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Effect of the Manufactured Bacterial Preparation on Some Cellular and Biochemical Blood Characteristics of Laying Hens

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Ahmed Al-Salhi [1,*], Sabah Al-Shatty [2]

Abstract: This experiment was conducted in the field of laying hens affiliated with the Department of Animal Production at the College of Agriculture at Basrah University during the period from 27/12/2021 to 21/2/2021. It was applied to 90 experimental units (a 45-week-old laying hens) of Lohmann Extra, randomly allocated to five treatments. Each treatment has three replicates, with six chickens per replicate. The treatments included: - T1: Negative control treatment (basal diet without Addition) T2: Positive control treatment, the addition of dried skim milk at a level of 1 g / kg of feed. T3, T4, T5: add the manufactured bacterial preparation at a level of (0.5, 1, and 2) g/kg feed, respectively. To study the effect of manufactured bacterial preparation on some cellular and biochemical blood characteristics of laying hens. There was a significant (P≤0.05) improvement in cellular blood parameters, packed cell volume (PCV) and biochemical parameters of blood serum (cholesterol, total protein, albumin, globulin) in laying hens, and there were no significant differences in the ratio of heterophile cells to lymphocytes(H/L), glucose, and liver enzymes Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT).

Keywords: Lactobacillus, Laying Hens, Blood Parameters, Bacterial, biosecurity.

Introduction

It is necessary to popularize the concept of biosecurity. Because of its great role in preventive medicine and its economic impact, the term "biosecurity" is very broad, and it is difficult to limit it to a specific definition. Still, it is developing according to its use, and it means in poultry farming projects: raising poultry inside the house isolated from harmful microorganisms as much as possible except that obtained by birds through water or feed, which are often different from the microorganisms that grow in the intestines of poultry (Hwang and Singer, 2020).

Because of the difficulty of controlling microorganisms, specifically pathogenic ones, many of those interested in poultry farming projects have contributed to the use of antibiotics to reduce pathogenic bacteria, and enhance biosecurity. However, this was negatively reflected in the emergence of bacterial species resistant to antibiotic treatments such as *Salmonella*and coliform bacteria. Which are endemic to most poultry farms (Alnajjarand Alemadi, 2017). Because it has resistance to some types of antibiotics. The World Health Organization prohibited the use of some types of these antibiotics in poultry farming for fear of passing them on to the consumer (Agboola*et al.*, 2015).

This lead many researchers to conduct various studies on the use of vital enhancers from different sources. To enhance the concept of biosecurity, Afsari*et al*. (2014) indicated that the use of the commercial Yeasture® bio-enhancer in the ration of laying hens at a level of 0.06 g / kg, led to a significant increase in the concentration of cholesterol in the blood serum and did not affect the ratio of heterophile cells to lymphocytes(H/L) in his study on 144 experimental units of Lohmann Lite laying hens, which lasted for seven weeks.

In a study conducted on 120 experimental units of laying hens of Lohmann Brown breed of 32 weeks of age, for 56 days in which a probiotic was used, which was isolated from the colon area in cattlewas used, at three levels (0.20, 0.40 and 0.60) g of probiotic/kg of feed, While Bidura et al. (2016) observed, that the prohibitic reduced cholesterol concentration in blood serum compared to the control treatment.

Tang et al. (2017) studied the effect of using 0.1% of the commercial probiotic (Primalac®) on the blood parameters of laying hens, which included: PCV, H/L, cholesterol, glucose and liver enzymes AST and ALT on 160 laying hens between 36 and 52 weeks of age, where the results indicated a significant decrease in cholesterol, ALT, H/L, and there was no significant difference in each of AST enzyme, total protein, glucose, and PCV.

The findings of Fathi*et al.* (2018) indicated that the probiotic in laying hens' diets did not affect cellular blood parameters and blood biochemical parameters that including total protein, albumin, and globulin but caused a reduction in serum cholesterol compared to the control treatment. Their study was, conducted on 216 experimental units of laying hens of 32 weeks of age under heat stress conditions for 90 days, in which the probiotic was used at a level of (0, 200and,400) g/ton of feed. In a study conducted on laying hens at the age of 70 weeks, in which a commercial probiotic (Protexin®) prepared by Probiotics UK International was used in drinking water at a concentration of 85 mg/L, no significant differences were observed in the concentration of cholesterol, AST and ALT (Anwar *et al*. 2018).

[1] Department of Animal Production, College of Agriculture and Marshes- University of Thi-Qar -Iraq
ahmed.a.k.alsalhi@gmail.com
[2] Department of Food Sciences, College of Agriculture, University of Basrah, Iraq

Hassan *et al*. (2019) in a study conducted on 360 experimental units of Lohmann Light laying hens for 21 weeks, showed that the use of the probiotic at a level of 0.1% did not affect the concentration of cholesterol in blood serum and liver enzyme AST compared to the treatment of control that is not added.

Naseem *et al.* (2021) confirmed that the probiotic does not affect the concentration of total protein and albumin in his study on white Leghorns W-36 laying hens at 32 weeks of age for eight weeks, in which the probiotic was used at1×10^{12}(cfu/ g) per kg of ration, which consists of Lactobacillus spp. species (*L. paracasei* + *L. plantarum* + *L. rhamnosus*).

In continuation to previous studies, the current research aimed to study the effect of using a manufactured bacterial preparation, one gram of which contains no less than 16×10^{9}(cfu/ g), of seven different local strains of lactic acid bacteria, added to skim milk, in the form of fine powder (Al-Salhi, 2022), on some cellular and biochemical blood characteristics of laying hens.

Materials and Methods

Experimental design

This experiment was applied to 90 hens from the Lohmann strain age of 45 weeks. The experimental units were randomly allocated to five treatments in a completely randomized design (CRD) Each treatment had three replicates, with six hens per replicate. The treatments were as follows:

T1: Negative control treatment (basal diet without any addition).

T2: Positive control treatment, adding dried skim milk at a level of 1g/kg of feed.

T3,T4,T5: Adding the Manufactured Bacterial Preparation at a level of (0.5, 1 and, 2) g/kg feed, respectively.

Flock management

Layering hens were raised in 1 x 1 m^2cages and a height of 60 cm. Feed was provided to the birds in the amount of 130 g / chicken divided into two meals per day, the first at eight o'clock in the morning and the second at four o'clock in the afternoon. The feed was placed in longitudinal metal troughs allocated to each refiner, using the suspended automatic manifold system to be available throughout the day. The feed (productive ration) contained a crude protein percentage of 17.64% and representative energy of 2765 kcal/kg feed. Table (1) shows the percentages of the ingredients and the calculated chemical composition of the layer ration during the production period. All necessary measures were prepared to maintain the temperature, ventilation, and lighting inside the hall and within the appropriate bird conditions, based on the management manual for the Lohmann brown chicken breed.

Blood traits

Blood samples were collected from birds twice during the experiment, at 48 and 52 weeks of age. Blood samples were taken from the wing veins of six birds from each treatment using a medical syringe. Blood was poured into two types of tubes: the first was a capacity of 6 ml that did not contain anticoagulant, placed horizontally to get rid of the clot (fibrinogen proteins); to measure the biochemical parameters of the blood, the other 3 ml contains anticoagulant (EDTA); To measure cellular blood parameters, blood samples that do not have anticoagulant were placed in a centrifuge at 3000 rpm for 15 minutes, then the serum was transferred to new plastic tubes marked with repeater codes; for laboratory analysis.

Table (1) The chemical composition of the productive ration.

Ingredients	(%)
Yellow corn	42
Wheat	15
Soybean meal (48%)	25
Premix (6%)	2.5
Limestone	9.8
Vegetable oil	1.5
Salt	0.3
Filler	3.9
Total	100
Calculated chemical composition	
Crude protein	17.64
The energy represented as kcal/kg of feed	2765
The ratio of energy to protein	156.72
Crude fiber	3.22
Calcium	4.32
Available phosphorous	0.63

-The premix produced by the Iraqi Laymix Company in Erbil governorate contains 6% crude protein, and 4331.57 kcal/kg represented energy, lysine 1.50%, methionine 5.90%, methionine and cysteine 5.00%, calcium 24.05%, available phosphorous 10.20% and threonine 0.85%.

-Chemical analysis according to NRC (1994).

Packed cell volume (PCV)

The micro-hematocrit method was used, which involves drawing blood by capillary action into the microtubules, from one end until two-thirds of the tube's length is filled with blood, then closing one end of the tube immediately after collecting the blood with artificial mud, then placing the tubes in a centrifuge, at a speed of 12000 rpm for 5 minutes. The readings were recorded using a special ruler Micro-hematocrit reader (Al-Darajiet al., 2008).

The ratio of heterophylliesand lymphocytes (H/L)

Swabs of blood were made on glass slides by placing a drop of blood on a slide. Another glass slide was placed on the blood drop, pulled in one direction and at an angle of 45 degrees, and left for 10 minutes for the purpose of blood drying, and stained with a mixture of two Wright-Giemsa dyes, then examined with a light microscope) Burton and Guion,1968), after which heterophyllies cells and lymphocytes were counted, and from them, the (H/L) ratio was calculated according to the following equation mentioned by Shen and Paterson (1983).

$$\text{ratio H/L} = \frac{heterophyllis\ cells\ in\ H/100\ cells}{lymphocyte\ cells\ in\ \ L/100\ cells}$$

Measurement of the concentrations of biochemical parameters

The concentrations of the biochemical parameters of the blood serum (cholesterol, glucose, total protein, albumin and globulin) were measured using ready-made (kits) supplied by the French company BIOLABO. Then the following equation (Young ,1997), was applied to calculate the concentrations.

$$concentration\ (x) = \frac{Sample\ absorbance}{absorbance\ of\ standard\ solution} \times Standard\ solution\ con.$$

AST and AL enzymes

The concentrations of AST and ALT enzymes in the blood serum were measured using a kit supplied by the French company BIOLABO. The analyzes were carried out according to the instructions provided by the company. The absorbance of the samples and the standard solution was measured in the Spectrophotometer at the wavelength of 505 nm. Then the activity of the enzymes was estimated by reading the absorbance on graph paper included with the ready-made kit (Reitman and Frankel, 1957).

Statistical Analysis

The statistical analysis was performed using SPSS software (SPSS, 2018). Analysis of Variance (ANOVA) was carried out and differences between the means of treatments were tested by Duncan's Multiple Range test (1955) using a significance level of 0.05.

Results and Discussion

Cellular Characteristics of Blood

Table (2) shows the effect of using the manufactured bacterial preparation on some cellular traits of blood: PCV, and heterophile cells to lymphocytes (H/L). There was a significant improvement (P≤0.05) in the treatments of the synthetic product, specifically the fifth treatment (T5), had significantly higher PCV% (33%) compared to the two control treatments (T1, T2), which recorded a percentage of 27.00%and27.66 %, for both treatments, respectively. No significant differences were found among the experimental treatments in the ratio of heterophils to lymphocytes (P≤0.05).

Table (2) Effect of using the manufactured bacterial preparation on some cellular blood characteristics of laying hens for the period from 48-52 days (mean ± standard error).

Treatments	Age in weeks			
	48	52	48	52
	PCV%		H/L	
T1	27.00±1.15 [b]	28.00 ±1.73 [c]	0.43±0.04	0.39±0.03
T2	27.66 ±1.76 [b]	29.00 ±1.52[c]	0.44±0.03	0.38±0.02
T3	29.00 ±1.00 [ab]	31.00 ± 0.57[bc]	0.38±0.04	0.37±0.03
T4	31.00 ±2.00 [ab]	1.15 ± 34.00 [ab]	0.35±0.04	0.37±0.05
T5	33.00 ±1.73 [a]	36.33 ±1.66 [a]	0.34±0.02	0.36±0.02
Significance	*	*	N. S	N. S

T1: Negative Control Treatment. T2: Positive Control Treatment, addition of powdered skim milk at a level of (1) g/kg feed. T3, T4, T5: add the manufactured bacterial preparation at (0.5, 1, and 2)g/kg feed, respectively. N.S: Indicates that there are no significant differences between the averages of the transactions. * The different letters within the same column indicate significant differences between the treatments at the 0.05 probability level.

The reason for the improvement in the PCV in the bacterial treatments is due to the strains of lactic acid bacteria present in the manufactured bacterial preparation, which contributed to improving the absorption of nutrients, including iron, copper, and folic acid, which are essential elements in building blood cells. The red cells are ultimately reflected in the PCV. This result did not agree with what was observed by Tang *et al.* (2017). Who found no significant differences in PCV, when using 0.1% of the commercial probiotic (Primalac®), on 160 experimental units of laying hens at 36 and 52 weeks of age. At the same time, our results are in agreement with what was observed by Afsari*et al.* (2014) that the ratio of heterotrophic cells to lymphocytes H/L was not affected by the use of the probiotic in the ration of laying hens at a level of 0.06 g / kg in the study conducted on laying hens, which lasted for seven weeks.

Biochemical Traits

Glucose and Cholesterol

Table (3) shows the effect of using the manufactured bacterial preparation on some biochemical blood traits (glucose and cholesterol concentration) for laying hens at the age of 48 and 52 weeks. For glucose concentration, there were no significant differences between all experimental treatments. Concerning the cholesterol concentration, an apparent significant decrease was observed in the treatments of the manufactured product compared to the two control treatments, noting that there were no significant differences between the treatments of the preparation on one hand and the two control treatments on the other hand.

Table (3) The effect of using the manufactured bacterial preparation on the concentrations of glucose and cholesterol of laying hens for the period from 48-52 days (mean ± standard error).

Treatments	Age in weeks			
	48	52	48	52
	Glucose (mg \100 ml)		Cholesterol (mg \100 ml)	
T1	220.18±15.19	231.14±16.81	172.58±3.35[a]	174.53±3.17 [a]
T2	226.99±22.02	235.20±12.50	175.96±4.07[a]	178.39±6.98 [a]
T3	213.31±11.12	218.14±5.66	146.21±7.21[b]	140.89± 5.78[b]
T4	206.83±10.04	214.48±7.84	141.19±9.52[b]	135.39±5.39[b]
T5	201.52±10.15	206.51±6.82	137.96±6.28[b]	127.94±4.10 [b]
Significance	N. S	N. S	*	*

T1: Negative Control Treatment. T2: Positive Control Treatment, addition of powdered skim milk at a level of (1) g/kg feed. T3, T4, T5: add the manufactured bacterial preparation at (0.5, 1, and 2) g/kg feed, respectively. N.S: Indicates that there are no significant differences between the averages of the transactions. * The different letters within the same column indicate significant differences between the treatments at the 0.05 probability level.

The reason for the decrease in cholesterol concentration in the treatments of manufactured bacterial preparations in Table (3) to the role played by lactic acid bacteria in possessing the enzyme Bile Salt Hydrolase, which works to unbind bile salts, reducing their solubility in the acidic medium and thus inhibits the process of cholesterol molecule formation, after which it is removed from the body with faeces (Ma *et al.*,2019). The results agreed with what was observed by Bidura*et al.* (2016) in a study conducted on 120 experimental units of laying hens at 32 weeks of age, for 56 days in which the probiotic was used, where that it lowered the concentration of cholesterol in the blood serum compared to the control treatment. Also, the results agreed with the findings of Fathi*et al.* (2018) that the inclusion of the probiotic in laying hens' diets leads to a lowering of cholesterol in the blood serum compared to the control treatment. They also agreed with the findings of Tang *et al.*(2017). who found no effect on glucose concentration when using 0.1% of the commercial probiotic (Primalac®), in his study conducted on laying hens at 36 and 52 weeks of age.

Serum Proteins

Table (4) shows the effect of using the Manufactured Bacterial Preparation on blood serum proteins: Total Protein, Albumin, and Globulin, for laying hens at the age of 48and 52 weeks. A significant improvement (P≤0.05) was observed in the fifth treatment T5, (which included the addition of 2 g of product / 1 kg of feed) in comparison with the two control treatments, in the concentrations of total protein, albumin, and globulin, while there were no significant differences between T4 and T5 at 48 and 52 weeks of age.

Table (4) Effect of using the manufactured bacterial preparation on total protein, albumin, and globulin of laying hens for the period from 48-52 days (mean ± standard error).

Treatments	Age in weeks			
	48	52	48	52
	Total Protein (g \100 ml)		Albumin (g \100 ml)	
T1	4.07±0.10 c	4.21±0.03 c	2.31±0.05 c	2.34±0.10 c
T2	4.36±0.00 b	4.43±0.01 c	2.48±0.03 b	2.54±0.01bc
T3	4.58±0.07 b	4.87±0.19 b	2.52±0.06 b	2.59±0.15bc
T4	4.88±0.13 a	5.21±0.08 ab	2.63±0.05 ab	2.83±0.07 ab
T5	5.14±0.09 a	5.42±0.13 a	2.77±0.05 a	2.98±0.06 a
Significance	*	*	*	*

Treatments	Globulin (g \100 ml)	
	48	52
T1	1.76±0.04 c	1.86±0.11 b
T2	1.87±0.02 c	1.89±0.02 b
T3	2.06±0.03 b	2.27±0.04 a
T4	2.25±0.08 a	2.37±0.00 a
T5	2.36±0.04 a	2.44±0.08 a
Significance	*	*

T1: Negative Control Treatment. T2: Positive Control Treatment, addition of powdered skim milk at a level of (1) g/kg feed. T3, T4, T5: add the manufactured bacterial preparation at (0.5, 1, and 2) g/kg feed, respectively. * The different letters within the same column indicate significant differences between the treatments at the 0.05 probability level.

The improvement that occurred in blood serum proteins, which included: total protein, albumin, and globulin, in Table (4), occurred due to the lactic acid bacteria present in the manufactured bacterial preparation, which works to increase protein synthesis by increasing the availability of food elements, including protein, in addition to an increase in the absorption of the amino acid lysine secreted by some types of lactic acid bacteria, which is reflected in the increase in total protein in the blood serum of laying hens, which in turn increases the concentrations of albumin and globulin. The results did not agree by Fathi*et al.* (2018) who found that the inclusion of the probiotic in laying hens' diets did not affect the total protein, albumin, and globulin, in a study conducted on 32-week-old laying hens under heat stress conditions, for 90 days, in which the probiotic was used at (0, 200, and 400) g/ton of Feed. Our results also differ with what was observed by Naseem *et al*. (2021), where the probiotic used at an amount of 1×10^{12} (cfu/g) per kg of feed for a period of 8 weeks did not affect the concentration of total protein and albumin in his study on laying hens at 32 weeks of age.

Liver enzymes

Table (5) shows the effect of using the manufactured bacterial preparation on liver enzymes (AST and, ALT) for laying hens at the age of 48 and52 weeks. No significant differences were found between all experimental treatments (P≤0.05).

Table (5) Effect of using the manufactured bacterial preparation on AST and ALT liver enzymes for laying hens for the period from 48-52 days (mean ± standard error).

Treatments	Age in weeks			
	48	52	48	52
	AST (U/L)		ALT (U/L)	
T1	50.33±1.66	55.00± 4.00	19.66±2.66	21.00±2.30
T2	46.66±3.17	55.33±6.00	21.00±2.30	22.33±2.66
T3	49.00±5.29	50.33±1.66	21.00±2.30	23.66±1.33
T4	52.66±3.48	55.66±7.68	23.66±1.33	22.33±2.66
T5	47.33±6.64	53.33±7.53	22.33±1.33	23.66±1.33
Significance	**N. S**	**N. S**	**N. S**	**N. S**

T1: Negative Control Treatment. T2: Positive Control Treatment, addition of powdered skim milk at a level of (1) g/kg feed. T3, T4, T5: add the manufactured bacterial preparation at (0.5, 1, and 2) g/kg feed, respectively. N.S: Indicates that there are no significant differences between the averages of the transactions.

This result is in agreement with what Anwar et al. (2018)who also did not find significant differences in liver enzymes AST and ALT during his study on laying hens at 70 weeks of age, in which the commercial protexin® was used in drinking water at a concentration 85 mg/L. Hassan et al. (2019) noted that using the probiotic at a level of 0.1% in the ration of laying hens for 21 weeks does not affect liver enzymes compared to the control treatment; because it does not contain harmful substances that cause inflammation or damage to liver cells, as a result of which the rate of these enzymes increases, and significant differences appear in table.

Conclusion

The Manufactured Bacterial Preparation contributed to a significant improvement in the PCV and biochemical parameters of the blood serum (cholesterol, total protein, albumin, globulin) in laying hens. It did not affect the ratio of heterophile cells to lymphocytes (H/L), the concentration of liver enzymes AST, ALT and glucose sugar. A significant improvement in some blood parameters will contribute to the improvement of the Laying Hens' health status, which will be reflected positively in increased production.

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