Research Article

The Effects of Herbs as Feed Additives Through Feed and Drinking Water on Broiler Blood Parameters

Imbang Dwi Rahayu[1], Ali Mahmud[1*], Wahyu Widodo[1], Adi Sutanto[1], Apriliana Devi Anggraini[1], Devi Dwi Siskawardani[2], Wisnu Nurcahyo[3], Tri Untari[3]

[1]Department of Animal Science, Faculty of Agriculture and Animal Science, University of Muhammadiyah Malang, Malang 651444, East Java, Indonesia; [2]Department of Food Technology, Faculty of Agriculture and Animal Science, University of Muhammadiyah Malang, Malang 651444, East Java, Indonesia; [3]Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia.

Abstract | Using herbs as feed additives in poultry can improve the health that affects the quality of broiler chicken. This study aimed to determine the effects of giving herbs as feed additives through feed and drinking water on the biochemical parameters of broiler blood. In this study, 175 chickens were used and divided into seven levels of treatments; T0 (feed without herbs), T1 (feed + 1% herbs), T2 (Feed + 2% herbs), T3 (Feed + 3% herbs), T4 (drinking water +1% herbal), T5 (drinking water + 2% herbal), T6 (drinking water + 3% herbal). The data were analyzed using a one-way analysis of variance. As a result, the treatments did not affect (p>0.05) the total plasma protein (TPP), globulin, cholesterol, high-density lipoprotein (HDL), aspartate aminotransferase (AST), and all parameters of the blood profile. However, the herbs' addition significantly (p<0.05) affected albumin, triglycerides (TG), low-density lipoprotein (LDL), alanine aminotransferase (ALT), and malondialdehyde (MDA). Adding herbs through drinking water gave lower TG and LDL levels than through feed or the control. The control had the lowest level of ALT, which increased along with adding 2% herbs through drinking water. Adding 3% herbs through the feed reached the highest ALT level. Adding herbs through feed and drinking water up to 3% could maintain normal blood parameters (total plasma protein, globulin, cholesterol, HDL, AST, erythrocyte count, leukocyte count, PCV value, and hemoglobin level). On the other hand, the 2% herb addition could maintain the normal levels of albumin and ALT.

Keywords | Blood Biochemistry, Serum Protein Profiles, Serum Fat Profiles

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***Correspondence** | Ali Mahmud, Department of Animal Science, Faculty of Agriculture and Animal Science, University of Muhammadiyah Malang, Malang 651444, East Java, Indonesia; **Email:** alimahmud58@umm.ac.id
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INTRODUCTION

Indonesia has tremendous natural resources in the forms of potential biodiversities of herbal plants, such as garlic (*Allium sativum* L.), ginger (*Zingiber officinale* Rosch.), sand ginger (*Kaempferia galanga* L.), galangal (*Alpinia galangal*), turmeric (*Curcuma longa* Linn), curcuma (*Curcuma xanthorrhiza*) and cinnamon (*Cinnamomum verum*) (Ardiansyah et al., 2022). Parts of herbal plants, including leaves, flowers, stems, and roots (rhizome), can be used to improve poultry health and productivity; these parts contain various active compounds with varying types and levels. These active compounds can act as antimicrobial, antioxidant, anti-inflammatory, anthelmintic, and anti-oxidative properties (Denli and Demirel, 2018).

The use of herbs as feed additives in poultry aims to replace the antibiotic growth promoters (AGP), which have been banned by the government of the Republic of Indonesia Number 14/PERMENTAN/PK.350/5/2017 (Adli et al., 2023). AGP causes a negative impact, namely bacterial resistance to antibiotics, including tetracycline, erythromycin, streptomycin, and amoxicillin (Untari et al., 2021; Sholikin et al., 2023). In addition, the ban on the use of antibiotics is because antibiotics have prophylactic activity and reduce the health of broilers (Al-Khalaifah et al., 2022). In contrast, herbs have a positive effect because their bioactive compounds can stimulate the growth of beneficial bacteria, such as *Lactobacilli* and *Bifidobacteria*, and inhibit the growth of pathogenic bacteria, such as *Salmonella* spp. and Coliforms (Giannenas et al., 2018; Hussein et al., 2020). The lavender essential oil inhibits *Escherichia coli* and Coliform bacteria's growth while increasing probiotic bacteria's growth (Adaszynska and Szezerbinska, 2019).

In the body, the active substances contained in herbs undergo many processes, including absorption, distribution, metabolism (biotransformation), and elimination (Yekti et al., 2023). The speed of the biological process depends on the type and form of the compounds, the way to enter the body, and the condition of the tissues during the process (Yekti et al., 2022). After the absorption, the original compounds and metabolites would be carried by the blood and distributed to all body parts. Metabolism occurs in the organs, cells, and tissues. Elimination can be carried out by the excretory organs, especially the kidneys, in urine and through the intestines in feces. This study aimed to determine the effect of herbs as feed additives added to feed and drinking water on broiler blood parameters.

MATERIALS AND METHODS

Experimental design

A total of 175 broiler chickens were used in this experiment. The animals were divided into seven levels of treatment with a completely randomized design (CRD). The herbal supplementation was given through feed and drinking water and five replications (each replication consisted of 5 broiler chickens). Approximately 3 mL of blood samples were collected from vena axillaris from each chicken at age 35 days.

The feed formulation for the starter and finisher phases of the broiler chickens is shown in Table 1. The feed consists of a mixture of feedstuff such as rice bran, yellow corn, fish flour, bone flour, meat flour, soybean pulp, coconut oil, calcium, salt, lysine, and methionine following the recommendation from Rahayu et al. (2019). The treatments consisted of T0 (feed without herbs), T1 (feed + 1% herbs), T2 (feed + 2% herbs), T3 (feed + 3% herbs), T4 (drinking water +1% herbs), T5 (drinking water + 2% herbs), T6 (drinking water + 3% herbs).

Table 1: Feed formulation of the treated broiler chickens.

Feedstuff	Starter	Finisher
	Amount (%)	Amount (%)
Feed formulation		
Rice bran	0.6	0.4
Yellow corn	59.22	65.11
Fish flour	2.8	2.31
Bone flour	1	2
Meat flour	4.42	-
Soybean pulp	29	27
Coconut oil	1.22	1.12
Calcium	0.35	0.5
Salt	0.5	0.5
Lysine	0.7	0.9
Methionine	0.19	0.16
Total (%)	100.00	100.00
Feed composition		
Metabolic energy (kcal/kg)	3000.00	3000.00
Crude protein (CP) (%)	23	20
Crude fat (CF) (%)	4.00	4.00
Crude fibre (CFB) (%)	3.55	3.47
Ca (%)	0.89	0.76
P (%)	0.42	0.36
Na (%)	0.15	0.15
Amino acids		
Arginine (%)	0.88	0.98
Histidine (%)	0.35	0.38
Isoleucine (%)	0.77	0.82
Leucine (%)	0.98	1.02
Lysine (%)	0.99	1.00
Methionine (%)	0.46	0.40
Phenylalanine (%)	0.76	0.82
Threonine (%)	0.66	0.69
Tryptophan (%)	0.31	0.26
Valine (%)	1.23	1.09

Sources: Rahayu *et al*., 2019

Herbs materials and preparation

The herbs used in this study are a mixture of eight types of herbals, vitamins, and flour as a mixing ingredient. The composition and proportion of the herbal mixture are shown in Table 2. The rhizomes were initially sorted out to separate from any foreign materials; then, it was washed, drained, sliced, and dried in an oven at a temperature of 45°C until the water content reached 10% (Rahayu et al., 2019).

Table 2: Herbs mixture composition used in this study.

No	Ingredients	Proportion (%)
1	Aromatic ginger (*Kaempferia galanga L.*)	10
2	Garlic (*Allium sativum* L.)	10
3	Ginger (*Zingiber officinale* Rosc)	5
4	Galangal (*Alpinia galangal*)	5
5	Turmeric (*Curcuma longa Linn*)	5
6	Curcuma (*Curcuma zanthorrhiza*)	5
7	Betel leaf (*Piper betle Linn*)	2.5
8	Cinnamon (*Cinnamomum verum*)	2.5
9	Vitamin	5
10	Mixing ingredient (flour)	50
Total		100

Hematological and biochemical analysis

The measured variables were protein profiles (total plasma protein (TPP), albumin, and globulin levels), fat profiles (triglycerides (TG), low-density lipoprotein (LDL), and high-density lipoprotein (HDL), hepatoprotective profile (alanine aminotransferase (ALT), aspartate aminotransferase (AST), and malondialdehyde (MDA)), and blood profiles (red blood cell (RBC), white blood cell (WBC), packed cell volume (PCV), and hemoglobin).

The hemoglobin concentration is evaluated by matching acid hematin solution against a standard-colored solution in Shal's hemoglobin meter. The capillary hematocrit method calculates the packed cell volume (PCV). The hemocytometer method determined the total red blood cell (RBC) count and the total leukocytes (Taweya et al., 2020).

The method of measuring total plasma protein, albumin, and globulin concentrations was carried out according to Taweya et al. (2020), while the process used to determine the activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and Malondialdehyde (MDA) was determined according to Attia et al. (2020).

Data analysis

The research data were tabulated in the Excel program. First, the serum biochemistry data were analyzed using analysis of variance (ANOVA). Then, the Duncan Multiple Range Test (DMRT) was applied when the results were significantly affected among treatments. The following model was used:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where Yij was the parameters observed, μ was the overall mean, Ti was the treatment effect, and eij was the error number (Adli, 2021).

RESULTS AND DISCUSSION

Protein profile

The effect of herbal supplementation on broiler chicken's protein profile is presented in Table 3. The different method of herbal supplementation (through feed or drinking water) has given no differences in TPP and globulin values. Therefore, the two routes are suspected of providing the same ability in broilers in protein consumption, metabolic rate, and intensity of serum protein formation (Tóthová et al., 2019). The statistical result showed that the treatment level did not affect the TPP and globulin level, but significantly affected the albumin level.

Table 3: The protein profile of broiler chicken treated with herbs addition.

Treatment	TPP (g/dL)	Albumin (g/dL)	Globulin (g/dL)
T0	6.34 ± 1.28	3.53 ± 0.64[c]	6.52 ± 0.36
T1	6.16 ± 0.85	3.52 ± 0.71[c]	6.70 ± 0.51
T2	6.00 ± 1.00	3.41 ± 0.84[c]	7.50 ± 0.90
T3	6.46 ± 0.70	4.49 ± 0.43[b]	6.76 ± 0.62
T4	7.28 ± 0.28	5.06 ± 0.28[ab]	6.61 ± 0.34
T5	6.95 ± 0.16	4.44 ± 0.15[b]	6.46 ± 0.43
T6	6.86 ± 1.00	5.39 ± 0.35[a]	6.66 ± 0.30

TPP, total plasma protein. Different superscript within the same column indicated significant differences ($p < 0.05$).

Adding herbs influenced the albumin level increase in T3 – T6 treatments (ranging from 4.44 to 5.39 g/dL). However, the supplementation of 1% and 2% herbs (3.52±0.71 g/dL and 3.41±0.84 g/dL, respectively) through feed showed similar albumin levels with those of the control group (3.53±0.64 g/dL). This study indicated that the supplementation of herbs through feed up to 2% had a better effect since it has the lowest albumin level. It is suspected that the active substances contained in herbs given through feed are more durable in the digestive tract. Furthermore, improved liver digestive process, nutrient absorption, and protein metabolism, including albumin, were achieved. Albumin is the most abundant protein in blood plasma and has various physiological roles, including the transportation of fatty acid and bilirubin through the osmotic pressure of blood (Kajal and Pathania, 2021). It was in line with Liu et al. (2015), who stated that albumin determined the growth rate of chickens and was also used as an antibody and protein deposition.

In the control group, the control group's TPP, albumin, and globulin (T0) were 6.34 ± 1.28 g/dL, 3.53 ± 0.64 g/dL, and 6.52 ± 0.36 g/dL, respectively. The TPP values were higher than reported in the previous study; 31.57±0.99 g/L (Khabirov et al., 2020) and 33.2±1.89 g/L (Tothova et al., 2019). Different measurement methods of the TPP might cause differences. In this study, we calculate the total protein in plasma, but the comparative study measured it in serum level. As it is known that the proteins contained in plasma include albumin, globulin, and fibrinogen, so the level is higher than in serum. The albumin and globulin levels in this study were also higher than according to Khabirov et al. (2020): 48.6 ± 3.12 g/L and 31.8 ± 2.88 g/L, respectively. This result might be due to the different ages in blood sampling collection. In this study, the blood samples were taken at the age of 35 days, whereas Khabirov et al. (2020) collected the blood samples at 42 days.

The three variables were interrelated: TPP, albumin, and globulin. Either the concentration of albumin and globulin influenced an increase or decrease of TPP in the blood. Both the increase and decrease in TPP concentration form an abnormality. The results showed that the globulin levels among treatments were not significantly different from those of the control. It indicated that adding herbs through feed or drinking water gave the same globulin levels. Following the research results by Alabi et al. (2019), high globulin values indicate increased immunoglobulin production.

Lipid profiles

The results of the DMRT test on the effects of providing herbs on the levels of TG, cholesterol, LDL, and HDL are presented in Table 4. The results of ANOVA indicated that the treatment had no significant effect on cholesterol and HDL but affected TG and LDL. Supplementing herbs through drinking water gave a lower mean of TG and LDL than those provided through feeds or the control.

Table 4: Herbs supplementation effects on the broiler's lipid profiles.

Treatment	TG (mg/dL)	Cholesterol (mg/dL)	LDL (mg/dL)	HDL (mg/dL)
T0	24.86±4.04[a]	134.10±8.39	48.50±5.81[ab]	77.58±0.73
T1	24.90±2.03[a]	126.47±5.51	45.05±12.56[ab]	76.02±1.08
T2	21.52±5.33[ab]	128.56±9.75	53.92±11.05[a]	76.34±7.80
T3	22.85±2.66[a]	121.20±3.35	42.50±4.90[b]	81.57±5.86
T4	16.90±2.43[bc]	144.38±23.25	22.02±2.27[c]	75.60±6.18
T5	16.37±1.94[c]	138.25±19.72	18.47±4.56[c]	81.72±7.31
T6	18.06±2.23[bc]	124.50±19.39	20.04±3.03[c]	75.78±3.08

TG, Triglycerides; LDL, Low-Density Lipoprotein; HDL, high-density lipoprotein. Different superscript within the same column indicated significant differences ($p < 0.05$).

Based on the Table 4, there was a tendency for the average level of TG and LDL to be lower than that of either the feed group or the control. Researchers suspect that there is a role for water in the process of lipid metabolism. Orakpoghenor et al. (2021) state that water is essential for poultry, makes about 70-80% lean body mass, and plays critical roles in poultry metabolism and thermal homeostasis. Lack of drinking water in poultry caused a decrease in feed efficiency as water involved in the metabolic, circulated food essences to all parts of the body, and regulated livestock's body temperature. The function of water was to form body fluids, transport nutritional elements, control body heat, and transport residual oxidation from the body. Waheed et al. (2017) reported that limonene compounds such as monoterpenes and polyphenols in *Z. zerumbet* could reduce cholesterol by suppressing cholesterol absorption and increasing bile acid excretion. Bile acid production is the essential cholesterol catabolic pathway; cholesterol conversion to bile acids in the liver prevents the body from burdening it with cholesterol. Gao et al. (2020) stated that adding astaxanthin from *Haematococcus pluvialis* at a concentration of 50-100 mg/kg positively affected regulating fat metabolism.

The low percentage of TG and LDL in this study proved that the bioactive substances in herbs in the form of essential oils and curcumin had successfully demonstrated function in improving digestion and fat metabolism. Kuralkar and Kuralkar (2021) state that various herbs play a vital role in the production of animals and act as feed additives and growth promoters through different pharmacologically active ingredients. This statement is supported by Jachimowicz et al. (2022) that herbs and their bioactive components added to chicken diets can improve the broiler chicken meat by altering the content of fatty acids

Essential oils and curcumin in herbs could increase the activity of the pancreas to release lipase enzymes that could break down fat into fatty acids and glycerol so that the amount of fat reduced, including TG and LDL. Essential oils have an advantageous effect on broiler chicken through their role on many metabolic pathways, including lipid metabolism, stimulating digestive enzyme secretion and activity, acting as antimicrobial, and enhancing the gut integrity of chicken, leading to improved broiler performance (Puvača et al., 2022).

ALT, AST and MDA levels

The ALT, AST, and MDA values for each treatment's level are provided in Table 5. The ANOVA result showed that the herbal addition significantly impacted the ALT and MDA levels but did not affect the levels of AST. The control had the lowest ALT level, followed by the herbal feed at 1%-2%. The supplementation of herbs achieved the highest level via feeds.

Based on Table 5, the administration of herbs to broiler chickens, both through feed and drinking water, showed higher serum ALT levels than the control group but did not cause macroscopic changes to the liver. This organ's microscopic damage is unknown because this study did not conduct a histopathological examination, as it is known that ALT, among other enzymes, is a marker of liver function (Visaria et al., 2020). The results of this study are similar to the report of Arczewska-Wlosek et al. (2018), that the activity of the ALT enzyme was reduced in poultry fed a diet with normal or increased CP (21.6% or 23.6%) and without being given herbal extracts, but grew to almost two-fold in poultry given herbal extracts (*Echinacea purpurea*, *Salvia officinalis*, *Thymus vulgaris*, *Rosmarinus officinalis*, *Allium sativum*, *Origanum vulgare*) of 2g/kg feed.

Table 5: Herbs supplementation effects on hepatoprotective profile.

Treatment	ALT(U/L)	AST (U/L)	MDA (nmol/L)
T0	3.35 ± 0.55[d]	3.04 ± 0.60	1.74 ± 0.23[a]
T1	4.40 ± 0.83[c]	2.54 ± 0.48	1.75 ± 0.56[a]
T2	4.73 ± 0.59[bc]	2.54 ± 0.29	1.59 ± 0.07[a]
T3	5.83 ± 0.54[a]	3.15 ± 0.29	1.46 ± 0.20[a]
T4	5.12 ± 0.74[ab]	2.63 ± 0.32	1.13 ± 0.07[b]
T5	5.67 ± 0.76[ab]	2.85 ± 0.29	1.12 ± 0.08[b]
T6	5.51 ± 0.49[ab]	2.55 ± 0.21	1.11 ± 0.07[b]

ALT, alanine amino transferase; AST, aspartate amino transferase; MDA, Malondialdehyde. Numbers followed by the same letter in the column indicated no significant difference in Duncan's 5% Test. The letter ab in the ALT column means no significant difference in ALT levels at 1%, 2%, and 3% herbal addition via drinking water. The letter bc implied no significant difference in ALT levels at the 2% and 1% levels of herbs supplementation via feed.

Herbs supplementation to broilers through drinking water provides a lower MDA level than feed or control group supplementation. It is believed that the antioxidants contained in herbs play a role in suppressing oxidative stress. This explanation is to the statement of Skomorucha and Muchacka (2020) that adding herbal extracts to drinking water reduced MDA levels in broiler leg muscles. This finding may be related to increased superoxide dismutase (SOD) activity in broiler leg muscles. The low MDA level in chicken muscle treated with an enriched antioxidant diet was also reported by Giannenas et al. (2018) and Sierżant et al. (2018).

RBC, WBC, PCV, and hemoglobin level

The blood profiles (RBC, WBC, PCV, and hemoglobin level) of the chicken treated with herbal addition are shown in Table 6. The statistical analysis described that supplementation herbs, either feed or drinking water, did not significantly affect the broilers' blood's erythrocytes, leukocytes, PCV, and hemoglobin.

Table 6: Herbs supplementation effects on blood profiles.

Treat-ment	RBC (x 10^6/mm^3)	WBC (x 10^3/mm^3)	PCV (%)	HB (g/dL)
T0	2.68±0.18	15.36±3.43	29.91±2.19	7.54±0.37
T1	2.65±0.16	16.37±2.15	33.78±8.78	8.12±0.63
T2	2.71±0.16	15.14±2.92	30.51±1.77	8.03±1.21
T3	2.81±0.44	15.95±2.54	29.08±3.61	7.64±0.65
T4	2.56±0.11	15.98±1.41	30.07±1.38	8.40±0.60
T5	2.65±0.78	16.97±0.64	29.93±1.27	8.10±0.55
T6	2.77±0.70	15.92±4.31	30.70±1.18	8.47±0.43

RBC, red blood cell; WBC, white blood cell; PCV, packed cell volume; HB, Hemoglobin.

Table 6 shows that the average number of erythrocytes, leukocytes, PCV, and hemoglobin levels were not significantly affected than the control. The average hematological values for chickens are 2.0-4.0 x 10^6/mm^3 for RBC, 27-42% for PCV, and 7.0-11.0 g/dl for Hb (Joshua et al., 2022). It showed that the herbal supplementation, either through feed or drinking water, had a positive impact on the blood biochemistry of broiler chickens. Normal RBC, PCV, and Hb values in chickens show that erythropoiesis runs efficiently (Ezihe and Dagih, 2019). The supplementation of ginger and garlic herbs increases hematological blood components such as PCV, RBC, and WBC; this supports the health of poultry by increasing active immunity (Abd El-Hady et al., 2020).

Erythrocytes mainly function as hemoglobin carriers that bind oxygen from the lungs and circulate it to all tissue cells. This function is believed to be supported by the active substances in herbs, namely essential oils. Widjastuti et al. (2021) stated that the presence of essential oils in red ginger could help digestion by stimulating the secretory nervous system to secrete gastric juice, which contains enzymes such as lipase, amylase, and trypsin into the stomach and intestines so that the chicken can break down fat, amylose, and protein into easily absorbed compounds. Rahayu et al. (2019) also described that the essential oil in *Zingiber zerumbet* could increase digestibility as the crucial oil caused relaxation and reduced peristalsis of the small intestines. Therefore, the digestion and absorption of nutrients could be optimal.

The herbs from feed or drinking water improved the chicken's digestive system. The digestion of nutrients and absorption occurred like usual, including protein, iron, folic acid, and vitamin B_{12} necessary for synthesizing erythrocyte cells. As a result, the hemoglobin levels for oxygen transport to all body tissues were also fulfilled. Most of the oxygen transported from the lungs to the tissues was bound to hemoglobin; only a tiny amount of oxygen was dissolved in plasma and blood cells.

The average WBC count in this study (15.14 -16.97 x 10^3/mm^3) was higher than the standard in the Joshua et al. (2022), which ranging 3.0 - 6.0 x 10^3/mm^3, thought to be related to an increase in the immune response. This result can be explained as follows, phagocytic cells that play an essential role in the immune system are components of white blood cells (leukocytes) called monocytes. Monocytes are present in blood circulation and then migrate to the tissues, where they will differentiate into macrophages (Dillasamola et al., 2019). Macrophages are cells that play an essential role in the immune system against pathogens. One of the primary roles of macrophages in the natural immune system is the function of phagocytosis, which aims to eliminate extracellular particles and damaged or dead cells (Dillasamola et al., 2019; Song et al., 2021).

CONCLUSIONS AND RECOMMENDATIONS

It could be concluded that the herbal supplementation as feed additives had the significant potency to maintain the health of the biochemical parameters of broiler chickens' blood. Herbs' addition through feed and drinking water could maintain normal blood parameters, including total plasma protein, globulins, cholesterol, HDL, AST, erythrocyte count, leukocyte count, PCV, and hemoglobin level. Giving herbs through feed maintained the normal albumin levels and ALT. Herbs supplementation through drinking water resulted in the normal range for LDL and MDA levels.

NOVELTY STATEMENT

The government has outlawed antibiotic growth promoters (AGP). Thus, using herbs as feed additives in chicken tries to replace them. Various active ingredients found in herbs have been shown to have antimicrobial, antioxidant, anti-inflammatory, anthelmintic, and anti-oxidative properties. This study found that up to 3% of herbs added to feed and water could keep blood parameters regular. Giving herbs through feed in amounts up to 2% preserved the normal ALT and albumin levels. LDL and MDA levels returned to normal when 3% of herbs were added to drinking water.

AUTHOR'S CONTRIBUTION

IDR and AM designed and coordinated the study. AM and WW supervised the experiment. AS, ADA, and DDS experimented, analyzed the data, and drafted the manuscript. IDR, AM, WN, and TU took part in preparing and critically checking this manuscript. All authors read and approved the final manuscript.

Ethical approval

All methods involving animals in the study followed national or institutional criteria for animal care and use and the institution's or practice's ethical standards. This study has been authorized and approved with the Ethical Clearance Certificate of The Institutional Animal Care and Use Committee (IACUC) of the Brawijaya University, with the approval number: 155-KEP-UB-2022.

Conflict of interest

The authors have declared no conflict of interest.

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