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Effect of combination magnetic technology and encapsulated probiotics in the drinking water on blood profile and immunity of laying hens

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Abstract
This study aimed to determine the health status of laying hens using a combination of magnetic technology and probiotics in drinking water. A total of two hundred and eighty-eight (288), 57-week-old ISA Brown laying hens were assigned to six treatments with nine replicates in drinking water. Statistical data analysis followed a completely randomized design with treatments including water as control, magnetic technology (MT), 0.6% probiotic (PRO), 0.6% probiotic and magnetic technology (PRO+MT), 0.6% encapsulated probiotic (EPRO), and 0.6% encapsulated probiotic and magnetic technology (EPRO+MT). The laying hens received treatments for six weeks (42 d). The encapsulated probiotic was used to improve probiotic durability from intestinal damage. The health status consisted of blood and immunity profiles, including leukocytes, erythrocytes, haemoglobin, and haematocrit, CD8+, CD4+, malondialdehyde, and B cells. The water quality was improved by the use of magnetic technology. Magnetic technology effectively decreased the $CD8^+$, $CD4^+$, malondialdehyde, and B cells in the blood of laying hens compared to treatments without magnetic technology. The combination of magnetic and encapsulation technology showed maximum results in improving blood profile and immune response.

Keywords: blood profile, encapsulated probiotic, laying hens, magnetic technology
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Introduction
Several factors that influence laying hen production include management, feed, and health status. The provision of feed and drinking water to laying hens is an important factor in increasing production. Management of water quality in laying hens is important because the quality of drinking water influences the health status. The presentation of drinking water using magnetic technology is an effort to improve the quality of water. Generally, drinking water contains minerals such as Mg^{2+}, Ca^{2+}, CO_3^{2-}, and HCO_3^- (Nihlgård *et al.*, 2020). The dissolved ions that have bonds are the ions formed by Ca^{2+} and CO_3^{2-}. Magnetic technology releases Ca^{2+}, which is used as an antibacterial compound to reduce the population of bacteria in drinking water. Immune response in poultry has been enhanced in water treatment using magnetic technology containing electrolytes, glucose, and citric acid (El Hadri *et al.*, 2004). The content of Ca and Mg in water will substantially affect the immune response, blood profile, and antioxidants of laying hens (Khajali *et al.*, 2008; Attia *et al.*, 2015).

In addition, the use of antibiotic growth promoters (AGPs) is an option to maintain the production and health status of laying hens. However, the use of AGPs has been banned as a feed additive for poultry (Nuningtyas *et al.*, 2021). One solution to overcome this problem is to use natural

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growth promoters (NGPs). In a free range system, the addition of 1% yeast autolysate in the diet is recommended for partridge breeding (Bolacali *et al.*, 2018). Furthermore, the addition of 0.3 and/or 0.4% synbiotic has a positive effect on quails by improving performance and enhancing some serum lipids and protein parameters (Tufan & Bolacali, 2017). The pathway of probiotics and prebiotics to decrease absorption in the gastrointestinal tract by the synthesis of cholesterol in the liver can lead to a reduction in the serum cholesterol of broiler chickens fed probiotic- and prebiotic-supplemented diets. Probiotics, one of the NGPs, will adapt and symbiose with bacteria in the intestinal system and will not affect resistance. Probiotics are used as natural growth promoters, for microflora modulation in the intestine, as inhibitors of pathogenic bacteria, for immunomodulation, and meat quality improvement (Zhang *et al.*, 2012; Sobczak & Kozłowski, 2015). According to Ayasan (2016), inclusion of probiotics at 1,5 kg/ton decreases the average body weight of quail. Probiotics as feed supplements can improve the balance of microorganisms in the gut, prevent the growth of pathogenic microbes, support digestion, lower cholesterol, and improve immune function (Xiang *et al.*, 2019). The use of probiotics in feed supplementation improves health status by increasing the concentration of immunoglobulins and eliminating pathogenic bacteria in laying hens (La Fata *et al.*, 2018).

A probiotic powder has a problem, namely the low level of homogeneity, so the effectiveness of probiotics in the digestion of chickens is also constrained. Powdered probiotics are also quite expensive compared to liquid probiotics. Liquid probiotics are also problematic when served in drinking water. Liquid probiotics have a low shelf-life, lasting only 2–4 h after serving in drinking water. The low resistance of probiotics in drinking water will affect the effectiveness of probiotics in laying hens. Encapsulation technology is the process of liquid-coating probiotics to protect probiotics from damage and increase probiotic durability. Encapsulation technology aims to protect the active substance in feed by adding encapsulation as a coating, protecting it from damage (Natsir *et al.*, 2010; Lee *et al.*, 2020; Liu *et al.*, 2020). This study aims to evaluate the combination of magnetic technology and encapsulated probiotics in the drinking water on blood profile and immunity of laying hens.

Materials and Methods

All laying hens in this experiment were approved by the Animal Care Committee of Universitas Brawijaya, Indonesia, with the ethical clearance number, 066- KEP-UB-2020, which was signed by the Head of the Ethic Committee.

Two hundred and eighty-eight (288) ISA Brown laying hens of 57-weeks-old were used in this study and reared in battery cages (40 cm × 35 cm × 30 cm) that contained one laying hen per cage. The magnetic technology used level 2700 Gauss, which was counted by Gaussmeter in the Electrical Engineering Laboratory of the Faculty of Engineering, Universitas Brawijaya. A three-channel Gauss-meter with a three-axis probe was used to measure the magnetization.

Probiotics that served in this study contained *Lactobacillus* sp., *Bacillus* sp., *Saccharomyces* sp., and *Pseudomonas* sp. at 1.8 x 10^7 cfu/ml. The encapsulation technology used two coating processes, which were conducted in the Animal Feed Industry Laboratory of the Faculty of Animal Science, Universitas Brawijaya. The first encapsulation used chitosan, and the second encapsulation used Arabic gum and whey protein as encapsulants. The feed was analysed in the Animal Nutrition and Feed Laboratory of the Faculty of Animal Science, Universitas Brawijaya. Feed in this experiment was self-mixed using corn, rice bran, soybean meal, meat bone meal, grit, lysine, methionine, premix, salt, and monocalcium phosphate. The feed used was free from antibiotic growth promotors. The ingredients and calculated nutrient level of the feed are shown in Table 1.

Table 1 Ingredients and nutrient content of feed for 57-week-old ISA Brown laying hens

Ingredients	**Value (%)**	**Nutrient Content**	**Value (%)**
Corn	52.7	Dry Matter	90.28
Rice brain	13.95	Metabolism Energy (kcal/kg)	2959
Soybean meal	24.5	Crude Protein	19.44
Meat bone meal	4.7	Crude Fiber	2.95
Grit	3.1	Crude Fat	4.93
Lysine	0.1	Ash	7.99
Methionine	0.15		
Premix[1]	0.2		
Salt	0.2		
Monocalcium Phosphate	0.4		
Total	100		

[1] Premix from PT. MITRAVET (Composition/kg: vitamin A: 2 000 000 IU, vitamin D3: 400 000 IU, vitamin E: 3 000 mg, vitamin K: 400 mg, vitamin B12: 4 mcg, thiamin HCI/B1: 400 mg, riboflavin HCI/B2: 1 200 mg, pyridoxin HCI/B6: 800 mg, Ca-d-pantothenate: 2 160 mg, niacinamide: 8 000 mg, folic acid: 200 mg, biotin: 4 mg, L-Carnitine : 10 000 mg, copper sulphate: 4 000 mg, cobalt sulphate: 300 mg, ferro sulphate: 10 000 mg, Mn oxide: 20 000 mg, sodium selenite: 150 mg, carrier ad: 1 000 mg)
[2]Nutrient contents expressed as % unless otherwise stated

The laying hens were divided into six groups with four replications, where every replication consisted of twelve laying hens. The treatments were conducted in drinking water, including water as a control, magnetic technology (MT), 0.6% probiotic (PRO), 0.6% probiotic and magnetic technology (PRO+MT), 0.6% encapsulated probiotic (EPRO), and 0.6% encapsulated probiotic and magnetic technology (EPRO+MT). The treatments were conducted for six weeks (42 d). The feed was presented once a day with a total intake of 120 g/head/day. The blood samples was taken in the last period (after 6 w) through the vena pectoralis using a vacuum tube. The blood samples were analysed using isolation cells and the flow cytometry method to determine CD8+, CD4+, malondialdehyde (MDA), and B cells. An improved Neubauer method was used to determine leukocytes, erythrocytes, haemoglobin, and haematocrit (Su *et al*., 2014; Liermann *et al*., 2019).

The data was analysed as a completely randomized design using SPSS software (version 26, IBM, USA). The biological model of this experiment was:

$$Y_{ij} = \mu + T_i + \varepsilon_{ij} \quad (1)$$

where:
Y_{ij} = observed value in treatment i, repetition j
μ = common mean
T_i = effect of treatment i
E_{ij} = random effect (trial error) on treatment i and repetition j

Significant differences were established at P <0.05. Moreover, probability vales were calculated using Duncan’s test if there were significant differences (P <0.05).

Results and Discussion

Table 2 describes the effect of using magnetic technology and probiotics in drinking water on blood profiles in various treatments. The treatments in this study included a control, magnetic technology, probiotics, probiotics + magnetic technology, encapsulated probiotics, and encapsulated probiotics + magnetic technology. The use of magnetic technology and probiotics in drinking water had an effect (P <0.01) on the blood profile, including leukocytes, erythrocytes, haemoglobin, and haematocrit. The use of magnetic technology and probiotic encapsulation improved the blood profile (Table 2).

Table 2 The effect of using magnetic technology and probiotics in drinking water on blood profile in laying hens

Treatments	**Leukocyte (10^3/µL)	**Erythrocytes (10^6/µL)	** haemoglobin (g/dL)	** haematocrit (%)
Control	18.00^e	$2.27b^c$	$6.40b^c$	30.00^a
MT	15.80^d	2.41^c	5.20^a	28.00^a
PRO	7.70^b	1.90^{ab}	5.20^a	26.00^a
PRO+MT	6.90^b	2.65^c	7.20^d	34.00^b
EPRO	4.50^a	2.42^c	6.80^c	29.00^a
EPRO+MT	11.70^c	1.84^a	6.00^b	26.00^a
SEM	1.04	0.07	0.17	0.74
p	<0.01	<0.01	<0.01	<0.01

MT: magnetic technology, PRO: non-encapsulated probiotic, EPRO: encapsulated probiotic
$^{a-e}$ Different letters indicate significant differences between the means
**indicates a highly significant difference (*P* <0.01)

The statistical results showed that the combination of magnetic technology and encapsulated probiotics in the drinking water had an effect on leukocytes, erythrocytes, haemoglobin, and haematocrit of laying hens (*P* <0.01). Increasing leukocytes are a parameter of immunity and decreased leukocytes are an indication of infection or the presence of pathogenic bacteria in the body. Leukocytes are white blood cells that fight infection and are used for body immunity. The highest leukocytes were found in the control treatment at 18.00 103/µL. Erythrocytes are red blood cells that carry oxygen from the lungs to all body tissues. PRO+MT delivered the best result for the erythrocyte value in laying hens. This is because the use of magnetic technology improved the activity of erythrocytes. The use of magnetic technology and probiotics in drinking water positively affected the blood profile of laying hens, including leukocytes, erythrocytes, haemoglobin, and the haematocrit. This is contrary to previous research, which reported that the use of probiotics could improve blood haematology (leukocytes, erythrocytes, and haemoglobin) in 52-week-old laying hens (Tang *et al.,* 2017). Kuo *et al.* (2020) reported that animal health could be measured from total leukocytes. Haemoglobin is a protein in red blood cells that functions in binding oxygen. PRO+MT produced the best result for the haemoglobin value in laying hens. The haematocrit is the percentage of red blood cells to blood volume. Red blood cells have an important role in the health of the body, namely as a carrier of oxygen and nutrients to all parts of the body. Blood haematocrit levels showed the highest value in the PRO + MT treatment. This difference may be because blood leukocyte levels are influenced by sex, hormones, drugs, and disease in chickens (Forsblad-d'Elia *et al.,* 2009). The profile of white blood cells consists of total leukocytes, including the percentage of heterophils, eosinophils, basophils, lymphocytes, and monocytes. Magnetic technology and probiotics in drinking water markedly increased the blood erythrocyte levels of laying hens. Magnetic technology generally improved blood erythrocyte levels compared to the treatments without magnetic fields. PRO+MT was the best result for the erythrocyte value of layer hens. This was affected by probiotic fermentation, which acidifies the gastrointestinal tract and results in better absorption from the small intestine. Erythrocytes are in charge of transporting nutrients from digestive processes for metabolism. An increase in blood erythrocyte levels indicates an increase in the metabolism of layer hens, which means an increase in the immune response. The number of erythrocytes is induced by biological supplementation of the feed digested by hens (Çetin *et al.,* 2005). Probiotics improve the balance of intestinal conditions by increasing the growth of non-pathogenic bacteria. This condition improves absorption of nutrients in the digestive system of laying hens.

PRO+MT increased haemoglobin in the blood of laying hens (*P* <0.01). There was an increase in haemoglobin levels caused by the use of probiotics in drinking water. These results follow previous studies that reported that probiotic supplementation markedly increased the erythrocyte count, haemoglobin concentration, and haematocrit value in Turkish chickens (Cetin *et al*., 2005). In contrast, Torshizi *et al.* (2010) stated that blood haemoglobin was not affected by probiotic treatment. This is possible because of the addition of magnetic technology, which improved the quality of drinking water in this study so that it could optimize the effect of using probiotics in laying hens. Increased haemoglobin concentration indicates a better health status. Blood haematocrit levels were highest in the PRO + MT treatment (P <0.01), in agreement with previous studies in which the haematocrit value changed with probiotic treatment (Çetin *et al.*, 2005; Khajali *et al.*, 2006). Capcarova *et al.* (2010) reported that probiotics in feed substantially decreased the haematocrit of laying hens. This difference may be due to differences in the types of probiotics used in the study. Increased levels of haemoglobin and blood haematocrit indicated an increase (P <0.01) in the health status of laying hens. The use of magnetic technology and probiotics in drinking water increased the number of erythrocytes, haemoglobin, and haematocrit (P <0.01).

The use of magnetic technology and probiotics in drinking water on immunity is reported in Table 3. An immune response is evidenced in the total CD8+, CD4+, MDA, and B cells. Magnetic technology and probiotics given through drinking water had an effect (P <0.01) on the immunity of laying hens.

Table 3 Effect of using magnetic technology and probiotics in drinking water on immunity of laying hens

Treatments	**$CD8^+$ (% gated)	**$CD4^+$ (% gated)	**Malondialdehyde (% gated)	**B Cells (% gated)
Control	8.62^d	11.50^a	12.21^d	1.86^c
MT	8.43^d	11.75^a	5.65^b	1.54^b
PRO	4.31^b	34.75^c	16.74^f	1.27^b
PRO+MT	4.13^b	12.25^a	8.90^c	0.89^a
EPRO	5.85^c	12.00^a	14.21^e	2.70^d
EPRO+MT	1.69^a	17.50^b	2.21^a	0.90^a
SEM	0.51	0.02	1.05	0.13
p	<0.01	<0.01	<0.01	<0.01

MT: magnetic technology, PRO: non-encapsulated probiotic, EPRO: encapsulated probiotic
$^{a-f}$ Different letters indicate significant differences between the means
**indicates a highly significant difference (P <0.01)

The use of EPRO+MT could reduce the total CD8+ and MDA content. The lower CD8+ value indicates the health of the laying hens. The low weight of MDA means a low value of oxidative stress in the body. The PRO treatment showed that the CD4+ value was the highest (P <0.01) of the treatments. In addition, the EPRO treatment showed the highest B cells value compared to other treatments. Magnetic technology and probiotics in drinking water had a marked effect on the CD8+ and CD4+ populations of blood lymphocytes. The treatments produced a numerical decrease in the $CD8^+$ population in blood lymphocytes. Mudroňová *et al.* (2020) stated that a decrease in the $CD8^+$ population in blood lymphocytes indicates better health in laying hens. This is because the $CD8^+$ population is a transmembrane protein that functions as a co-receptor on T killer cells. When T killer cells recognize the antigen complex, MHC I, $CD8^+$ as a co-receptor amplifies the transduction signal so that T killer cells are activated (Torshizi *et al.*, 2010). EPRO+MT produced the lowest values for the CD8+ population in blood lymphocytes. The addition of magnetic technology to improve water quality and encapsulation technology to protect probiotics in the digestive system can improve the health of laying hens, as seen from the smaller $CD8^+$ population of blood lymphocytes compared to other treatments.

EPRO had the highest $CD4^+$ population in blood lymphocytes in the laying hens. There was a numerical increase in the percentage of the $CD4^+$ population in blood lymphocytes in the current study (Table 3). An increase in the percentage of the $CD4^+$ population in blood lymphocytes indicates an improved immune response in laying hens. The $CD4^+$ population has a co-receptor function on helper T cells to recognize the antigen complex, MHC II, by amplifying the transduction signal so that T helper cells are activated. A decreased percentage of $CD4^+$ cells in blood lymphocytes will result in a decrease in T helper cell activity (Shete *et al.*, 2010). This circumstance is inversely proportional to the results of the $CD8^+$ cells, which have a positive impact on the health of laying hens. The ratio of $CD4^+$ to $CD8^+$ is a marker of immune stimulation. Healthy animals usually have larger $CD4^+$ T cell counts than CD8+. Certain conditions with disease infection will drastically reduce the $CD4^+$ T cells, and T lymphocytes will be dominated by $CD8^+$ T cells (Mudroňová *et al.*, 2020). EPRO+MT had the highest ratio of $CD4^+$ to $CD8^+$ (10.36), suggesting improved immune function in the hens. EPRO+MT increased $CD4^+$ values and decreased CD8⁺ cells. This suggests that EPRO+MT enhances immune function and improves the ability to resist pathogens. Cytokines produced by CD4⁺ T-helper cells promote humoral immunity and cell-mediated immunity (Liu *et al.*, 2020).

Magnetic technology and probiotics in drinking water improved MDA content in the blood of laying hens (P <0.01) (Table 3). The use of magnetic technology in drinking water consistently produced lower results compared to treatment without magnetic technology in drinking water. This concurs with previous research using magnetized water in rabbit bucks, which resulted in a decrease in the lipid peroxidation biomarker, MDA, and thiobarbituric acid-reactive (TBA) substances (Attia *et al.*, 2015). There was a positive correlation between the addition of a magnetic field (to improve the quality of drinking water) with the percentage of metabolic MDA in laying hens. MDA is a product of the process of lipid peroxidation and indicates oxidative cell damage (Tsikas, 2017). Lipid peroxidation will increase the production of MDA. Lipid peroxidation occurs when polyunsaturated fatty acids (PUFAs) are attacked by free radicals (Ayala *et al.*, 2014). PUFAs, which have hydrogen–carbon double bonds, are weakened by free radicals. The released hydrogen atoms form lipid radicals and produce lipid peroxyl radicals when oxidized. One of the end products of this process is MDA. Lipid peroxidation will disrupt membrane permeability physiology and inhibit metabolic reactions. A decreasing MDA value indicates that the metabolism of laying hens has improved. The addition of magnetic technology improved the quality of drinking water by reducing lipid peroxidation in the blood of laying hens by stimulating phagocyte cells to respond to cellular immunity. This will stabilize the increase in reactive oxygen species (ROS) and reduce MDA in the blood.

The use of magnetic technology and probiotics in drinking water had an impact on the B cells in blood lymphocytes. EPRO shows the highest yield in the percentage of B cells in blood lymphocytes (Table 3). The results in this variable were correlated with CD4⁺ cells. This is because B cells and T cells are related to performance. The lymphocytes conduct a role in cellular immune responses through cytotoxic T cells and T helper (Th) cells. The humoral immune response is conducted by B lymphocytes, producing antibodies. The entrance of an antigen in the body will produce the formation of antibodies by B cells (Honda *et al.*, 2015). The process of forming antibodies, when antigen fragments in cells bind to MHC II, affects the immune response, and the effective humoral immunity depends on B cell responses, which are dependent on CD4⁺ T cells (Akkaya *et al.*, 2020). The bond is channelled to the surface of the cell, where the combination of the MHC molecule and the antigen fragment is recognized by the Th cell. The cells activate B cells to produce antibodies. The results of the current study indicate that the use of encapsulated probiotics is successful in increasing immune power by increasing the percentage of B cells to produce antibodies for laying hens.

Conclusions

In conclusion, the combination of magnetic technology and encapsulation technology has shown beneficial results in improving the blood profile and immune response. This finding will be of use in improving the health status of laying hens.

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Authors' contributions

MHN (ORCID: 0000-0003-4830-7928) planned the experiment and statistically analysed the data; OS (ORCID: 0000-0003-0115-3853) critically and controlled the experiments; AM (ORCID: 0000-0001-6871-0525) helped in preparing the data, YFN (ORCHID:0000-0002-3095-1515) drafted the manuscript for the journal, FM helped in the *in vivo* research and in drafting the manuscript. All of the authors have read and approved this manuscript.

Conflict of interest declaration

There is no potential conflict of interest to this article.

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