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Effect of dietary supplementation of postbiotics produced by lactic acid bacteria on laying hens performance, egg quality, and serum biochemical parameters

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ABSTRACT

The study investigated the impact of postbiotics generated from *Lactobacillus acidophilus* (Lap) and *Lactiplantibacillus plantarum* (Lpp) on the productive performance, egg quality, and serum biochemical parameters of laying hens. At 40 weeks of age, 126 Lohmann hens were randomly assigned to seven treatments with three replications of six birds each. The basal diet (T1) was administered without supplements (negative control) or supplemented with tetracycline (T2) at 0.02% (positive control). The other five groups: T3, T4 (basal diet supplemented with postbiotics (Lap) 0.35%, and (Lap) 0.70% produced from *Lactobacillus acidophilus* bacteria respectively); T5, T6 (basal diet supplemented with postbiotics (Lpp) 0.35%, and (Lpp) 0.70% produced from *Lactiplantibacillus plantarum* bacteria respectively); T7 (basal diet supplemented with postbiotics (0.35% Lap + 0.35% Lpp). Postbiotics and tetracycline (TET) did not affect ($P \geq 0.05$) in body weight, feed intake, feed conversion ratio (FCR), egg weight, egg mass, egg quality or serum total protein, albumin and globulin. Egg production and egg number were greater ($P \leq 0.05$) in the postbiotics (Lap 0.70%, Lpp 0.70%, and mixture (0.35% Lap + 0.35% Lpp), and TET supplemental group as compared to the control (T1). While cholesterol and triglycerides (except 0.35% Lap, 0.35% Lpp), were decreased significantly ($P \leq 0.05$) than T1. Superoxide dismutase and catalase activity (except 0.35% Lap, 0.35% Lpp) improved as compared to T1. The results indicate that the supplementation of postbiotics has a positive effect on laying hens performance and some biochemical parameters.

تأثير اضافة مستويات مختلفة من مستحضر البوستبيوتكس المنتج من بكتيريا حامض اللاكتيك في أداء الدجاج البياض وجودة البيض والمؤشرات الكيموحيوية في مصل الدم

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الخلاصة

هدفت الدراسة الحالية لمعرفة تأثير إضافة مستحضر البوستبيوتكس المنتج من بكتيريا *Lactobacillus acidophilus* (Lap) و *Lactiplantibacillus plantarum* (Lpp) الى علائق الدجاج البياض في الأداء الإنتاجي وجودة البيض والمعايير الكيموحيوية للدم. عند عمر 40 أسبوعًا، تم توزيع 126 دجاجة لوهمان عشوائيًا على سبع معاملات بثلاثة مكررات ولكل منها ستة طيور. تم تغذية الطيور في (T1) على عليقة اساسية بدون اضافات (سيطرة سالبة)، أو مضافاً اليها المضاد الحيوي التتراسيكلين (T2) بنسبة 0.02 % (سيطرة موجبة)، المجموعات الخمس الأخرى: T3، T4 (عليقة اساسية مضافا اليها 0.35 % Lap، و 0.70 % Lap على التوالي)؛ T5، T6 (عليقة اساسية مضافا اليها 0.35 % Lpp، و 0.70 % Lpp على التوالي)؛ T7 (عليقة اساسية مضافا اليها 0.35 % Lpp + 0.35 % Lap). بينت النتائج عدم وجود فروقات معنوية ($P≥0.05$) في وزن الجسم، استهلاك العلف، معامل التحويل الغذائي، وزن وكتلة البيض. كما لم يتأثر تركيز البروتين الكلي، الألبومين والكلوبيولين في مصل الدم. فيما لوحظ وجود ارتفاع معنوي ($P≤0.05$) في عدد ونسبة البيض المنتج، وفي تركيز انزيمات الاكسدة للمعاملات (T7, T6, T4, T2) مقارنة بمعاملة السيطرة (T1)، كما لوحظ وجود انخفاض معنوي ($P≤0.05$) في تركيز الكولسترول في جميع معاملات اضافة مستحضر البوستبيوتكس، وانخفاض في تركيز الدهون الثلاثية لمصل الدم في المعاملات (T7, T6, T4) مقارنة بمعاملة السيطرة (T1)، حيث أشارت النتائج إلى أن إضافة مستحضر البوستبيوتكس له تأثير إيجابي على أداء الدجاج البياض وبعض المؤشرات الكيموحيوية لمصل الدم.

الكلمات المفتاحية: المضادات الحيوية، مضادات الأكسدة، إنتاج البيض، بكتريا حامض اللاكتيك، الدجاج البياض.

INTRODUCTION

Many additives are used in poultry water and feed to improve performance (Abbas and Khauoon, 2021; Saed *et al*., 2023; Ali and Abdulrazaq, 2023) or promoting health (Al-Zuhairi *et al*., 2023; Majeed and Mustafa, 2023; Mustafa and Othman, 2024), and it is considered an integral nutritional component of poultry nutrition. Overuse of antibiotics is also widespread to treat or prevent diseases and promote poultry health, and tetracycline was the most widely used (83.63%) of the antibiotics family in poultry (Soromou *et al*., 2020), while additives that improve the digestive tract functions of bird are the most important feed additives (Stadnicka *et al*., 2023). Probiotic supplements have been introduced into poultry feed and results have shown their effectiveness in many functions, but one of the major limitations of using probiotics in poultry feed is the inability to survive and remain stable during feed manufacturing and drying due to the need for high temperatures for feed pelleting, which can kill or weaken probiotics bacteria (Wang *et al*., 2021). However, studies have shown that nonviable microorganisms and their cellular components and metabolic products have a positive effect on health (Vinderola *et al*., 2022).

Postbiotics is produced from microorganisms and can confer health benefits to the host, without requiring compliance with food safety regulations applicable to probiotics (Scott *et al*., 2022), and postbiotics also have advantages over probiotics as they have a longer storage period of up to five years, higher safety dose limits, clear chemical composition, and similar health benefits to probiotics without the need to manage live microorganisms that are not always safe (Rafique *et al*., 2023). Additionally, postbiotics do not contain live microorganisms, but they show beneficial health effects through similar mechanisms to probiotics while reducing the risks associated with their intake, as postbiotics lack serious side effects while maintaining similar efficacy to probiotics (Żółkiewicz et al., 2020).

Dietary additives of postbiotics to broilers feed leads to positive changes in the environment of the digestive tract by improving its tissue structure, increase in villus height and depth (Danladi *et al*., 2022), increase the digestibility and nutrients passage rate, balance of the intestinal microbiota and prevent the growth of pathogens (Khayoon *et al*., 2024), which leads to improved productive performance. However, few studies have investigated the effect of supplementation of different postbiotics types on laying hens as potential alternatives to antibiotics.

Therefore, this study aimed to evaluate the impact of postbiotics produced from two kinds of lactic acid bacteria on laying hens performance, egg quality, and serum biochemical parameters.

MATERIAL AND METHODS

Ethical Approve

The study was approved by the research ethics committee of University Basrah, Iraq, with approval number 13-37-2024.

Bacteria Source and Preparation of Postbiotics (Lap + Lpp)

The lactic acid bacteria (L. acidophilus and L. plantarum) were obtained from a Chinese Company (Herbasea Biotechnology). The postbiotics were prepared as described in Khayoon et al., (2024).

Experimental Design

This experiment was conducted in the Poultry Field of the College of Agriculture, University of Basrah, Iraq, for a duration of 12 weeks, from October 14, 2023 to January 5, 2024. One hundred and twenty-six Lohman laying hens at 40 weeks-old were randomly assigned to seven treatment groups, each group had three replicates, containing six birds each. Feed was provided twice a day, at 6 am and 2 pm at 130 g/bird/day. All birds were acclimated to a basal diet for one week. The temperature (27 ºC), and 16 h lighting /8 h dark. Water was provided *ad libitum* during the study period. The experiment involved different dietary treatments: basal diet (T1) administered without supplements (negative control) or supplemented with tetracycline (T2) at 0.02% (positive control). The other five groups: T3, T4 (basal diet supplemented with postbiotics (Lap) 0.35%, and (Lap) 0.70% produced from *Lactobacillus acidophilus* bacteria respectively); T5, T6 (basal diet supplemented with postbiotics (Lpp) 0.35%, and (Lpp) 0.70% produced from *Lactiplantibacillus plantarum* bacteria respectively); T7, (basal diet supplemented with postbiotics (0.35% Lap + 0.35% Lpp). The composition of the basal diet provided to the hens is listed in Table 1.

Table 1: Percentage and calculated composition of the experimental diets.

Ingredient (%)	(%)
Yellow corn	62.00
Wheat	5.00
Soybean meal (48%)	22.00
Vegetable oil	1.30
Limestone	8.00
Di-calcium phosphate	1.20
Vitamin and mineral premix*	0.25
Salt (NaCl)	0.25
Total	100
Calculated nutrients**	
Metabolizable energy (kcal/kg)	2884
Crude protein	16.53
Crude fat	2.65
Crude fiber	2.01
Calcium	3.40
Available phosphorus	0.33
Lysine	0.83
Methionine + Cysteine	0.30

*Layer Vitamin-mineral premix each 1 Kg consists of: Vit. A, 8000 IU; Vit. D3, 1300 ICU, Vit. E 5 mg; Vit. K, 2 mg; Vit B1, 0.7 mg; Vit. B2, 3 mg; Vit. B6, 1.5 mg; Vit. B12, 7 mg; Biotin, 0.1 mg; Pantothenic acid, 6 g; Niacin, 20 g; Folic acid, 1 mg, Manganese, 60 mg; Zinc, 50 mg, Copper, 6 mg; Iodine, 1 mg, Selenium, 0.5 mg; Cobalt, 1 mg.

**The calculation was based on the chemical composition of the feedstuff found in NRC, (1994).

Serum Biochemical Parameters

Blood samples were collected from the wing vein of one bird per replicate when it was 52 weeks old. The samples were collected in tubes without EDTA for serum separation. The tubes were placed in a centrifuge at 3000 rpm for 15 minutes at 25°C. The concentration of total protein (TP), albumin, cholesterol (CHOL), and triglycerides (TAG) was estimated using available commercial kits (Biolabo SAS, France), and serum globulin was calculated as the difference between TP and albumin. The activity of superoxide dismutase (SOD) and catalase (CAT) enzymes in serum was assayed using a standard ready-made kit produced by ABO Swiss Company.

Statistical Analysis

Data were analyzed as a completely randomized design by using SPSS program software (2017), and were compared by Duncan's multiple range test ($P \leq 0.05$).

RESULTS AND DISCUSSION

The productive performance responses to postbiotics or antibiotic (TET) supplementation in the entire trial are summarized in Table 2. No significant differences ($P \geq 0.05$) were found between the experimental treatments in initial and final body weight, body weight change, feed intake, FCR, egg mass, and egg weight among the different treatments. Previous studies on laying hens have shown that the administration of postbiotics derived from *L. plantarum* had no significant effect on their body weight, overall feed intake, and FCR (Loh *et al*., 2014; Farran *et al*., 2024). The results also indicated that all dietary groups, except T3 and T5, showed significant improvements ($P \leq 0.05$) in egg production (%) and egg number when postbiotics (Lap and Lpp) or TET were included in the diet, compared to the control group (T1). According to the results, a higher level of postbiotics (Lap 0.70% or Lpp 0.70%, and the mixture 0.35% Lap and 0.35% Lpp) had a numerically higher value in egg production percentage and egg number compared to the TET group. However, this difference was not statistically significant ($P \geq 0.05$). The increased egg production can be attributed to improved health and immunity of laying hens and increased nutrient utilization, as postbiotics can strengthen immune responses and promote gut health (Kaouk, 2024).

Table 2: Effect of experimental diets on production performance of laying hens for 40-52 weeks.

Parameters	**Dietary treatments**							**Significant level**
	T_1	**T_2**	**T_3**	**T_4**	**T_5**	**T_6**	**T_7**	
Initial body weight (g)	1874.45 ± 6.39	1869.73 ± 6.91	1862.22 ± 8.38	1859.61 ± 7.33	1876.12 ± 6.35	1878.88 ± 7.30	1876.61 ± 6.56	NS
Final body weight (g)	1905.87 ± 5.40	1902.98 ± 7.93	1893.57 ± 7.16	1893.69 ± 6.38	1908.69 ± 4.89	1914.50 ± 6.27	1910.93 ± 7.75	NS
Body weight change (g)	31.42 ± 1.15	33.25 ± 1.02	31.35 ± 1.21	34.08 ± 1.02	32.57 ± 1.60	35.62 ± 1.89	34.32 ± 1.66	NS
Feed intake (g/d)	127.50 ± 0.57	128.59 ± 0.26	127.55 ± 0.39	128.49 ± 0.22	127.47 ± 0.51	128.55 ± 0.10	128.57 ± 0.31	NS
FCR (g feed/g egg)	2.24 ± 0.029	2.19 ± 0.030	2.23 ± 0.035	2.19 ± 0.026	2.22 ± 0.022	2.17 ± 0.028	2.19 ± 0.041	NS
Hen day egg production (%)	86.24^{c} ± 1.01	90.21^{ab} ± 0.92	86.51^{c} ± 1.03	89.88^{ab} ± 0.80	87.10^{bc} ± 1.15	90.61^{a} ± 1.23	90.34^{ab} ± 0.92	$P \leq 0.05$
Egg weight (g)	65.90 ± 0.06	65.23 ± 0.17	66.05 ± 0.36	65.38 ± 0.24	65.97 ± 0.34	65.26 ± 0.16	65.11 ± 0.67	NS
Egg number (egg/hen)	72.43^{c} ± 0.84	75.78^{ab} ± 0.78	72.67^{c} ± 0.87	75.49^{ab} ± 0.67	73.16^{bc} ± 0.96	76.11^{a} ± 1.03	75.89^{ab} ± 0.78	$P \leq 0.05$
Egg mass (g)	56.83 ± 0.61	58.84 ± 0.69	57.14 ± 0.81	58.77 ± 0.64	57.46 ± 0.48	59.13 ± 0.82	58.83 ± 0.91	NS

$^{a-c}$ means within a row for each parameter with different superscripts are significantly different ($P \leq 0.05$), NS: non-significant.

Postbiotics also stimulate the growth of important probiotic bacteria such as *Lactobacillus* (Khayoon *et al*., 2024), there may be an effect of probiotics on ovary size and egg production, Zhou *et al*., (2020) observed that adding probiotics to the diet of laying hens increased FSH and estradiol levels, which resulted in increased ovarian weight and enhanced egg production. While Xu *et al*., (2023) noted in their study that adding probiotics to laying hens diets led to ovarian development and increased egg production as a result of increased levels of FSH and estradiol in the blood serum. Hameed *et al*., (2020) also suggested that probiotics may have an impact on the function of the pituitary glands, improving hormone function, specifically FSH and LH, as FSH plays a role in increasing follicle size and LH increasing egg production and ovulation rate (Prastiya *et al*., 2022). Additionally, the improvement of bird health in the treatment of tetracycline supplement (T2) related to tetracycline activity as antioxidant and anti-inflammatory, it has been proven to be highly effective as an antimicrobial by the bacteriostatic action of tetracycline based on their inhibition of protein biosynthesis of target pathogens (Ramachanderan and Schaefer, 2021). Studies conducted by Loh *et al*., (2014) and Farran *et al.*, (2024) also showed an increase in egg production percentage in laying hens when fed postbiotics produced from *L. plantarum*.

Egg Quality Criteria

The parameters regarding egg quality measured at 52 weeks have been presented in Table 3. The results indicate that there were no significant variations observed among the experimental groups in terms of eggshell thickness, the percentage of shell, yolk and albumen, yolk height, yolk diameter, yolk index, albumen height, albumen diameter, albumen index, and Haugh unit.

Table 3: Effect of experimental diets on egg quality criteria measured at 52 weeks of age.

Parameters	Dietary treatments							Significant level
	T_1	T_2	T_3	T_4	T_5	T_6	T_7	
Eggshell thickness (mm)	0.617 ±0.061	0.662 ±0.082	0.642 ±0.079	0.701 ±0.071	0.677 ±0.054	0.884 ±0.169	0.799 ±0.118	NS
Shell (%)	9.88 ±0.28	9.79 ±0.35	9.92 ±0.63	10.24 ±0.38	9.42 ±0.46	9.85 ±0.59	10.21 ±0.47	NS
Yolk (%)	22.43 ±1.22	23.96 ±1.73	22.45 ±1.05	22.84 ±1.42	22.77 ±090	23.78 ±1.03	23.73 ±0.91	NS
Albumen (%)	67.69 ±1.12	66.25 ±1.44	67.63 ±092	66.92 ±1.15	67.81 ±0.71	66.37 ±1.52	66.06 ±1.28	NS
Yolk Height (mm)	11.93 ±0.27	12.68 ±1.53	12.38 ±1.44	12.36 ±1.64	13.74 ±1.10	12.45 ±1.14	13.61 ±0.41	NS
Yolk diameter (mm)	28.98 ±0.38	29.71 ±0.64	29.65 ±0.92	29.64 ±0.82	30.62 ±0.63	30.44 ±0.68	31.20 ±0.97	NS
Yolk index	0.410 ±0.012	0.423 ±0.045	0.417 ±0.038	0.413 ±0.044	0.453 ±0.041	0.410 ±0.036	0.437 ±0.027	NS
Albumen Height (mm)	5.18 ±0.06	5.37 ±0.23	5.51 ±0.19	5.49 ±0.14	5.60 ±0.10	5.29 ±0.18	5.40 ±0.24	NS
Albumen diameter (mm)	44.08 ±2.34	45.18 ±3.24	48.04 ±2.43	48.50 ±2.21	49.30 ±1.25	44.72 ±3.55	45.39 ±2.84	NS
Albumen index	0.118 ±0.006	0.119 ±0.006	0.115 ±0.003	0.113 ±0.003	0.114 ±0.003	0.119 ±0.003	0.119 ±0.006	NS
Haugh unit score	84.67 ±0.23	85.69 ±1.14	86.5 ±1.24	86.46 ±0.89	86.76 ±0.51	84.4 ±1.5	85.15 ±1.2	NS

$^{a-c}$ means within a row for each parameter with different superscripts are significantly different ($P≤0.05$), NS: non-significant.

These results coincide with the results of Loh *et al*., (2014) who reported that adding a postbiotics prepared from *L. plantarum* to the diet of laying hens (Lohmann) at 23 weeks of age, did not affect the Haugh unit. As well as with Farran *et al.*, (2024) reported that including postbiotics *L. plantarum* in the diet of laying hens did not affect yolk weight percentage and shell thickness, which is similar to our research results. However, in contrast to our results, they found a significant reduction in the percentage of egg white weight.

The biochemical constituents of laying hens are summarized in Table 4. The results showed no significant ($P \geq 0.05$) effect on serum TP, albumin, and globulin, among all experimental treatments, while there was a significant ($P \leq 0.05$) effect on CHOL, TAG, SOD, and CAT activity. Postbiotics supplementation resulted in significantly lower CHOL levels ($P \leq 0.05$) compared to the control group (T1). However, there was no significant difference ($P \geq 0.05$) in CHOL between the negative (T1) and positive control (T2) or between control (T2), T3 (0.35% Lap), and T5 (0.35% Lpp).

It was observed that the use of postbiotics supplements resulted in a significant decrease ($P \leq 0.05$) in triglyceride levels, as compared to the control group (T1), with the exception of groups T3 (0.35% Lap) and T5 (0.35% Lpp). Furthermore, no significant differences were observed in the concentration of TAG between the negative and positive control groups.

SOD and CAT activity were significantly improved ($P \leq 0.05$) in all dietary groups, except T3 and T5, compared to the negative control (T1). According to previous studies, the presence of exopolysaccharides (EPS) in postbiotics preparations can lead to a decreased serum CHOL levels. EPS produced by lactic acid bacteria has been found to have cholesterol lowering effects by inhibiting CHOL absorption (Gezginç *et al*., 2022; Dilna *et al*., 2015).

Table 4: Effect of experimental diets on serum biochemical parameters of laying hens.

Parameters	**Dietary treatments**							**Significant level**
	T_1	**T_2**	**T_3**	**T_4**	**T_5**	**T_6**	**T_7**	
TP (g/100 ml)	4.87 ± 0.09	5.01 ± 0.06	4.93 ± 0.07	5.02 ± 0.03	4.94 ± 0.08	5.04 ± 0.09	5.07 ± 0.05	NS
Albumin (g/100 ml)	2.32 ± 0.07	2.29 ± 0.02	2.27 ± 0.06	2.32 ± 0.03	2.26 ± 0.02	2.30 ± 0.05	2.34 ± 0.04	NS
Globulin (g/100 ml)	2.55 ± 0.06	2.72 ± 0.04	2.66 ± 0.08	2.70 ± 0.03	2.68 ± 0.05	2.74 ± 0.06	2.73 ± 0.09	NS
CHOL (mg/100ml)	139.69^{a} ± 3.34	134.62^{ab} ± 2.76	128.96^{bc} ± 2.58	123.68^{c} ± 2.37	126.77^{bc} ± 3.49	120.42^{c} ± 4.14	122.47^{c} ±3.61	$P \leq 0.05$
TAG (mg/100 ml)	125.70^{a} ± 3.86	123.64^{ab} ± 2.96	122.47^{ab} ± 2.45	111.61^{c} ± 3.47	124.15^{ab} ± 4.13	113.53^{bc} ± 3.42	108.67^{c} ± 2.89	$P \leq 0.05$
SOD (µmol/l)	2.53^{b} ± 0.12	2.98^{a} ± 0.17	2.69^{ab} ± 0.15	3.03^{a} ± 0.13	2.86^{ab} ± 0.11	3.12^{a} ± 0.09	3.09^{a} ± 0.12	$P \leq 0.05$
CAT (µmol/l)	3.72^{b} ± 0.10	4.15^{a} ± 0.14	3.82^{ab} ± 0.08	4.19^{a} ± 0.14	3.94^{ab} ± 0.15	4.24^{a} ± 0.12	4.21^{a} ± 0.13	$P \leq 0.05$

$^{a-c}$ means within a row for each parameter with different superscripts are significantly different ($P \leq 0.05$), NS: non-significant.

The decrease in serum cholesterol (CHOL) level may be due to an increase in the population of lactic acid bacteria, where these bacteria induce the production of bile salt hydrolase, which converts long-chain fatty acids into medium and short-chain fatty acids. This process can reduce blood serum cholesterol levels by inhibiting the reabsorption and excretion of bile acids. As a result, serum cholesterol is converted by the liver into bile acids, leading to lower blood serum cholesterol levels (Agustono *et al*., 2023). It has been observed that *Lactobacillus* bacteria can boost the production of high density lipoprotein CHOL while reducing the levels of low density lipoprotein CHOL and TAG, which may be attributed to the presence of short-chain fatty acids that possess statin-like effects by inhibiting the synthesis of CHOL precursors (Aggarwal *et al*., 2022). As a result, the reduction in TAG could be associated with presence of short-chain fatty acids in postbiotics or produced by *Lactobacillus* bacteria, which play a crucial role in energy and fat metabolism. The significant increase in the level of the antioxidant enzyme (SOD, CAT) is attributed to the ability of postbiotics to inhibit the oxidation process, by the formation of pyrrole compounds and cyclic compounds (Chang *et al*., 2021). Additionally, the tetracycline supplement (T2) increased antioxidant activity due to the presence of a dimethyl amino group at the C4 carbon, which leads to increase in antioxidant activity (Murakami *et al*., 2020). A previous study on laying hens found that all postbiotics treatments significantly decreased serum CHOL levels compared to the negative control (Choe *et al*., 2012; Loh *et al*., 2014). Alaqil *et al*., (2020) noted that supplementing *L. acidophilus* bacteria to the diet of laying hens led to a significant decrease in CHOL and triglyceride concentrations in their blood serum, in comparison to the control group.

CONCLUSION

It could be concluded that the inclusion of postbiotics, which are produced from either *L. acidophilus* (0.70%) or *L. plantarum* (0.70%), or a combination of both (0.35% Lap + 0.35% Lpp), can result in an improvement in egg production percentage, egg number, SOD and CAT activity, and also lead to a reduction in CHOL and TAG concentration. Hence, postbiotics can serve as a substitute for antibiotics, without any impact on the health of the birds or their productive performance.

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