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Evaluating the effects of *Lactobacillus plantarum, Bacillus subtilis* and mannan-oligosaccharides (MOS) on serum protein and lipid profiles in broiler chicken[#]

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Abstract

This study assessed the influence of dietary supplementation with Lactobacillus plantarum, Bacillus subtilis, mannan-oligosaccharides (MOS) and their combinations on serum lipids and protein profiles and immune organ weights in broiler chicken. A total of 216-day-old Cobb broiler chicks were assigned to six treatment groups with each group comprising three replicates of 12 birds each, ensuring a completely randomised design (CRD): T1 (control), T2 (L. plantarum), T3 (B. subtilis), T4 (MOS), T5 (L. plantarum + MOS) and T6 (B. subtilis + MOS). Data collected were analysed by one-way ANOVA using SPSS (version 24.0). Significant differences in triglycerides (p = 0.04), LDL (p = 0.04), and VLDL (p = 0.03) levels were observed, with T5 (L. plantarum + MOS) and T6 (B. subtilis + MOS) groups showing reduced lipid levels compared to other treatments. No significant differences were found in total cholesterol, HDL, total protein, albumin, globulin levels, or the weight of immune organs (bursa and spleen). These results indicate that probiotics and prebiotics combinations may have beneficial effects on lipid metabolism in broilers, while their influence on protein levels and immune organ development is minimal.

Keywords: *Bacillus subtilis, Lactobacillus plantarum, Mannan-oligosaccharide, lipid profile, broiler*

The poultry industry is increasingly adopting natural feed additives like probiotics and prebiotics to improve growth, health and productivity while reducing reliance on antibiotics. Probiotic supplementation has been shown to improve intestinal morphology and overall gut health in broiler chickens (Supriya *et al.,* 2021). Among the most promising candidates of feed additives *Lactobacillus plantarum* (*L. plantarum*), *Bacillus subtilis* (*B. subtilis*) and mannan oligosaccharides (MOS) have demonstrated significant benefits for gut health, immune function and nutrient absorption. These additives help to optimise digestive efficiency, boosts the immune system and support a healthier gut environment, which all contribute to enhanced performance in broilers.

Lactobacillus plantarum, a lactic acid bacterium, promotes beneficial gut microbiota, improves nutrient digestibility and strengthens the intestinal barrier while *B. subtilis,* a spore-forming bacterium, enhances digestive enzyme

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activity and gut morphology (Wang *et al*., 2018; Chen *et al.*, 2024). Similarly, MOS, derived from yeast cell walls, acts as a prebiotic by binding harmful pathogens, fostering beneficial microorganisms, and improving intestinal immunity (Spring *et al*., 2000; Baurhoo *et al.,* 2007). These additives, when used individually or in combination, show potential for synergistic effects on broiler health and productivity.

This study investigates the effects of *L. plantarum, B. subtilis,* and MOS on serum lipid profiles, protein utilisation, and immune organ weights in broiler chicken, thus providing actionable insights into optimising poultry nutrition and health management practices, paving the way for sustainable and efficient poultry production systems.

Material and methods

The experiment was conducted at the Department of Poultry Science, College of Veterinary and Animal Sciences, Kerala Veterinary and Animal Sciences University (KVASU), Mannuthy, Thrissur. A total of 216-day-old Cobb 430Y broiler chicks were procured from Ayisha Hatchery, Koduvayoor, Palakkad. The chicks were wing-banded for individual identification and weighed to record their initial body weights. They were then randomly divided into six treatment groups, with each group consisting of three replicates of 12 birds, following a completely randomised design (CRD). The experiment spanned for about 42 days, starting from the chicks' first day of age to 42 days.

Treatment groups

The treatment groups and their diets are described in Table 1. Each treatment group received their respective diets throughout the experimental period.

Table 1. Treatment groups and their respective diets

Treatments	Diets
T1	Basal diet (Control)
T2	Basal diet supplemented with *L. plantarum* (10^8 CFU/kg)
T3	Basal diet supplemented with *B. subtilis* (10^8 CFU/kg)
T4	Basal diet supplemented with MOS (1g/ kg)
T5	Basal diet supplemented with *L. plantarum* (10^8 CFU/kg) and MOS (1 g/kg)
T6	Basal diet supplemented with *B. subtilis* (10^8 CFU/kg) and MOS (1 g/kg)

Experimental diets

The birds were fed a pre-starter diet up to seven days of age, a starter diet from eight to twenty-one days of age and a finisher diet up to 42 days of age. Standard basal diets were formulated to meet the nutrient requirements of broilers as per BIS (2007) guidelines. The ingredients and chemical composition of the basal diet are presented in Table 2. The experimental diets were prepared by incorporating the respective supplements into the basal diets.

Table 2. Ingredient composition of the basal diets (%)

Ingredients	Pre starter	Starter	Finisher
Yellow maize	51.90	52.80	57.20
Soyabean meal	41.50	39.2	33.6
Rice bran oil	2.64	4.10	5.10
Dicalcium phosphate	1.80	1.80	1.80
Calcite	1.40	1.40	1.40
Salt	0.38	0.36	0.31
L-Lysine	0.14	0.24	0.20
DL -Methionine	0.17	0.18	0.25
Total	100	100	100

Serum lipid profile and protein estimation

Blood samples were collected from two randomly selected birds per replicate across three replicates in each treatment groups at 42nd day and serum were separated by centrifugation (at 2000 rpm for 5 min) and then stored at –20°C until analysed. Total cholesterol, triglycerides and high-density lipoprotein cholesterol (HDL-C), total protein, albumin and globulin values were measured using an automated biochemical analyser (SELECTRA Pro SLite). Very low-density lipoprotein cholesterol (VLDL-C) values were calculated by dividing triglyceride values by five and low-density lipoprotein cholesterol (LDL-C) values by subtracting total values of HDL-C and VLDL-C from total cholesterol, according to the method described by Panda *et al*. (2006).

Weight of immune organs

The bursae of Fabricius and spleen were collected, weighed and recorded individually.

Results and discussion

Serum lipid profile and protein values

The data on mean total cholesterol, triglycerides, high density lipoprotein, low density lipoprotein and very low-density lipoprotein in serum of broilers in different treatments at six weeks of age are presented in Table 3.

Serum lipid profile

The differences in total cholesterol levels among the treatment groups were not statistically significant. The T3 group (*B. subtilis*) exhibited the highest cholesterol level (131.21 mg/dL), while the T1 group (control) showed a lower level (113.02 mg/dL). These findings align with previous studies, such as those by Hung *et al.* (2012) and Karunanayaka *et al.* (2020), who reported no significant effects of probiotics like *B. subtilis* on serum cholesterol levels in broilers. Similarly, Hussein and Selim (2018) suggested that the impact of probiotics on cholesterol may vary based on specific strains and dosages used. In contrast with the findings our study, Abdel-Hafeez *et al.* (2017) observed that prebiotics (MOS) significantly reduced total cholesterol in broiler chicken.

There were statistically significant differences in triglyceride levels among the treatment groups (p = 0.04). The T2 group (*L. plantarum*) recorded the highest triglyceride level (30.33 mg/dL), which was significantly greater than the T5 *L. plantarum* + MOS) and T6 (*B. subtilis* + MOS) groups, with levels of 18.37 mg/dL and 17.77 mg/dL, respectively. The T1 (control), T3 (*B. subtilis*) and T4 (MOS) groups had intermediate levels that were not significantly different from the highest or lowest groups. These findings suggest that combining probiotics with MOS reduce triglyceride levels in broilers. This is supported by studies of Gong *et al*. (2018) and Yalçinkaya *et al.* (2008), who noted that probiotics and MOS supplementation could lower serum triglycerides by improving lipid metabolism and enhancing fatty acid utilisation.

The differences in HDL levels among the treatment groups were not statistically significant (p = 0.10). The T3 group (*B. subtilis*) showed the highest HDL level (98.20 mg/dL), while the T4 group (MOS) had the lowest (80.70 mg/dL). This outcome aligns with findings from Jahanian and Ashnagar (2015), who reported that MOS supplementation could increase HDL levels significantly. However, the variability in HDL responses might depend on the specific strains of probiotics or prebiotics used, as observed by Hedayati *et al.* (2015).

Statistically significant differences were found in LDL levels among the treatment groups (p = 0.04). The T3 (*B. subtilis*) group (28.66 mg/dL) and T4 (MOS) group (28.05 mg/dL) had significantly higher LDL levels compared to the T6 (*B. subtilis* + MOS) group (21.70 mg/dL). The T1 (control), T2 (*L. plantarum*), and T5 (*L. plantarum* + MOS) groups had intermediate levels. These results suggest that the combination of *B. subtilis* with MOS (T6) reduce LDL levels in broilers compared to other treatments. Jahanian and Ashnagar (2015) also reported that MOS supplementation could reduce LDL levels significantly.

Statistically significant differences were also observed in VLDL levels among the treatment groups (p = 0.03). The T2 group (*L. plantarum*) had the highest VLDL level (6.06 mg/dL), significantly greater than the T6 (*B. subtilis* + MOS) and T5 (*L. plantarum* + MOS) groups, with levels of 3.46 mg/dL and 3.78 mg/dL, respectively. The T1 (control), T3 *(B. subtilis*) and T4 (MOS) groups had intermediate levels. These findings suggest that the combinations of probiotics with MOS may beneficially reduce VLDL levels in broilers.

The results revealed that certain dietary treatments significantly influenced components of the lipid profile, particularly triglycerides, LDL and VLDL levels, while no significant differences were observed for total cholesterol or HDL levels. These findings suggest that some probiotic and prebiotic combinations may beneficially affect lipid metabolism in broilers, although their impact on protein levels (total protein, albumin, globulin) was minimal lipid profile of broilers in different dietary treatment groups at six weeks of age.

Serum protein values

For the serum protein levels, no statistically significant differences were observed among the treatment groups in total protein (p = 0.20), albumin (p = 0.85) or globulin (p = 0.13) levels (Table 4). The T5 group (*L. plantarum* + MOS) showed the highest total protein and globulin levels, while the T6 (*B. subtilis* + MOS) group had the lowest, but these differences were not significant.

Table 3. Serum lipid profile of broilers in different dietary treatment groups at six weeks of age

Parameters	T1 (control)	T2 (*L. plantarum*)	T3 (*B. subtilis*)	T4 (MOS)	T5 (*L.plantarum* + MOS)	T6 (*B. subtilis* + MOS)	p -value
Total cholesterol (mg/dL)	113.02±3.78	122.51±4.70	131.21±1.42	113.74±8.35	117.51±1.93	122.36±0.36	0.09
Triglycerides (mg/dL)	25.47^{ab}±3.41	30.33^{a}±4.72	22.37^{ab}±0.97	24.97^{ab}±1.93	18.37^{b}±0.52	17.77^{b}±0.39	0.04
HDL (mg/dL)	86.00±6.35	89.00±3.33	98.20±1.06	80.70±7.44	85.70±0.85	95.43±0.98	0.10
LDL (mg/dL)	26.23^{ab}±0.83	27.45^{ab}±1.61	28.66^{a}±2.24	28.05^{a}±1.33	27.33^{ab}±2.90	21.70^{c}±0.35	0.04
VLDL (mg/dL)	5.42^{ab}±0.74	6.06^{a}±0.93	4.41^{abc}±0.22	4.99^{abc}±0.39	3.78^{bc}±0.12	3.46^{c}±0.14	0.03

Different superscripts (a, b, c) within a row indicate significant differences between treatments at p < 0.05, **N=6**

Table 4. Serum protein values of broilers in different dietary treatment groups at six weeks of age (N=6)

Parameters	T1 (control)	T2 (*L. plantarum*)	T3 (*B. subtilis*)	T4 (MOS)	T5 (*L.plantarum* + MOS)	T6 (*B. subtilis* + MOS)	p -value
Total protein (g/dL)	3.03±0.03	3.00±0.10	2.93±0.07	2.83±0.24	3.23±0.09	2.80±0.06	0.20
Albumin (g/dL)	1.13±0.03	1.07±0.03	1.10±0.02	1.13±0.07	1.10±0.06	1.10±0.01	0.85
Globulin (g/dL)	1.88±0.02	1.93±0.10	1.85±0.06	2.07±0.19	2.16±0.11	1.75±0.05	0.13

These findings are consistent with studies like Abdel-Fattah and Fararh (2009) and Erdoğan *et al.* (2010), who reported no significant changes in total protein or albumin levels with probiotic or prebiotic supplementation. However, some studies, such as Al-Baadani *et al.* (2018), Abdel Fattah and Fararh (2009) and Dev *et al.* (2020) those by noted that probiotic or synbiotic supplementation could enhance serum protein levels, indicating that the effects of probiotics and prebiotics on protein metabolism may vary depending on the specific strains, dosages and experimental conditions.

In conclusion, the results of this study indicate that certain combinations of probiotics and prebiotics, particularly *L. plantarum* + MOS and *B. subtilis* + MOS, can significantly reduce triglyceride, LDL and VLDL levels in broilers at six weeks of age, suggesting potential benefits for lipid metabolism. However, no significant effects were observed on total cholesterol, HDL, total protein, albumin, or globulin levels. These findings highlight the potential for specific dietary interventions to improve lipid profiles in broilers, although their impact on protein levels appears minimal. Further research is needed to explore the mechanisms underlying these effects and to optimise the use of probiotics and prebiotics for improving both lipid and protein metabolism in broilers.

Weight of immune organs

The weight of immune organs (bursa and spleen) of broilers was expressed as a percentage of body weight at six weeks of age across different dietary treatments (Table 5). No statistically significant differences were observed in the weights of the immune organs among the treatment groups, suggesting that the dietary treatments did not have a substantial impact on immune organ development under the conditions of this study.

Table 5. Mean (±SE) weight immune organs of broilers in different dietary treatments at six weeks of age, per cent

Treatments	Bursa	Spleen
T1 (control)	0.22±0.06	0.07±0.04
T2 (*L. plantarum*)	0.19±0.04	0.11±0.01
T3 (*B. subtilis*)	0.16±0.05	0.11±0.01
T4 (MOS)	0.16±0.02	0.12±0.02
T5 (*L. plantarum* + MOS)	0.19±0.02	0.09±0.01
T6 *(B. subtilis* + MOS)	0.11±0.06	0.11±0.02
p -value	0.57	0.61

The bursa weight as a percentage of body weight showed no significant differences among the treatment groups (p = 0.57). The T1 (control) group had the highest mean bursa weight, while the T6 group (*B. subtilis* + MOS) had the lowest, but these differences were not statistically significant (Table5). This finding aligns with studies such as Zhang *et al.* (2013) and Al-Khalaifa *et al.* (2019), who reported no significant effects of probiotic or prebiotic supplementation on the bursa weight of broilers. Although some studies, like those by Biswas *et al.* (2019), found that MOS supplementation could increase bursa weight, this study did not observe such effects, indicating that the impact of dietary treatments on bursa development may depend on the specific formulations and dosages used.

Similarly, the spleen weight as a percentage of body weight also showed no significant differences among the treatment groups. The T4 (MOS) group had the highest mean spleen weight, while the T1 (control) group had the lowest, but these differences were not statistically significant. These results are consistent with studies by Reis *et al.* (2017) and Kirkpinar *et al.* (2018), who reported no significant effects of probiotic or synbiotic supplementation on bursa and spleen weight. However, other research, such as that by Manafi *et al.* (2017), noted significant increases in spleen weight with *B. subtilis* supplementation, suggesting that the effects of probiotics on immune organs may vary depending on the strains and conditions of the study.

Overall, the results of this study indicate that the dietary treatments, including probiotics and prebiotics, did not have a statistically significant effect on the development of immune organs (bursa and spleen) in broilers at six weeks of age. Although some numerical variations were observed, particularly in the spleen weights of the T4 (MOS) group and bursa weights of the T1 (control) group, these differences were not statistically significant. These findings suggest that the immune organ weights were not markedly influenced by the dietary interventions in this study, supporting the results from previous research by Zhang *et al.* (2013) and Al-Khalaifa *et al.* (2019). Further studies may be required to explore whether different dietary formulations, probiotic strains, or longer feeding periods could have more pronounced effects on immune organ development in broilers.

Conclusion

The study demonstrated that specific dietary treatments, particularly the combinations of probiotics and prebiotics (T5: *L. plantarum* + MOS and T6: *B. subtilis* + MOS), significantly reduced triglyceride, LDL, and VLDL levels in broilers, indicating their potential lipid-lowering effects. However, no significant differences were observed in total cholesterol, HDL, serum protein levels, or immune organ development among the treatment groups. These findings suggest that dietary combinations of probiotics and prebiotics can positively influence lipid metabolism in broilers, although their impact on other health parameters remains minimal.

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Conflict of interest

The authors declare that they have no conflict of interest

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