Iranian Journal of Livestock Science, 2024, VOL. 1, NO. 1: 41-48

Iranian Journal of Livestock Science
journal homepage: www.ijls.yu.ac.ir

Comparative Effects of *Serendipita indica* and *Lactobacillus* spp. on Performance Parameters and Intestinal Microbial Populations in Broiler Flocks

Mahdi Hedayati[1*], Maryam Safari[1], Saeed Khalaji[1], Mehdi Ghabooli[2]

[1] *Department of Animal Science, Faculty of Agriculture, Malayer University, Malayer,Iran.*
[2] *Department of Plant Production and Genetics, Faculty of Agriculture, Malayer University, Malayer,Iran.*

Article Info

Iranian Journal of Livestock Science, 2024, VOL. 1, No. 1: 41-48
https://doi.org/10.61186/IJLS.1.1.41

Article History
Received: December 7th 2023
Revised: May 27th 2024
Accepted: June 8th 2024
Published: June 27th 2024

ABSTRACT

A study was conducted to examine the comparative impacts of *Serendipita indica* (**SI**), Lactobacillus spp. probiotics (**LP**), and autoclaved *Serendipita indica* (**AS**) on the performance, biochemical factors, and intestinal microbial populations of 240 one-day-old male Ross 308 chicks. The chicks were randomly allocated into four experimental groups, each with six replications of ten chicks: Group 1 (control) received a basal diet without feed additives; Group 2 received a basal diet supplemented with 0.6% SI; Group 3 received a basal diet with 0.6% AS; and Group 4 received a basal diet with 0.2% LP. The study spanned from day 1 to day 42. The findings indicated that including SI and LP significantly increased body weight, while the LP group exhibited a notable increase in feed intake. The feed conversion ratio (**FCR**) differed significantly compared to the control group ($P < 0.05$). Blood serum protein analysis showed no significant disparities among groups. Regarding the weight of immune organs, the LP group had the highest weight, whereas the control group showed the lowest. Additionally, the LP group demonstrated a significant rise in immune titers against Newcastle disease after 35 days. In terms of cecal bacterial populations at 28 and 35 days, the probiotic group had the lowest counts, followed by the AS group, while the control group exhibited the highest counts. Overall, the group receiving probiotics showed the most significant improvements in performance traits, with the autoclaved *Serendipita indica* group following closely behind. This study highlights the potential benefits of probiotics and prebiotics in poultry nutrition.

Keywords: Broiler, *Lactobacillus*, Performance, *Serendipita indica*

INTRODUCTION

The use of medicinal herbs as safe additives in poultry feed for treating animal diseases is considered cost-effective and environmentally friendly, with no adverse effects or complications (Sevim & Ayasan, 2020). *Serendipita indica* (synonym: *Piriformospora indica*) is a fungal species belonging to the order Sebacinales. This root endophytic fungus, abundant in mannan oligosaccharides, was isolated from the rhizosphere soil of plants thriving in the extreme heat of the Thar Desert in Rajasthan, India, including orchid plants. *S. indica* can be cultivated on various substrates and has been shown to enhance plant growth through its mutualistic symbiotic relationships with a wide range of plants (Varma *et al.*, 1999). Despite this, limited research has been conducted on the impact of this fungus on poultry performance. Soleimanpour *et al.* (2020) revealed that *P. indica*, as a promising prebiotic candidate, could help broilers achieve high-performance levels while maintaining gut health and morphology.

***Corresponding author**
Postal Address: Malayer University, Kilometer 4, Malayer-Arak Road, Malayer, 65719-95863
E-mail: mahvet77@gmail.com or hedayati@malayeru.ac.ir

[DOI: 10.61186/ijls.1.1.41]

Introducing beneficial probiotic microorganisms into the diet of chicks during this crucial early stage may enhance overall health and performance throughout the production cycle (Shivaramaiah *et al.*, 2011). The growing consumer demand for poultry products free from chemical residues has led to global efforts to identify environmentally friendly and healthy alternatives to enhance animal health and performance (Wolfenden *et al.*, 2010). These alternative approaches include reducing antibiotic use and improving livestock gut health. Probiotics are live microorganisms that confer health benefits to their hosts when consumed in adequate amounts (Hedayati *et al.*, 2022). Among the available probiotic species, Lactobacillus, Saccharomyces, Bacillus, Streptococcus, and Aspergillus have been shown to play beneficial roles in poultry nutrition (Zhang *et al.*, 2005). The use of probiotic Bacillus bacteria as feed additives has been widely considered due to their ability to withstand various environmental stresses, including storage, transport, and feed pelleting processes, thanks to their aerobic and endospore-forming nature (Wu *et al.*, 2011). Additionally, studies have shown that probiotic yeasts like Saccharomyces can enhance the immune system of chicks without compromising their growth performance (Bai *et al.*, 2013).

In this investigation, we aim to examine the effects of autoclaved *Serendipita* fungi, non-autoclaved *Serendipita* fungi, and a commercial probiotic (Parsi Lact®) on performance, biochemical parameters, and intestinal microbial populations.

MATERIALS AND METHODS

The Animal Care Committee of the Iranian Council of Animal Care approved the procedures and guidelines for the experiments conducted (1995, NO. IACUC95). The study involved 240 one-day-old male Ross 308 broilers, which were randomly assigned to four experimental groups with six replications. Each replicate consisted of ten chickens fed a corn-soybean diet for five weeks. The diet was formulated using WUFFDA software based on the breeding method for Ross 308 broilers manual (2014; Table 1).

Table 1. Ingredients and chemical compositions of the basal diet in two periods

Item	Grower diet (0-21 d)	Finisher diet (22-35 d)
Ingredient (%)		
Corn	58.78	63.70
Soybean meal	34.62	29.32
Soybean oil	2.78	3.39
Dicalcium phosphate	1.63	1.46
Limestone	0.78	0.74
Sodium chloride	0.30	0.30
Sodium bicarbonate	0.10	0.10
L-Lysine-HCl (98%)	0.15	0.16
Dl-Methionine (98%)	0.28	0.26
l-Threonine	0.08	0.07
Vitamin and mineral premix[1]	0.50	0.50
Calculated composition (%, unless otherwise stated)		
Metabolizable energy (MJ/kg)	12.60	13.02
Crude protein	20.80	18.90
Calcium	0.84	0.78
Available phosphorous	0.42	0.38
Sodium	0.16	0.16
Lysine	1.11	1.00
Methionine	0.56	0.52
Methionine+cysteine	0.84	0.78
Threonine	0.75	0.67
Dietary cation anion balance (mEq/kg)	224	200

[1]Supplied per kg diet: Vitamin A 9000 IU, vitamin D_3 2000 IU, vitamin E 18 IU, vitamin K_3 2 mg, riboflavin 6.6 mg, pantothenic acid 10 mg, pyridoxine 3 mg, folic acid 1 mg, thiamin 1.8 mg, B_{12} 15 µg, biotin 0.1 mg, niacin 30 mg, choline 500 mg, selenium 0.2 mg, iodine 1 mg, copper 10 mg, iron 50 mg, zinc 85 mg, manganese 100 mg.

[DOI: 10.61186/ijls.1.1.41]

The experimental groups were as follows: group 1 served as the control group, receiving a basal diet without any feed additives; group 2 received a basal diet with 0.6% *Serendipita indica* (**SI**); group 3 received a basal diet with 0.6% autoclaved SI (**AS**); and group 4 received a basal diet with 0.2% Lactobacillus probiotic (**LP**). During the study, Parsi Lact® (Pardis Roshd Mehregan, Shiraz, Iran) was used as a probiotic, containing 6 × 10^9 cfu/g of Bacillus and Lactobacillus spp.

Piriformospora indica was cultured according to the method outlined by Ghabooli *et al.* (2013). The cultures were grown on a complex agar medium (15 g/L agar) at 28 ± 1°C. For liquid culture, discs from the actively growing edges of the petri dish culture were transferred to 250 mL Erlenmeyer flasks containing 100 mL of complex medium without agar and placed on a gyratory shaker at 150 rpm for 6–7 days. After the growth phase, the culture medium was combined with varying amounts of wheat bran (100, 200, 400, 600, and 800 mL) and incorporated into different diets to create diets with varying levels of *S. indica* (Babamir *et al.*, 2018; Ghabooli *et al.*, 2013).

Performance traits examined in this research included body weight (**BW**), feed consumption (**FC**), and feed conversion ratio (**FCR**), which were monitored weekly. For serum biochemical analysis, two chicks were selected from each pen, and individual blood samples were collected from the brachial vein into non-heparinized tubes at 4 and 5 weeks of age. The serum was assessed for biochemical parameters such as total protein, globulin, and albumin using commercial kits (Boehringer Mannheim Hitachi 704 automatic analyzer, Japan). Antibody titers against Newcastle disease (**ND**) and Avian Influenza (**AI**) viruses were determined using the hemagglutination inhibition (**HI**) test on samples from two birds in each replicate at days 28 and 35.

To analyze cecal bacteria, one gram of cecal digesta from two birds in each replicate was homogenized in test tubes with 9 mL of phosphate-buffered saline, and a series of tenfold dilutions was prepared. Selective agar media were used to quantify *E. coli*, total coliform bacteria (Eosin Methylene Blue and MacConkey agar, Quelab, UK), and Salmonella (*Salmonella-Shigella* Agar, Quelab, UK). Results were expressed as log10 colony-forming units (**CFU**) per gram of cecal digesta, following procedures outlined by Li *et al.* (2009). Data were analyzed using SAS software (version 9.2; 2009). Treatment mean comparisons were conducted using Duncan's multiple range test (1995), with a significance level set at $P \leq 0.05$.

RESULTS

The weekly BW outcomes during the initial five weeks exhibited significant variations compared to the control group and other treatments. The impact of SI autoclaved AS, and LP on BW indicated that the inclusion of these additives notably influenced BW from day 1 to day 35 ($P < 0.05$). The probiotic group (**T4**) experienced the highest body weight gain, while the control group (**T1**) had the lowest, both significantly ($P < 0.05$; Table 2).

Table 2. The effect of *Serendipita indica*, autoclaved *Serendipita indica*, and lactobacilli probiotic on body weight (BW) (g/bird) of broilers from 1 to 35 days of age

Treatment	1-7d	1-14d	1-21d	1-28d	1-35d
T1	165.75^{c}	384.75^{c}	759.50^{d}	1217.50^{d}	1847.50^{d}
T2	174.00^{b}	400.00^{b}	799.00^{c}	1335.0^{c}	1875.00^{c}
T3	173.00^{b}	415.00^{a}	823.75^{b}	1380.00^{b}	2037.50^{b}
T4	182.25^{a}	420.00^{a}	866.00^{a}	1427.50^{a}	2060.00^{a}
SEM	0.973	4.797	3.540	11.592	8.906
p-value	0.001	0.009	0.001	0.001	0.001

T1: basal diet (based on corn and soybeans without any feed additives), T2: basal diet with 0.6% of *Serendipita indica* (SI), T3: basal diet with 0.6% of autoclaved *Serendipita indica* (AS), T4: basal diet with 0.2% of lactobacilli probiotic (LP).
Mean values within a row with different superscript letters (a, b, and c) were significantly different ($P<0.05$).

From days 0 to 35, the LP group significantly increased FI ($P < 0.05$). In contrast, the SI group

[DOI: 10.61186/ijls.1.1.41]

showed a significant decrease in FI during the first week, and subsequently, the control group exhibited reduced FI ($P < 0.05$; Table 3).

Table 3. The effect of *Serendipita indica*, autoclaved *Serendipita indica*, and lactobacilli probiotic on feed intake (FI) (g/bird) of broilers from 1 to 35 days of age

Treatment	1-7d	1-14d	1-21d	1-28d	1-35d
T1	107.50^{b}	447.50^{d}	999.82^{d}	1688.72^{d}	2620.50^{d}
T2	103.40^{c}	465.70^{c}	1012.10^{c}	1747.62^{c}	2891.25^{c}
T3	125.80^{a}	477.12^{b}	1099.95^{b}	1878.25^{b}	3092.62^{b}
T4	126.10^{a}	490.57^{a}	1115.17^{a}	1906.97^{a}	3155.50^{a}
SEM	1.24	2.83	5.49	8.07	3.39
p-value	0.001	0.001	0.001	0.001	0.001

T1: basal diet (based on corn and soybeans without any feed additives), T2: basal diet with 0.6% of *Serendipita indica* (SI), T3: basal diet with 0.6% of autoclaved *Serendipita indica* (AS), T4: basal diet with 0.2% of lactobacilli probiotic (LP).
Mean values within a row with different superscript letters (a, b and c) were significantly different (P<0.05).

The FCR during the first to fourth weeks did not show significant differences from the control group. However, by day 35, significant differences were observed ($P < 0.05$), with the SI, LP, and AS groups having higher FCR than the control group. At day 35, the control group exhibited the lowest FCR ($P < 0.05$; Table 4).

Table 4. The effect of *Serendipita indica*, autoclaved *Serendipita indica*, and lactobacilli probiotic on FCR, 1 to 35 days of age

Treatment	1-7d	1-14d	1-21d	1-28d	1-35d
T1	0.78	1.15	1.31	1.43	1.42^{b}
T2	0.72	1.16	1.28	1.36	1.54^{a}
T3	0.76	1.18	1.34	1.41	1.51^{a}
T4	0.76	1.17	1.27	1.37	1.53^{a}
SEM	0.030	0.027	0.022	0.035	0.051
p-value	0.500	0.872	0.170	0.474	0.013

T1: basal diet (based on corn and soybeans without any feed additives), T2: basal diet with 0.6% of *Serendipita indica* (SI), T3: basal diet with 0.6% of autoclaved *Serendipita indica* (AS), T4: basal diet with 0.2% of lactobacilli probiotic (LP).
Mean values within a row with different superscript letters (a, b, and c) were significantly different (P<0.05).

The levels of total protein, albumin, and globulin in serum were not affected by the addition of Lactobacillus probiotics or *Piriformospora indica* in the diets (Table 5).

Table 5. The effect of *Serendipita indica*, autoclaved *Serendipita indica*, and lactobacilli probiotic on biochemical parameters in broilers at 28 and 35 days of age (mg/dl)

Treatment	28d			35d		
	Total protein	Albumin	Globulin	Total protein	Albumin	Globulin
T1	3.33	1.42	1.91	3.37	1.55	1.82
T2	3.28	1.40	1.88	3.47	1.57	1.90
T3	3.30	1.40	1.90	3.47	1.57	1.90
T4	3.32	1.40	1.92	3.42	1.53	1.89
SEM	0.094	0.082	0.047	0.106	0.054	0.208
p-value	0.993	0.890	0.610	0.618	0.793	0.853

T1: basal diet (based on corn and soybeans without any feed additives), T2: basal diet with 0.6% of *Serendipita indica* (SI), T3: basal diet with 0.6% of autoclaved *Serendipita indica* (AS), T4: basal diet with 0.2% of lactobacilli probiotic (LP).
Mean values within a row with different superscript letters (a, b, and c) were significantly different (P<0.05).

The findings indicated that supplementing diets with SI, AS, and Lactobacillus probiotics significantly impacted the immune organs in the control group. The Lactobacillus probiotics

[DOI: 10.61186/ijls.1.1.41]

increased the weight of immune organs at 28 and 35 days, while the control group exhibited lower weights of the bursa of Fabricius, thymus, and spleen at these ages (Table 6).

Table 6. The effect of *Serendipita indica*, autoclaved *Serendipita indica*, and lactobacilli probiotic on immune organs weight in broilers at 28 and 35 days of age (g/100gBW)

Treatment	28d			35d		
	Bursa of fabricius	Thymus	Spleen	Bursa of fabricius	Thymus	Spleen
T1	0.15^{b}	0.24^{c}	0.08^{c}	0.12^{c}	0.37^{b}	0.08^{d}
T2	0.18^{a}	0.26^{b}	0.11^{a}	0.18^{a}	0.39^{b}	0.12^{b}
T3	0.18^{a}	0.28^{b}	0.10^{b}	0.16^{b}	0.33^{c}	0.10^{c}
T4	0.18^{a}	0.30^{a}	0.12^{a}	0.18^{a}	0.41^{a}	0.13^{a}
SEM	0.004	0.004	0.002	0.006	0.010	0.002
p-value	0.002	0.001	0.001	0.001	0.001	0.001

T1: basal diet (based on corn and soybeans without any feed additives), T2: basal diet with 0.6% of *Serendipita indica* (SI), T3: basal diet with 0.6% of autoclaved *Serendipita indica* (AS), T4: basal diet with 0.2% of lactobacilli probiotic (LP).
Mean values within a row with different superscript letters (a, b, and c) were significantly different ($P<0.05$).

Results for humoral immunity against ND and AI showed that supplementation with SI, AS, and Lactobacillus probiotics did not significantly affect the control group at 28 days. However, at 35 days, the Lactobacillus probiotic increased the ND titer compared to the other groups (Table 7).

Table 7. The effect of *Serendipita indica*, autoclaved *Serendipita indica*, and lactobacilli probiotic on Antibody in broilers at 28 and 35 days of age (log2)

Treatment	28d		35d	
	AI	ND	AI	ND
T1	3.50	1.65	3.00	2.00c
T2	3.80	1.85	2.85	2.05b
T3	3.90	1.95	2.90	2.05b
T4	3.75	1.90	2.75	2.20a
SEM	0.279	0.714	0.994	0.176
p-value	0.55	0.489	0.659	0.001

T1: basal diet (based on corn and soybeans without any feed additives), T2: basal diet with 0.6% of *Serendipita indica* (SI), T3: basal diet with 0.6% of autoclaved *Serendipita indica* (AS), T4: basal diet with 0.2% of lactobacilli probiotic (LP).
Mean values within a row with different superscript letters (a, b, and c) were significantly different ($P<0.05$).

The cecum of broilers at days 28 and 35 showed a significant decrease in populations of coliforms, E. coli, and Salmonella across all treatment groups compared to the control group ($P < 0.05$; Table 8).

Table 8. The effect of *Serendipita indica*, autoclaved *Serendipita indica*, and lactobacilli probiotic on colony count of cecum in broilers at 28 and 35 days of age (log10 cfu/g)

Treatment	28d			35d		
	salmonella	e.coli	coliforms	salmonella	e.coli	coliforms
T1	7.11a	6.94a	7.20a	7.45a	8.22a	8.18a
T2	5.93c	6.89b	6.52c	7.21b	8.12b	8.10b
T3	6.00b	5.69c	6.88b	6.76c	8.06c	7.23c
T4	5.86d	6.65d	6.45d	5.59d	7.71d	7.11d
SEM	0.009	0.011	0.019	0.015	0.020	0.025
p-value	0.001	0.001	0.001	0.001	0.001	0.001

T1: basal diet (based on corn and soybeans without any feed additives), T2: basal diet with 0.6% of *Serendipita indica* (SI), T3: basal diet with 0.6% of autoclaved *Serendipita indica* (AS), T4: basal diet with 0.2% of lactobacilli probiotic (LP).
Mean values within a row with different superscript letters (a, b, and c) were significantly different ($P<0.05$).

[DOI: 10.61186/ijls.1.1.41]

DISCUSSION

The group that received *Piriformospora indica* spores experienced a significant increase in body weight gain, as noted by Babamir *et al.* (2018). Similarly, Soleimanpour *et al.* (2020) found that adding *Piriformospora indica* at a dosage of 0.4 g/kg of diet also resulted in increased body weight gain. In a study by Farmani *et al.* (2022), broiler diets supplemented with *Piriformospora indica* at a dosage of 10 mg/kg showed significant improvements in body weight gain at 5 and 6 weeks of rearing. These findings suggest that probiotic supplementation with *Piriformospora indica* may enhance final body weight due to its ability to eliminate harmful bacteria, promote beneficial bacteria growth, compete for colonization sites and nutrients, reduce toxic compounds, modulate the mucosal immune system, and enhance dietary digestibility, leading to improved nutrient absorption (Applegate *et al.*, 2010).

Zhang *et al.* (2005) reported similar positive effects on performance and feed efficiency in broilers with probiotic supplementation. However, Soleimanpour *et al.* (2020) found that the addition of *Piriformospora indica* at 0.4 g/kg did not significantly affect feed consumption compared to other groups. In contrast, Farmani *et al.* (2022) observed a significant increase in feed consumption when *Piriformospora indica* was added to the diets between 35 and 42 days.

Regarding FCR, Babamir *et al.* (2018) reported that adding *Piriformospora indica* spores did not significantly impact FCR. However, Soleimanpour *et al.* (2020) found a decreased FCR with the same dosage of 0.4 g/kg, while Farmani *et al.* (2022) demonstrated a decrease in FCR with the addition of *Piriformospora indica* at 10 mg/kg.

The concentration of different blood components varies under the influence of various factors such as age, sex, physiological status, nutrition, and genetics. In this experiment, all factors except nutrition were fixed. Another study showed that there are no differences between treatments on glucose, cholesterol, triglyceride, and LDL on day 14, however at the end of 42 days of age blood glucose was enhanced, and cholesterol, triglyceride, and LDL was reduced in chicks fed diets containing *P.indica* (Soleimanpour *et al.*, 2020). Farmani *et al.* (2022) added the PI to diets and total protein, albumin, and globulin decreased by SI.

The recent findings indicated that the inclusion of *Serendipita indica*, autoclaved *Serendipita indica*, and lactobacilli probiotics in the diets had a significant impact on the immune organs in the control group. Specifically, the lactobacilli probiotics led to an increase in the weight of the immune organs at 28 and 35 days. Conversely, the control group exhibited lower weights of the bursa Fabricius, thymus, and spleen at 28 and 35 days of age. Furthermore, the humoral immunity response to ND and AI revealed that the supplementation of the diets with *Serendipita indica*, autoclaved *Serendipita indica*, and lactobacilli probiotics did not have a significant effect on the control group at 28 days. However, at 35 days, the Lactobacilli probiotic increased the ND titer compared to the other groups. Notably, the Lactobacilli spores demonstrated a significant enhancement in the immune response against infectious bursal disease and infectious bronchitis. These findings highlight the potential of Lactobacilli spores to effectively influence antibody titers against these diseases. Similar results were observed for the Newcastle disease titer in birds that were administered with Lactobacillus spp. (Hedayati *et al.*, 2022).

The number of bacteria present in the cecum of broilers at day 28 and 35d in all treatment groups exhibited a significant decrease in coliforms, *E. coli*, and Salmonella when compared to the control group. The addition of probiotics resulted in a noteworthy reduction (P <0.05) in coliforms, *E. coli*, and Salmonella spp. in comparison to all other dietary treatments. Nevertheless, various factors, as highlighted by Hedayati *et al.* (2022), can either enhance or diminish the effectiveness of probiotics, including the composition and viability of species, dosage, type of administration (e.g. live bacteria or spores), frequency of application, diet composition, age and strain of the birds, as well as the health and hygiene conditions of the farm. The intestinal health outcomes of the present

[DOI: 10.61186/ijls.1.1.41]

study indicate a decrease in E. coli, Salmonella, and coliform populations in the caecum. This discovery suggests that Lactobacillus spores may have a positive impact on the host animal by enhancing the balance of its intestinal microbiota.

CONCLUSION

In this study, it was observed that in performance traits except FCR, the group that received probiotics performed better in performance parameters. A significant difference was observed in the immune titer and immune organs of the group receiving probiotics. The largest decrease in the population of cecum bacteria was seen in the group receiving probiotics. While none of the *Serendipita indica* treatments performed better than the probiotics, but autoclaved AS was second to the probiotic group in most of the traits studied.

ACKNOWLEDGEMENTS

The authors would like to thank Malayer University for the scientific and technical support of the current study.

CONFLICT OF INTEREST

No potential conflict of interest was reported by the authors.

AUTHOR CONTRIBUTIONS

MS and MH established the experimental shed, arranged materials, and performed the methodology. MS and MH contributed to sample collection, and take data. MGH has prepared *Serendipita indica* for this study. SKH analyzed the data, and MH wrote the manuscript.

DATA AVAILABILITY

The datasets generated during and/or analyzed during the current study are presented in the manuscript or are available from the corresponding authors on reasonable request.

ETHICAL CONSIDERATIONS

Ethical issues (including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy) have been checked by all the authors.

FUNDING

No funding was received for conducting this study.

REFERENCES

Applegate T J, Klose V, Steiner T, Ganner A. & Schatzmayr G. (2010). Probiotics and phytogenics for poultry: Myth or reality? Journal of Applied Poultry Research, 19, 194-210.

Babamir M, Hedayati M, Khalaji S, Yari M & Ghabooli M. (2018). Study of using the *Piriformospora indica* spores as a new candidate of prebiotic in broiler chickens. Animal Science Journal, 31(120), 267-281. DOI: 10.22092/asj.2017.110911.1463 (in Persian).

Bai S P, Wu A M, Ding X M, Lei Y, Bai J, Zhang K Y & Chio, J. S. (2013). Effects of probiotic-supplemented diets on growth performance and intestinal immune characteristics of broiler chickens. Poultry Science, 92(3), 663-670.

Farmani A, Hedayati M, Yari M & Ghabooli M. (2022). Influence of a *Piriformospora indica* plant-specific fungus and clove essence on broiler health and performance parameters during oxidative stress. South African Journal of Animal Science, 52 (6), 860-866. DOI: 10.4314/sajas.v52i6.11.

Ghabooli, M., Khatabi, B., Ahmadi, F.S., Sepehri, M., Mirzaei, M. & Amirkhani A. (2013). Proteomics study reveals the molecular mechanisms underlying water stress tolerance induced by *Piriformospora indica* in barley. Journal of Proteomix, 94, 289–301. doi: 10.1016/j.jprot.2013.09.017.

Hedayati M, Khalaji S & Manafi M. (2022). Lactobacilli spp. and *Zataria multiflora* essence as antibiotic substituent on broiler health and performance parameters, Italian Journal of Animal Science, 21(1), 1–7. DOI:10.1080/1828051X.2021.2013738.

Li S P, Zhao X J & Wang J Y. (2009). Synergy of *Astragalus polysaccharides* and probiotics (Lactobacillus and Bacillus cereus) on immunity and intestinal microbiota in chicks. Poultry Science, 88(3), 519-525.

SAS (2003). SAS users guide: Statistics. Version 9.1 ed. Cary, NC: SAS Inst. Inc.

Sevim B & Ayasan T. (2020) The Use of Jatropha (*Jatropha curcas*) in Poultry Nutrition. Turkish Journal of Agriculture Research, 7(2), 227-232. DOI: org/10.19159/tutad.684358.

Shivaramaiah S, Pumford N R, Morgan M J, Wolfenden R E, Wolfenden A D, Torres-Rodríguez A. Hargis B M & Téllez G. (2011). Evaluation of Bacillus species as potential candidates for direct-fed microbials in commercial poultry. Poultry Science, 90(7), 1574-1580.

Soleimanpour A, Khalaji S, Yari M & Ghabooli M. (2020). Evaluating the efficacy of plant-specific fungus (*Piriformospora indica*) rich in mannan oligosaccharides as a microbial feed additive on growth performance, protein digestibility, plasma characteristics, intestinal microflora, and morphology in chicks. Journal of Applied Animal Research, 48(1), 460–469. DOI:10.1080/09712119.2020.1826324.

Varma A, Verma S, Sahay N, Bütehorn B & Franken P. (1999). *Piriformospora indica*, a cultivable plant-growth-promoting root endophyte. Applied Environmental Microbiology, 65, 2741–2744. DOI: 10.1128/aem.65.6.2741-2744.1999.

Wolfenden R E, Pumford N R, Morgan M J, Shivaramaiah S, Wolfenden A D, Tellez G & Hargis B M. (2010). Evaluation of a screening and selection method for Bacillus isolates for use as effective direct-fed microbials in commercial poultry. International Jounal of Poultry Science, 9(4), 317-323.

[DOI: 10.61186/ijls.1.1.41]

Wu B Q, Zhang T, Guo L Q & Lin JF. (2011). Effects of *Bacillus subtilis* KD1 on broiler intestinal flora. Poultry Science, 90(11), 2493-2499.

Zhang A W, Lee B D, Lee S K, Lee K W, An G H, Song K B & Lee CH. (2005). Effects of yeast (*Saccharomyces cerevisiae*) cell components on growth performance, meat quality, and ileal mucosa development of broiler chicks. Poultry Science, 84(7), 1015-1021.

[DOI: 10.61186/ijls.1.1.41]