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MORALITY BEFORE KNOWLEDGE

# Application of Moringa leaves (*Moringa oleifera*), acidifiers, and probiotics as natural growth promoters to improve broiler chicken growth performance: A review

**Titis Putri Dika Amalia[1*], Syifa Annisa[1]**
[1] *Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia;*
*Correspondence: titis.putri.dika.amalia@mail.ugm.ac.id

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## ABSTRACT

**Background:** The use of AGP as a feed additive is 96.97% utilized to stimulate growth and suppress infections by pathogenic microorganisms in the digestive tract. Long-term use of AGP can cause genetic mutations by pathogenic agents, resulting in decreased effectiveness of antibiotic therapy. Moringa is a plant that grows well in tropical areas and is widely known as a vegetable and traditional medicine containing various active compounds such as alkaloids, flavonoids, steroids, triterpenoids, and tannins. **Methods:** The research method is a literature study by analyzing secondary data based on reviews from several research journals related to the potential of moringa leaves, acidifiers, and probiotics in improving the growth performance of broiler chickens. **Findings:** These compounds act as antioxidants, antibacterials, and hepatoprotective agents, improving broiler chickens' carcass quality. This composition can be supported by the administration of acidifiers in the form of organic acids to inhibit the growth of pathogenic bacteria in the digestive tract, thereby optimizing the growth process of broiler chickens. Digestive bacterial balance can be achieved by administering probiotics, which play a role in enhancing immunity, health, and growth at all ages and classes of poultry, improving the balance of healthy bacteria in the digestive tract, promoting intestinal integrity and maturation, preventing inflammation, increasing feed intake and digestion by enhancing digestive enzyme activity, reducing bacterial enzyme activity, lowering ammonia production, neutralizing enterotoxins, and stimulating immune function. **Conclusion:** The combination of these three compositions is expected to provide optimal results for broiler chicken performance. **Novelty/Originality of this Article:** This article highlights a combined approach using moringa leaf compounds, acidifiers, and probiotics as an alternative to AGP in broiler feed, aiming to achieve optimal growth performance while avoiding the risks associated with antibiotic resistance.

**KEYWORDS**: acidifier, AGP, moringa leaf, probiotic.

## 1. Introduction

Poultry is a major sector of the national economy that is rapidly developing and has become a promising business in the livestock sector. According to the Indonesian Feed Producers Association (APPI), the poultry industry employs up to twelve million people and has a value of more than USD 34 billion (Ferlito & Respatiadi, 2018). The high global population impacts the demand for nutritious food products to maintain a healthier and more prosperous standard of living. Consumption patterns have gradually increased the intake of animal protein, including broiler chicken farming (Adi et al., 2017). Broiler chickens are one type of poultry that has high growth potential, growing quickly within 5-7 weeks with a harvest weight of 1.3-2.5 kg. The production and consumption of broiler

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chicken meat at the national level continue to increase, reaching 6.048 kg per capita per year in 2022 (Prasetyo et al., 2020).

Based on estimates by the Center for Agricultural Data and Information Systems, the broiler chicken population in Indonesia is projected to reach 3.20 billion heads in 2025. This result is supported by the global perspective that poultry meat production accounted for nearly 40% of global meat production in 2020 (Darmawan, 2023). The increase in production is accompanied by significant challenges that raise concerns about food security. Results from the national socio-economic survey show that per capita consumption of broiler chicken meat tends to continue increasing by 7.87% per year. According to data from the National Food Agency (Bapanas), the average Indonesian consumes 7.46 kg of broiler chicken meat per capita per year in 2023. The substantial demand for broiler chicken meat creates opportunities for farmers to accelerate growth rates and achieve ideal weight within a limited time. One of the methods is using AGP (antibiotic growth promoters) as the most common and economical way to improve broiler chicken performance (Sihombing & Fajri, 2024).

The use of AGP as a feed additive is 96.97% intended to stimulate growth and suppress infections from pathogenic microorganisms in the digestive tract. The use of AGP is also aimed at increasing body weight by 3.9%, feed efficiency by 2.9%, growth by 3.8-11.1%, and reducing mortality. The use of AGP has been banned in various countries since 2006 because it potentially leaves antibiotic residues in food products absorbed by consumers, which results in increased bacterial resistance and chemical residues in humans (Prasetyo et al., 2020). Based on research conducted by Affandi et al. (2024), 48.48% of farmers have a supportive attitude toward the use of AGP, while 51.52% are less supportive. The compliance rate of farmers in using AGP correctly and according to therapeutic purposes is only 21.22%, whereas 45.45% use it to stimulate growth and increase the body weight of broiler chickens. Some farmers use commercial feed containing AGP that is not recommended by the government, such as penicillin, kanamycin, erythromycin, and oxytetracycline. The study shows that most broiler chicken farmers violate AGP usage regulations due to concerns about decreased broiler performance and feed efficiency.

The working mechanism of AGP is to modulate the immune system and gut microflora of broiler chickens, leading to increased feed efficiency. Immune system modulation occurs by accelerating the maturation of immune cells, such as macrophages and lymphocytes, making the broiler chicken's body response to pathogens more sensitive. The use of AGP in feed also affects the gut microflora by suppressing pathogenic bacteria, improving microflora balance (eubiosis), and producing antimicrobial compounds. Antibiotic growth promoters work by inhibiting the growth of pathogenic bacteria in the digestive tract, such as *Clostridium* sp. and *Salmonella* sp., allowing beneficial bacteria populations, such as *Bifidobacteria* sp. and *Lactobacilli* sp., to thrive. This maintains a balance between beneficial and pathogenic bacteria in the gut with an ideal composition of 85% beneficial bacteria and 15% pathogenic bacteria. This ideal composition can prevent microflora imbalance (dysbiosis) that can reduce livestock productivity. Additionally, AGP stimulates the production of antimicrobial compounds and free fatty acids that create an environment unfavorable for the growth of pathogenic bacteria. Long-term use of AGP will cause genetic mutations by pathogenic agents, resulting in decreased effectiveness of antibiotic therapy. This factor became the basis for the ban on the use of AGP in 2018, regulated under the Livestock and Animal Health Law Number 18 of 2009 in conjunction with Number 41/2014. The risks arising from this ban include decreased broiler chicken performance, increased mortality and feed conversion ratio (FCR), as well as decreased function of the liver, gizzard, and heart organs (Prasetyo et al., 2020).

Food security will be affected if the use of AGP continues to be practiced by broiler chicken farmers. Eating animal-based foods, such as broiler chicken meat containing antibiotic residues, can cause health problems. The dangers of these residues can be direct hazards in the short term and indirect hazards in the long term. Short-term direct hazards can include allergies, digestive disorders, skin problems, anaphylaxis, and hypersensitivity, while long-term indirect hazards can include microbiological resistance, carcinogenic effects, mutagenic effects, teratogenic effects, and reproductive disorders (Marlina et al., 2015). That is in line with the research that has been conducted by Prasetyo et al. (2020), the cessation of AGP in feed causes feed wastage of up to 2.5%, stunted growth, higher mortality, and broiler chickens become more susceptible to coccidiosis infection.

Moringa is a plant that grows well in tropical regions and is widely known as a vegetable and traditional medicine (Apriantini et al., 2022). The phytochemical test results of moringa leaves show the presence of alkaloids, flavonoids, steroids, triterpenoids, and tannins. Alkaloid compounds are used as stimulants for the nervous system, cough medicine, eye drops, malaria medicine, cancer medicine, antibiotics, and sedatives. Alkaloids can also accelerate wound healing by increasing TGF-α (transforming growth factor-α1) and EGF (epidermal growth factor) (Putra et al., 2016). Flavonoids act as antimicrobials through their action in causing damage to the permeability of bacterial cell walls, microsomes, and lysosomes. Some types of flavonoids also have hepatoprotective properties by inhibiting prostaglandin synthesis (Widowati et al., 2014). Furthermore, flavonoids can also increase the expression of IGF-1 (insulin-like growth factor-1) (Mistiani et al., 2020). Steroids can increase the body weight of broiler chickens with a physiological effect in the form of increased lean muscle mass (Andriyanto et al., 2015). Triterpenoids are known to have antibacterial activity capable of killing pathogenic bacteria, such as *Salmonella* sp., *Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas fluorescens* (Putra et al., 2016). Tannins at low concentrations can inhibit bacterial growth, whereas at high concentrations they act as antibacterial agents by coagulating bacterial protoplasm due to the formation of stable bonds with bacterial proteins (Wiryawan et al., 2007). Saponin is capable of increasing the permeability of the intestinal wall, increasing nutrient absorption, which affects body weight gain (Khodijah et al., 2012). Saponin is often used to inhibit fat accumulation, thereby improving the carcass quality of broiler chickens (Rudi et al., 2021).

Acidifiers are organic acids added to feed or drinking water to inhibit the growth of pathogenic bacteria in the digestive tract, thereby optimizing the growth process of broiler chickens. The organic acids used can include citric acid, lactic acid, propionic acid, acetic acid, or a mixture of organic acids (Pasi et al., 2024). The effect of organic acids on gut microflora involves specific influences of acid anions on enzymes or cell membranes, internal pH value, buffering capacity, the amount of ATP used in proton pumping, and the transport of acid molecules (Hidayat et al., 2018). One organic acid with potential as an acidifier is lime (*Citrus aurantifolia*). Lime contains flavonoids, saponins, and essential oils (Mona & Syukma, 2023). The antibacterial mechanism of flavonoids in lime involves forming complex compounds with extracellular proteins to damage bacterial cell membranes. Saponins act as antibacterials by reducing surface tension and interacting with sterol membranes of bacterial cells (Berlian et al., 2016). The influence of essential oils on broiler performance is linked to their role in lipid metabolism pathways, stimulating secretion and activity of digestive enzymes, acting as antimicrobials, and enhancing intestinal integrity (Puvaˇca et al., 2022).

Probiotics are living microorganisms that provide benefits by improving the balance of intestinal microorganisms, and their activity can maintain the integrity of the intestinal mucosal membrane (Dewi et al., 2021). Probiotics function to enhance immunity at all ages and poultry classes, promote intestinal integrity and maturation, prevent inflammation, increase feed intake and digestion by boosting digestive enzyme activity, reduce bacterial enzyme activity, lower ammonia production, neutralize enterotoxins, and stimulate immune function. The species of microorganisms commonly used as probiotics include *Lactobacillus bulgaricus*, *Lactobacillus plantarum*, *Streptococcus thermophilus*, *Bifidobacterium bifidum*, *Aspergillus oryzae*, and other species. These microorganisms do not leave residues in animal products such as meat, milk, and eggs (Rehman et al., 2019).

## 2. Methods

This research method is a literature study by analyzing secondary data based on reviews from several research journals related to the potential of moringa leaves, acidifiers, and probiotics in improving the growth performance of broiler chickens. The sources or references obtained were determined by inclusion and exclusion criteria. The inclusion criteria are data in the form of journals (national and international), textbooks, and scientific articles published after 2015. The exclusion criteria are data in the form of journals (national and international), textbooks, and scientific articles published before 2015. Based on the results of the literature study, various research variables related to the application of moringa leaves, acidifiers, and probiotics as natural growth promoters to improve the growth performance of broiler chickens were obtained.

## 3. *Results and Discussion*

### *1.1 Moringa leaves*

According to Apriantini et al. (2022), the following is the scientific classification of moringa. Moringa leaves belong to the kingdom Plantae, which includes all green plants. The genus of Moringa leaves is Moringa. Within this genus, there are various species, one of which is *Moringa oleifera*. This plant is also included in the division Spermatophyta. Within this division, Moringa oleifera is classified in the subdivision Angiospermae, which indicates that it is a flowering plant. Furthermore, this plant belongs to the Class Dicotyledonae with the Subclass Dialypetalae, which consists of dicots with separate flower petals. The species Moringa oleifera also belongs to the Order Rhoeadales (Brassicales) and the family Moringaceae.

Fig. 1. Moringa leaf
(Redaksi Trubus, 2023)

Kelor is a plant that grows well in tropical regions and is widely known as a vegetable and a traditional medicine. This plant originates from the Himalayan region and India, then spreads to surrounding areas, including the African continent and West Asia, as well as Indonesia (Apriantini et al., 2022). The morphology of the kelor plant is a softwood tree with a diameter of about 30 cm and a height reaching 7-12 meters. Its stem is woody (lignified), upright, dirty white, with thin bark that is easily broken. The root bark of kelor has a sharp and spicy aroma, with the inner part pale yellow, finely lined, and oriented transversely. The kelor roots are not hard, irregularly shaped, with a somewhat smooth outer bark surface and a slightly fibrous inner surface; the wood part is light brown or cream-colored, with each fiber separated from one another. Kelor flowers are cream-colored (yellowish-white) or red. The flower bracts are green and emit a fragrant aroma. The characteristics of kelor leaves are pinnately compound but incomplete, small (about the size of a finger), and egg-shaped. The leaves range in color from light green to brownish green, with an inverted egg shape, 1-3 cm long, 4 mm to 1 cm wide, blunt leaf tips, rounded leaf bases, and smooth leaf edges (Marhaeni, 2021). Harvesting of kelor leaves can be done after the plant grows to 1.5-2 meters by plucking leaf stalks from the branches or cutting the branches 20-40 cm above the ground (Widowati et al., 2014).

Fig. 2. Moringa plant
(Redaksi Trubus, 2023)

Moringa is a long-lived (perennial) plant that can grow in both lowlands and highlands up to an altitude of approximately 1000 meters above sea level. This plant can be propagated both generatively (by seeds) and vegetatively (by stem cuttings). One of the advantages of moringa is its ability to tolerate the environment even under extreme conditions. Moringa can survive long dry seasons and grows well in areas with an annual rainfall ranging from 250 to 1,500 mm. The environmental parameters required for moringa to grow well include a tropical or subtropical climate, an altitude of 0-1000 meters above sea level, temperatures of 25-35°C, annual rainfall of 250-2.000 mm, sandy or sandy loam soil type, soil pH of 5-9, and good irrigation is necessary if rainfall is less than 800 mm (Putra et al., 2016).

Research conducted by Putra et al. (2016) reported that phytochemical tests on moringa leaves revealed the presence of alkaloids, flavonoids, steroids, triterpenoids, and tannins. Alkaloids are the most abundant organic compounds found in leaves that have a bitter taste. Each alkaloid compound has a specific biologically active site. Alkaloids in moringa plants are used as toxins to combat insects or plant-eating animals. These compounds are used as nervous system stimulants, cough medicines, eye drops, antimalarials, anticancer drugs, antibiotics, and sedatives in pharmacology. In addition, alkaloids can accelerate wound healing by increasing TGF-α and EGF.

Flavonoid compounds function in photosynthesis, antimicrobial, and antiviral processes. Flavonoids act as antimicrobials by causing damage to the permeability of bacterial cell walls, microsomes, and lysosomes due to the interaction between flavonoids and bacterial DNA. The lipophilic nature of flavonoids causes damage to bacterial cell membranes. Some types of flavonoids, such as silymarin and silybin, have been proven to be hepatoprotective by inhibiting prostaglandin synthesis (Widowati et al., 2014). The hepatoprotective mechanism also occurs by acting as an inhibitor of CYP3A activity and serving as a scavenger of free radicals that directly bind to reactive oxygen species (ROS) or reactive nitrogen species (RNS), then increasing endogenous antioxidant activity (glutathione) to suppress free radical production in liver cells. In addition, flavonoids can also increase the expression of IGF-1, which acts as a mediator in fibroblast proliferation and collagen synthesis, thereby triggering muscle mass growth (Mistiani et al., 2020).

Steroids are a group of compounds that have a cyclopentanoperhydrophenanthrene core structure with four fused rings. Steroids in plants have uses such as regulating growth (sesquiterpenoid abscisic acid and gibberellin) and assisting in photosynthesis (carotenoids) (Putra et al., 2016). One steroid that can increase the body weight of broiler chickens is testosterone. Testosterone is a steroid hormone that has anabolic and androgenic effects. One of testosterone's targets is muscle, with a physiological effect of increasing lean muscle mass. Testosterone stimulates the release of growth hormone (GH) from the anterior pituitary. Growth hormone influences physiological processes in the body, including fat, protein, and carbohydrate metabolism as well as bone growth. Growth hormone stimulates the release of IGF-1 from the liver to skeletal muscle, then supports satellite cell activation in proliferation and differentiation. Testosterone works in the cell nucleus by passively diffusing into muscle cells, then binding to receptors on the nuclear membrane and being transported into the nucleus. Testosterone stimulates DNA to transcribe into mRNA, which is then transported to the cytoplasm and translated into new proteins, thereby increasing cell size. Testosterone is circulated by steroid-binding globulins such as α-globulin. Broiler chickens stimulated with testosterone generally show an increase in body weight due to muscle mass growth and a decrease in fat mass, resulting in increased lean body mass (Satyaningtijas et al., 2014).

Triterpenoids are compounds whose carbon skeletons are derived from six isoprene units and are derived from acyclic hydrocarbons (squalene). These compounds appear as colorless crystals, have a high melting point, are optically active, and are difficult to characterize due to a lack of chemical reactivity. In plants, triterpenoids function as a defense against insect pests and as growth factors. Triterpenoids are known to have antibacterial activity capable of killing pathogenic bacteria such as *Salmonella* sp., *Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas fluorescens* (Putra et al., 2016). Research conducted by Wiryawan et al. (2007) showed that triterpenoids can inhibit the growth of bacteria that cause diarrhea, preventing *Escherichia coli* infection from reaching acute symptoms (severe diarrhea). The antibacterial mechanism of triterpenoids involves disrupting the phospholipid layer of the bacterial cell membrane, causing changes in the composition of fatty acids and membrane phospholipids, followed by cell swelling, which leads to increased permeability and loss of essential cellular components.

Tannins are a group of active plant compounds that are phenolic and are characterized by an astringent taste. Tannins are widely distributed in various plant species and play roles as pesticides and regulators of plant growth. They function as antioxidants and tumor growth inhibitors (Putra et al., 2016). Tannins exhibit antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, and *Streptococcus faecalis*. At low concentrations, tannins can inhibit bacterial growth, while at high concentrations, they act as antibacterial agents by coagulating bacterial protoplasm through the formation of stable bonds with bacterial proteins (Wiryawan et al., 2007). However, the tannin content in feed supplements must be carefully considered since these compounds are classified as antinutritional factors. Tannins can precipitate proteins, resulting in protein-tannin cross-links. These bonds make the feed difficult to digest by digestive enzymes (Khodijah et al., 2012).

Research conducted by Nikmah et al. (2022) added that moringa leaves also contain saponins. Saponins are complex glycoside compounds that form bases in water with a high molecular weight. Saponins have soap-like (foaming) properties that can clean and increase the permeability of the intestinal wall, thereby facilitating the absorption of large molecules in the body, which affects an increase in body weight (Khodijah et al., 2012). Saponins are also known to reduce fat levels in broiler chickens. Saponins are often used to inhibit fat accumulation, thereby improving the quality of broiler chicken carcasses. Fat accumulation is influenced by the composition of the ration, such as the energy level in the ration, the energy-protein ratio, and fat content (Rudi et al., 2021). However, excessively high levels of saponins can also act as antinutritional factors for broiler chickens. Saponins can reduce feed consumption because chickens are very sensitive to saponins. The inhibitory effect of saponins on growth is caused by their ability to inhibit the activity of digestive enzymes, such as trypsin and chymotrypsin (Wuntu et al., 2024).

Table 1. The effect of moringa leaf application on broiler chicken performance

| Author, Year | Findings |
|---|---|
| Setiadi et al., 2023 | One solution to replace the role of AGP is the utilization of moringa due to its antioxidant activity, which can act as an antibiotic. Moringa leaves, as a natural antioxidant, contain active compounds such as carotenoids, selenium, flavonoids, phenolics, and a relatively high protein content of around 24,5%, which can improve performance. The ability of these antioxidant substances to maintain the structure of basic biological macromolecules effectively inhibits oxidation and neutralizes reactive oxygen free radicals associated with diseases. |
| Taufik et al., 2017 | The high protein content in moringa leaves causes the use of moringa leaf powder to be considered more as a substitute for other protein sources rather than as a low-level feed supplement. The consumption of moringa leaf powder is not optimal when mixed into feed, so moringa leaves can be extracted and mixed into drinking water. |
| Astuti & Irawati, 2022 | Moringa leaf extract has a significant effect on the feed consumption of broiler chickens. This is because moringa contains antioxidants that can improve pancreatic function, protecting chickens from oxidative stress and providing good growth results by enhancing both body metabolism and nutrient absorption in the chickens. |
| Tirajoh et al., 2020 | The body weight of broiler chickens given additional moringa leaf powder is higher final weight. Moringa leaf powder contains various active substances, high energy concentration, and nutrients that support the metabolism and absorption of food substances, thereby accelerating muscle formation. The nutrients contained in moringa leaves include protein, calcium, potassium, magnesium, phosphorus, iron, and zinc, which are higher compared to other vegetables. |
| Desy et al., 2021 | The use of moringa leaf powder can reduce the fat content in broiler chicken meat, thus increasing the carcass percentage, especially in the breast and thigh parts. Moringa leaves are a strong antioxidant that can protect and maintain chickens from oxidative stress, resulting in better growth speed and body mass. |

### *1.2 Acidifier*

Acidifiers are organic acids added to feed or drinking water to inhibit the growth of pathogenic bacteria in the digestive tract, thereby optimizing the growth process of broiler chickens. Acidifiers can replace the role of antibiotics in balancing the digestive microflora and enhancing the absorption of nutrients in the small intestine. The organic acids used can include citric acid, lactic acid, propionic acid, acetic acid, or a mixture of organic acids (Pasi et al., 2024). The effect of organic acids on intestinal microflora involves specific influences of acid anions on enzymes or cell membranes, internal pH value, buffering capacity, the amount of ATP used in proton pumping, as well as the transport of acid molecules (Hidayat et al., 2018).

Fig. 3. Lime
(Siwi, 2018)

One organic acid with potential for use as an acidifier is lime (*Citrus aurantiifolia*). According to Rahmatullah et al. (2021), the following is the scientific classification of lime. Acidifier belong to the kingdom Plantae, which includes all green plants. The genus of Acidifier is *Citrus*. Within this genus, there are various species, one of which is *Citrus aurantifolia*. This plant is also included in the division Spermatophyta. Within this division, Acidifier is classified in the subdivision Angiospermae. Furthermore, this plant belongs to the Class Dicotyledonae with the Subclass Rosidae.

Lime is a small tree with a dense but irregular branching habit, with a height ranging between 1,5 to 5 meters. The plant has strong, fairly deep roots and can grow well in various types of soil. The branches and twigs of the lime tree have short, stiff, and sharp thorns. The leaves of the lime tree are dark green and glossy on the upper surface and light green on the underside (Rukmana, 2003). The lime leaves are alternately arranged, shaped from oblong to round, rounded at the base, blunt at the tip, with finely serrated edges, and have narrow winged petioles. The size of the lime leaves ranges from 4-8 cm in length and 2-5 cm in width (Sarwono, 2001). The lime plant produces fruit that is very sour, round to oval in shape, and has thin skin. The fruit diameter is 3-6 cm, and its surface has many glands. The lime fruit takes 5-6 months to develop. When ripe, the fruit changes color from green to yellow, then falls to the ground after fully ripening. The lime flowers are in short clusters and are located in the leaf axils on the tips that are just beginning to bloom. The number of flowers per cluster is about 1-10. The flower petals number 4-6 and are 8-12 cm long. The lime flowers have 20-25 stamens and a pistil stalk that is easily distinguishable from the ovary (Sarwono, 2001). Lime plants grow well under certain environmental characteristics, namely at altitudes of 200-1.300 meters above sea level, annual rainfall of 1.000-1.500 mm/year, air temperature of 20-30°C, moderate to high humidity (70-80%), moderate sunlight, and wind speed less than 40%. Lime plants require 5-6 months to grow and need the rainy season for flower and fruit development to keep the soil moist. This plant requires sufficient water, especially in July to August (Fanani, 2019).

Fig. 4. Lime plant
(Tani Maju Indonesia, 2018)

Lime contains flavonoids, saponins, and essential oils. The secondary metabolite flavonoids in lime consist of flavonols, flavones, and flavanones. Flavonoids are derivatives of phenol compounds with a C6-C3-C6 configuration, meaning they have fifteen carbon atoms. The hydroxyl groups commonly found in flavonoids influence their solubility in water (Mona & Syukma, 2023). The antibacterial mechanism of flavonoids in lime involves forming complex compounds with extracellular proteins to damage bacterial cell membranes. Flavonoids can damage cell membranes by inhibiting macromolecule synthesis. They can also depolarize cell membranes and inhibit the synthesis of DNA, RNA, and bacterial cell proteins. Additionally, flavonoids can inhibit the function of the cytoplasmic membrane and energy metabolism in bacteria (Berlian et al., 2016). Sirait (2007) added that the mechanism of flavonoids can also disrupt the activity of peptidoglycan transpeptidase, thereby interfering with cell wall formation and causing bacterial cell lysis.

The saponin content in lime consists of two groups, namely hydrophilic and hydrophobic groups. The hydrophilic group binds to polar compounds, which are the organic smear layer, while the hydrophobic group binds to nonpolar compounds, which are the inorganic smear layer (Widyastuti & Rini, 2022). Saponin also acts as an antibacterial agent by reducing surface tension, leading to increased permeability or leakage of bacterial cells, followed by releasing intracellular compounds. It can inhibit bacterial cell growth due to its interaction with membrane sterols. The main effect of this inhibition process is the release of proteins and enzymes from inside the bacterial cells (Berlian et al., 2016).

Another bioactive compound found in lime is essential oil. The physical characteristics of essential oil are that it is a clear yellowish liquid, with a specific gravity ranging from 0,85-0,87 g/mL (at 26°C), a refractive index in the range of 1,47-1,479 (at 26°C), and a pH of about 4-5 (Ulandari, 2022). Essential oil is an aromatic liquid characterized by its volatility and is mostly colorless. Its consistency is almost similar to water or alcohol. Essential oil is insoluble in water but soluble in all organic solvents, and it has a boiling point ranging from 150-280°C. Essential oil will thicken, darken, and become acidic when in contact with air for a long time. The effect of essential oil on the productive performance of broiler chickens is related to its role in lipid metabolism pathways, stimulating the secretion and activity of digestive enzymes, acting as an antimicrobial, and improving the intestinal integrity of chickens. The general mechanism associated with the stimulatory effects of essential oil on broiler characteristics is the reduction of undesirable bacteria. Essential oils can influence cecal microflora by stabilizing the gut, thereby reducing unwanted microbiological activity such as ammonia and biogenic amines. This can reduce microbiotic fermentation in the small intestine and increase the availability of essential nutrients (Puvaˇca et al., 2022). In addition, essential oil also exhibits hypocholesterolemic effects in broiler chickens by inhibiting enzymes involved in cholesterol and lipid synthesis, thus significantly lowering blood cholesterol levels. The mechanism works through the formation of insoluble saponin-cholesterol complexes in the digestive tract, as saponins inhibit the absorption of both endogenous and exogenous cholesterol in the intestine (Abdulkarimi et al., 2011). Jazi et al. (2018) further stated that the active compounds in essential oil have been reported to have cholesterol-lowering effects due to the expression of the protein 3-hydroxy-3-methyl-glutaryl-CoA reductase, which leads to a decrease in total cholesterol and LDL (low-density lipoprotein) concentrations. Other mechanisms occur through the activity of HMG-CoA reductase, cholesterol-7-hydroxylase, fatty acid synthase, and 6-amino-nicotinamide, which inhibit the activity of the pentose phosphate pathway in the liver.

Table 2. The effect of adding an acidifier as a feed additive on broiler chicken feed

| Author, Year | Findings |
|---|---|
| Prastiwi & Ferdiansyah, 2017 | Lime has been proven to have the ability to inhibit the growth of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Salmonella typhi*, and *Enterococcus faecalis*. The main components responsible for the antibacterial activity in lime are citric acid, malic acid, and tartaric acid. The inhibition mechanism by lime occurs by lowering the optimal pH for bacterial growth and inhibiting bacterial metabolism. |
| Berlian et al., 2016 | The polar nature of flavonoids in lime causes these compounds to more easily penetrate the cell wall of *Staphylococcus aureus* because the bacterial cell wall is single-layered and composed of peptidoglycan (proteins and sugars) and lipids in low amounts (1-4%). This ability of flavonoids enables them to act as natural antibacterial agents against *Staphylococcus aureus*. |
| Windisch et al., 2008 | Essential oils have beneficial effects on broiler chickens under stress conditions and enhance the absorption of essential nutrients that stimulate more intensive growth, achieving maximum genetic potential. The reduction of microbiological activity leads to decreased production of volatile fatty acids, which affects the stabilization of intestinal pH, thereby optimizing digestive enzyme activity. |

| | |
|---|---|
| Pasi et al., 2024 | The citric acid content in lime will enhance the performance of beneficial bacteria in the digestive tract of broiler chickens because the low pH will reduce the activity of harmful bacteria. Beneficial bacteria can work optimally to improve nutrient absorption in the small intestine. |

### *1.3 Probiotic*

Probiotics are live microorganisms that provide benefits by improving the balance of intestinal microorganisms and whose activity can maintain the integrity of the intestinal mucosal membrane. Microorganisms used as probiotics have common characteristics, such as originating from nonhuman sources, being nonpathogenic, resistant to technological processes, resistant to stomach acid, able to adhere to target epithelial tissues, capable of living in the gastrointestinal tract, producing antimicrobial substances, able to modulate the immune system, and capable of influencing metabolic activity (Dewi et al., 2021). Probiotics function to enhance immunity at all ages and classes of poultry, promote intestinal integrity and maturation, prevent inflammation, increase feed intake and digestion by enhancing digestive enzyme activity, reduce bacterial enzyme activity, lower ammonia production, neutralize enterotoxins, and stimulate immune function.

Microorganism species commonly used as probiotics include *Lactobacillus bulgaricus*, *Lactobacillus plantarum*, *Streptococcus thermophilus*, *Bifidobacterium bifidum*, and *Aspergillus oryzae*. These microorganisms do not leave residues in animal products such as meat, milk, and eggs. Probiotics modify the intestinal ecosystem by increasing the effectiveness of digestive enzymes and reducing digestive pH. Probiotics modulate gut microbiota and reduce pathogens, improve the immune sensory properties of broiler chickens, and enhance meat quality. Probiotic supplementation has significant effects on carcass yield as well as increased live weight and immune response. However, probiotic colonization in the gut is influenced by several factors, such as the availability of fermentation substrates (prebiotics), strain specificity to host dose, supplementation frequency, age, health, genetics, host nutritional status, intestinal pH, and stress (Rehman et al., 2019).

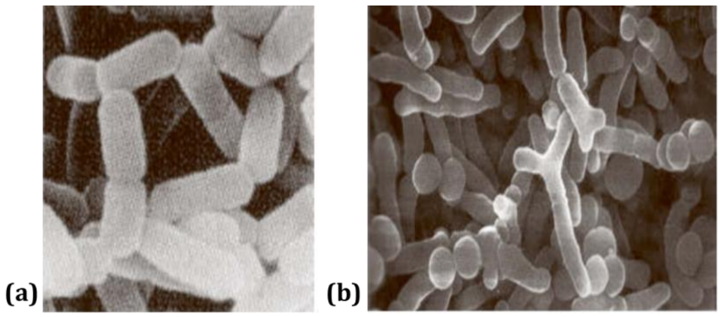


Fig. 5. Good bacteria in probiotics with (a) *Lactobacillus* and (b) *Bifidobacterium* (Winarno & Winarno, 2017)

Prebiotics are food ingredients that cannot be digested and are beneficial to the host's health by selectively stimulating the growth and activity of intestinal microorganisms. The main prebiotics commonly used consist of oligosaccharides, such as fructooligosaccharide (FOS), galactooligosaccharide (GOS), xylooligosaccharide (XOS), inulin, and fructans. An ideal prebiotic has several criteria: it is resistant to stomach acid, bile salts, and hydrolytic enzymes in the intestine; it cannot be absorbed by the digestive tract; and it is easily fermented by intestinal microflora (Cahyaningtyas & Wikandari, 2022). Prebiotics are necessary for the better survival of probiotics in the gut. Probiotics can thrive well in the digestive system with the help of prebiotics because they can tolerate anaerobic environments such as low oxygen levels, low pH, and temperature. Prebiotics serve as substrates for the survival and proliferation of probiotics in the gut, acting symbiotically.

Prebiotics have been proven to control pathogens like *Escherichia coli* and *Salmonella* sp. and stimulate the growth of *Lactobacilli* sp. and *Bifidobacteria* sp. A commonly used prebiotic is mannan oligosaccharide (MOS), derived from the outer cell wall of *Saccharomyces cerevisiae*, including 30% glucan, 30% mannan, and 12,5% protein. The protein is rich in serine, aspartic acid, glutamic acid, and methionine. Adding MOS to broiler chicken diets can have positive effects on growth. Mannan oligosaccharides contain ligands with high affinity for bacteria and provide competitive binding sites (Rehman et al., 2019).

The use of probiotics and prebiotics in poultry feed can enhance immune status. The antibody titer results for IBD (inflammatory bowel disease) are higher in poultry feed supplemented with probiotics. This higher antibody titer results from immune regulation by cytokines secreted by immune cells stimulated by probiotic microbes (Silva et al., 2009). Hakim et al. (2020) reported that probiotic supplementation can reduce fecal ammonia levels. This reduction in ammonia decreases ammonia pollution in the coop or the surrounding area. The mechanism involves lowering pathogenic bacteria in the gut, which leads to optimal growth of lactic acid bacteria (LAB), improving nutrient absorption in the intestine. Bacteriocin-producing lactic acid bacteria suppress pathogenic microbes, preventing fecal deamination and lowering $NH_3$ concentration.

Probiotics work by increasing the number of nonpathogenic bacteria in the intestines. Nonpathogenic bacteria can alter the intestinal environment, specifically the pH (hydrogen potential), making it more acidic, which enhances the immune function of the digestive tract. Probiotics function by producing bacteriocins and short-chain organic acids (lactate, acetate, propionate). These substances can inhibit the growth of harmful microbes. The adhesion ability of probiotics and endogenous microbes can act as a barrier against pathogens, thereby boosting immunity and nutrient absorption (Andriani et al., 2020). The mechanism of probiotics is also linked to the reduction of blood cholesterol levels. Cholesterol reduction occurs through the deconjugation of bile salts due to the bile salt hydrolase (BSH) activity possessed by *Lactobacillus* sp. Probiotic cells can deconjugate bile salts that are related to cholesterol in the digestive tract. Bile salts are deconjugated into free bile acids, which cannot be absorbed and are excreted with feces if probiotic cells have BSH activity. High BSH activity in deconjugating bile acids leads to increased excretion of bile acids. The body's compensation is to take cholesterol from the blood to be used as a precursor for the synthesis of new bile salts, resulting in a decrease in blood cholesterol levels (Astuti et al., 2009).

Table 3. The role of probiotics in optimizing broiler chicken performance

| Author, Year | Findings |
|---|---|
| Andriani et al., 2020 | Probiotic administration has been proven to reduce total cholesterol and LDL levels while increasing HDL levels in broiler chickens. Administering probiotics at a concentration of 10^8 CFU/g through feed and drinking water can influence the LDL and HDL ratio. The administration of *Lactobacillus* sp. probiotics yields optimal results in enhancing nutrient absorption and producing the enzyme BSH. The BSH enzyme can reduce cholesterol levels, lipase enzyme, and blood triglycerides without leaving residues and is easily absorbed by the intestines. |
| Ghorbani et al., 2011 | The use of probiotics containing *Lactobacillus casei* can improve energy and protein efficiency as well as reduce blood cholesterol levels. |
| Sumardi et al., 2016 | Probiotics given with feed can be optimally utilized because they stimulate the synthesis of the lipoprotein lipase enzyme, which catalyzes the breakdown of glycerol and fatty acids, leading to the breakdown of LDL. Probiotic administration can influence the reduction of LDL levels and the increase of HDL levels. |
| Asmara et al., 2019 | Broiler chickens treated with probiotics have total leukocyte counts above normal. *Lactobacillus bulgaricus* bacteria can increase |

| | |
|---|---|
| | macrophage production and activate phagocytes. Increased macrophage activity affects the release of antibodies in broiler chickens because macrophages supply antigens to lymphocytes. |
| Hossain et al., 2025 | Microbial analysis of the cecum in chickens treated with probiotics shows that the number of *Lactobacillus* spp. is higher compared to the significantly lower numbers of *Escherichia coli* and *Salmonella* spp. This intestinal microbial balance is associated with a reduced incidence of systemic inflammation, which can affect meat quality by minimizing the activity of proteolytic enzymes that degrade muscle protein. |

*1.4 Effect of Moringa leaves, acidifier, and probiotics on the slaughter weight of broiler chickens*

Cutting weight is the result of weighing chickens before slaughter, but they have been fasted from feed for eight hours while drinking water is available ad libitum (Jefri et al., 2020). The increase in cutting weight can be attributed to moringa leaves containing active substances such as calcium, potassium, magnesium, and phosphorus, as well as minerals like iron and zinc. The essential amino acids in moringa leaves include isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine, with a total protein percentage reaching 40% (Rimbawanto et al., 2022). The high nutrient content significantly contributes to muscle growth and carcass formation in broiler chickens by enhancing digestibility and metabolism. Improved digestibility occurs due to better permeability and motility of the intestinal cell membranes, making nutrient absorption more effective. Additionally, natural antioxidants in moringa leaves, such as ascorbic acid, carotenoids, selenium, flavonoids, polyphenols, phenolics, and quercetin, also influence the increase in chicken metabolism, reducing oxidative stress and boosting the immune system (Setiadi et al., 2023). The strength of the immune system plays a crucial role in broiler chickens' resistance to pathogenic infections, thus contributing to optimal weight gain. Valdivié-Navarro et al. (2020) reported that moringa leaves and stems can be safely used for nonruminant livestock such as poultry, pigs, and rabbits at slaughter ages of 35-60 days. The chemical composition at these slaughter ages contains protein and amino acids equivalent to alfalfa forage, but moringa leaf meal has higher crude fiber NDF (neutral detergent fiber) and ADF (acid detergent fiber) content.

Table 4. Research results on the effect of moringa leaves, acidifier, and probiotics on the slaughter weight of broiler chickens

| Author, Year | Findings |
|---|---|
| Dewi et al., 2021 | The administration of 5% moringa leaf extract *Moringa oleifera* through drinking water can increase the slaughter weight of broiler chickens aged 2-6 weeks compared to the control group. |
| Wanti et al., 2024 | Moringa leaf extract has a statistically significant effect on increasing the average slaughter weight. Increasing the dose used will optimize the phytochemical content, such as tannins, saponins, and flavonoids, in enhancing slaughter weight. |
| Tirajoh et al., 2020 | Broiler chickens given additional moringa leaf powder have a higher average weight compared to the untreated group. |
| Pasi et al., 2024 | The addition of 75 mL of lime extract can increase the slaughter weight of broiler chickens. This is due to improved palatability of the feed and the effectiveness of liver function in the absorption of fiber and protein. |
| Jaelani et al., 2014 | Mixing commercial feed with probiotics has a significant effect on the slaughter weight of 35-day-old broiler chickens. Adding probiotics up to 4,5 g/kg body weight in the feed can produce the best performance as seen from slaughter weight, carcass percentage, and abdominal fat percentage. |

The increase in broiler chicken slaughter weight can also be influenced by the addition of lime extract due to the improvement in the animals' palatability towards feed as a result of the lime extract. The addition of acidifiers has been proven to enhance absorption by optimizing the function of digestive enzymes, which affects the digestion of fiber and protein. The administration of citric acid as an acidifier can increase relative weight (Pasi et al., 2024). Mahardi et al. (2020) added that the inclusion of acidifiers in probiotic feed aims to optimize the growth of probiotics in the digestive tract.

Jaelani et al. (2014) reported that the addition of probiotics in broiler chicken feed can significantly increase the slaughter weight. The mechanism of probiotics is to digest fat, crude fiber, and protein in the feed into easily absorbable substances. Probiotics also enhance enzymatic activity, digestion, and nutrient absorption, leading to faster livestock growth and increased productivity. Probiotic bacteria help break down tissue structures that are difficult to decompose, allowing more nutrients to be absorbed and transformed into the broiler chicken's body. The higher the probiotic addition in the feed, the greater the increase in slaughter weight. Other factors influencing slaughter weight include chicken strain, sex, environment, and the amount of feed consumed (Bell & Weaver, 2002).

*1.5 The Effect of Moringa leaves, acidifier, and Probiotics on the carcass weight of broiler chickens*

The increase in carcass weight in broiler chickens is directly proportional to feed consumption, where higher feed intake is accompanied by an increase in carcass weight. A diet containing qualitative and quantitative nutritional elements is necessary to achieve optimal growth rates according to genetic potential; thus, there is a relationship between growth rate and feed consumption (Wahyu, 2004). Research conducted by Rahmawati et al. (2020) showed that broiler chickens given fermented moringa leaf treatment had a higher final carcass weight due to the protein content in fermented moringa leaves reaching 26,43%. The fermentation of moringa leaves enhances the activity of amylase, lipase, and trypsin enzymes to accelerate the breakdown and absorption of protein by forming peptide bond complexes and insoluble compounds in the digestive tract. The final product of protein digestion is amino acids absorbed through the villi in the walls of the small intestine. The absorbed amino acids enter the bloodstream through the portal vein and are carried to the liver. Some amino acids are stored in the liver, while others are transported via the bloodstream to body tissue cells, including muscles. The high protein intake from moringa leaves stimulates amino acid absorption, thereby increasing the carcass weight of broiler chickens.

Table 5. Research results related to the effect of moringa leaves and probiotics on the carcass weight of broiler chickens.

| Author, Year | |
|---|---|
| Desy et al., 2021 | The administration of 4% moringa leaf powder tends to yield better results in live weight, carcass weight, and abdominal fat. |
| Kelanasari et al., 2021 | The administration of moringa leaf extract can be used to optimize the percentage of carcass and noncarcass in 35-day-old broiler chickens. |
| Mahmud et al., 2021 | The administration of moringa leaf powder can increase carcass weight at a dose of 30% moringa leaf powder. |
| Sukmaningsih & Rahardjo, 2019 | The best average carcass weight of broiler chickens is obtained in those receiving a probiotic mixture. An optimal ecosystem condition in the digestive tract is achieved with the administration of a probiotic mixture as live microbes in sufficient quantities. |
| Ridhana & Fitri, 2019 | The addition of probiotics to broiler chicken feed results in a significant difference ($p<0,05$) in carcass weight. Probiotics act as catalysts in breaking down organic compounds, making the feed easier to digest. |

The active compounds in lime that act as acidifiers for broiler chicken growth include lactic acid, citric acid, ascorbic acid, vitamin C, flavonoids, and other antimicrobial compounds. The lactic acid and citric acid content improves the performance of beneficial bacteria in the broiler's digestive tract because the low pH reduces the activity of pathogenic bacteria. Additionally, the addition of organic acids has been shown to enhance fiber and protein absorption as well as digestive enzyme function (Pasi et al., 2024).

The addition of beneficial bacteria from probiotics to the composition of moringa leaves will maximize the inhibition process of pathogenic bacteria. Beneficial bacteria from probiotics such as *Lactobacillus casei*, *Lactobacillus acidophilus*, and *Lactobacillus plantarum* will colonize and compete to obtain nutrients and adhesion sites on the intestinal wall, as well as directly inhibit the defeated pathogenic bacteria (Putra & Humaidah, 2022). Diaz's (2008) research results show that the use of probiotics can reduce pathogenic bacteria in the small intestine because the villi sites in the intestinal mucosa are replaced by beneficial probiotic bacteria, allowing the small intestine to function more optimally. Beneficial bacteria also stimulate the production of the enzyme β-glucanase, which helps break down substrates and reduce digesta viscosity, thereby speeding up feed absorption. This aligns with the research conducted by Ali et al. (2019), where the addition of EM4 fermentation in broiler chicken rations resulted in higher body weight gain compared to pure rations without EM4 fermentation. This is because the feed quality improves after proper fermentation. The combination of acidifier and probiotic administration will increase the absorption surface on jejunum epithelial cells, allowing maximal absorption of amino acids, starch, fat, and vitamins in the feed (Malhan et al., 2024). Optimal absorption positively affects the increase in broiler carcass weight. Other factors influencing carcass weight include age, sex, slaughter weight, body size and conformation, body fat condition, feed quality and quantity, and the strain being raised (Subekti et al., 2012).

### *1.6 The Effect of Moringa leaves, acidifier, and probiotics on the carcass percentage of broiler chickens*

Table 6. Research results regarding the influence of moringa leaves, acidifiers, and probiotics on the carcass percentage of broiler chickens

| Author, Year | Findings |
|---|---|
| Kelanasari et al., 2021 | The administration of moringa leaf extract in drinking water at a 30% dose has been proven to optimize the carcass percentage of 30-day-old broiler chickens. |
| Listyowati et al., 2024 | The administration of 30% moringa leaf extract in the treatment group was statistically analyzed using Duncan's multiple range test, resulting in an increase in carcass percentage up to 59,40%. |
| Mahmud et al., 2021 | The increase in carcass weight and percentage can be enhanced by administering a 30% dose of moringa leaf powder. |
| Sibarani et al., 2014 | The addition of 1,6% citric acid acidifier in a double-step-down feed for broiler chickens can influence a higher carcass percentage compared to the group without citric acid administration. |
| Nadin et al., 2022 | Probiotic EM4 has a significant effect on the carcass percentage of broiler chickens. The microorganisms contained in the probiotic play a beneficial role in the absorption process of feed nutrients. |

Subekti et al. (2012) reported that the fat percentage affects the carcass percentage weight, where the lower the fat, the higher the carcass percentage weight produced. High protein content in the feed will also result in a high carcass percentage. This occurs because protein is a component that helps meet the broiler chicken's needs to produce a carcass. Carbohydrates play a role in calorie formation, so a high carbohydrate content in the feed will help increase the carcass percentage weight. The interaction of lysine and methionine in moringa leaves also positively influences the carcass percentage. Lysine is a precursor for carnitine biosynthesis, while carnitine stimulates the β-oxidation process of long-chain fatty acids occurring in mitochondria. The increased formation of carnitine due to lysine supplementation in the feed can cause more body fat to be oxidized, thus lowering the fat and cholesterol content in the meat (Pamungkas et al., 2020). The lysine content in moringa leaves needs attention because lysine is the primary limiting amino acid, followed by methionine as the second limiting amino acid. Lysine plays an important role in metabolism as it can synthesize proteins and assemble other important components needed in metabolism. Lysine functions to help calcium absorption required for bone formation, thereby enhancing growth (Si et al., 2001). Based on research by Sulasmi et al. (2013), adding 5% moringa leaf powder to commercial broiler feed can increase carcass weight percentage and reduce feed conversion.

The carcass percentage can increase due to higher digestion and absorption of feed nutrients in broiler chickens. Treatment with 0,25% citric acid and 0,25% butyric acid has a positive effect on feed efficiency. Good feed efficiency can occur because of an increased absorptive surface area on the jejunum epithelial cells. A wide absorptive surface can improve the digestibility of amino acids, starch, fat, and vitamins in the feed. One effect of acidifier administration is lowering the pH to 5-6, which inhibits the growth of pathogenic microbes and even kills them, while increasing the total lactic acid bacteria in the digestive tract. Lactic acid bacteria can convert carbohydrates into lactic acid and help digest crude fiber in the feed, thereby increasing the carcass percentage (Sibarani et al., 2014). Nadin et al. (2022) reported that probiotic administration effectively improves carcass quality and increases the percentage of breast and back meat in broiler chickens. The quality of life of broilers given probiotics in their ration can reach up to 75%. Other factors influencing carcass percentage include the final weight of the chicken, genetics, ration composition, and environmental temperature. Environmental temperature needs attention because broilers raised at high temperatures tend to reduce their appetite as they pant to release body heat (Nurhidayat et al., 2020).

## 4. Conclusions

The application of moringa leaf, acidifier, and probiotics can be an innovation as a natural growth promoter to improve the growth performance of broiler chickens. This application can be an alternative to the use of AGP without leaving residues in animal products. The bioactive compounds contained in moringa leaves can replace the role of AGP due to their antioxidant activity. The use of acidifiers as feed additives provides an optimal pH environment for digestive bacteria. The balance of digestive bacteria can be supported by the administration of probiotics. The combination of these three compositions is expected to provide optimal results for the performance of broiler chickens.

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## Author Contribution

T.P.D.A. and S.A. contributed to the literature search, interpretation, writing, and proofreading of the manuscript. All authors have read and agreed to the published version of the manuscript.

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## Biographies of Authors

**Titis Putri Dika Amalia,** Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia.

- Email: titis.putri.dika.amalia@mail.ugm.ac.id
- ORCID: N/A
- Web of Science ResearcherID: N/A
- Scopus Author ID: N/A
- Homepage: N/A

**Syifa Annisa,** Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia.

- Email: syifaannisa@mail.ugm.ac.id
- ORCID: N/A
- Web of Science ResearcherID: N/A
- Scopus Author ID: N/A
- Homepage: N/A