0.27		1927.19^{b} ± 24.89	1.84 ± 0.08	96.88^{a} ± 1.40
F- value	0.24		30.48	0.92	3.66
Significance	NS		**	NS	*

Means within column bearing different superscripts differ significantly

** – Highly significant (P<0.01)

Table 2: Mean (±SE) antibody titres against sheep RBC antigen (log 2) in broilers chickens at different age as influenced by *in ovo* supplementation of *Lactobacillus acidophilus*

Treatments		Days post inoculation of sheep RBC antigen		
		7^{th} day	14^{th} day	21^{st} day
Non injected control		1.39^{c} ± 0.00	2.08^{b} ± 0.00	2.08^{b} ± 0.00
In ovo injection of 0.2 ml of	Sterile water (Sham)	1.74^{b} ± 0.15	2.08^{b} ± 0.00	2.19^{b} ± 0.11
	L. acidophilus $1x10^{6}$cfu	2.77^{a} ± 0.00	3.12^{a} ± 0.16	4.28^{a} ± 0.16
	L. acidophilus $1x10^{9}$cfu	2.77^{a} ± 0.00	3.00^{a} ± 0.14	4.39^{a} ± 0.15
	L. acidophilus $1x10^{12}$cfu	2.77^{a} ± 0.00	3.24^{a} ± 0.14	4.51^{a} ± 0.15
F- value		95.00	24.34	107.15
Significance		**	**	**

No. of observations (N) = 6

Means within column bearing different superscripts differ significantly

** Highly significant (P<0.01)

Conclusion

In ovo injection of *Lactobacillus acidophilus* at the dose of $1x10^{6}$ and $1x10^{9}$ significantly improved body weight, body weight gain, livability and immune status but feed consumption and FCR were not affected in commercial broilers. Based on the results obtained from this experiment it can concluded that the *in ovo* delivery of *Lactobacillus acidophilus* improved growth performance, overall immune status and survivability of commercial broilers.

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References

1. Hajati H, Rezaei M. The application of probiotics in poultry production. Inter. J Poult. Sci. 2010; 9:298-304.
2. Ferket PR, Uni Z. Incubation and *in ovo* nutrition affects neonatal development. 33rd Annual Carolina Poultry Nutrition Conference, 2006.
3. Noor SM, Husband AJ, Widders PR. *In ovo* vaccination with *Campylobacter jejuni* establishes early development of intestinal immunity in chickens. Brit. Poult. Sci. 1995; 36:563-573.
4. BIS. Bureau of Indian Standards of poultry feed. Manak Bhawan, 9, Bahadur Shah Zafar Marg, New Delhi, India, 2007.
5. Van Der Zijpp AJ, Leenstra FR. Genetic analysis of the humoral immune response of White Leghorn chicks. Poult. Sci. 1980; 59(7):1363-9.
6. SPSS. Statistical software package for the social sciences SPSS, version 17. Int. USA, 1999.

7. De Oliveira JE, Van der Hoeven-Hangoor E, Van de Linde IB, Montijn RC, Van der Vossen JMBM. *In ovo* inoculation of chicken embryos with probiotic bacteria and its effect on posthatch Salmonella susceptibility. Poult. Sci. 2014; 93:818-829.
8. Edens FW, Parkhurst CR, Casas IA, Dobrogosz WJ. Principles of *ex ovo* competitive exclusion and *in ovo* administration of *Lactobacillus reuteri*. Poult. Sci.1997; 76:179-196
9. Wolfenden RE, Pumford NR, Morgan MJ, Shivaramaiah S, Wolfenden AD, Pixley CM *et al*. Evaluation of selected direct-fed microbial candidates on live performance and *Salmonella* reduction in commercial turkey brooding houses. Poult. Sci. 2011; 90:2627-2631
10. Chasity MP, Sungwon Kim, Tiffany D Potter, Miranda M Ritzi, Mark Young *et al*. *In Ovo* Supplementation of Probiotics and Its Effects on Performance and Immune-Related Gene Expression in Broiler Chicks. Poult Sci. 2005; 96(5):1052-1062.
11. Cox CM. *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, 2013.
12. Majidi-Mosleh A, Sadeghi AA, Mousavi SN, Chamani M, Zarei A. Ileal MUC2 gene expression and microbial population, but not growth performance and immune response, are influenced by *in ovo* injection of probiotics in broiler chickens. Brit. Poult. Sci. 2017; 58(1):40-45,
13. Zulkifli I, Abdullah N, Azrin NM, Ho YW. Growth performance and immune response of two commercial broiler strains fed diets containing *Lactobacillus* cultures and oxytetracycline under heat stress conditions. Br. Poult. Sci. 2000; 41:593-597.
14. Hosseini Amir Meimandipour, Fatemeh Alami, Ali Mahdavi, Maziar Mohiti-Asli, Houshang Lotfollahian, Deborah Cross. Effects of Ground Thyme and Probiotic Supplements in Diets on Broiler Performance, Blood Biochemistry and Immunological Response to Sheep Red Blood Cells. Ital J Anim. Sci. 2013; 12:19.
15. Hosein Nikpiran, Mehdi Taghavi, Ali Khodadadi, Seyyed Shamsadin Athari. Influence of Probiotic and Prebiotic on broiler chickens performance and immune status. J Nov. Appl Sci. 2013; 2(8):256-259.
16. Afsharmanesh M, Sadaghi B. Effects of dietary alternatives (probiotic, green tea powder ad Kombucha tea) as antimicrobial growth promoters on growth, ileal nutrient digestibility, blood parameters, and immune response of broiler chickens. Comp. Clin. Pathol. 2014; 23(3):717-724.
17. Haghighi HR, Gong J, Gyles CL, Hayes MA, Sanei B, Parvizi P *et al*. Modulation of Antibody-Mediated Immune Response by Probiotics in Chickens. Clin. Diagn. Lab. Immunol. 2005; 12(12):1387-1392.
18. Anjum MI, Khan AG, Azim A, Afzal M. Effect of dietary supplementation of multi-strain probiotic on broiler growth performance. Pak Vet J. 2005; 25:25-29.
19. Sohail Hassan Khan, BurhanYousaf, Asghar Ali Mian, Abdul Rehman, Muhammad Sabir Farooq. Assessing the effect of administering different probiotics in drinking water supplement on broiler performance, blood biochemistry and immune response. J Appl. Anim. Res. 2011; 39(4):418-428.