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Growth Response of Chickens on Performance and Liver Percentage Supplemented by Miana Leaf Based Eco Enzyme in Drinking Water

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ABSTRACT. *The aim of the research was to determine the influence of Miana leaf (ML)-based eco-enzyme onthe performance and liver percentage inbroilers. This research used a completely randomized design. 200-day-old broilers (DOC) were randomly divided into 4 treatments and 5 replications, with each replicate consisting of 10 chickens. The four treatments were: A (0% eco-enzyme based on ML), B (2% eco-enzyme based on ML), C (4% eco-enzyme based on ML), D (6% eco-enzyme based on ML) which is supplemented by drinking water. The parameters measured were feed consumption, average daily gain (ADG), feedconversion ratio (FCR) and liverpercentage. The results obtained showed that supplemented ML significantly (P<0,05) affected ADG. The supplementation of ML based eco enzyme did not have a significant effect (P>0,05) on feed consumption, feed conversion ratio and liver percentage. The supplementation of ML based eco enzyme could be tolerated up to 6% in drinking water of broilers.*

Key word : Miana leaf, body weight gain, consumption ratio, conversion ratio, percentage of liver weight

INTRODUCTION

The need for food sources is increasing as the world population continues to grow, with about 8 billion people (World Meter Info, 2023) and 276.36 million people in Indonesia (BPS, 2023). To achieve these targets, humans require food sources which rich in nutrients. One type of food is broiler chicken, a type of broiler chicken that is easily accessible and high in protein.

Broilers are very easy to care for and are loved by people of all ages. Broiler farmers often administer commercial antibiotics to increase the productivity of their livestock. This commercial antibiotic is administered with the aim of reducing mortality and increasing the growth rate of broilers.

Commercial antibiotics are widely used in modern chicken farming to prevent and treat disease. The goal of giving antibiotics is to prevent disease from occurring and thus reduce the risk of death. In addition, the supplementation of antibiotics is aimed at stimulating the growth of broilers. In 1960, breeders began using antibiotics in broiler rations as growth stimulants. Given the high risk of the use of commercial antibiotics leading to antibiotic resistance. However, due to the ability of bacteria to survive the effects of an antibiotic attack, the European Commission decided on January 1, 2006 to ban the administration of synthetic antibiotics that act as growth stimulants in animal feed. The ban on the administration of synthetic antibiotics has led researchers to look for solutions to replace these antibiotics with medicinal plants. One of these plants is miana leaves (ML). This plant is an herbal plant with bioactive properties such as phenols, anthocyanins, flavonoids, essential oils and organic acids that act as antibacterial agents, antioxidants, antifungal agents and natural dyes (Edi, 2020).

Auliawan & Cahyono (2014),reported that ML extract contains alkaloids, flavonoids, saponins and tannins and has a negative effect on steroid/triterpenoid tests. Supplementation of flavonoids and saponins has been reported to increase growth, feed efficiency and meat quality of non-ruminants (Miah *et al*., 2004b).

(Lisdawati *et al*., 2008) revealed that the phytochemicals of ML contain terpenoids, tannins, catecate tannins and flavonoids.

Previous research on the administration of ML as a feed additive in both drinking water and feed resulted in denser carcass structure, higher body weight gain and reduced feed conversion without affecting the appearance of broilers (Praptiwi & Indriastuti, 2015; Fati *et al*., 2019; Fati *et al*., 2020; Mahata *et al*., 2021).

The flavonoid content in ML is good for chickens and can be used as a natural feed additive,which play a role in maintaining the immune system of chickens and increase the utility and economic value of ML. In order for the feed additive made from ML to have a long shelf life, it can be made through a fermentation process, which is now better known as eco-enzyme.

Eco-enzyme is a chemical conversion of organic compounds by enzymes produced by microorganisms. This eco-enzyme can be stored for a long time and has the potential to be a natural feed additive that can replace chemical-based antibiotics and can be produced in large quantities without compromising the quality of the eco-enzyme.

Based on the background above the researcher conducted experiment entitled growth response of chickens on performance and liver percentage supplemented by Miana Leaves (ML) based eco enzyme as in drinking water.

MATERIALS AND METHODS

This research was carried out by the Quality Testing and Analysis Laboratory at Payakumbuh State Agricultural Polytechnic Animal Production Laboratory for 6 (six) months (March– September 2023)

Materials and tools for producing Miana leaf-based eco-enzymes

The materials used in making eco enzyme were: 1) Healthy and fresh ML, 2) Well water, 3) Molasses, while the tools used are: 1) Digital scale, 2) Measuring cups. 3) Conductor, 4) Basin, 5) Filter, 6). Blender, 7) thermometer.

Materials and tools used in the application of eco enzyme for broilers

The materials used were: a) 200 DOC non-sexing broiler, b) Eco enzyme based on ML, 3) Feed 311 produced by PT. Charoen Pokphan (From 1 day old to 7 days old, 4) Feed 511 produced by PT Charoen Pokphan (From 8 days old to 28 days old). The tools used were: 1) Cage (consisting of 20 units measuring 100 x 100 cm with a partition height of 60cm), 2) Baby chick feeder, 3) Hanging feeder, 4) Drinker with a capacity of 1 gallon, 5) Digital scale, 7) measuring cups.

Process for producing eco-enzyme based on miana leaves

Preparation of a Miana leaf based eco-enzyme by providing 10 liters of clean water in a canister and then adding 1 kg of molasses. Then stir until the molasses has dissolved in the water until a homogeneous mass is formed. 3 kg of cut and cleanly washed ML are placed in a mixture of water and molasses. In the production of eco-enzymes, a ratio of water: miana leaves: sugar = 10: 3: 1 is used (Rochyani et al., 2020). The input material must not be completely filled; an empty space is provided for the production of biogas. After mixing the ingredients, cover the canister to prevent outside air from entering. The produced eco-enzyme is stored in a place protected from sunlight. Fermentation lasts 3 months. During the first week after production, the bottle cap can be opened twice a day for a few seconds to release the resulting gas. After 3 months of storage, the liquid can be filtered from the sediment.

Cage preparation

First, the cage and equipment were prepared. Cleaning the cage, liming, adding lights, and cleaning the cage with bars are all steps needed to prepare the research cage. Each cage unit was filled with ten chicks housed in an insulated 100 x 100 cm cage. All cage units have places for food and water. Each cage up to two weeks old used shell bedding.

Placement of DOC in the cage unit

Before DOC arrives, they first supply each cage unit with sugar water. Ten chicks were randomly sampled, then each animal was weighed and the weight of the chick was recorded. The body weight of the chickens was weighed when they first arrived, this is the

initial weight data for the broiler. Initial body weight was measured for each cage unit. A total of 200 DOC broilers were randomly divided into 20 experimental units, each unit consisted of 10 DOC broilers, and each individual was randomly placed in each treatment cage unit.

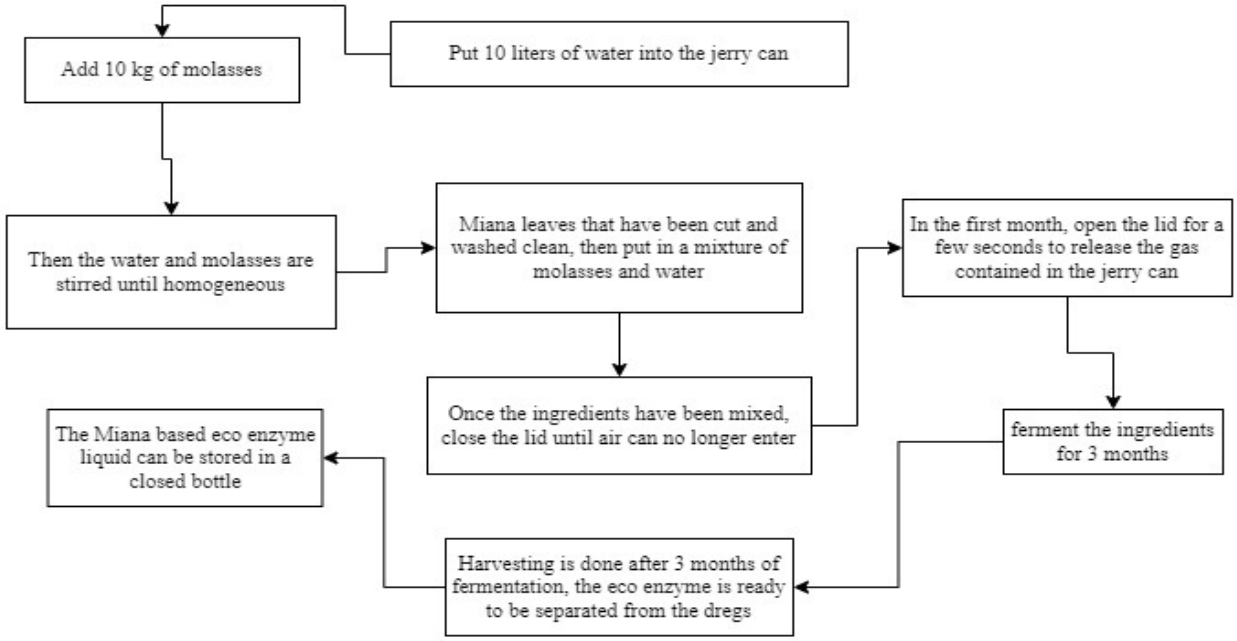

Figure 1. Chart for making eco enzyme based on miana leaves.

Application of an eco-enzyme based on ML in drinking water

The study was carried out on 200 broilers. In the first week of the research, the chicks were given drinking water without being given an eco-enzyme based on miana leaves. Administration of the ML-based eco-enzyme begins in week 2.

This research used a completely randomized design with 4 treatments and 5 replitions. The number of experimental units was 20 units, each unit consisting of 10 chickens. The dosages treatment for the administration of ML based eco-enzyme in drinking water were:

A1 = drinking water without eco enzyme based on ML.
A2 = 2% eco enzyme based on ML per liter of drinking water.
A3 = 4% eco enzyme based on ML per liter of drinking water.
A4 = 6% eco enzyme based on ML per liter of drinking water.

Data collection

Performance data collection included body weight gain, consumption ratio and feed conversion ratio, which are calculated per week. At the end of the study, body weight gain, consumption and change per day ratio were measured.

Liver weight percentage data were obtained from live weight and liver weight data. Chicken live weight and liver weight data were randomly collected from each cage unit at the end of the study, from chickens aged 28 days. Broilers were sampled sequentially, two broilers per treatment. At the end of the study, the chickens which innards had been removed were separated from the liver and then weighed to determine the weight of each organ. After the weight of each organ is determined, the weight was compared to the live weight and then multiplied by 100%.

Table 1. Eco enzyme phytochemical content based on miana leaves

Content	Eco enzyme based on miana *	ML powder **	ML liquid extract***
Total fenol	182.56 ppm	90.80 ppm	6360.00 ppm
Flavonoid	49.84 ppm		437.20 ppm
Anti oksidan (IC50)	1924.88 ppm	282.02 ppm	816.03 ppm
Saponin	Negative	Negative	Positive
Triterpenoid	Positive	Positive	Positive
Protease activity	45.76 µ/ml		

Ket : * Result analysis Laboratorium uji mutu dan analisis Politeknik Pertanian Negeri Payakumbuh (2023)
** Result analysis Laboratorium uji mutu dan analisis Pertanian Negeri Payakumbuh (2019)
*** Result analysis Laboratorium uji mutu dan analisis Politeknik Pertanian Negeri Payakumbuh (2019).

Observed Variables

Daily feed consumption

It is calculated using the method (Ojediran et al., 2017), namely the total amount of feed provided (g) minus the total remaining feed (a) divided by 28 days (g/head/day).

Daily body weight gain

It is calculated using the method (Ojediran et al., 2017), namely the average body weight of broiler chickens (g/head) at the end of the study minus the initial body weight (g/head) is divided by the length of maintenance for a period of 28 days (g/head /day).

Feed conversion

Feed conversion was calculated according to the method of (Ojediran et al., 2017), namely feed consumption (g/head/day) divided by daily body weight gain (g/head/day).

Liver weight percentage

The calculation of liver weight percentage was conducted using the method of (Kurniawan et al., 2021), namely weighing the liver weight (g) compared to the live weight (g) multiplied by 100%.

Data Analysis

The data obtained in this study was analyzed using analysis of variance (ANOVA) based on a unidirectional completely randomized design (CRD) to determine the effect on the measured variables. If significantly different results are obtained, then proceed with the Duncan Multiple Range Test (DMRT) to determine the differences among treatments (Steel & Torrie., 1993) .

RESULTS AND DISCUSSION

The results of miana leaf (ML) based eco enzyme addition to drinking water on average daily gain (ADG), feed consumption and feed conversion are presented in Table 2.

Table 2. Average body weight gain, feed consumption and feed conversion of4 weeks chicken

Treatment	Body Weight Gain (g/head/day)	Feed Consumption (g/head/day)	Feed Conversion	Liver Weight (%)
A (0%)	52.14 ± 1.66^{b}	67.54 ± 1.23	1.29 ± 0.05	2.08 ± 0.08
B (2%)	51.69 ± 2.09^{b}	70.87 ± 2,05	1.37 ± 0.07	2.10 ± 0.14
C (4%)	52.14 ± 1.26^{b}	69.80 ± 2.13	1.34 ± 0.06	1.99 ± 0.09
D (6%)	54.73 ± 0.85^{a}	70.22 ± 1.60	1.28 ± 0.04	1.92 ± 0.07

Note: Superscript in the same column are significantly different (P<0.05).

Weight Gain

Based on the analysis of variance presented in Table 2, it was found that the supplementation of ML-based eco enzyme significantly(P<0.05) affected BWG of broilers. Average daily gain body weight gain from A to D was A (52.14 ± 1.66 g/head), B (51.69 ± 2.09 g/head), C (52.14 ± 1.26 g/head), D (54.73 ± 0.85

g/head). The highest daily weight gain was treatment D. The supplementation of ML-based eco enzyme significantly (P<0.05) affected the daily body weight gain of broilers. The addition of ML-based eco enzyme significantly (P<0.05) increased daily body weight compared to without supplementation ML-based eco enzyme. The supplementation of 6% ML-based eco enzyme in water showed the highest increasement in daily body weight compared to other treatments (Table 2). Duncan's test results showed that daily body weight gain containing 6% eco enzyme ML in drinking water was higher compared to other treatments. Treatment A (0% eco enzyme based on ML was not significantly different (P>0.05) from treatment B (2% eco enzyme based on ML), treatment C (4% eco enzyme based on ML), while there was a significant difference (P<0.05) for treatment D (6% eco enzyme based on ML).

The addition of 6% eco enzyme based on miana leaves (ML) in drinking water can increase broiler body weight the highest compared to other treatments. The addition of 6% eco enzyme based on miana leaves in drinking water significantly (P<0.05) increased broiler body weight. The addition of ML-based eco enzyme has a positive effect on broiler daily weight gain. The results of research by (Fati et al., 2019), the addition of 4% ML flour in the ration can still be tolerated in the ration for weight gain broilers. The research results of (Fati et al., 2020) found that the addition of 0.125% ML water extract to drinking water can increase body weight. Meanwhile, research results from (Mahata et al., 2021) showed that the supplementation of ML flour up to 12.5% in the ration could increase the weight gain of broilers. Previous research results found that the supplementation of ML in different forms could increase the weight gain of broilers in line with the results of this study by adding ML-based eco enzyme to drinking water.

The increase in body weight added by miana leaf-based eco enzyme is thought to be due to the active compounds contained, namely triterpenoids, flavonoids, phenols, tannins, lignin and flavonoids (Table 1) which act as antibacterials which can cause bacterial cell growth to be inhibited, this was caused by surface tension. cell walls decrease, resulting in cell leakage. This active substance also inhibits the formation of bacterial cell wall polypeptides, so that they are not completely formed so that bacterial cell walls are easily lysed, this is the function of the tannin compound. Flavonoids function to inhibit the DNA replication process and inhibit cell membrane function. The function of bacterial cell membranes results in weaknesses and advantages (Mufti et al., 2017; Anita et al., 2019). Furthermore it was found that the administration of ML extract can inhibit the growth of E. coli so that broilers growth increased (Mpila et al., 2012). Nannapaneni et al., (2008), stated that medicinal plants have bioactive compounds which act as feed additives and function as growth promoters that can influence broiler growth. The feed additive's mechanism of action is to kill pathogenic microorganisms in the digestive tract. This feed additive can kill microorganisms and crusts that stick to the intestines, as a result, the walls of the small intestine become thinner, which increases the absorption of food substances.

The research found that ML-based eco enzyme contained a total of 182.56 ppm of phenol, IC50 of 1924.88 ppm, flavonoid content of 49.84 ppm, protease activity of 45.76 µ/ml and triterpenoids were positive. With the active substance contained in the eco enzyme based on ML, the condition of the chickens is healthier and there is an increase in body weight compared to those without the supplementation of eco enzyme based on ML.

Feed Consumption Ratio

Based on the results of the analysis of variance presented in Table 2, it is known that the addition of ML-based eco enzyme had no significant effect (P>0.05) on broiler consumption ratio per day. The average daily feed consumption per head from treatment A to D was A (67.54 ± 1.23 g/head), B (70.87 ± 2.05 g/head), C (69.80 ± 2, 13 g/head), D (70.22 ± 1.60 g/head). Numerical consumption ratio was higher for those given miana leaf (ML) based eco enzyme compared to those not given miana leaf (ML) based eco enzyme. Eco enzyme contains three enzymes, namely amylase, lipase and protease (Rochyani *et al*., 2020; Vama & Cherekar, 2020; (Rasit & Chee Kuan, 2018), where the amylase enzyme functions to break down carbohydrates, the protease enzyme

functions to break down proteins and the cellulase enzyme functions is to break down fiber. The enzymes produced from eco enzyme can help break down substances that are difficult to digest in the ration, such as fiber so that nutrients become more easily available to broilers. Giving eco enzymeencourages broilers to consume more rations than those not given eco enzymes, thus speeding up the process of digestion of feed substances, which has an impact on increasing broiler body weight.

This study also showed that chicken consumption ratio with the addition of Eco enzyme based on ML was higher than those without eco enzyme. This is also suspected to be the role of the active substance of ML contained in the eco enzyme, which also contains active substances. The results of the ML-based eco enzyme test from the Payakumbuh State Agricultural Polytechnic quality laboratory test (2023) showed that total phenols were 182.56 ppm, flavonoids 49.84 ppm, anti-oxidants (IC50) 1924.88 ppm, positive triterpenoids. According to Nugroho (2010) and (Mpila et al., 2012), active substances such as flavonoids, saponins, tannins, essential oils, eugenol, polyphenolic compounds, alkaloids, ethyl salicylate, calcium oxalate, rosmarinic acid compounds are active compounds as antimicrobials capable of kills pathogenic microorganisms such as Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa. The condition of the digestive tract will be healthy, so that the process of digestion and absorption of food substances will be optimal. Besides that, this active compound can improve digestion and absorption of food substances better than other treatments. The consumption ratio obtained from this study was in line with (Malvin et al., 2021), that the addition of fermented ML extract to drinking water can increase consumption ratio compared to the control. Meanwhile, the results of (Mahata et al., 2021), showed that the addition of miana leaf flour in the ratio was not significantly different from without the addition of miana leaf flour, the higher consumption ratio compared to the control was the addition of 5% miana leaf flour .

Feed Conversion

Table 2 shown the results obtained from the feed conversion of broiler rations by supplementation of ML-based eco enzyme per treatment 0%, 2%, 4% and 6% in drinking water during 28 days.. Based on Table 2, the average feed conversion from high to low was B (1.37 ± 0.07), C (1.34 ± 0.06), A (1.29 ± 0.05) and D (1.28 ± 0.04).

Based on the results of variance analysis, it was found that the addition of miana leaf (ML) based eco enzyme had no significant effect ($P > 0.05$) on conversion ratio. The results showed that the addition of miana leaf (ML) based eco enzyme had no significant effect ($P>0.05$) on conversion ratio. This means that adding miana leaf (ML) based eco enzyme to drinking water does not negatively affect on the ratio.

Feed conversion was numerically lower for those given miana leaf (ML) based eco enzyme at a dose of 6% compared to those not given miana leaf (ML) based eco enzyme. The addition of 6% miana leaf (ML) based eco enzyme in water showed lower daily conversion ratio than other treatments (Table 2). Eco enzyme contains the enzymes protease, lipase and amylase (Vama & Cherekar, 2020; Rasit & Chee Kuan, 2018), which are digetive enzymes that can help break down complex nutrients in rations into simpler forms, making them easier for broilers to digest. This can increase nutrient absorption and reduce undigested nutrient losses. Due to increased digestion and absorption of nutrients, broilers can grow faster and reach the desired harvest weight in a shorter time. Miana leaf-based eco enzyme, besides containing enzymes which are very important in helping the digestive process, also contains active substances such as flavonoids, total phenols and anti-oxidants. Flavonoids have been reported to increase feed efficiency and growth (Miah et al., 2004; Magdalena et al., 2014). The active substances such as flavonoids, tannins, and saponins may cause damage to the bacterial cell membrane. This causes the bacterial cells to swell, lose stability, and ultimately lyse the bacterial cells. This process can also result in the release of various bacterial cell components, including proteins, nucleic acids, amino acids, and nucleotides, which can affect bacterial cell function and ultimately cause bacterial cell death. Thus bacterial cells cannot grow and develop (Ismarani, 2012; (Maftuhah et al., 2015). The results of research from (Basir et al., 2020),

show that ML can be used as a natural antibiotic and have antibacterial activity equivalent to the antibiotic oxytetracycline. The addition of ML-based eco enzyme will inhibit the development of E.coli bacteria and pathogenic bacteria so that it can speed up nutrient digestionand increase nutrient absorption which has an impact on increasing body weight and increasing feed efficiency.

The conversion ratio produced in this study was 1.45 + 0.05 to 1.53 + 0.02. The results of research (Ulupi & Inayah, 2015), the addition of feed additive in the form of betel powder using commercial rations was 1.72 + 0.13 to 1.74 + 0.11 with a research duration of 5 weeks, while the results of research (Sukmaningsih & Rahardjo, 2019) with the addition of a mixture of probiotics and herbs resulted in a conversion ratio of 1.47 to 1.6. The differences in the resulting feed conversion were related to differences in the form and method of adding feed additives to the broiler.

Liver Weight Percentage

The results obtained from the percentage of broiler liver weight by administering miana leaf (ML) based eco enzyme doses per treatment sequentially 0%, 2%, 4% and 6% in drinking water during 28 day maintenance can be seen in Table 3. Based on Table 3, the average -The average percentage of broiler liver weight from lowest to highest was D (1.92 ± 0.07), C (1.99 ± 0.09), A (2.08 ± 0.08) and B (2.10 ± 0.14).

Based on the results of analysis of variance, it was found that the addition of ML-based eco enzyme had no significant effect ($P > 0.05$) on the percentage of broiler liver weight. The results of this research showed that the addition of ML-based eco enzyme did not affect the percentage of broiler liver weight. This means that the addition of ML-based eco enzyme does not have a negative effect on the percentage of broiler liver organs, because one of the functions of the liver is to detoxify toxic substances that enter the body, either through food, drink or metabolic processes, which are converted into easier forms. Excreted, if continue to consume toxic substances, the liver will become damaged and the liver will enlarge from its normal size and the color of the liver will also change to yellow (McLelland, 1990 cit. (Suyanto et al., 2013), whereas from the results of this study the size of the liver is still in normal size, namely 1.98 – 2.10% of live weight. According to (Putnam, 1991), the percentage of liver weight ranges from 1.7 – 2.8% of live weight. The results of research (Fati et al., 2018) which added *bangun-bangun* leaf extract to the ration showed that the percentage of liver weight was 2.5 - 2.77% of live weight, while the results of research (Simanjuntak & Robinson, 2021) obtained the percentage of liver weight is 1.85 – 2.49% with the addition of black vegetable flour. Researchers stated that by increasing the percentage of black vegetable flour, the percentage of liver weight also increases. However, it can still be tolerated by the chicken's body so that liver swelling does not occur. Meanwhile, the results of this research showed that giving up to 6% of the ration resulted in a reduction in the percentage of liver weight but it was still within normal liver weight limits, meaning that the supplementation of ML-based eco enzyme did not produce dangerous toxins and could still be tolerated by the chicken's body so that the liver did not swell.

CONCLUSION

The results of the research showed that adding 6% ML-based eco enzyme to drinking water increases body weight. The addition of miana leaf-based eco enzyme could be tolerated up to 6% in drinking water based on consumptionand conversion ratio, alsobroiler liver weight percentage. There is a need for further research on the addition of ML-based eco enzyme in terms of frequency of supplementation during maintenance so that optimal use can be found in broiler drinking water.

CONFLICT OF INTEREST

The authors state that they have noconflict of interest regarding the material discussed in this publication.

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