International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2018; 6(4): 1919-1922

Received: 10-05-2018
Accepted: 13-06-2018

Survase Swapnil H
PG Scholar, Department of Poultry Science, Madras Veterinary College, Chennai, Tamil Nadu, India

Srinivasan G
Professor and Head, Department of Poultry Science, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Vepery, Chennai, Tamil Nadu, India

Kumanan K
Director, Centre for Animal Health Studies, Tamil Nadu Veterinary and Animal Sciences University, Vepery, Chennai, MMC, Chennai, Tamil Nadu, India

Kanagaraju P
Assistant Professor, Department of Poultry Science, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Vepery, Chennai, Tamil Nadu, India

Rathnapraba S
Assistant Professor, Department of Animal Biotechnology, Madras Veterinary College, Chennai, Tamil Nadu, India

Correspondence
Survase Swapnil H
PG Scholar, Department of Poultry Science, Madras Veterinary College, Chennai, Tamil Nadu, India

Effect of *in ovo* delivery of *Lactobacillus acidophilus, Mannan oligosacchrides* (MOS) and their combination on growth performance of commercial broiler chicken

Survase Swapnil H, Srinivasan G, Kumanan K, Kanagaraju P and Rathnapraba S

Abstract
In the post-antibiotics era, probiotic, prebiotic and their combination are proposed as alternatives to antibiotic growth promoters in the modern poultry production. The objective of the present study was to evaluate the effect of *in ovo* injection of *Lactobacillus acidophilus, MOS* and their combination on the production performance of commercial broilers. On d 18 of incubation, 720 eggs were randomly divided into 5 experimental groups each with four replicates of 30 eggs each and were injected with different bioactive compounds viz., 0.2 ml of *Lactobacillus acidophilus* $3x10^7$ cfu (T3); 0.2 ml of 0.5% Mannan-oligosaccharide (T4); 0.2 ml of *Lactobacillus acidophilus* $3x10^7$ cfu + 0.5% Mannan-oligosaccharide (T5). The *in ovo* treatment groups were compared with non-injected control (T1); sham control group injected with 0.2 ml of Physiological saline (T2). After hatching, 20 day-old broiler chicks from each replicate was utilized for biological study. All chicks were reared under deep litter system up to five weeks of age under standard and uniform manage mental conditions in an environmentally controlled broiler house throughout the experimental period. Birds were fed with *ad libitum* pre starter, starter and finisher mash prepared as per as per BIS (2007) from 0-14 d, 15-21 d and 22-35 d of age, respectively. The data on bi-weekly body weight, body weight gain, feed consumption, feed conversion ratio (FCR) and liveability were recorded and statistically analysed. The results of the present study revealed that 5^{th} week cumulative body weight and weight gain were significantly (P<0.01) improved in all *in ovo* treated broilers irrespective of the bioactive compound when compared to control birds. But, there was no significant difference between *in ovo* treatment groups. The cumulative feed consumption, FCR and liveability of broilers up to five weeks of age were not influenced by *in ovo* supplementation of *Lactobacillus acidophilus, MOS and Lactobacillus acidophilus + MOS* between treatments. But there was marginal improvement in the liveability of broilers. Based on the results, it can be concluded that the *in ovo* supplementation of *Lactobacillus acidophilus* and *MOS* either independently or in combination has beneficial effect on growth and survivability of commercial broilers.

Keywords: *In ovo*, probiotic, prebiotic, synbiotic, growth performance, broilers

Introduction
Antibiotics have been used since the 1940s to sustain the overall health and well-being of animals (Consumers Union, 2014) [1]. The use of antibiotics in sub therapeutic doses in livestock feed and/or water to promote growth and improve feed efficiency was eliminated with effective from January 1, 2017, as the Food and Drug Administration (FDA) has amended the new animal drug regulations to implement the veterinary feed directive (VFD) [2]. Increasing concern due to the emergence of antibiotic resistant bacteria has led researchers to look for alternatives to using antibiotics in poultry. Use of Probiotic, prebiotic and synbiotic is the one of the alternate way to curtail the use of antibiotics in poultry. Probiotics, cultures of a single bacteria strain or mixture of different strains, are being studied in livestock and poultry extensively as a production enhancer. Prebiotics are non-digestible carbohydrates and it works as a competitive binding site, as bacteria bind to it rather than the intestine and are carried out of the body.

Earlier *in ovo* vaccination technology has allowed the poultry industry to improve broiler health. *In ovo* feeding is the administration of exogenous nutrients into the amnion of late term avian embryo (Uni and Ferket, 2003) [3].

As the avian embryo orally consume the amniotic fluid prior to piping the air cell (about 18th day of incubation), *in ovo* feeding will improve the energy status and gut development of the perinatal chick by supplementing the amnion with nutrient. The *in ovo* administration of probiotics, i.e. lactic acid bacteria, was performed on the 18th day of incubation (Casas-Perez and Edens 1995) [4]. In fact, *in ovo* method has been successfully used for prebiotic and synbiotic injections (Bednarczyk *et al*. 2011 [5]; Maiorano *et al*. 2012 [6]; Madej and Bednarczyk 2016 [7]). However, studies on the *in ovo* injection of probiotics, prebiotics and synbiotics on the production performance and immune competence of broilers are limited. Hence, the present study was carried out to evaluate the effects of *in ovo* injection of probiotic, prebiotic and synbiotic supplement on growth performance of commercial broilers.

Material and Methods

Incubation and *in ovo* injection procedure

Eight hundred fertile eggs with uniform weight were collected from 35 weeks old broiler breeder flock (Cobb 400) and divided in to five groups with four replicates of 30 eggs each. The eggs were incubated under standard incubation management conditions. On 18th day of incubation, six hundred hatching eggs with live embryo were selected through candling and divided into 5 experimental groups and the fertile eggs were injected with one of the following treatments viz., 0.2 ml of *Lactobacillus acidophilus* $3x10^7$ cfu (T3); 0.2 ml of 0.5% Mannan Oligosacchrides (T4); 0.2 ml of *Lactobacillus acidophilus* 3x107 cfu + 0.5% Mannan-oligosaccharide and were compared with T1 - non injected control; T2 - 0.2 ml of Physiological saline; *In ovo* injection procedure was carried out as per modified method recommended by Uni and Ferket (2003) [3] method. Probiotic bacteria *Lactobacillus acidophilus* (MTCC 10307) was purchased from The Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh, India and the prebiotic mannan-oligosaccharide (Lot I3I00Ib) was purchased from M/s.Exotic Biosolution Private Limited, Mumbai, India respectively.

Biological Experiment

On day of hatching 80 day-old broiler chicks were utilized for each treatment with 20 chicks in each replicate. The biological study was conducted for 35 days at semi environmentally controlled poultry house, Department of Poultry Science, Madras Veterinary College, Chennai, India. Birds were reared on a deep litter system and standard manage mental practices were followed. Birds were provided with clean, potable drinking water and fed *ad libitum* with pre starter, starter and finisher mash as per BIS (2007) [8] from 0-7 d, 8-21 d and 22-35 d of age, respectively. The lighting programme was 23 h of light and 1 hour of darkness with the intensity of 10 lux from 1 to 7 days and 18 h of light and 6 hours of darkness with 5.5 lux intensity from 8 to 35 days. The birds were immunized against Ranikhet and Infectious Bursal Diseases as per recommended vaccination schedule.

Parameters Measurement

Body weight and feed intake recorded bi-weekly interval by using an electronic balance with 0.1 g accuracy. Feed conversion ratio was calculated by dividing average feed consumption by average body weight gain. Feed efficiency was calculated at 2nd, 4th and 5th week of age. Also mortality recorded bi-weekly interval. Bi-weekly body weight gain recorded as, weight of bird in gram minus hatch weight in gram and Feed conversion ratio (FCR) was calculated as a feed consumption (in kg) to body weight (in kg). All the statistical analysis was performed by using statistical Package for Social Science (SPSS, 1999) [9] software (version 20.0). The mean were compared by one way ANOVA for significant difference among treatment.

Animal ethics committee approval

The birds were reared according to the Institutional Animal Ethics Committee (IAEC) of the Tamil Nadu Veterinary and Animal Sciences University, Chennai, India. The minimum invasive protocol as approved by the IAEC, TANUVAS, Chennai (No.2140/SA/DFBS/IAEC/2017 dated 30.10.2017) was also followed.

Results and Discussion

The effect of *in ovo* injection of *Lactobacillus acidophilus, MOS and Lactobacillus acidophilus + MOS* on body weight, bodyweight gain, feed consumption, feed conversion ratio and livability are presented in Table 1.

Table 1: Mean (±SE) body weight, body weight gain, feed consumption, FCR and livability of broiler chicken as influenced by *in ovo* injection of *Lactobacillus acidophilus*, MOS *and their combination* on 18th day of incubation

Treatment	Body weight (g)	Body weight gain (g)	Feed consumption (g)	FCR	Cumulative Liveability (%)
Non injected control	2003.94^{b} ± 28.31	1965.43^{b} ± 28.32	3313.37 ± 53.61	1.62 ± 0.02	95.00 ±2.04
0.2 ml of Physiological saline (Injected control)	2078.61^{ab} ± 22.76	2038.69^{ab} ± 22.75	3385.65 ± 45.99	1.63 ± 0.02	98.75 ±1.25
0.2 ml of *Lactobacillus acidophilus* $3x10^7$ cfu	2105.32^{a} ± 25.70	2065.04^{a} ± 25.69	3407.17 ± 128.39	1.62 ± 0.07	96.25 ±1.25
0.2 ml of 0.5% MOS	2123.03^{a} ± 25.64	2083.25^{a} ± 25.67	3346.13 ± 103.38	1.58 ± 0.05	98.75 ±1.25
0.2 ml of *Lactobacillus acidophilus* $3x10^7$ cfu + 0.5% of MOS	2136.67^{a} ± 24.93	2097.12^{a} ± 24.97	3471.66 ± 97.61	1.62 ± 0.05	96.25 ±3.75
Significant	**	**	NS	NS	NS

Means with at least one common superscript in the same row do not differ significantly
NS-Not significant, ** (P<0.01).
Mannan-oligosaccharide (MOS)

In ovo delivery of *Lactobacillus acidophilus,* MOS *and Lactobacillus acidophilus* + MOS did not have significant influence on day-old body weight of broilers and it ranges from 39.5 to 40 g. However, the cumulative body weight at 5th week of age was more significantly (P>0.01) affected by different *in ovo* treatments. All the *in ovo* treatments recorded significantly higher marketing body weight at the 35 d and ranged from 2105.32 to 2136.67 g compared to non-injected control (2003.94 g) with no significant difference among *in ovo* treated groups. Whereas, the sham control birds recorded intermediary body weight of 2078.61 g. Similar trend was observed in body weight gain also with significantly (P>0.01) higher body weight gain *in ovo* treated broilers compared to sham and non-injected control birds. The positive influence of *in ovo* probiotics on marketing body weight was confirmed by Chasity *et al*. (2017) [10] and De Oliveira *et al*. (2014) [11] who reported that *in ovo* supplementation of probiotic bacteria significantly increased the body weight of broilers when

compared to the birds of negative control and sham control (P = 0.01). Contrarily, Maiorano *et al*. (2017) [12] reported non-significant improvement in the body weight and body weight gain of birds received *in ovo* prebiotic and synbiotic treatment compared with the control group.

Pre hatch delivery of *Lactobacillus acidophilus,* MOS *and Lactobacillus acidophilus* +MOS did not have significant influence on the 5th week cumulative feed consumption in broilers. However, the *in ovo* treated broilers consumed numerically more feed when compared to non-injected control. The results of the present study on feed consumption are in line of agreement with those of Maiorano *et al.* (2017) [12] who found that the *in ovo* treatment with prebiotics did not significantly affect the feed intake in broilers. However, Pruszynska-Oszmale *et al*. (2015) [13] found that *in ovo* administration of prebiotic and synbiotic marginally increased (statistically not significant) the mean daily feed intake per bird with compare to Control group.

In ovo supplementation of *Lactobacillus acidophilus,* MOS *and Lactobacillus acidophilus* +MOS did not affect the feed conversion ratio in broilers and the value ranged from 1.58 (*In ovo* MOS) to 1.63 (Injected control). The present findings are in agreement with Pender *et al.* (2017) [14] who reported that the *in ovo* injection of Primalac® through amniotic route did not significantly affect the FCR when compared to sham and negative control birds. Contrarily, Bednarczyk *et al*. (2016) [15] reported that broilers from the control group showed significantly (P<0.05) better FCR compared with birds received *in ovo* prebiotics Bi^{2}tos (3.5 mg/embryo group) and DiNovo® (0.88 mg/embryo group).

Per cent cumulative liveability of broiler chicken up to five weeks of age was not significantly (P<0.05) changed by *in ovo* injection of *Lactobacillus acidophilus,* MOS *and* their combination of *Lactobacillus acidophilus* and MOS. Similar results were observed by Adrianna *et al.* (2017) [16] who found that the injection of Di Novo® prebiotic did not have any significant impact on liveability up to 42 d of rearing. Similarly, Pender *et al.* (2017) [14] observed no significant effect on mortality of broilers injected with commercial probiotic Primalac® on 18th day of incubation. Whereas, Aleksandra *et al.* (2017) [17] observed numerically lower mortality in the *in ovo* synbiotic-treated groups compare to control.

The beneficial effects of in ovo supplementation of *Lactobacillus acidophilus,* MOS *and Lactobacillus acidophilus* +MOS compared to control might be due to increase in the population of beneficial microflora on the day of hatch, and leads to a high and stable level of Biffido bacteria throughout the broiler chickens growing period as witnessed by Martinez-Villaluenga *et al*. 2004 [18]. Also, it can be inferred that the bio active substances injected in a very low doses are effective compared to dietary supplementation of *Lactobacillus acidophilus,* MOS, *Lactobacillus acidophilus* +MOS and antibiotics in the diet which is also confirmed by Bednarczyk *et al*. 2011 [19].

Conclusion

Based on the results obtained from this biological study, it can be concluded that the *in ovo* supplementation of *Lactobacillus acidophilus* and MOS either independently or in combination has beneficial effect on growth and survivability of commercial broilers. But, the economic feasibility under Indian field conditions depends upon the availability, affordability and usage of commercial *in ovo* injection equipment.

Acknowledgement

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

Conflict of interest statement

The author expresses no conflict of interest with any other individual or organisation regarding the information discussed in the manuscript.

References

1. Consumers Union. The overuse of antibiotics in food animals threatens public health. http://consumersunion.org/news/theoveruse- of-antibiotics-in-food-animals-threatens-public-health-2/.2014; Accessed April 15, 2014.
2. Veterinary Feed Directive (VFD) Basics. Www.avma.org. Retrieved 2017-03-14.
3. Uni Z, Ferket PR. Enhancement of development of oviparous species by *in ovo* feeding. US Regular Patent US. 2003; 6:B2.
4. Casas-Perez IA, Edens FW. *In ovo* method for delivering *Lactobacillus reuteri* to the gastrointestinal tract of poultry. Biogaia, A.B. assignee. US Pat. No. 5, 1995; 458-875.
5. Bednarczyk M, Urbanowski M, Gulewicz P, Kasperczyk K, Maiorano G, Szwaczkowski T. Field and *in vitro* study on prebiotic effect of raffinose family oligosaccharides in chickens. Bulletin of the Veterinary Institute in Pulawy. 2011; 55:465-469.
6. Maiorano G, Sobolewska A, Cianciullo D, Walasik K, Elminowska-Wenda G, Sławi nska A *et al*. Influence of *in ovo* prebiotic and synbiotic administration on meat quality of broiler chickens. Poult. Sci. 2012; 91:2963-2969.
7. Madej JP, Bednarczyk M. Effect of *in ovo*-delivered prebiotics and synbiotics on the morphology and specific immune cell composition in the gut-associated lymphoid tissue. Poult. Sci. 2016; 95:19-29.
8. BIS. Bureau of Indian Standards of poultry feed. Manak Bhawan, 9, Bahadur Shah Zafar Marg, New Delhi, India, 2007.
9. SPSS. Statistical software package for the social sciences SPSS, version 17. Int. USA, 1999.
10. Chasity Pender M, Sungwon Kim, Tiffany Potter D, Miranda Ritzi M, Mark Young, Rami Dalloul A. *In ovo* supplementation of probiotics and its effects on performance and immune-related gene expression in broiler chicks. Poult. Sci. 2017; 96:1052-1062.
11. De Oliveira JE, Hoeven-Hangoor EVD IB, Van de Linde, Montijn RC, Van der Vossen JMBM. *In ovo* inoculation of chicken embryos with probiotic bacteria and its effect on post hatch Salmonella susceptibility. Poult. Sci. 2014; 93:818-829.
12. Maiorano G, Stadnicka K, Tavaniello S, Abiuso C, Bogucka J, Bednarczyk M. *In ovo* validation model to assess the efficacy of commercial prebiotics on broiler performance and oxidative stability of meat. Poultry Science. 2017; 96:511-518.
13. Pruszynska-Oszmalek E, Kolodziejski PA, Stadnicka K, Sassek M, Chalupka D, Kuston B *et al*. *In ovo* injection of prebiotics and synbiotics affects the digestive potency of the pancreas in growing chickens. Poult. Sci. 2015; 94:1909-1916.

14. Pender CM, Kim S, Potter TD, Ritzi MM, Young M, Dalloul RA. Effects of *in ovo* supplementation of probiotics on performance and immunocompetence of broiler chicks to an Eimeria challenge. Benef. Microbes. 2017; 7(5):699-705.
15. Bednarczyk M, Stadnicka K, Kozłowska I, Abiuso C, Tavaniello S, Dankowiakowska A *et al*. Influence of different prebiotics and mode of their administration on broiler chicken performance. Animal. 2016; 10(8):1271-1279.
16. Adrianna Sobolewska, Gabriela Elminowska-Wenda, Joanna Bogucka, Agata Dankowiakowska, Anna Kułakowska, Agata Szczerba *et al*. The influence of in ovo injection with the prebiotic Di Novo® on the development of histomor phological parameters of the duodenum, body mass and productivity in large-scale poultry production conditions. J Anim Sci Biotechnol. 2017; 8:45.
17. Aleksandra Dunislawska, Anna Slawinska, Katarzyna Stadnicka, Marek Bednarczyk, Piotr Gulewicz, Damian Jozefiak *et al*. Synbiotics for broiler hickensð *in vitro* design and evaluation of the influence on host and selected microbiota populations following *in ovo* delivery. Journal. Pone. 2017; 12(1):e0168587.
18. Martinez-Villaluenga C, Frias J, Gulewicz K, Vidal-Valverde C. An improved method to obtain pure α-galactosides from lupin seeds. J Agric. Food Chem. 2004; 52:6920-6922.
19. Bednarczyk M, Urbanowski M, Gulewicz P, Kasperczyk K, Maiorano G, Szwaczkowski T. Field and *in vitro* study on prebiotic effect of raffinose family oligosaccharides in chickens. Bull. Vet. Inst. Pulawy. 2011; 55:465-469.