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Effects of applying combination extracts of cherry (*Muntingia calabura* L.) leaves and soursop (*Annona muricata* L.) leaves as antioxidants on the digestibility of dry matter, organic matter, and crude protein in broiler chickens

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Abstract. Broiler chickens are one of the livestock from poultry species that are susceptible to heat stress due to high ambient temperatures and will trigger oxidative stress that affects their productivity. Efforts made to overcome heat stress in broilers are the provision of antioxidants contained in cherry leaves and soursop leaves by suppressing excess oxidants. The primary objective of this study was to evaluate the impact of antioxidants added to drinking water, specifically cherry leaves extract (CLE) and soursop leaves extract (SLE), on the digestibility of dry matter, organic matter, and crude protein in broiler chickens. This study used complete randomised design with treatment P0 (control), P1 (9% CLE), P2 (9% SLE), P3 (6% CLE + 3% SLE) and P4 (3% CLE + 6% SLE). Data on feed digestibility was collected using the total excreta collection method for broiler chickens. The P3 treatment delivered the greatest results in terms of dry matter digestibility (84.77%), organic matter digestibility (92.99%), and crude protein digestibility (91.68%). The conclusion of this study is the addition of a combination of 6% CLE and 3% SLE in drinking water gives a good effect on feed digestibility in broiler chickens.

1. Introduction

In Indonesia, broiler farming is one of the largest contributors to the provision of animal protein. Broiler chicken is a type of poultry that grows very fast and can be harvested within 5 weeks. However, the superiority of its genes needs to be supported by optimal environmental conditions. Broiler chickens are classified as animals that find it difficult to dissipate their body heat into the environment so that they are easily exposed to heat stress which can result in lower productive performance [1]. Oxidative stress is part of the broiler stress response that can be triggered by inappropriate environmental temperatures that can result in metabolic disorders that result in stunted broiler growth [2]. Oxidative stress plays a crucial role in the stress response of broilers, often triggered by exposure to suboptimal environmental temperatures. These temperature extremes, whether too high or too low, can disrupt the broiler's physiological balance, leading to metabolic disorders. Such metabolic imbalances, in turn, compromise the broilers' growth, contributing to stunted development. The underlying mechanisms involve an overproduction of reactive oxygen species (ROS) that overwhelm the broiler's antioxidant defence system, exacerbating tissue damage, impairing nutrient absorption, and reducing overall productivity.



This chain of events highlights the importance of maintaining optimal environmental conditions to prevent oxidative stress and ensure healthy broiler growth.

Providing antioxidants is a method to prevent oxidative stress caused by free radicals influenced by high environmental temperatures. Antioxidants are compounds that can prevent excessive oxidation due to free radical attack by neutralising them. The use of natural ingredients that contain antioxidant compounds when combined and used with the right concentration will produce better potential antioxidant activity, due to the effect of the combination of ingredients that are able to complement each other. Natural antioxidants can produce higher potential antioxidant activity when made in combination [3]. Some of them are cherry and soursop plants. Cherry and soursop are fruiting plants that have high antioxidant content, especially in the leaves. Cherry leaves contain terpenoids, saponins, flavonoids and polyphenols, while soursop leaves contain saponins, flavonoids, alkaloids and terpenoids. These active compounds have antioxidant activity that can capture free radicals. Digestibility in livestock can describe the part that has been absorbed by livestock [4]. Digestibility shows the high and low utilisation of feed consumed by livestock. Livestock species, environmental temperature, feed composition, physical form of feed, feeding, and age of livestock affect digestibility. Water additives that are intentionally added to drinking water can affect antioxidant activity that can increase livestock productivity and can reduce microbial populations in the digestive organs of livestock and can increase the digestibility of feed ingredients [5]. The use of cherry leaves and soursop leaves in extract form is still rarely found, especially with the many benefits of the two types of leaves that can be used as additives, especially the content of compounds that have antioxidant properties. The antioxidant compounds found in both types of leaves are expected to prevent high heat stress which reduces digestibility in the broiler chicken digestive system.

2. Materials and methods

2.1. Research materials

The following materials required for the study: 125 day-old chicks (DOC), commercial feed, distilled water and 70% ethanol as extract solvents, cherry and soursop leaves as raw materials for extracts, and boric acid (H_3BO_3) as a nitrogen binder during excreta collection processes [5].

The tools used were experimental cages that had a size of 50 cm × 100 cm × 100 cm as many as 25 cage plots, measuring cups, measuring flasks, erlenmeyers, mortars, cups, filter paper, analytical scales, pasteur pipettes, ovens, furnaces, digesters, vapodest, markers, plastic samples, tissues, thermometers, cutting boards, tarpaulins and excreta collection containers.

2.2. Research method

This study used a completely randomised design (CRD) consisting of 5 treatments and 5 replicates. Each replicate consisted of 5 broiler chickens. The treatments that will be studied are as follows:

P0 = Drinking water without giving extract (Control)

P1 = Drinking water with added 9% Cherry Leaf Extract

P2 = Drinking water with added 9% Soursop Leaf Extract

P3 = Drinking water with added combination 6% Cherry Leaf Extract + 3% Soursop Leaf Extract

P4 = Drinking water with added combination 3% Cherry Leaf Extract + 6% Soursop Leaf Extract

2.3. Preparation of cherry leaf and soursop leaf extracts

The washed cherry leaves and soursop leaves were dried and made into simplisia powder. The simplisia was then soaked with 70% ethanol in a closed soaking container for 5 days and stirred every day. After the soaking process is complete, the extract is filtered, the dregs of the remaining soaking are washed with 70% ethanol and then filtered again. Next, the macerate was evaporated at 40°C using a rotary evaporator and then concentrated with a freeze dryer until a thick extract was obtained, then diluted using distilled water.

2.4. Research implementation and data collection

Cherry leaf and soursop leaf extracts were administered as antioxidants to chickens aged 15-35 days. The extracts were added to drinking water. The amount of drinking water given during treatment was 500 ml/plot with 45 ml/plot of extract. After the treatment extract runs out, drinking water is provided ad libitum.

Digestibility data were collected using the total collection method. Excreta collection begins with the chicken not being fed within 24 hours but still given drinking water ad libitum. The total collection method is done by collecting excreta in a container. Excreta collection was done within 3 days and during the collection period, H₃BO₃ (boric acid) was sprayed to capture excreta nitrogen. The collected excreta were dried and ground and analysed for dry matter digestibility, organic matter digestibility and crude protein digestibility and then calculated using the formula.

$$\text{Dry matter digestibility (\%)} = \frac{\text{Dry matter consumption} - \text{Excreta dry matter}}{\text{Dry matter consumption}} \times 100\% \quad (1)$$

$$\text{Organic matter digestibility (\%)} = \frac{\text{Organic matter consumption} - \text{Organic dry matter}}{\text{Organic matter consumption}} \times 100\% \quad (2)$$

$$\text{Crude Protein digestibility (\%)} = \frac{\text{Crude protein consumption} - \text{Crude protein matter}}{\text{Crude protein consumption}} \times 100\% \quad (3)$$

2.5. Ethical statement

The animal use protocol was reviewed and approved by Animal Research Ethics Committees/AREC, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara (Ref: 0855/KEPH-FMIPA/2025). This approval confirms that the proposed research involving the use of animals has been thoroughly reviewed and meets the ethical standards required for animal welfare, scientific integrity, and compliance with institutional as well as national guidelines. The committee's decision ensures that the study will be conducted with proper ethical considerations, including minimizing animal distress, applying humane treatment, and justifying the scientific value of the work in relation to its potential benefits.

3. Results and discussion

Antioxidant features of cherry leaf and soursop leaf extracts in drinking water showed an increase in the amounts of digestibility of dry matter, organic matter, and crude protein of broiler chickens. The average results of the study can be seen in table 1.

Table 1. Average dry matter, organic matter and crude protein digestibility (%)

Treatment	Dry Matter Digestibility	Organic Matter Digestibility	Crude Protein Digestibility
P0	82.37 ^b ± 0.46	91.74 ^b ± 0.09	88.59 ^d ± 0.53
P1	82.97 ^b ± 1.52	92.52 ^{ab} ± 0.99	90.57 ^{cd} ± 0.98
P2	82.49 ^b ± 1.75	91.86 ^b ± 0.69	89.60 ^{bc} ± 0.84
P3	84.77 ^a ± 1.11	92.99 ^a ± 0.58	93.03 ^a ± 1.29
P4	83.93 ^{ab} ± 0.67	92.72 ^a ± 0.21	91.68 ^b ± 0.33

Notes: Different *superscripts* indicate significant difference (P<0.05)

3.1 Dry matter digestibility

Dry matter digestibility refers to the proportion of feed dry matter that is digested and absorbed by the animal, rather than excreted as waste. This metric is closely tied to the amount of dry matter consumed in the feed and the corresponding dry matter found in the excreta. A higher digestibility reflects a more efficient breakdown and absorption of nutrients, leaving less undigested material to be excreted. This balance between intake and excretion is crucial for evaluating the nutritional efficiency of a given feed.

The dry matter in the feed used was 86%. The results in table 1 show the average dry matter digestibility of each treatment is 82.37% (P0), 82.97% (P1), 82.49% (P2), 84.77% (P3), 83.93% (P4). Based on these averages, it is known that the digestibility of dry matter in all treatments has increased when compared to the control. P3 had the highest average dry matter digestibility (84.77%), whereas P1 had the lowest (82.97%) average. The results of analysis of variance explained that the use of cherry leaf extract and soursop leaves as antioxidants had a significant effect ($P < 0.05$) on the digestibility of broiler dry matter.

Duncan's further test showed that dry matter digestibility in P3 and P4 treatments was significantly different compared to P0, P1, and P2, but P3 and P4 treatments as well as P0, P1, and P2 did not have significant differences. Thus, these results indicate that the use of cherry leaf and soursop leaf extracts in combination gives almost the same impact as the use of single extracts of both types of leaves. At the same treatment level, P0, P1 and P3, as well as between P3 and P4, showed similar effects on broiler dry matter digestibility. These findings reflect that the use of 9% cherry leaf and soursop leaf extracts as antioxidants can have a good effect on broiler dry matter digestibility, especially when used in combination with a ratio of 6% cherry leaf extract and 3% soursop leaf extract (P3), which resulted in the highest level of dry matter digestibility.

The increase in dry matter digestibility in this study is supported by research on a 0.50% diet system in the form of a powder mixture of medicinal plant leaves (*Anacardium occidentale* 60%, *Psidium guajava* 20% and *Morinda citrifolia* 20%) which shows changes in dry matter digestibility and dry matter digestibility in the small intestine in broilers [6]. Natural additives from plants help the growth process to be better, free from free radicals and multiply important nutrients in the intestine, in this case extracts of cherry leaves and soursop leaves as antioxidants play a role in increasing the digestibility of dry matter from broilers. Although the extract levels in all treatments (P0, P1, P2, P3 and P4) were equal at 9%, the P3 and P4 treatments consisting of combined extracts resulted in the highest increase in dry matter digestibility. The combination of cherry leaves and soursop leaves can produce a synergistic effect, the increase in antioxidant activity of each ingredient is due to the synergistic effect [7].

Heat stress in the cage environment can affect the digestibility of chickens. The size of the duodenal villi can decrease due to the attack of free radicals derived from heat stress [8]. P3 produces the highest average dry matter digestibility because the combination of cherry leaf and soursop leaf extracts in P3 contains antioxidants such as flavonoids that are more than other treatments. The 2:1 ratio of extracts increases the effectiveness of active compounds that work as antioxidants in the extracts [9]. The content of flavonoid compounds in the extract increases the duodenal villi of broilers which results in a wider surface area for nutrient absorption and more optimal nutrient absorption. Better nutrient absorption results in increased digestibility, including dry matter digestibility [10]. The use of cherry leaf extract and 9% soursop leaves, especially in combination, resulted in more optimal dry matter digestibility compared to research using 2-8% cherry leaf flour [11] with an average dry matter digestibility of 70-79%.

3.2 Digestibility of organic matter

Organic matter digestibility reflects the extent to which the organic matter content of feed can be digested by livestock. The digestibility of organic matter includes the feed components' digestion, including vitamins, proteins, carbs, and lipids. Organic matter digestibility is expressed as the ratio between the total organic matter consumed and the amount of organic matter excreted, divided by the total organic matter that has been consumed. The organic matter of the feed consumed was 92%. Table 1 displays the average digestibility of organic matter from this study's results.

The results showed the average organic matter digestibility of each treatment was 91.71% (P0), 92.52% (P1), 91.86% (P2), 92.99% (P3), 92.72% (P4). The average value of organic matter digestibility was highest in P3, which was 92.99%, while the lowest was in P2 at 91.86%. The average digestibility of organic matter from this study showed an increase compared to the control (P0). The application of cherry leaf and soursop leaf extracts as antioxidants significantly ($P < 0.05$) improved the broiler chickens' ability to digest organic materials, according to an analysis of variance.

Duncan's further test in this study indicated a significant difference between the mean digestibility of organic matter in P3 and P4 compared with P0 and P2. Between P3, P4 and P1 there were no significant differences, as well as between P0, P2, and P1 were not significantly different. The digestibility of organic matter is in line with the digestibility of dry matter, in accordance with the concept of increasing dry matter will affect the increase in organic matter, and vice versa [12]. The same principle additionally remains regarding dry matter digestibility; as dry matter digestibility rises, so will organic matter digestibility. As organic matter comprises almost all of dry matter's components and is a component of both, variables impacting dry matter's high and low digestibility will likewise affect organic matter's high and low digestibility [13]. The P3 treatment demonstrated the highest average organic matter digestibility, primarily due to the optimal combination of cherry leaf and soursop leaf extracts in a 2:1 ratio. This specific formulation was particularly effective in producing a potent blend of antioxidant compounds, with flavonoids being the most prominent. Flavonoids are known for their capacity to improve digestive health by enhancing nutrient absorption and protecting the gastrointestinal tract from oxidative damage. The synergy between the cherry and soursop leaf extracts likely maximized the bioavailability and efficacy of these bioactive compounds, promoting a more efficient breakdown and utilization of organic matter. As a result, the P3 treatment not only optimized antioxidant activity but also contributed to improved feed efficiency and overall metabolic performance in the test subjects.

Flavonoid compounds act to contain free radicals in intestinal cells, thus protecting the lipid layer and preventing damage to intestinal cells [8]. This mechanism allows the intestines to function efficiently and optimally in absorbing nutrients, which in turn has a positive impact on enhancing organic matter digestibility. The proper functioning of the intestinal tract is crucial for maximizing the breakdown and assimilation of nutrients from the feed, contributing to improved overall metabolic health. Additionally, maintaining a healthy excretory tract supports the effective utilization of these feed nutrients by facilitating the smooth elimination of waste products. This dual role promoting nutrient absorption while ensuring waste elimination further increases the digestibility value of organic matter, ultimately optimizing feed efficiency and contributing to better animal growth and performance. Phenol compounds such as flavonoids have the ability to break down proteins and act as antibacterials, thereby increasing non-pathogenic bacteria and reducing organic matter in excreta [14]. The lower the excreted organic matter, the higher the digestibility of organic matter. A decrease in the amount of excreted organic matter is directly associated with an improvement in the digestibility of organic matter. This relationship indicates that a greater proportion of the organic matter present in the feed is being efficiently broken down and absorbed by the digestive system, rather than being excreted as waste. Higher digestibility reflects an enhanced ability of the animal to utilize the available nutrients in the feed, leading to improved metabolic processes and overall feed efficiency. This reduction in excreted organic matter not only signifies better nutrient retention but also contributes to reduced environmental waste from livestock production, making it a key factor in sustainable agricultural practices.

3.3 Crude protein digestibility

The protein content in the feed plays a crucial role in determining the digestibility of crude protein, as it directly influences the extent to which protein can be broken down and absorbed by the digestive system. A higher protein concentration in the feed provides more substrate for enzymatic action, potentially improving the efficiency of protein digestion. Conversely, suboptimal protein levels may limit the availability of essential amino acids and reduce the overall digestibility of crude protein. Therefore, ensuring an appropriate balance of protein in the diet is essential for optimizing protein digestibility, which directly affects nutrient utilization, animal growth, and overall production efficiency. Protein digestibility is the part of food substances that have been adsorbed by livestock [15]. The feed utilised contains a protein amount that is 21.5%. For every treatment in this investigation, the average crude protein digestibility was 88.59% (P0), 90.57% (P1), 89.60% (P2), 93.03% (P3), and 91.68% (P4) (table 1). The average crude protein digestibility of this study increased compared to the control. In order, the highest average crude protein digestibility was P3, P4, P1 and P2. The highest crude protein digestibility was found in P3 which was 91.68% while the lowest was found in P0 (control) which was

88.59%. The results of the analysis of variance showed the use of cherry leaf extract and soursop leaves in broiler chickens gave a significant effect ($P < 0.05$) on the digestibility of crude protein of broiler chickens. The digestibility of crude protein P3 with P4, P1, P2, and P0 varied significantly, according to the findings of Duncan's further test in this study. There were no significant differences between P4 and P1, but there were between P4 and P2 and P0 and P1, P4, P2, and P0 and P1 and P0 were considerably different, and between P2 and P0 there were no significant differences. There was an increase in crude protein digestibility in this study, especially for the P3 treatment.

Nitrogen retention affects crude protein digestibility; better nitrogen retention levels reflect more optimal protein digestibility. Nitrogen retention reflects that unabsorbed nitrogen will be excreted. Aspects that can affect protein digestibility are age, sex, growth rate, disease, energy level, species, strain and environmental conditions [15]. Increased absorption of nutrients contained in the ration causes protein digestibility to increase. Efficient utilisation of protein by livestock can be seen through evaluation of protein digestibility and feed consumption, because the amount of feed consumption illustrates how much protein is consumed [16]. The content of antioxidants such as flavonoids contained in cherry leaf extract and soursop leaves can increase appetite thereby increasing protein digestibility and protein efficiency [17]. Flavonoids have the potential to increase crude protein digestibility due to the ability of flavonoids to stimulate the growth of chicken duodenal villi which results in a wider nutrient absorption surface [10]. As the duodenal villi grow and elongate, the efficiency of nutrient uptake from the feed improves, optimizing the digestion process. The increase in length and surface area of the intestine in poultry will optimise the absorption of consumed feed nutrients [18]. This enhanced nutrient absorption capacity ultimately leads to improved feed utilization and growth performance in poultry, highlighting the significance of flavonoids as functional bioactive compounds that can support gut health and feed efficiency in livestock production systems.

In accordance with research on the utilisation of herbs for poultry [19], the combined treatment of cherry leaves and soursop leaves with a level of 9% in this study showed the best results for crude protein digestibility [19]. Combined extracts produce more optimal antioxidant activity when compared to single extracts. This is due to the synergistic effect that arises due to the combination of extracts [9]. Crude protein digestibility can increase due to higher phenol compound content prevents the onset of heat stress. Phenol compounds can damage protein structures and disrupt the integrity of bacterial cell membranes, as a result the number of non-pathogenic bacteria can increase so that the protein excreted through excreta decreases [14]. This disruption can interfere with the normal functioning of pathogenic bacteria, reducing their populations and allowing non-pathogenic bacteria to proliferate. As a result, there is a shift in the microbial composition within the gut, which can positively influence nutrient absorption and metabolism. Consequently, this microbial balance leads to a reduction in protein excretion through the excreta, as more protein is efficiently utilized by the host organism. This phenomenon underscores the role of phenolic compounds in modulating microbial ecosystems and enhancing nutrient retention, which can have important implications for improving feed efficiency and overall animal health

4. Conclusions

Through the application of soursop (*Annona muricata* L.) and cherry (*Muntingia calabura* L.) leaf extracts as an antioxidant capable of preventing high heat stress, broiler chickens' digestibility of dry matter, organic matter, and crude protein can be increased. The use of a combination extract between cherry (*Muntingia calabura* L.) and soursop (*Annona muricata* L.) leaves at the 9% level (cherry leaf 6% + soursop leaf 3%) obtained the highest average result for the digestibility of organic matter, crude protein, and dry matter in broiler chickens.

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