Open Access

Check for updates

Research Article

Journal of Veterinary and Animal Sciences

ISSN (Print): 0971-0701, (Online): 2582-0605

https://doi.org/10.51966/jvas.2025.56.1.53-59

Effect of *in ovo* inoculation and dietary supplementation of *Lactobacillus plantarum,* mannan oligosaccharide and its combination on growth performance of broiler chicken#

K. K. Malini[1], S. Prasoon[2*], Binoj Chacko[1], S. Harikrishnan[3], Vimal Antony Muttathettu[1] and N. S. Sunilkumar[4]

[1]Department of Poultry Science, [4]Department of Veterinary Anatomy, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, [2]College of Avian Sciences and Management, Thiruvazhamkunnu, [3]University Poultry and Duck Farm, Mannuthy, Kerala Veterinary and Animal Sciences University, Pookode, Wayanad, Kerala, India

Citation: Malini, K.K., Prasoon, S., Binoj, C., Harikrishnan, S., Vimal, A.M. and Sunilkumar N.S. 2024. Effect of in ovo inoculation and dietary supplementation of *Lactobacillus plantarum*, mannan oligosaccharide and its combination on growth performance of broiler chicken*. J. Vet. Anim. Sci.* **56** (1):53-59

Received: 24.09.2024 Accepted: 07.12.2024 Published: 31.03.2025

Abstract

The experiment was conducted at Avian Research Station, Thiruvazhamkunnu, to study the effects of in ovo inoculation and dietary supplementation with Lactobacillus plantarum (L. plantarum) and mannan oligosaccharide (MOS) on broiler growth performance from day-old to 42 days. A total of 420 eggs were assigned to seven treatment groups, each with 60 eggs: T1 (0.2 mL normal saline, control), T2 (0.2 mL of 10^7 CFU L. plantarum), T3 (0.2 mL of 0.5 per cent MOS), T4 (combination of L. plantarum and MOS), T5 (dietary 10^7 CFU L. plantarum/kg feed), T6 (dietary 0.5 per cent MOS), and T7 (dietary combination of L. plantarum and MOS). Hatch weights were significantly higher in the in ovo inoculated groups. By six weeks, the dietary synbiotic group (T7) and in ovo synbiotic group (T4) had similar body weights, which was significantly ($p<0.05$) higher than all other groups. Cumulative body weight gain was higher in synbiotic groups (T4 and T7). The in ovo prebiotic group (T3) showed growth performance similar to the synbiotic groups throughout the study period. However, cumulative feed consumption and feed conversion ratio (FCR) were unaffected by the treatments. The study concludes that in ovo inoculation of prebiotic and synbiotic is as effective as their dietary supplementation for enhancing growth performance of broiler chicken.

Keywords: *Lactobacillus plantarum, MOS, synbiotic, in ovo, growth performance*

Commercially chicken have become key food producing animals in the global food system. Newly hatched chicks undergo various procedures *viz.*, pull out, grading, storage and transportation before housing in the brooder, which introduces a gap in the access to feed and water (Kadam *et al*., 2013). This gap dampens the potential for microflora inoculation and prevents proper development of microbiome, gastrointestinal system and innate immunity. As a consequence, the industrial production of broiler chicken with a poor microbial profile leads to infectious disease outbreaks. The delay dampens the stimulation of intestinal villi and establishment of beneficial gut microflora, which leads to delayed development of gastrointestinal system, and innate immunity (Das *et al*., 2021). Consequently, broiler chickens with a poor microbial profile are more susceptible to disease outbreaks in industrial production.

Probiotics, prebiotics and synbiotics are used widely in poultry feeds as alternatives to antibiotic growth

Part of MVSc thesis submitted by first author to Kerala Veterinary and Animal Sciences University

**Corresponding author: prasoon@kvasu.ac.in, Ph. 9446997834*

promoters. Probiotics and prebiotics must be administered as early as possible to achieve the desired efficacy of gut maturation. The composition of gut microbiota can be modified through the use of suitable probiotics (Reshma *et al*., 2023). Research showed that *in ovo* inoculation of nutrients during late incubation helps in the early development of intestinal villi in the chicks which results in enhanced nutrient absorption and thereby increased final body weight of broiler chicken (Panda *et al*., 2006). Studies on the administration of *L. plantarum* and MOS through *in ovo* route in broiler chicken are scanty. Hence, the present study is designed to compare the growth performance of broiler chicken supplemented with *L. plantarum*, MOS and their combination through *in ovo* inoculation and dietary route.

Materials and methods

A total of 600 fertile Vencobb-430Y broiler chicken eggs were procured from Venkateshwara Hatcheries Private Ltd. and incubated in a setter maintained at 37.7°C with 55 per cent relative humidity. On the 18th day, the eggs were candled and embryonated eggs were allotted into seven treatment groups of 60 eggs each. The experimental groups T2, T3 and T4 were *in ovo* inoculated with *L. plantarum*, MOS and their combination in 0.2 mL normal saline (NS) as described in the Table 1. T1 served as control and was inoculated with 0.2 mL NS. After inoculation, all the eggs were immediately transferred to a hatcher at 37.2°C and 65 per cent relative humidity. Upon hatching, healthy chicks were randomly selected, wing banded, weighed, and allotted to three replicates of 10 birds each in respective treatment groups. Experimental groups T5, T6 and T7 received dietary supplementation of *L. plantarum,* MOS and their combination, respectively, until 42 days of age as described in the Table 1, while the *in ovo* treated groups were fed a standard broiler diet formulated as per BIS (2007). All birds were given ad libitum access to feed and water, and feed samples analysed for proximate composition (AOAC 2016).

MOS was procured from Provet Pharma, Chennai and the culture of *Lactiplantibacillus plantarum* (formerly known as *Lactobacillus plantarum*), strain IDK 120 (VTCDM 648 B), was obtained from the starter culture laboratory of the Dairy Microbiology Division of Verghese Kurien Institute of Dairy and Food Technology, Mannuthy, was used for the study. Probiotic preparations were prepared by the method described by Sun *et al.* (2022).

On the 18th day of incubation, 240 viable embryonated eggs were collected for *in ovo* inoculation as described by Uni and Ferket (2003). Each egg was candled, and the air cell marked. The inoculation site was sterilized with 70 per cent isopropyl alcohol, and a hole was made at the broad end using a sterilised 18-gauge needle. Probiotic, prebiotic, and synbiotic solutions in normal saline (0.2 mL each) were inoculated into the amnion using a sterile insulin syringe with a 24-gauge needle. All procedures were conducted aseptically under laminar airflow, and the hole was sealed with sterile molten paraffin wax post-inoculation.

The body weight (g) of individual birds were recorded at weekly intervals from day-old to six weeks of age and body weight gain was calculated. Feed consumed (g) per bird in each replicate was recorded weekly up to six weeks of age and cumulative feed consumption for the entire period was calculated. FCR (kg of feed consumed per kg weight gain) was calculated for each replicate using the data on body weight gain and feed consumption. Statistical analysis was performed by one-way ANOVA using SPSS version 24.0.

Results and discussion

Body weight

The effects of experimental treatments on body weight of broilers are represented in Table 2. The body weight of broilers at weekly intervals in different treatment groups (T1 to T7) showed significant differences across various age intervals, indicating that both *in ovo* inoculation and dietary supplementation had variable impacts on the growth performance of broilers. The *in ovo* synbiotic group (T4) exhibited the highest day-old body weight, comparable to the control (T1) and MOS *in ovo* group (T3), while non-inoculated groups (T5, T6, T7) had lower weights, suggesting that *in ovo* inoculation

Table 1. Description of the experimental groups

Treatment groups	Treatment Particulars	
	***In ovo* inoculation (day 18 of incubation)**	**Dietary supplementation (0-42 days of age)**
T1 (Control)	Normal saline	Nil
T2	*L. plantarum* (10^7 CFU/egg)	Nil
T3	MOS (0.5% solution)	Nil
T4	*L. plantarum* (10^7 CFU/egg) + MOS (0.5% solution)	Nil
T5	Nil	*L. plantarum* (10^7 CFU/kg)
T6	Nil	MOS (5g/kg)
T7	Nil	*L. plantarum* (10^7 CFU/kg) + MOS (5g/kg)

Table 2. Body weight (Mean ±SE) of broilers at weekly intervals in different treatments, g

Age (weeks)	Treatments							p- value
	T1	T2	T3	T4	T5	T6	T7	
Day-old	45.08^{a} ±0.53	43.77^{ab} ±0.80	44.81^{a} ±0.48	45.32^{a} ±0.67	42.32^{b} ±0.76	42.12^{b} ±0.58	42.25^{b} ±0.48	0.001
1	147.48^{bc} ±3.63	152.01^{abc} ±3.72	157.27^{ab} ±2.73	153.73^{abc} ±3.47	145.58^{c} ±3.36	160.20^{a} ±3.57	154.35^{abc} ±3.66	0.04
2	386.86^{a} ±9.56	359.96^{b} ±8.40	410.58^{a} ±10.33	395.73^{a} ±5.81	335.79^{c} ±9.27	410.76^{a} ±6.89	388.75^{a} ±7.59	0.001
3	691.07^{ab} ±17.31	668.79^{b} ±18.22	685.02^{ab} ±10.56	699.61^{ab} ±11.20	594.07^{c} ±12.69	686.12^{ab} ±14.71	729.73^{a} ±15.24	0.001
4	1079.14^{bc} ±27.58	1071.65^{c} ±20.56	1143.01^{ab} ±23.13	1182.73^{a} ±18.86	1055.51^{c} ±21.91	1153.17^{a} ±22.56	1177.72^{a} ±26.06	0.001
5	1573.70^{b} ±38.49	1580.52^{b} ±30.99	1682.10^{a} ±33.42	1718.51^{a} ±23.63	1560.30^{b} ±30.03	1642.84^{ab} ±31.37	1739.86^{a} ±34.56	0.001
6	1975.21^{c} ±42.20	2005.62^{bc} ±46.64	2089.39^{bc} ±38.35	2128.23^{ab} ±32.11	1978.61^{c} ±42.85	2102.16^{bc} ±40.94	2229.11^{a} ±45.18	0.001

Mean values bearing different superscripts within a row differ significantly ($p<0.05$)

effectively improves hatch weight. These findings are in alignment with the observations of Dunislawska *et al*. (2017) who reported higher hatch-weight in broiler chicken *in ovo* inoculated with a combination of *L. plantarum* and Raffinose Family Oligosaccharide. Similarly, Swapnil *et al*. (2021) reported improved day-old body weigh in broilers *in ovo* inoculated with MOS and its synbiotic combination. By one week, the dietary MOS group (T6) outperformed the *L. plantarum* group (T5) and control (T1), indicating that MOS enhances early growth. By two weeks, both the MOS *in ovo* group (T3) and dietary MOS group (T6) maintained superior weights and was comparable with the synbiotic groups. As the study progressed to weeks three through six, synbiotic treatments consistently yielded higher body weights compared to probiotic groups. These findings are in agreement with the observations of Swapnil *et al*. (2021) who reported improved fifth week body weight as a result of *in ovo* inoculation of both prebiotic and synbiotic, while Slawinska *et al*. (2020) and Stasiak *et al*. (2021) reported increased final body weight in the broiler chicken *in ovo* inoculated with prebiotics. Similarly, Abd-El-Latif and Omar (2023) observed that the dietary synbiotic supplementation improved the body weight of broiler chickens than the control group, while was comparable between the dietary probiotic and prebiotic supplemented groups.

Contrary to the current findings, Stasiak *et al*, (2021) stated that *in ovo* inoculation of synbiotic reduced the body weight in broiler chicken compared to the control. This variation may be due to the difference in the probiotic strains used.

Body weight gain

The mean body weight gain in weekly intervals are presented in Table 3. During the first week, the dietary MOS group (T6) showed the highest weight gain, significantly outperforming the control (T1) and dietary *L. plantarum* group (T5). In the second week, both *in ovo* and dietary MOS groups (T3 and T6) continued to demonstrate superior body weight gain followed by *in ovo* and dietary synbiotic groups. By the third week, the dietary synbiotic group (T7) had the highest weight gain, suggesting a synergistic effect from combining *L. plantarum* and MOS. During fourth week, the prebiotic groups (T3 and T6), *in ovo* synbiotic group (T4) and dietary probiotic group (T5) showed higher weight gain followed by the dietary synbiotic group (T7). In weeks five and six, the dietary combination group consistently showed the greatest gains, highlighting the sustained benefits of synbiotics. Cumulatively, the highest weight gains were in the dietary combination group (T7), followed by *in ovo* synbiotic group (T4), while the lowest were in the dietary *L. plantarum* and control groups, indicating that single additives may be less effective. In agreement with present findings, Sohail *et al*. (2013) stated increased early stage body weight gain in dietary synbiotic supplemented groups. Similary, Ghasemi and Taherpour (2013), Bogucka *et al*. (2019), Karimian and Rezaeipour (2020) and Song *et al*. (2022) reported higher body weight gain in broiler chicken dietary supplemented with synbiotics. Swapnil *et al*. (2021) reported higher body weight gain in *in ovo* prebiotic and synbiotic groups than *in ovo* probiotic groups. Higher body weight gain in MOS dietary supplemented groups were reported by Bozkurt *et al*. (2008), Barros *et al*. (2015) and Shah *et al*. (2019). *In ovo* probiotic group had lowest body weight gain in this study, which is in alignment with Guo *et al*. (2023) who observed lower weight gain in *in ovo* probiotic groups than normal saline supplemented control.

Contrary to the present findings, McCann *et al*. (2006) reported reduced weight gain in synbiotic group and Peng *et al*. (2016) and Wang *et al*. (2017) reported increased body weight gain in dietary *L. plantarum* supplemented groups. Also, Pender *et al*. (2017) and Abdel-Moneim *et al*. (2020) reported higher early stage body weight gain in *in ovo* probiotic groups.

Feed consumption

The mean feed consumption of birds at weekly intervals is presented in Table 4. During the first two weeks, no significant differences in feed intake were observed, indicating that *in ovo* inoculation or dietary supplementation had little effect during early stages, consistent with findings of Benites *et al*. (2008) and Kamran *et al*. (2013), who reported similar feed intake in MOS dietary supplemented groups during early stages. By the third week, significant differences emerged, with the highest feed intake in the dietary synbiotic group (T7). Similar trends were noted in the fourth week, where groups received synbiotic (T4 and T7) and prebiotic (T3 and T6) showed higher feed consumption compared to the *L. plantarum in ovo* group (T2). However, by the fifth and sixth weeks, differences in feed intake diminished and the cumulative feed consumption was similar among the treatment groups. Supporting to these findings, Barros *et al*. (2015) reported increased feed intake in dietary MOS supplemented group during early stage and Duan *et al*. (2021) who reported improved feed intake in *in ovo* synbiotic groups. Similarly, the cumulative feed intake was not affected by the supplementations in the present study is in alignment with the observations of Elangia *et al.* (2024) who reported similar cumulative feed intake in broilers fed with *L. planarum*, MOS and their combination. The probiotic *in ovo* and dietary supplemented groups and control group had similar feed intake which was in agreement with Majidi-Mosleh *et al*. (2017) and Pender *et al*. (2017) in *in ovo* probiotic supplementation and Wang *et al*. (2015) and Lenkova *et al*. (2019) in dietary *L. plantarum* supplementation, which was contrary to Liu *et al*. (2023) who reported higher feed intake in *L. plantarum* dietary supplemented group.

Feed conversion ratio (FCR)

The mean feed conversion ratio of broilers in different treatment groups at weekly intervals and cumulative period (Day-old to 6 weeks) presented in Table 5 did not show any significant difference between the treatment groups throughout the experimental period except for the second week. By the second week, *in ovo* probiotic (T2) and dietary probiotic groups (T5) demonstrated higher FCR, reflecting lower efficiency, while the control (T1), *in ovo* MOS (T3), and dietary MOS (T6) groups had better FCR. The improved FCR with dietary MOS supplementation during early stage aligns with the results of Kamran *et al*. (2013) who reported improved FCR on dietary MOS supplementation. In contrast to these findings, Benites *et al*. (2008) and Barros *et al*. (2015) reported no significant effects of dietary MOS on FCR. Cumulatively, while no significant differences were found, the dietary synbiotic group (T7) had the numerically lowest FCR, indicating the best overall efficiency, whereas the *in ovo* synbiotic group (T4) exhibited slightly lower efficiency. This observation aligns with the findings of Karimian and Rezaeipour (2020) who reported that dietary prebiotic supplementation can effectively enhance feed efficiency under specific conditions.

Conclusion

The dietary synbiotic supplementation improved the body weight and weight gain during the study period followed by *in ovo* synbiotic supplementation. *In ovo* inoculation of prebiotic and synbiotic resulted in increased hatch weight and had a performance similar to dietary groups during most of the weeks. The study

Table 3. Body weight gain (Mean ±SE) of broilers at weekly intervals in different treatments, g

Age (weeks)	Treatments							p- value
	T1	T2	T3	T4	T5	T6	T7	
1	102.40[b] ±3.48	108.24[ab] ±3.50	112.45[ab] ±2.64	108.41[ab] ±3.15	103.26[b] ±3.10	118.08[a] ±3.29	112.10[ab] ±3.45	0.01
2	239.38[a] ±7.61	207.95[b] ±7.19	253.32[a] ±9.24	242.00[a] ±3.24	189.88[b] ±6.71	250.56[a] ±5.34	234.39[a] ±5.26	0.001
3	304.21[b] ±11.40	308.83[ab] ±16.52	274.43[bc] ±12.03	303.88[b] ±10.20	258.28[c] ±10.56	275.36[bc] ±10.41	340.98[a] ±11.81	0.001
4	388.07[c] ±15.73	402.86[bc] ±20.58	457.99[a] ±16.40	483.12[a] ±12.59	461.44[a] ±16.76	467.05[a] ±16.69	447.99[ab] ±23.99	0.001
5	494.56[bc] ±17.32	508.87[bc] ±17.22	539.10[ab] ±14.89	535.79[abc] ±12.01	504.80[bc] ±16.44	489.67[c] ±13.34	562.14[a] ±17.03	0.01
6	401.52[b] ±17.41	425.10[b] ±25.12	407.29[b] ±16.17	409.72[b] ±20.31	418.31[b] ±20.76	459.32[ab] ±18.02	489.25[a] ±15.40	0.01
Cumulative (0-6)	1930.14[d] ±7.06	1961.85[cd] ±38.78	2044.58[bc] ±30.45	2082.91[b] ±34.08	1935.96[d] ±5.66	2060.04[bc] ±48.03	2186.86[a] ±34.63	0.001

Mean values bearing different superscripts within a row differ significantly (p<0.05)

Table 4. Feed consumption (Mean ±SE) of broilers at weekly intervals in different treatments, g

Age (weeks)	Treatments							p-value
	T1	T2	T3	T4	T5	T6	T7	
1	155.78 ±5.21	156.45 ±8.91	156.04 ±9.24	148.90 ±14.64	130.51 ±11.05	159.08 ±14.37	152.50 ±14.14	0.65[ns]
2	334.10 ±5.50	335.26 ±8.58	339.25 ±6.03	364.27 ±10.84	325.38 ±16.48	348.66 ±11.59	341.96 ±1.81	0.21[ns]
3	506.12[ab] ±11.28	494.77[ab] ±5.83	479.73[b] ±3.49	491.48[ab] ±1.94	428.19[c] ±4.38	484.53[b] ±29.66	535.44[a] ±19.56	0.01*
4	575.31[bc] ±14.59	529.12[c] ±26.91	644.82[a] ±33.43	669.86[a] ±14.66	602.12[ab] ±22.08	647.11[a] ±18.05	648.09[a] ±11.35	0.01*
5	938.76 ±6.35	936.29 ±26.40	878.09 ±9.18	1023.72 ±19.64	945.97 ±71.21	905.84 ±58.68	1007.62 ±25.74	0.17[ns]
6	940.49 ±47.63	872.15 ±76.51	835.77 ±25.7	949.70 ±64.52	893.07 ±93.16	887.33 ±20.08	848.10 ±25.96	0.75[ns]
Cumulative (0-6)	3450.56 ±54.58	3324.04 ±132.57	3333.70 ±37.79	3647.94 ±58.90	3325.24 ±120.50	3432.55 ±43.63	3533.72 ±48.71	0.09[ns]

Mean values bearing different superscripts within a row differ significantly ($p<0.05$)

Table 5. Feed conversion ratio (Mean ±SE) of broilers at weekly intervals in different treatments

Age (weeks)	Treatments							p- value
	T1	T2	T3	T4	T5	T6	T7	
1	1.53 ±0.12	1.45 ±0.07	1.39 ±0.09	1.37 ±0.07	1.26 ±0.08	1.34 ±0.05	1.35 ±0.05	0.36
2	1.40[c] ±0.05	1.62[ab] ±0.1	1.34[c] ±0.04	1.51[bc] ±0.07	1.72[a] ±0.06	1.39[c] ±0.08	1.46[bc] ±0.02	0.01
3	1.67 ±0.07	1.61 ±0.07	1.76 ±0.10	1.63 ±0.11	1.66 ±0.03	1.76 ±0.07	1.59 ±0.11	0.67
4	1.49 ±0.05	1.31 ±0.05	1.41 ±0.00	1.39 ±0.06	1.31 ±0.07	1.39 ±0.05	1.50 ±0.18	0.57
5	1.90 ±0.02	1.84 ±0.04	1.63 ±0.08	1.91 ±0.07	1.87 ±0.08	1.86 ±0.15	1.79 ±0.02	0.26
6	2.35 ±0.12	2.05 ±0.14	2.07 ±0.12	2.32 ±0.13	2.15 ±0.25	1.95 ±0.12	1.74 ±0.04	0.11
Cumulative (0-6)	1.79 ±0.02	1.69 ±0.04	1.63 ±0.01	1.75 ±0.04	1.72 ±0.06	1.67 ±0.06	1.62 ±0.03	0.10

Mean values bearing different superscripts within a row differ significantly ($p<0.05$)

concludes that *in ovo* supplementation of prebiotics and synbiotics resulted in better early growth performance having persistent effects till market age and had similar performance with dietary prebiotic and synbiotic groups. Therefore, it is recommended that *in ovo* inoculation of prebiotic and synbiotic can be used as an alternative for its dietary supplementation in broiler chicken to improve early growth performance. Further research needs to be conducted to evaluate the combined beneficial effects of both *in ovo* and dietary supplementation in broiler chicken.

Acknowledgements

The authors are very thankful to Kerala Veterinary and Animal Sciences University for the facility provided.

Conflict of interests

The authors declare that they have no conflict of interest.

References

Abd-El-Latif, M.A. and Omar, M.O. 2023. Productive performance, digestibility, blood parameters and intestine microbiology of broiler chicks affected by prebiotic, probiotic and synbiotic addition. *Egypt. Poult. Sci. J.* **43**: 217-237.

Abdel-Moneim, A.M.E., Elbaz, A.M., Khidr, R.E.S. and Badri, F.B. 2020. Effect of *in ovo* inoculation of *Bifidobacterium spp.* on growth performance, thyroid activity, ileum histomorphometry, and microbial enumeration of broilers. *Probiotics Antimicrob. Proteins*. **12**: 873-882.

AOAC [Association of Official Analytical Chemists]. 2016. *Official Methods of Analysis*. (20th Ed.). Association of Official Analytical Chemists International, Rockville, Maryland, 1885p.

Barros, V.R.S.M.D., Lana, G.R.Q., Lana, S.R.V., Lana, Â.M.Q., Cunha, F.S.A. and Neto, J.V.E. 2015. β-mannanase and mannan oligosaccharides in broiler chicken feed. *Ciênc. Rural*. **45**: 111-117.

Benites, V., Gilharry, R., Gernat, A.G. and Murillo, J.G. 2008. Effect of dietary mannan oligosaccharide from Bio-MOS or SAF-Mannan on live performance of broiler chickens. *J. Appl. Poult. Res.* **17**: 471-475.

BIS [Bureau of Indian Standards]. 2007. *Poultry Feed Specification*. (5th Ed.). Bureau of Indian Standards, New Delhi, 30p.

Bogucka, J., Ribeiro, D.M., Bogusławska-Tryk, M., Dankowiakowska, A., da Costa, R.P.R. and Bednarczyk, M. 2019. Microstructure of the small intestine in broiler chickens fed a diet with probiotic or synbiotic supplementation. *J. Anim. Physiol. Anim. Nutr*. **103**: 1785-1791.

Bozkurt, M., Küçükyilmaz, K., Çatli, A.U. and Çinar, M. 2008. Growth performance and slaughter characteristics of broiler chickens fed with antibiotic, mannan oligosaccharide and dextran oligosaccharide supplemented diets. *Int. J. Poult. Sci*, **7**: 969-977.

Das, R., Mishra, P. and Jha, R. 2021. *In ovo* feeding as a tool for improving performance and gut health of poultry: a review. *Front. Vet. Sci*. **8**: 754246.

Duan, A.Y., Ju, A.Q., Zhang, Y.N., Qin, Y.J., Xue, L.G., Ma, X., Luan, W.M. and Yang, S.B. 2021. The effects of *in ovo* injection of synbiotics on the early growth performance and intestinal health of chicks. *Front. Vet. Sci.* **8**: 658301. https://doi.org/10.3389/fvets.2021.658301

Dunislawska, A., Slawinska, A., Stadnicka, K., Bednarczyk, M., Gulewicz, P., Jozefiak, D. and Siwek, M. 2017. Synbiotics for broiler chickens-*in vitro* design and evaluation of the influence on host and selected microbiota populations following *in ovo* delivery. *PloS one*. **12**: 168587. https://doi.org/10.1371/journal.pone.0168587

Elangia, N., Senthil Murugan, S., Chacko, B., Ananth, D. and Vergis, J. 2023. Nutrient digestibility and growth promoting potential of *Lactobacillus plantarum* culture as probiotics in broilers. *J. Vet. Anim. Sci*. **55**: 330-337.

Ghasemi, H.A. and Taherpour, K. 2013. Comparative effects of probiotic, prebiotic and synbiotic supplements on performance, jejunal morphology, serum lipid profile and antibody response of broiler chicks. *J. Livest. Sci. Technol*. **1**: 20-27.

Guo, W., Wang, C., Qin, K., Shi, H., Yang, X. and Yang, X. 2023. *Lactobacillus plantarum* injection at the embryonic stage alters the early growth performance and lipid metabolism of broilers by specific genera of bacteria. *Poult. Sci*. **102**: 102522. https://doi.org/10.1016/j.psj.2023.102522.

Kadam, M.M., Barekatain, M.R., Bhanja, S.K., Iji, P.A. 2013. Prospects of *in ovo* feeding and nutrient supplementation for poultry: the science and commercial applications- a review. *J. Sci. Food Agric*. **93**: 3654-3661.

Kamran, Z., Mirzaa, M.A., Ahmad, S., Samad, H.A., Sohail, M.U. and Saadullahb, M. 2013. Performance of broiler chickens fed mannan oligosaccharides as alternatives to antibiotics from one to twenty-two days of age. *J. Anim. Plant Sci*. **23**: 1482-1485.

Karimian, R.A. and Rezaeipour, V. 2020. Effects of dietary Mannan-oligosaccharides and phytase supplementation alone or in combination on growth performance, serum metabolites, cecal microbiota activity and intestinal morphology in broiler chickens. *Poult. Sci. J.* **8**: 27-32.

Lenkova, T., Nikonov, I., Kuznetsov, Y., Karpenko, L. and Balykina, A. 2019. Development of the probiotic feed supplement based on *Lactobacillus plantarum* to increase the broiler productivity. *Int. J. Innov. Technol. Explor. Eng*. **9**: 2452-2454.

Liu, L., Li, L., Li, C., Wang, H., Zhang, X., Ren, Q., Zhang, H., Jin, N., Li, C. and Zhao, C. 2023. Effects of *Lactiplantibacillus plantarum* LPJZ-658 supplementation on the production, meat quality, intestinal morphology, and caecal microbiota of broilers chickens. *Microorganisms*. **11**:1549.https://doi.org/10.3390/microorganisms11061549

Majidi-Mosleh, A., Sadeghi, A.A., Mousavi, S.N., Chamani, M. and Zarei, A. 2017. Effects of *in ovo* infusion of probiotic strains on performance parameters, jejunal bacterial population and mucin gene expression in broiler chicken. *Braz. J. Poult. Sci.* **19**: 97-102.

McCann, M.E.E., Newell, E., Preston, C. and Forbes, K. 2006. The use of mannan-oligosaccharides and/or tannin in broiler diets. *Int. J. Poult. Sci.* **5**: 873-879.

Panda, A.K., Shyam Sunder, G., Rama Rao, S.V. and Raju, M.V.L.N. 2006. Early nutrition enhances growth and speeds up gut development. *Wld. Poult.* **22**: 15-16.

Pender, C.M., Kim, S., Potter, T.D., Ritzi, M.M., Young, M. and Dalloul, R.A. 2017. *In ovo* supplementation of probiotics and its effects on performance and immune-related gene expression in broiler chicks. *Poult. Sci.* **96**: 1052-1062.

Peng, Q., Zeng, X.F., Zhu, J.L., Wang, S., Liu, X.T., Hou, C.L., Thacker, P.A. and Qiao, S.Y. 2016. Effects of dietary *Lactobacillus plantarum* B1 on growth performance, intestinal microbiota, and short chain fatty acid profiles in broiler chickens. *Poult. Sci.* **95**: 893-900.

Reshma, R., Chacko, B., Anitha, P., Prasoon, S. and Chandrasekhar, L. 2023. Effect of probiotics and rice on ileal microbial count and intestinal morphometry in broiler chicken. *J. Vet. Anim. Sci.* **54**: 443-449

Shah, R., Paswan, V., Alolofi, A. and Yadav, S. 2019. Effect of mannan oligosaccharide prebiotic supplementation on growth and production performance of broiler chickens. *Int. J. Livest. Res*. **9**: 85-91.

Slawinska, A., Zampiga, M., Sirri, F., Meluzzi, A., Bertocchi, M., Tavaniello, S. and Maiorano, G. 2020. Impact of galactooligosaccharides delivered *in ovo* on mitigating negative effects of heat stress on performance and welfare of broilers. *Poult. Sci*. **99**: 407-415.

Sohail, M.U., Ijaz, A., Younus, M., Shabbir, M.Z., Kamran, Z., Ahmad, S., Anwar, H., Yousaf, M.S., Ashraf, K., Shahzad, A.H. and Rehman, H. 2013. Effect of supplementation of mannan oligosaccharide and probiotic on growth performance, relative weights of viscera, and population of selected intestinal bacteria in cyclic heat-stressed broilers. *J. Appl. Poultry Res.* **22**: 485-491.

Song, D., Li, A., Wang, Y., Song, G., Cheng, J., Wang, L., Liu, K., Min, Y. and Wang, W. 2022. Effects of synbiotic on growth, digestibility, immune and antioxidant performance in broilers. *Animal*. **16**: 100497. https://doi.org/ 10.1016/j.animal.2022.100497

Stasiak, K., Slawinska, A. and Bogucka, J. 2021. Effects of probiotics, prebiotics and synbiotics injected *in ovo* on the microstructure of the breast muscle in different chicken genotypes. *Animals*. **11**: 2944. https://doi.org/10.3390/ani11102944

Sun, Y., Zhang, S., Li, H., Zhu, J., Liu, Z., Hu, X. and Yi, J. 2022. Assessments of probiotic potentials of *Lactiplantibacillus plantarum* strains isolated from Chinese traditional fermented food: phenotypic and genomic analysis. *Front. Microbiol.* **13**: 895132. https://doi.org/10.3389/fmicb.2022.895132

Swapnil, S., Kanagaraju, P., Srinivasan, G., Kumanan, K. and Rathnapraba, S. 2021. Effect of *in ovo* feeding of probiotic, prebiotic and synbiotic to Broiler embryos on growth performance, Mucin-2 gene expression and gut colonization of microbiota. *Indian J. Anim. Sci*. **91**: 51-57.

Uni, Z. and Ferket, P.R. 2003. Enhancement of development of oviparous species by *in ovo* feeding. US Patent No. *6592878*.

Wang, S., Peng, Q., Jia, H.M., Zeng, X.F., Zhu, J.L., Hou, C.L., Liu, X.T., Yang, F.J. and Qiao, S.Y. 2017. Prevention of *Escherichia coli* infection in broiler chickens with *Lactobacillus plantarum* B1. *Poult. Sci.* **96**: 2576-2586. ■