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Research Article
Effect of Probiotic Consortium Administration in Improving Organ Function and Blood Biochemistry in Laying Chickens

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Abstract

Background and Objective: Probiotics have been known as a potential alternative to replace antibiotic growth promotors and have many benefits for poultry health. This research investigated that how administering a combination of probiotics affects the health and physiological parameters of laying chickens. This includes understanding if and how probiotics can enhance organ function and influence blood biochemistry profiles in these birds, therefore they are used to increase the production of laying hens. **Materials and Methods:** A Completely Randomized Design (CRD) with 56 hens was used. The treatments with two consortium yogurt B1 (*Bifidobacterium* spp. and *L. acidophilus*) and B2 (*L. bulgaricus*, *S. thermophilus*, *L. acidophilus* and *B. bifidum*) consisted of a control group that was not given the same treatment as the control group (T0), Group-1 was treated with WSPE probiotic B1 2% (T1), Group-2 was treated with WSPE probiotic B2 2% (T2), Group-3 was treated with 2% probiotic B1 powder (T3), Group-4 was treated with 3% probiotic B1 powder (T4), Group-5 were treated with 2% B2 probiotic powder (T5) and Group-6 were treated with 3% B2 probiotic powder (T6), data were analyzed using Analysis of Variance (ANOVA) and followed by Duncan's multiple range test. **Results:** The giving consortium probiotics to laying hens has a significant effect on uric acid levels as well as decreased SGOT, SGPT and creatinine levels also increasing total protein, albumin and globulin levels. **Conclusion:** The use of probiotics 2% increased organ function, namely an increase in total protein, albumin and globulin levels.

Key words: Laying hens, probiotic yogurt, blood biochemistry, organ function, physiology

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Currently, antibiotics are no longer used as additives in feed, so many smallholder farmers replace the use of antibiotics with feed additives in the form of (probiotics, phytobiotics and acidifiers). Probiotics is a term that refers to microorganisms that provide benefits to humans and animals. Probiotics have been widely used to improve production and body condition of livestock. These microorganisms play a role in the balance of intestinal microbes and are important in maintaining health. Probiotics also produce antibacterial compounds that can control intestinal pathogens as they compete for nutrients and adhesion sites[1]. The addition of a certain amount of probiotic powder can increase hemoglobin, hematocrit, erythrocyte and leukocyte levels, as well as reduce blood cholesterol and triglyceride levels, Gamma-Glutamyl Transferase (GGT), creatinine, uric acid, SGOT, SGPT, protein total, albumin and globulin[2]. Probiotics enhance intestinal villi repair by enlarging their surface area, thereby boosting nutrient absorption. Additionally, they stimulate metabolism and hormone synthesis, promoting increased productivity in laying hens. Yogurt, containing lactic acid, bacteriocins and antioxidants, enhances digestibility and production in hens by inhibiting pathogenic bacterial growth. Sufficient supplementation can thus impart health benefits to livestock[3,4].

This research used bacteria with strains of *Lactobacillus acidophilus*, *Streptococcus thermophilus* and *Bifidobacterium bifidum*. Probiotic yogurt powder containing these bacteria can increase egg production at certain doses. Previous research using probiotic yoghurt can improve blood fat content in broiler chickens[5]. The greater the quantity of microorganisms employed, the quicker the fermentation of probiotic yogurt occurs, consuming more substrate. Following the drying process, the total count of lactic acid bacteria reached 1.6×10^7 CFU/g[6]. Apart from using powdered yogurt, this research also used WSPE from liquid yogurt with the same content. Water-soluble peptide extract (WSPE) found in probiotic yogurt is derived from yogurt and consists of water-soluble peptides. The WSPE exhibits antibacterial properties similar to bacteriocins, contributing to its health-promoting benefits[7]. Bacteriocins are peptides or protein compounds that are released extracellularly by lactic acid bacteria and have bacteriostatic and bactericidal effects on pathogenic bacteria[8]. This bacteriocin compound can control intestinal pathogens through competition for nutrients and attachment sites[9]. Some researchers use liquid or dry forms of yogurt. Using probiotic yogurt powder is better than liquid because it mixes well and doesn't leave residue in the feed area. The results of drying probiotics produce lower microbiota than the liquid form. So this research uses these two methods to determine the effect of probiotic yogurt on organ function and blood biochemistry of poultry.

MATERIALS AND METHODS

This research was carried out in June-August, 2023 at the Biochemistry and Food Chemistry Laboratory, Department of Chemistry, Faculty of Animal Husbandry, Padjadjaran University and Test Farm, Faculty of Animal Husbandry, Padjadjaran University, Indonesia.

Making probiotic yogurt powder: The bacteria utilized were sourced from the Nanobio Laboratory. Two consortia, designated as B1 and B2, were employed in the study. Consortium B1 consisted of *Bifidobacterium* spp. and *Lactobacillus acidophilus*, while consortium B2 included *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, *Lactobacillus acidophilus* and *Bifidobacterium bifidum*. These bacteria were inoculated at a concentration of 7.5% (v/v) into 250 mL of De Man Rogosa and Sharpe (MRS) media and then incubated at 37°C for 24 hrs. About 1 L fresh milk from the North Bandung Milk Cooperative (KSBU Lembang) underwent pasteurization at 80°C. After cooling to 45°C, 7.5% of the consortium bacteria was added and homogenized. The fermentation process lasted 14 hrs. In the production of probiotic powder, the probiotic yogurt was mixed with an encapsulating agent (maltodextrin), added to sterile distilled water (making up half of the total solution volume) and stirred and homogenized.

Once homogeneous, the mixture is dried using a spray dryer with an inlet temperature of 160°C and an outlet of 65-70°C to produce probiotic yogurt in powder form. To make WSPE liquid yogurt, the yogurt sample is centrifuged at 10,000 rpm at a temperature of 4°C for 10 min. The supernatant obtained was filtered using filter paper to obtain WSPE.

Experimental procedures

Livestock preparation: Fifty-six laying hens, 31 weeks old, female bred at Sapta Karya Megah and Intama. Laying hens are maintained at a temperature of 24-27°C under 24 hrs light and 60% relative humidity, with feed and drinking water ad libitum for 6 weeks at the Test Farm, Faculty of Animal Husbandry, Padjadjaran University. Laying hens were randomly divided into seven treatment groups. The control group was not given the same treatment as the control group (P0), Group-1 was treated with WSPE probiotic B1 (*Bifidobacterium* spp. and *L. acidophilus*) 2% (P1), Group-2

Table 1: List of probiotic yogurt treatment in feed

Category	Treatment
T0	Only basal feed (without probiotics)
T1	Basal feed+2% WSPE probiotic B1
T2	Basal feed+2% WSPE probiotic B2
T3	Basal feed+2% powder probiotic B1
T4	Basal feed+3% powder probiotic B1
T5	Basal feed+2% powder probiotic B2
T6	Basal feed+3% powder probiotic B2

B1: *Bifidobacterium* spp.+*L. acidophilus* and B2: *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, *Lactobacillus acidophilus* and *Bifidobacterium bifidum*

was treated with WSPE probiotic B2 (*L. bulgaricus*, *S. thermophilus*, *L. acidophilus* and *B. bifidum*) 2% (P2), Group-3 was treated with 2% probiotic B1 powder (P3), Group-4 was treated with 3% probiotic B1 powder (P4), Group-5 was treated 2% probiotic B2 powder (P5) and Group-6 was given 3% probiotic B2 powder (P6). All laying hens were adapted for 2 weeks before treatment.

Treatment: This treatment was carried out for 6 weeks. Laying hens for probiotic powdered yogurt and WSPE were given seven treatments and repeated four times. Each cage contains 2 laying hens and is labeled with treatment and repetition numbers to facilitate observation and data collection. Probiotic mixed feed is given twice, namely in the morning and evening, at 120 g/head/day and drunk *ad libitum*. Eating and drinking places are always monitored and cleaned to prevent disease. The treatments used can be seen in Table 1.

Blood sample collected: After maintenance for 6 weeks a blood sample was taken using a syringe inserted from the pelvic vein (external pectoral vein) in the amount of 3 mL. The blood sample is then placed in a container containing EDTA which is then shaken gently and then stored in the refrigerator before the blood is analyzed.

Sample analysis: After completing treatment, chicken blood was taken from the external pectoral vein using a venoject tube containing 3 mL of EDTA anticoagulant. The blood was centrifuged at a speed of 3500 rpm for 15 min, to collect the plasma fluid. After that, the plasma was separated and collected into a 3 mL complementary tube, using a micropipette. Plasma is used for analysis of Gamma GT, SGOT, SGPT, total protein, albumin, globulin, creatinine and uric acid. This blood biochemical analysis uses the spectrophotometric method with analysis techniques based on the instructions in the Biolabo kit[10].

Statistic analysis: All data were analyzed statistically using Analysis of Variance (ANOVA) and followed by Duncan's multiple range test using SPSS 22.0. Statistical significance was set at $p>0.05$.

RESULTS AND DISCUSSION

From Table 2, it is known from the results of statistical analysis that giving probiotic yogurt has a significant effect on uric acid and has no significant effect on Gamma GT, SGOT, SGPT, total protein, albumin, globulin and creatinine. The uric acid content of T1 has a lower content than T0, while the other treatments are higher than T0. Even though statistical calculations, giving probiotics does not have a significant effect, it can be seen that giving probiotics in low doses can decrease Gamma GT, SGOT, SGPT and creatinine levels and increase total protein, albumin and globulin levels.

From the treatment used, it is known that probiotic yogurt has a no significant effect on Gamma GT, SGOT and SGPT levels. Giving probiotic yogurt at T1 2% or giving low doses of probiotics shows a decrease in uric acid levels, meaning that low doses of yogurt can improve kidney function. In the results of this study, the levels of Gamma Glutamyl Transpeptidase (γ-GT) in the blood plasma of treated laying hens decreased at low doses, this shows that there is a positive correlation with the administration of probiotic yoghurt. The addition of 2% probiotic yogurt at T1 reduced Gamma GT levels compared to without treatment by 58,327 IU/L. Several enzymes can be indicators of liver performance besides Gamma GT, namely serum Glutamate Oxaloacetate Transminase (SGOT) and Serum Glutamate Pyruvate Transaminase (SGPT). Transaminases are a group of enzymes that act as catalysts in the process of transferring amino groups from alpha-amino acids and alpha-keto acids[11]. Both serums are indicators that indicate liver damage if the values are high. The SGOT reflects more general damage such as muscle cells, brain cells, liver cells, heart cells, etc. while SGPT in liver cells. The normal ranges of SGPT and SGOT have standards. However, the range can vary slightly, depending on the testing method in the laboratory. From this, it can be concluded that the physiological conditions of livestock blood differ according to their environment and giving this probiotic yogurt can reduce levels SGPT and SGOT livestock. Giving probiotics can function as an antioxidant so that it can improve health because it can ward off free radicals[12]. The results of this study showed that giving probiotics can decrease SGOT and SGPT levels compared to the control, indicating that free radicals can be prevented by probiotics

Table 2: Blood test result on laying hens given probiotic yogurt

Parameter	T0	T1	T2	T3	T4	T5	T6	p-value
Gamma GT	454.9545	396.627	474.6798	337.239	413.595	282.093	451.773	0.18631
SGOT	14.144	7.514	8.545	3.094	11.492	7.514	12.376	0.71446
SGPT	8.398	7.072	6.7773	1.768	3.978	7.072	8.84	0.2459
Total protein	5.266	5.763	4.782	4.909	5.019	5.563	5.327	0.48766
Albumin	2.161	2.354	2.076	2.161	2.182	2.264	2.122	0.80818
Globulin	3.105	3.408	2.706	2.747	2.837	3.298	3.205	0.71753
Creatinine	0.274	0.207	0.259	0.244	0.267	0.244	0.211	0.48644
Uric acid *	6.21^{a}	5.67^{a}	6.80^{ab}	10.03^{c}	7.15^{ab}	8.49^{bc}	6.48^{ab}	0.04144

*Shows that the treatment has a significant effect, ^{abc}Different superscript letters behind the experimental mean, indicating different responses significantly ($p<0.05$), GT: Glutamyl transferase, SGOT: Serum Glutamic Oxaloacetic Transaminase and SGPT: Serum Glutamic Pyruvic Transaminase

because some of the peptides in probiotics are antioxidants which can donate nitrogen atoms to hydroxyl to be neutralized, thereby preventing the formation of free radicals[13].

From the treatment used, it is known that probiotic yogurt has a significant effect on improving uric acid. On the parameters of creatinine, total protein, albumin and globulin in laying hens, the administration of this probiotic did not have a significant effect. Using a probiotic consortium is more beneficial than using only one species because it contains more active compounds. Administration of various combinations of probiotics often affects NH_3 concentrations in blood serum. Even though giving probiotic yogurt does not have a real effect, giving this probiotic can reduce creatinine levels at a low dose of T1 2% compared to the control. It can also increase levels of total protein, albumin and globulin at a low dose of T1 2%, so giving this probiotic is good for kidney function. According to research by Yeo and Kim[14], probiotics break down amino acids into NH_3, which are subsequently metabolized into uric acid (UA) in the liver of chickens. Li *et al.*[15] also reported a reduction in serum NH_3 and UA concentrations following probiotic supplementation. Administering feed with *Lactobacillus plantarum*, *Lactobacillus bulgaricus*, *L. acidophilus*, *Streptococcus thermophilus* and *Bifidobacterium bifidum* to male White Leghorn laying hen breeders led to increased total protein (TP) and albumin (ALB) levels in the serum[16]. Another research by Abramowicz *et al.*[17] stated that there was a decrease in uric acid and creatinine in laying hens given probiotics. Probiotics increased protein metabolism which allows better utilization of nitrogen, so that uric acid and creatinine or other toxins can be used as nutrients for the growth of probiotic bacteria. Low creatinine levels can be an indication of increased protein metabolism and have a protective effect on kidney function[12].

Blood serum proteins which include total protein, albumin and globulin will experience improvement due to the presence of lactic acid bacteria in bacterial preparations produced by probiotics which function to increase protein synthesis. The increase in absorption of the amino acid lysine which is secreted by several types of lactic acid is reflected in the increase in total protein in laying hens, followed by the concentration of albumin and globulin[18]. The results of this study showed that administration of probiotics had no significant effect on total protein, albumin and globulin levels, but at P1 results tended to increase levels compared to treatment. This was in line with previous research that giving probiotics to laying hen feed did not affect total protein, albumin and globulin in 32 week old laying hens under heat stress conditions[19]. Some researcher also stated that probiotics used in feed for 8 weeks did not affect total protein and albumin[20]. In this study, although these values did not have a statistically significant effect, administration of probiotic yogurt tended to increase total protein, albumin and globulin at P1 and P5. This happens because the yogurt consortium produces better protease enzymes, yogurt with consortium bacteria has high proteolytic activity which will produce a low pH or high acidity level. Increasing levels of this protease enzyme will cause an increase in protease enzyme activity in the digestive tract. Protease enzymes break down the peptide chains in feed protein to release amino acids that the body needs[21]. In the future, the use of probiotics 2% is expected to show an increase in organ function, namely an increase in total protein, albumin and globulin levels. The implications of giving probiotics can increase total blood protein which will result in the final result of blood protein content being closely related to the protein in egg yolk. In its application, you can use probiotic yoghurt in liquid form or in powder form which has almost the same benefits, but in practice using powder will be easier to apply because it can be mixed with feed.

CONCLUSION

Giving probiotic consortium B1 (*Bifidobacterium* spp.+ *Lactobacillus acidophilus*) and B2 (*Lactobacillus bulgaricus*, *Streptococcus thermophilus*, *Lactobacillus acidophilus*, *Bifidobacterium bifidum*) at a low dose of 2% T1 can

improve total protein, albumin and globulin although there is no significant difference as evidenced by the increase in total protein, albumin and globulin levels. Likewise for kidney function, namely uric acid and creatinine, proven by a decrease at a low dose of 2% T1. Meanwhile, for liver function parameters, all treatments were within the normal range.

SIGNIFICANCE STATEMENT

Providing a probiotic consortium to laying hens has good benefits for livestock health. This research shows the effect of giving yogurt consortium on organ function and blood biochemistry of laying hens. No research report previously showed the effect yoghurt containing lactate bacterium consortium and its effect on functional organs. In this study, giving probiotics had a significant effect on uric acid levels. Although the other ingredients do not have a significant effect, probiotics can decrease SGOT, SGPT and Gamma GT and also increase total protein, albumin and globulin in laying hens.

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REFERENCES

1. Adriani, L., D. Latipudin, N. Mayasari, A. Mushawwir, C. Kumalasari and T.I. Nabila, 2024. Consortium probiotic fermented milk using *Bifidobacterium* sp. and *Lactobacillus acidophilus* protects against *Salmonella typhimurium* and repairs the intestine. Asian J. Dairy Food Res., 43: 216-218.
2. Adriani, L., C. Kumalasari, E. Sujana and R. Lesmana, 2023. Probiotic powder supplementation in haematology and biochemistry blood late-phase laying hens. Adv. Anim. Vet. Sci., 11: 364-370.
3. Hill, C., F. Guarner, G. Reid, G.R. Gibson and D.J. Merenstein *et al*., 2014. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat. Rev. Gastroenterol. Hepatol., 11: 506-514.
4. Widjastuti, T., L. Adriani, I.Y. Asmara, I. Setiawan, Abun and L. Nurlaeni, 2021. Effect of mangosteen peel extract (*Garcinia mangostana* L.) with supplemental zinc and copper on performance and egg quality of sentul laying chicken. Jordan J. Biol. Sci., 14: 1015-1020.
5. Adriani, L., A. Andiany, D. Latipudin, T. Benito and C. Cahyani, 2018. Effect of fermented cow and soybean milk with probiotic in improving blood cholesterol and triglyceride levels on broilers. Int. J. Poult. Sci., 12: 600-604.
6. Adriani, L., D. Latipudin, I.M. Joni, C. Panatarani and G. Sania, 2021. Hematological status and egg production of laying hen with probiotic powder as feed supplements. IOP Conf. Ser.: Earth Environ. Sci., Vol. 902. 10.1088/1755-1315/902/1/012032.
7. Taha, S., M. El-Abd, C. de Gobba, M. Abdel-Hamid, E. Khalil and D. Hassan, 2017. Antioxidant and antibacterial activities of bioactive peptides in buffalo's yoghurt fermented with different starter cultures. Food Sci. Biotechnol., 26: 1325-1332.
8. Kim, K., J.J. Kim, R. Masui, S. Kuramitsu and M.H. Sung, 2011. A commensal symbiotic interrelationship for the growth of *Symbiobacterium toebii* with its partner bacterium, *Geobacillus toebii*. BMC Res. Notes, Vol. 4. 10.1186/1756-0500-4-437.
9. Servin, A.L., 2004. Antagonistic activities of lactobacilli and bifidobacteria against microbial pathogens. FEMS Microbiol. Rev., 28: 405-440.
10. Adriani, L., R. Permana and D. Latipudin, 2024. Dried probiotics enhances immunity and liver function of final production laying hens maintained at upper temperatures zone. IOP Conf. Ser.: Earth Environ. Sci., Vol. 1292. 10.1088/1755-1315/1292/1/012009.
11. Hartoyo, B., N. Iriyanti and E.A. Rimbawanto, 2020. Liver function and blood glucose levels of broiler chickens with various types of acidifiers as feed additives in feed containing probiotics [In Indonesian]. Rosiding Semin. Nasional Teknologi Agribisnis Peternakan, 7: 651-662.
12. Adriani, L., A. Darmadi, D. Latipudin, D. Rusmana and T. Widjastuti, 2022. Improving the level of serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate piruvate transaminase (SGPT) due to the addition of probiotic powder in phase layer chicken. Anim. Food Sci. J. Iasi, 77: 191-196.
13. Kamil, K.A., D. Latipudin, A. Mushawir, D. Rahmat and R.L. Balia, 2020. The effects of ginger volatile oil (GVO) on the metabolic profile of glycolytic pathway, free radical and antioxidant activities of heat-stressed cihateup duck. Int. J. Adv. Sci. Eng. Inf. Technol., 10: 1228-1233.
14. Yeo, J. and K.I. Kim, 1997. Effect of feeding diets containing an antibiotic, a probiotic, or yucca extract on growth and intestinal urease activity in broiler chicks. Poult. Sci., 76: 381-385.
15. Li, W.F., I.R. Rajput, X. Xu, Y.L. Li, J. Lei, Q. Huang and M.Q. Wang, 2011. Effects of probiotic (*Bacillus subtilis*) on laying performance, blood biochemical properties and intestinal microflora of shaoxing duck. Int. J. Poult. Sci., 10: 583-589.
16. Khaliq, T., A. Iftikhar, Zia-Ur-Rahman, H. Anwar and J.A. Khan *et al*., 2016. Effect of vitamins, probiotics and low protein diet on lipid profile, hormonal status and serum proteins level of molted white leghorn male layer breeders. Pak. J. Life Soc. Sci., 14: 18-23.

17. Abramowicz, K., M. Krauze and K. Ognik, 2019. The effect of a probiotic preparation containing *Bacillus subtilis* PB6 in the diet of chickens on redox and biochemical parameters in their blood. Ann. Anim. Sci., 19: 433-451.
18. Al-Salhi, A. and S. Al-Shatty, 2023. Effect of the manufactured bacterial preparation on some cellular and biochemical blood characteristics of laying hens. An-Najah Univ. J. Res. (Nat. Sci.), 37: 33-38.
19. Fathi, M., I. Al-Homidan, A. Al-Dokhail, T. Ebeid, O. Abou-Emera and A. Alsagan, 2018. Effects of dietary probiotic (*Bacillus subtilis*) supplementation on productive performance, immune response and egg quality characteristics in laying hens under high ambient temperature Ital. J. Anim. Sci., 17: 804-814.
20. Naseem, S., N. Willits and A.J. King, 2021. Varying combinations of *Lactobacillus* species: Impact on laying hens' performance, nitrogenous compounds in manure, serum profile, and uric acid in the liver. Transl. Anim. Sci., Vol. 5. 10.1093/tas/txab018.
21. Situmeang, J., L. Adriani, D. Saefulhadjar and S. Ishmayana, 2024. Protease and lipase enzyme activity of probiotic yogurt and its effect on protein, lipid, and cholesterol level of chicken egg yolk. Adv. Anim. Vet. Sci., 12: 873-878.