OPEN ACCESS
Advances in Animal and Veterinary Sciences

Research Article

Effect of Moringa Leaves (*Moringa oleifera*) Oil Microcapsules (MOM) on Growth and Plasma Metabolites Related to Inflammation in Sentul Chickens

Heni Natalia Aritonang[1], Lovita Adriani[2], Andi Mushawwir[2]*

[1]Animal Science Doctoral Programme, Faculty of Animal Science, Universitas Padjadjaran, Indonesia; [2]Department of Nutrition and Feed Technology, Faculty of Animal Science, Universitas Padjadjaran, Indonesia.

Abstract | This study aims to investigate the effects of microencapsulated moringa leaf essential oil (MOM) on body weight gain, plasma metabolites, and liver inflammation in Sentul chickens. The microcapsules, which contain the natural active compounds of moringa leaf essential oil (*Moringa oleifera*), were prepared using a complex coacervation method with alginate coatings, a glutaraldehyde crosslinker, and a polysorbate emulsifier. The study utilized a completely randomized design. A total of 280 Sentul chickens were randomly assigned to four treatment groups with seven replicates each, where each replicate consisted of ten chickens. The treatments were administered when the chickens were two weeks old. The groups were as follows: T0 (basal feed as the control), T1 (basal feed + 150 mg/kg MOM), T2 (basal feed + 250 mg/kg MOM), and T3 (basal feed + 350 mg/kg MOM). The results indicated significant differences ($P < 0.05$) in body weight gain, as well as levels of glucose, triglycerides, cholesterol, total protein, albumin, globulin, SGOT, SGPT, and gamma GT among the treatment groups. In conclusion, the study found that moringa leaf essential oil (Moringa oleifera) can enhance body weight, glucose levels, total protein, albumin, globulin, and SGPT, while reducing triglycerides, cholesterol, SGOT, and gamma GT levels.

Keywords | Microencapsulation, Essential oil, Moringa, Sentul chicken, Growth, Metabolism

Received | October 22, 2024; **Accepted** | November 21, 2024; **Published** | March 18, 2025
***Correspondence** | Andi Mushawwir, Department of Nutrition and Feed Technology, Faculty of Animal Science, Universitas Padjadjaran, Indonesia; **Email:** mushawwir@unpad.ac.id
Citation | Aritonang HN, Adriani L, Mushawwir A (2025). Effect of moringa leaves (*Moringa oleifera*) oil microcapsules (MOM) on growth and plasma metabolites related to inflammation in sentul chickens. Adv. Anim. Vet. Sci. 13(4): 835-842.
DOI | https://dx.doi.org/10.17582/journal.aavs/2025/13.4.835.842
ISSN (Online) | 2307-8316; **ISSN (Print)** | 2309-3331

INTRODUCTION

High growth rates, productivity, and immune responses are crucial in the chicken farming industry. The use of supplements and medications, such as antibiotics, can result in harmful chemical residues in the products and contribute to antibiotic resistance in harmful bacteria within the chickens (Founou *et al*., 2016; Pazla *et al*., 2023).

To enhance the productivity of Sentul chickens, it is essential to pay attention to their physiological conditions, which are closely linked to the metabolic processes within their bodies. The liver plays a crucial role in various functions, including glucose and lipid metabolism, digestion, fat and fat-soluble vitamin absorption, and detoxifying harmful substances. Damage to the liver can occur due to increased metabolic activity during the starter phase of Sentul chickens, producing free radicals. These free radicals can cause cellular damage and apoptosis or necrosis, ultimately decreasing liver function. This impairment raises the risk of liver tissue damage, negatively impacting the immune response.

Moreover, the metabolic processes within the body require ATP (adenosine triphosphate) as an energy source, which is produced through oxidation-reduction reactions in the mitochondria. Therefore, maintaining optimal liver health is vital for the overall well-being and productivity of Sentul chickens. Damage and death of liver cells are indicated by an increased presence of transaminases, which are enzymes that transfer amine groups. Specifically, the levels of SGOT (Serum Glutamic Oxaloacetic Transaminase) and SGPT (Serum Glutamic Pyruvic Transaminase) rise in the bloodstream. As a result, the levels of SGPT and SGOT in blood plasma increase.

One feasible alternative is to use feed additives derived from herbal plants to protect livestock and consumers from antibiotic residues while ensuring a safe supply of protein and promoting sustainable animal production. These plants contain natural active compounds that can be beneficial (Uyanga *et al*., 2022; Wanapat *et al*., 2015).

Moringa leaves contain approximately 65% water, 28% carbohydrates (primarily fructans), 2.3% organosulfur compounds, 2% protein (specifically alkaloid), 1.2% free amino acids (such as arginine), and 1.5% fiber (Santhosha *et al*., 2013). These leaves exhibit various health benefits, acting as antifungal, antiviral, antibacterial, anticancer, anthelmintic, antihypertensive, antiatherosclerosis, antiseptic, and anti-inflammatory agents (Bhatwalkar *et al*., 2021).

Previous studies indicate that certain active substances in flavonoids possess antibacterial properties that can enhance digestive function by relaxing the small intestine and inhibiting harmful microorganisms within the digestive tract. The active compounds found in this natural extract can improve digestion by promoting the growth of beneficial intestinal flora, which may lead to increased body weight (Mushawwir *et al*., 2021a).

Furthermore, some active substances derived from natural ingredients may serve as additives to boost immunity and improve blood profiles (Mushawwir *et al*., 2018). They have also been shown to reduce cell death (Korivi *et al*., 2012; Lee *et al*., 2014). The active compounds in moringa leaves can decrease hemorrhage in liver tissue (Cortes-Coronado *et al*., 2017), and their antioxidant content-particularly the diallyl component-helps mitigate oxidative stress, which can negatively impact the immune system.

The administration of active plant compounds in local chickens often yields ineffective results due to the instability of these compounds. This instability is primarily caused by exposure to oxygen, high humidity, gastric HCl damage (in the proventriculus of poultry), and the degradation of monomers before absorption (Chawda *et al*., 2017; Julaeha *et al*., 2022).

One identified weakness in research involving Moringa leaves is the standard method of administration, which typically relies on extracts or flour. These methods do not ensure that the active compounds are effectively delivered to the target cells. To overcome this issue, coating technology using microbeads and microcapsules can be utilized. This technique encapsulates the core ingredients of herbal extracts within a polymer matrix, employing irradiated chitosan and alginate as wall materials. With this information in mind, this study aims to investigate the effects of feeding microencapsulated Moringa leaf essential oil as a substitute for antibiotics on body weight gain, blood plasma metabolite levels, and liver inflammation.

MATERIALS AND METHODS

The material used in this study was moringa leaves essential oil (*Moringa oleifera)*. The dressing materials used were sodium alginate, glutaraldehyde crosslinker, and tween 80 emulsifier.

Preparation of the Microcapsules

Preparation of microcapsules using the complex coacervation method involved the following steps. Moringa leaves were distilled at 100°C for 3 hours. A gelatin solution was prepared by stirring 140 mL of distilled water at 60±1°C; then, 0.8 g of Tween-80 was added, followed by 3.5 g of essential oil extracted from the lime peel. In a separate container, 0.8 g of sodium alginate was dissolved in 40 mL of distilled water and stirred for 15 minutes at 60°C with a stirring rate of 600 rpm. Glacial acetic acid was added to adjust the pH to 3.75. The solution was cooled to 5-10°C, and 1.25 mmol of glutaraldehyde was slowly introduced. The temperature was then raised to 35°C, and the mixture was stirred using a magnetic stirrer for 3 to 4 hours. After mixing, the solution was allowed to cool to room temperature while continuing to stir. The resulting microcapsules were filtered and washed with water. Finally, they were dried to achieve a solid state and ground into a powdery consistency.

Animal Sample and Treatments

The study involved 280 Sentul chicken day-old chicks (DOCs) that were not sex-separated. These chicks were raised in semi-closed housing-type cages until they reached the developmental phase and were randomly selected for each experimental group. The research was conducted using a completely randomized design (CRD).

The chicks were divided into four treatment groups based on different essential oil feeding patterns, with each treatment placed in seven replicate cages. Each cage measured 2 × 1 meters and was equipped with feed and water containers and lamps for heating and lighting. Each experimental unit consisted of ten chickens.

At 14 days of age, the chicks were administered microcapsules of essential oil from moringa leaves (MOM) at varying concentrations. The treatment design was as follows: T0: Basal ration without MOM (control); T1: Basal ration + 150 mg/kg MOM; T2: Basal ration + 250 mg/kg MOM; and T3: Basal ration + 350 mg/kg MOM.

Analysis of Body Weight Gain

The body weight of the Sentul was measured at the beginning of the study and every week after that until the end.

Analysis of Blood Plasma Metabolites in Sentul Chickens

Circulating metabolites can provide insights into cellular metabolic phenomena. The metabolites measured include glucose, triglycerides, cholesterol, total protein, and globulin levels. Plasma metabolites will be analyzed using the UV spectrophotometer technique, following the instructions and protocols outlined in the Biolabo Kit from France and the Randox S2DE Kit from the UK.

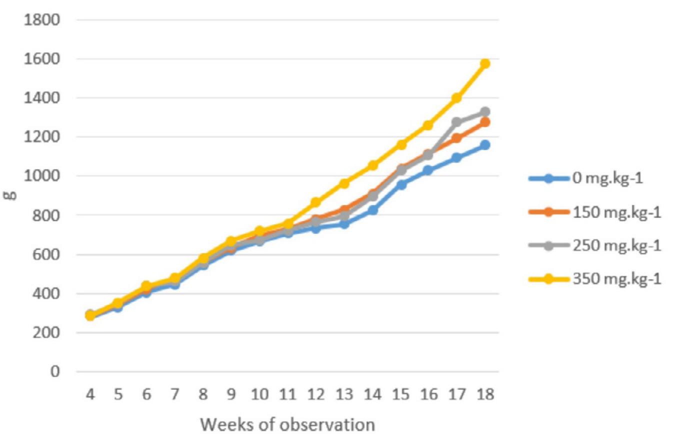

Figure 1: Effect of MOM on Body Weight Gain of Sentul Chicken from Starter Phase to Developer Phase.

Liver Inflammation Analysis

SGOT, SGPT, and Gamma GT levels were analyzed using spectrophotometer techniques, following measurement procedures based on the Biolabo KIT analysis protocol.

Statistical Analysis

Data on body weight gain, blood plasma metabolite levels, and liver inflammation were analyzed using a completely randomized design with analysis of variance (ANOVA). Duncan's multiple range test was employed to explore significant differences between treatment groups, with a significance level set at $P < 0.05$. The SAS program (SAS Institute Inc.) conducted the data analysis.

RESULTS AND DISCUSSION

Effect of MOM on Body Weight Gain

The impact of MOM in the feed on the body weight gain of Sentul chickens during the starter-to-developer phase is illustrated in Figure 1.

The results indicate that higher levels of MOM administration from the starter phase to the developer phase significantly affect the weight gain of Sentul chickens ($P < 0.05$). Duncan's multiple range test (Figure 1) further shows that the average body weight gain of Sentul chickens significantly differed among the treatment groups ($P < 0.05$).

Moringa leaves contain active compounds, specifically flavonoids, which enhance the height of intestinal villi and the depth of intestinal crypts. This development expands the nutrient absorption area and improves digestion. The growth of intestinal villi is beneficial for both intestinal function and the overall growth of Sentul chickens (Qaisrani *et al.*, 2015; Rahman *et al.*, 2021). Scordinin, a compound found in Moringa leaves, also aids in accelerating and enhancing body cell formation, contributing to optimal final weight (Dahlan and Haqiqi, 2012). Trisnadi (2014) suggests that scordinin acts as a growth promoter by facilitating the binding and breakdown of proteins within the body.

Peinado *et al.* (2013) discovered that compounds in Moringa leaves can increase nutrient digestibility and enhance the activity of intestinal mucosal enzymes in Cobb strain broilers. The essential oils in Moringa leaves, including scordinin, also offer strength and promote growth.

Moringa leaves also contain mitogenic gurwich rays, which stimulate body cell growth while rejuvenating various bodily functions (Syamsiah and Tajudin, 2004). Scordinin promotes muscle growth, while allicin inhibits the formation of fatty tissue. This leads to increased weight gain attributed to muscle mass rather than fat accumulation (Hidayati, 2005; Mushawwir *et al.*, 2022). Figure 2.

Effect of MOM on Blood Plasma Metabolite Levels of Sentul Chicken

The study summarizes the effect of incorporating MOM in the feed on the blood plasma metabolites of Sentul chickens in Table 1.

Based on the study's findings, it is clear that higher concentrations of Moringa oleifera leaves (MOM) lead to an increase in glucose levels. Results from Duncan's difference test (Table 1) indicated that the average glucose levels in Sentul chickens were significantly different ($P < 0.05$) across the treatment groups. Moringa leaves possess antioxidant and antibacterial properties that can reduce oxidative stress, contributing to better regulation of blood glucose levels. This regulation is crucial for improving glucose metabolism in Sentul chickens.

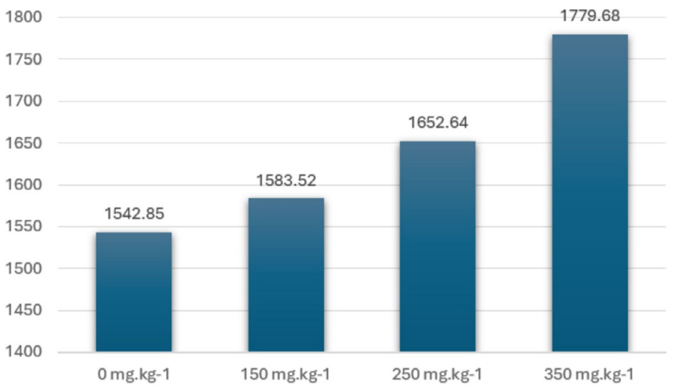

Figure 2: Effect of MOM on body weight of Sentul chicken in the Developer Phase.

Table 1: The average blood plasma metabolite levels (mg/dL) of Sentul chicken fed with MOM.

Treatment	Glucose	Triglyceride	Cholesterol	Total Protein	Albumen	Globulin
T0	185.98[a]	927.42[a]	117.23[a]	6.31[a]	1.69[a]	4.31[a]
T1	195.87[a]	794.23[b]	109.45[b]	8.53[b]	1.83[b]	6.74[ab]
T2	199.76[a]	753.11[c]	83.64[c]	8.63[b]	2.74[c]	6.63[ab]
T3	192.79[a]	578.05[d]	74.83[d]	8.86[bc]	2.78[c]	6.37[bc]

Mean followed by superscript of different alphabetical elements showed significant difference ($P<0.05$).

Blood glucose levels are regulated by hormonal mechanisms, specifically hormones produced by the pancreas: insulin, secreted by beta cells, and glucagon, produced by alpha cells. Glucose is synthesized into glycogen through the process of gluconeogenesis, and this glycogen is stored in the liver and muscles. When blood glucose levels decrease, glycogen is converted back into glucose through glycogenolysis. The glucose then enters cells and undergoes phosphorylation to form glucose-6-phosphate, facilitated by the enzyme hexokinase. Glucose-6-phosphate subsequently undergoes glycolytic metabolism to produce pyruvate and ATP, which serve as energy sources (Prata, 2018).

The triglyceride profile showed a decrease as the concentration of MOM increased. This reduction in triglyceride levels suggests that the sulfur compounds found in moringa leaves, particularly allicin, inhibit enzymes involved in cholesterol and triglyceride synthesis in the liver, such as HMG-CoA reductase (Rahmania *et al.*, 2022). Consequently, there is a reduction in endogenous triglyceride production. Additionally, moringa leaves can enhance the activity of lipoprotein lipase, an enzyme responsible for breaking down triglycerides in the bloodstream. This increased activity leads to a faster breakdown of triglycerides into free fatty acids, which can be used as energy sources.

The cholesterol profile improved as the concentration of microcapsules containing moringa leaf (Moringa oleifera) essential oil increased. This essential oil reduces cholesterol levels, serum triglycerides, very low-density lipoprotein (VLDL), and low-density lipoprotein (LDL) while increasing high-density lipoprotein (HDL). As a result, the ratio of LDL to HDL decreases, which is attributed to the presence of the active compound alkaloid. Alkaloids inhibit the enzyme 3-Hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase, which plays a crucial role in cholesterol biosynthesis. By inhibiting HMG-CoA reductase, cholesterol synthesis decreases, leading to an increase in the number of LDL receptors (Mushawwir *et al.*, 2021; Rahmania *et al.*, 2022; Firmansyah *et al.*, 2024).

The total protein profile increases with higher Moringa oleifera leaves (MOM) concentrations. Moringa leaves contain the active compound allicin, which has antimicrobial and anti-inflammatory properties. Allicin can combat infections caused by gram-negative and gram-positive bacteria and prevent damage to the small intestine (Tanuwiria *et al.*, 2023), thereby optimizing protein absorption from the intestine. The increase in total protein is also influenced by nutritional status (Kharazi *et al.*, 2022). Higher concentrations of moringa leaf powder in feed can reduce the levels of Salmonella typhimurium and improve feed conversion in broiler chickens (Mushawwir *et al.*, 2024), leading to enhanced protein intake and increased plasma protein levels.

Additionally, moringa leaves contain active compounds that stimulate the growth of body cells and rejuvenate overall bodily functions, including those of the liver. Since most plasma proteins are synthesized in the liver, optimal liver function contributes to increased plasma protein levels.

The albumin profile increased as the concentration of microencapsulated essential oil from Moringa oleifera increased, compared to the control group that received no treatment. The sulfur compounds present in moringa leaves—such as allicin, ajoene, S-allyl cysteine (SAC), alkaloid, S-methyl cysteine sulfoxide, and S-allyl cysteine sulfoxide—have therapeutic benefits. Previous studies indicate that diallyl disulfide inhibits the enzyme HMG CoA reductase in microsomes and reduces hepatocyte cholesterol levels. Furthermore, the administration of essential oils can suppress the inflammatory response related to dietary albumin, increasing the plasma albumin profile (Nurfauziah *et al.*, 2024).

Similarly, the globulin profile also increased with higher concentrations of microencapsulated essential oil from moringa leaves compared to the control group. The essential oil derived from moringa leaves is known for its immune-boosting properties. Incorporating moringa leaf powder into the feed has enhanced gamma globulin levels in broiler chickens (Tanuwiria and Mushawwir, 2020; Manin *et al*., 2024). This effect is believed to arise from the increased activity of lymphoid cells, which produce globulin and support immune strength as a defense mechanism. The active compound scordinin influences immune function and has phagocytic properties. Moringa leaves act as immunomodulators by promoting the production of T lymphocytes (Adriani *et al*., 2024). As lymphocyte activity increases, so do the concentrations of various components associated with the immune system, including globulins. Helper T cells play a crucial role in regulating immune functions by producing protein mediators known as lymphokines (Tanuwiria *et al*., 2022).

Effect of MOM on Liver Inflammation of Sentul Chicken

The impact of MOM in feed on the liver inflammation of Sentul chickens is shown in Table 2. The results of Duncan's difference test (Table 2) showed that the average SGOT and SGPT levels of Sentul chickens were significantly different ($P < 0.05$) across each treatment group. The findings indicated that higher levels of *Moringa oleifera* extract (MOM) administration decreased SGOT and SGPT profiles. MOM contains active compounds that possess antioxidant and anti-inflammatory properties, which can reduce liver inflammation, subsequently lowering SGOT and SGPT levels.

Table 2: The Effect of MOM on Sentul Chicken Liver Inflammation.

Treatment	Inflammatory Biomarkers (IU/L)		
	SGOT	SGPT	Gamma GT
T0	182.39^{a}	40.33^{a}	17.45^{a}
T1	123.43^{a}	33.41^{b}	11.11^{b}
T2	83.18^{b}	23.17^{c}	7.28^{c}
T3	52.28^{a}	16.26^{d}	5.51^{d}

^{a,b}Averages followed by different notations in the same column indicate significant differences ($P < 0.05$).

Previous research on natural extracts has demonstrated that MOM positively impacts kidney histology (Ansar *et al*., 2014; Eyng *et al*., 2015; Kharazi *et al*., 2022; Setiawan *et al*., 2024). Specifically, it reduces damage to kidney cells and tissues and decreases cell death caused by free radicals, thereby lowering necrosis and apoptosis (cell death) rates while improving immune function. According to Mushawwir *et al*. (2017), increasing essential oil concentration can enhance nutrient absorption and utilization, reduce SGOT and SGPT levels, and improve liver cell function. Other studies have also indicated that the administration of active ingredients, such as essential oils, is linked to reduced cellular inflammation (Sirovina *et al*., 2013; Muhammad *et al*., 2023; Chatterjee *et al*., 2015; Dudi *et al*., 2023). This results in decreased oxidative stress, improved tissue metabolism, and reduced apoptosis or cell death (Figure 3), and these results, similar to previous research reports (Rahmania *et al*., 2022; Mushawwir *et al*., 2024).

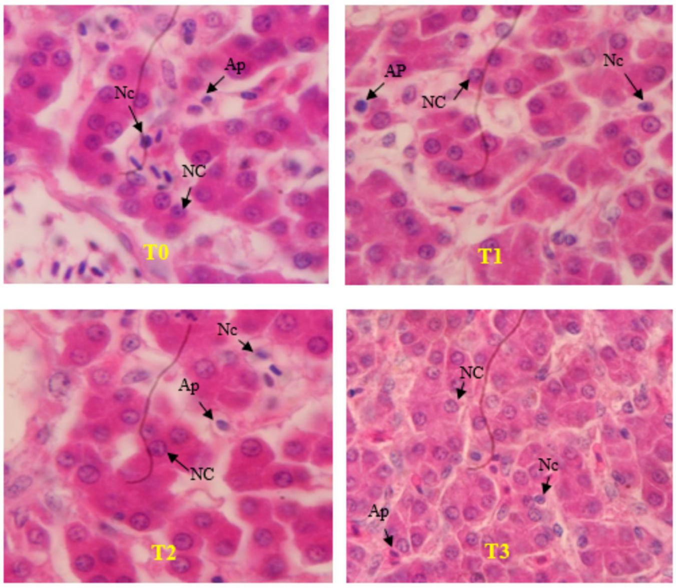

Figure 3: Liver Histology of Sentul Chicken without and with MOM treatment. NC: Normal Cells, Ap: Apoptosis, Nc: Necrosis. Object magnification 100x.

Data analysis in Table 2 revealed that the levels of gamma-glutamyl transpeptidase (γ-GT) in the blood plasma of treated Sentul chickens decreased compared to the control group (untreated). This finding indicates a positive correlation with the administration of MOM. Significant differences were also observed in the average levels of gamma-glutamyl transpeptidase (γ-GT) ($P < 0.05$) among the treated groups, as presented in Table 2.

In this experiment, MOM was able to reduce the death of Sentul chicken liver cells, both apoptosis and necrosis (Table 3 and Figure 3). Table 3 also shows an increase in normal cells and a decrease in the number of cell deaths (apoptosis and necrosis) as the level of MOM in the diet increased. This means that the administration of MOM can reduce damage to liver cells and prevent the migration of SGOT, SGPT, and Gamma-glutamyl transpeptidase into the blood plasma.

Gamma-glutamyl transpeptidase (γ-GT) plays a crucial role in glutathione metabolism and detoxification. Elevated levels of γ-GT in the blood often indicate stress and liver damage in livestock. Liver function impairment can result from various factors, including free radicals in the body, which increase with rising environmental temperatures (Mushawwir and Latipudin, 2013; Mushawwir *et al*., 2021a, b; 2023; 2024). When free radical production surpasses the body's antioxidant capacity, oxidative stress, apoptosis, or necrosis can occur in cells, including hepatocytes.

Table 3: Liver Cell Profile in Sentul chickens without and with MOM Administration (one field of view, with 10x object magnification).

Treatment	Liver Cell Profile		
	Normal	Apoptosis	Necrosis
T0	535.53^{b}	107.26^{b}	64.44^{b}
T1	654.66^{c}	86.25^{c}	47.358^{b}
T2	603.27^{d}	75.63^{d}	41.23^{a}
T3	617.40^{e}	63.42^{ad}	29.35^{c}

Mean followed by superscript of different alphabetical elements showed significant difference (P<0.05).

As chickens age, organ function typically declines due to increased cell degeneration—*Moringa oleifera* essential oil exhibits antibacterial and anti-inflammatory properties. Several studies have shown that consuming moringa leaves can enhance animal health and positively influence enzyme profiles, particularly gamma-glutamyl transpeptidase (γ-GT). The anti-inflammatory properties of moringa leaves may lead to improved liver health, the primary organ responsible for producing γ-GT. Improved liver function may contribute to better regulation of γ-GT levels, and previous researchers have reported similar findings (Adriani *et al*., 2024; Purwanti *et al*., 2024; Aritonang *et al*., 2024).

CONCLUSIONS AND RECOMMENDATIONS

The results of the current study indicate that higher concentrations of moringa leaves essential oil (Moringa oleifera) during the developmental phases of Sentul chickens significantly affect body weight gain (P < 0.05). As the moringa essential oil (MOM) concentration increased, the average blood plasma metabolite levels, such as triglycerides and cholesterol, decreased. Conversely, glucose, total protein, albumin, and globulin levels increased with higher concentrations of MOM. Additionally, mean liver inflammation decreased as the concentration of MOM increased.

ACKNOWLEDGEMENTS

The authors would like to express their gratitude to the funders of this research, the Ministry of Education, Culture, Research and Technology of the Republic of Indonesia. They also thank the research team for their collaborative efforts in completing this study and the accompanying article.

NOVELTY STATEMENT

Herbal extracts often have poor stability due to particle size, type of solvent, temperature, extraction time, pH, and the ratio of solvent to plant material. Additionally, these extracts are prone to degradation through oxidation and photolysis. In particular, extracts that use non-polar solvents can be challenging for oral administration, as they typically have low solubility and bioavailability. One effective way to address these stability issues is through microencapsulation. This technology isolates the active ingredients of essential oils within microcapsules, protecting them from external conditions and enabling controlled release.

AUTHORS' CONTRIBUTIONS

All authors contributed equally to the writing of this manuscript.

Ethical Approval

All procedures for this study have received approval from the Ethical Review Committee for Animal Experiments, Research and Technology Division, Agency for Agricultural Technology, under number 6353/PT.Riset.ttRE/08/24.

Conflict of Interest

The authors have declared no conflict of interest.

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