

The effect of lime leaf extract (*Citrus aurantifolia*) through drinking water on the chemical and physical quality of broiler meat

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Abstract

This study aims to investigate the impact of supplementing lime leaf extract (EDJ) in broiler chickens' drinking water on both the chemical and physical qualities of their meat. A total of 128 New Lohmann MB 202 strain broiler roosters were subjected to the same basal feed regimen but received distinct drinking water treatments over a 35-day rearing period. The drinking water treatments included plain water without additives (negative control; T0), water supplemented with 50 ppm Tetracycline antibiotics (positive control; T1), water enriched with 15 ml/liter EDJ (T2), and water infused with 30 ml/liter EDJ (T3). The basal diet consisted of corn and soybean flour, with a crude protein content of 22.01% and a metabolic energy level of 3113.12 kcal/kg. Feed and drinking water are provided ad libitum during the rearing period. The parameters observed in this study were the chemical and physical quality of the meat, which included variables: moisture content, ash content, protein content, fat content, pH value, water holding capacity, cooking loss, and tenderness. The data obtained were then analyzed for variance (ANOVA) using a one-way Complete Randomized Design based on a P value of less than 5%. The findings of the study indicated that the supplementation of EDJ through drinking water did not produce significant alterations in water content, protein content, fat content, cooking loss, or tenderness of the broiler meat. However, a notable outcome was observed: the addition of 30 ml/liter of EDJ to the drinking water led to a substantial increase in water holding capacity ($P < 0.05$) while simultaneously reducing the meat's pH value ($P < 0.05$). These results suggest that EDJ supplementation has the potential to enhance the physical quality of broiler meat, primarily by increasing water retention and decreasing pH values, which can contribute to improve meat texture and juiciness.

Keywords: broiler chickens; chemical quality; lime leaf extract; physical quality

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1. Introduction

Broiler chicken meat holds a central role in global food consumption, primarily due to its nutritional richness, including a high protein content (Saputra, 2014), and its versatility in culinary applications, offering an appealing taste, aroma, smooth texture, and cost-effectiveness (Suradi, 2006). Its widespread popularity is underpinned not only by its nutritional significance but also by its economic importance within the meat industry. As the demand for poultry products continues to rise, modern broiler production systems have continuously evolved, with a specific focus on optimizing growth rates and meat quality.

A critical aspect of this evolution is the incorporation of feed additives, which plays a pivotal role in enhancing broiler health, fostering growth performance, and improving overall meat quality. Additional feed additives encompass ingredients added to livestock feed to elicit specific effects on livestock, distinct nutrient content, and include substances like phytobiotics, antibiotics, probiotics, prebiotics, enzymes, and organic acids. These

additives yield substantial benefits, including heightened feed efficiency, accelerated growth performance, and improvements in animal health and microbial food safety in poultry (Broderick et al., 2020).

However, concerns about the emergence of antibiotic-resistant bacteria and the presence of antibiotic residues in meat products have prompted a significant shift in the poultry industry. To address these concerns, there is a growing trend towards using natural additives known as phytobiotics. These plant-based feed additives are valued for their active compounds that enhance the health and growth of livestock. Phytobiotics can come from various parts of plants, such as leaves, stems, flowers, and roots, and can be derived from whole plants or specific plant materials (Grashorn, 2010). These herbal extracts can stimulate appetite, enhance enzyme production, and possess antimicrobial, coccidiostat, or anthelmintic properties in monogastric animals (Ferdous et al., 2019).

Among these phytobiotics, lime leaf extract (*Citrus aurantifolia*) has garnered attention as a promising candidate for enhancing broiler meat quality. Lime plants, native to tropical and subtropical regions in Asia, have gained global recognition for their phytochemical richness, including flavonoids with antioxidant and antimicrobial properties (Seleem et al., 2016). These antioxidants found in lime leaves effectively combat free radicals in living organisms (Fajarwati, 2015). This has led to the exploration of lime leaf extract as a natural alternative to enhance broiler meat quality.

The addition of lime leaves as a phytobiotic in broiler chicken feed has a potential to influence both the chemical and physical quality of the meat. Lime leaves contain antioxidants that can prevent fat oxidation by breaking the chain of oxidation (Fassah et al., 2012). Additionally, lime contains citric acid, sulfur, and phosphorus, which could influence the meat's pH. Therefore, research is needed to understand the impact of lime leaf extract on the chemical and physical quality of broiler chicken meat.

In Indonesia, broiler chicken meat is popular due to its affordability and high demand (Badan Pusat Statistik, 2022). Broiler populations continue to increase, supported by fast growth and proper management, including the use of feed additives. Historically, antibiotics were used as growth promoters but were banned due to residue concerns (Wiyana et al., 1999). Consequently, the search for natural alternatives to antibiotics is crucial.

This research aims to investigate the effects of lime leaf extract (*Citrus aurantifolia*) administered through drinking water on the chemical and physical quality of broiler meat. By examining these parameters, we can better understand the potential of lime leaf extract as a phytobiotic in enhancing broiler meat quality, offering a natural alternative to traditional antibiotics.

2. Methods

2.1. Time and Location

This research was conducted at the Faculty of Animal Husbandry, Gadjah Mada University (UGM) Yogyakarta, from December 1, 2020, to January 30, 2021. Lime leaf extract production took place at the Animal Feed Science Laboratory, Faculty of Animal Husbandry, UGM. Broiler chicken maintenance was carried out in a closed house at PT. Japfa Comfeed Indonesia, located at the UGM Faculty of Animal Husbandry. Physical content testing of broiler chicken meat was conducted at the Laboratory of Meat Science and Technology, Faculty of Animal Husbandry, UGM. Chemical content testing of chicken meat was performed at the Animal Feed Science Laboratory, Faculty of Animal Husbandry, Gadjah Mada University, Yogyakarta.

2.2. Experimental Set up and Sample

This study involved 128 DOC (Day-Old Chick) male broiler chickens of the Lohman MB-202 Platinum strain. The chickens were housed in a closed cage system with 16 cages, each measuring (1x1) m² with steel screens. The litter in the cages consisted of dry rice husk, replenished weekly. Each cage was equipped with a drinker, feeder, and lamp.

The diet used in this study (Table 1) was a basal ration formulated to meet the dietary requirements of broiler chickens, following the formulation guidelines from (Hartadi et al., 2005). The feeding process was ad libitum, and the ration was primarily corn-based with a protein content of 4.79%, supplemented with soybean flour with a protein content of 14.18%.

Table 1. Composition and nutrient content of the basal ration

Material	Ingredients (%)	Nutrient and Energy Content								
		PK (%)	ME (kcal/kg)	Crude Fat (%)	Coarse Fiber (%)	Ca (%)	P (%)	Lys (%)	Met (%)	Thr (%)
Corn	55.00	4.79	1826.55	2.20	1.21	0.01	0.13	0.16	0.10	0.20
Soybean meal	30.30	14.18	671.45	0.33	1.33	0.09	0.18	0.78	0.15	0.49
Bone meal	6.00	2.80	115.38	0.50	0.06	0.57	0.28	0.16	0.04	0.09
Rice Bran	2.00	0.24	57.74	0.21	0.10	-	0.03	0.01	-	0.01
Palm oil	5.00	-	442.00	5.00	0.85	0.01	0.02	-	0.01	-
Premix Mineral-Vitamin ¹	0.25	-	-	-	-	0.09	-	-	-	-
L-Lysine HCl	0.10	-	-	-	-	-	-	0.08	-	-
DL-Methionine	0.15	-	-	-	-	-	-	-	0.15	-
Limestone	1.00	-	-	-	-	0.34	-	-	-	-
Salt	0.25	-	-	-	-	-	-	-	-	-
Total	100.00	22.01	3113.12	8.24	3.55	1.10	0.64	1.18	0.45	0.78

*PK = crude protein, ME = mobilized energy, Ca = Calcium, P = Phosphor, Lys = Lysine, Met = methionine, Thr = threonine. ¹Premiks mineral-vitamin contains: Ca = 32.5%; P = 1.0%; Fe = 0.6g, Mn = 4g; Iodine = 0.075g; Zn = 3.75g; Vitamin B12 = 0.5mg; Vitamin A = 300,000 IU; Vitamin D3 = 50,000 IU

2.3. Experimental and Sample Collection

Each chicken received one of four types of drinking water treatments, as follows: drinking water without additives (negative control; T0), drinking water with the addition of 50 ppm Tetracycline antibiotics (positive control; T1), drinking water with the addition of 15 ml/liter EDJ (T2), and drinking water with the addition of 30 ml/liter EDJ (T3). These treatments were assigned according to a Completely Randomized Design with a unidirectional pattern. Each treatment consisted of four replicates, each containing eight chickens.

During the starter maintenance period, broiler chickens were fed with commercial feed Broiler 1 Comfeed until they reached seven days of age. Afterward, the commercial ration was replaced with a basal ration in the form of crumbles and pellets. Weekly additions of husks were made to the coop to mitigate ammonia from chicken manure affecting the chickens' performance. At day 35, two chickens with body weights close to the median from each cage group were selected and slaughtered following Islamic Sharia guidelines, with three canals in the front of the neck. Breast meat samples were then collected for subsequent chemical and physical quality testing.

2.4. Lime Extract Preparation

Lime leaf extract was prepared by cleaning and grinding lime leaves, followed by drying at room temperature for three days. After drying, the leaves were sieved through a 1 ml filter and stored in a sealed container. Maceration was employed for extraction using 100 g of lime leaves with 500 ml of ethanol, covered with aluminum foil for three days. The solution

was filtered through filter paper and then evaporated in a water bath at 45 degrees Celsius to obtain lime leaf extract. The extract was dissolved using 1 g of lime leaf extract, 1 ml of polysorbate 80, and 50 ml of 70% ethanol, followed by dilution with distilled water to reach a final volume of 100 ml.

3. Results and Discussion

3.1. Chemical Quality of Meat

In this study, the chemical quality of chicken meat was assessed, focusing on moisture, ash, protein, and fat content, which were analyzed in vitro and presented in Table 2.

Table 2. Chemical quality of broiler chicken meat given lime leaf extract drinking water (%)

Variable	Treatment				SEM	P Value
	T0	T1	T2	T3		
Water content ^{ns}	73.27	73.98	73.99	74.13	0.38	0.504
Ash content ^{ns}	1.34	1.43	1.46	1.63	0.04	0.121
Protein levels ^{ns}	20.53	22.48	23.35	25.05	0.82	0.279
Fat level ^{ns}	1.09	1.3	1.38	1.46	0.98	0.639

*(T0; negative control) : without adding additives, (T1; positive control) : 50 ppm antibiotics Tetracycline, (T2) : 15ml/liter EDJ (T2), dan (T3) : 30ml/liter EDJ. ^{ns}: non significant

3.1.1. Moisture Content

Statistical analysis of the data regarding the water content of chicken meat (Table 2) revealed that the addition of lime leaf extract to drinking water had no significant impact on the moisture content of the meat, with values ranging from 73.27% to 74.14%. These values are consistent with normal ranges found in similar studies by (Attia et al., 2011), indicating that the moisture content of broiler chicken meat typically falls between 73.9% - 75.4% when non-antibiotic feed additives are used. The absence of an effect on moisture content aligns with findings from (Lan et al., 2020), who reported that additional feed ingredients did not influence meat moisture content.

The lack of a significant difference in water content can be attributed to the inability of lime leaf extract to enhance nutrient digestibility, particularly for protein and fat. Muscle protein, being hydrophilic, influences water content through hydrogen bond formation, as suggested by (Soeparno, 2011). In our study, the analysis of fat and protein content did not show significant differences, thus supporting the lack of variation in water content. The influence of intramuscular fat and meat protein content on water content was also underscored by (Soeparno, 2011).

3.1.2. Ash Content

Analysis of variance for ash content in chicken meat (Table 2) demonstrated that the addition of lime leaf extract at a concentration of 30mL/L through drinking water did not affect the ash content of chicken meat. The data from this study falls within the normal range, consistent with the findings of (Bianchi et al., 2007), (Rukmini et al., 2019), and (Putra, 2021), where the ash content of chicken meat exhibited similar ranges. Lime leaf extract supplementation did not influence meat ash content since the antioxidant compounds from the extract did not impact the digestibility of feed nutrients. This aligns with (Liur, 2020), who stated that higher feed digestibility results in increased meat ash content, while the nutritional content of broiler chicken carcasses is affected by chicken age, as affirmed by (Qurniawan et al., 2016).

3.1.3. Protein Content

Statistical analysis of water content data (Table 2) indicated that adding lime leaf extract to drinking water did not affect the protein content of chicken meat. This observation was reinforced by the data from the positive control (T2), involving antibiotics, which failed to significantly differ from the negative control (T1) involving plain water. Both antibiotics and

phytobiotics did not influence meat protein content, consistent with (Attia et al., 2011), who reported that additional feed ingredients did not affect the protein content of fresh broiler meat.

Antioxidant compounds in lime leaf extract were expected to play a role in regulating protein levels. However, our study found that these compounds did not impact feed protein digestibility or meat protein deposition rates. This lack of influence on meat protein content can be attributed to protein intake, as highlighted by (Suripta, et al., 2006), where protein levels in meat are closely linked to protein content in the diet.

3.1.4. Fat Content

Statistical analysis of the data on fat content in chicken meat (Table 2) demonstrated that adding lime leaf extract to drinking water did not affect the fat content of chicken meat. The research data fell within the normal range, consistent with (Fassah et al., 2012), who reported that broiler chickens fed antioxidant-based additives (tea leaves) had fat content levels within a similar range. The insignificant influence on fat content was attributed to the bioactive compounds in lime leaf extract, such as saponins, which can hinder fat absorption. However, the low saponin content in lime leaf extract did not cause significant fat reduction. This phenomenon is explained by (Nurhayati et al., 2020), who discussed the role of saponins in binding bile salts and reducing fat absorption.

In summary, the incorporation of lime leaf extract into broiler chicken diets exhibited no substantial alterations in the chemical quality of the meat, including moisture, ash, protein, and fat content. This lack of significant impact on the meat's chemical composition can be viewed as a positive outcome. The favorable aspect lies in the fact that lime leaf extract, with its antioxidant compounds, including saponins, did not compromise the nutritional quality of the meat. The limited influence on fat content, attributed to saponins' ability to impede fat absorption, suggests that the addition of lime leaf extract does not adversely affect the meat's chemical attributes while potentially offering other benefits related to meat quality.

3.2. Physical Quality of Meat

Physical quality is one of the aspects of meat products that encompass various parameters, including pH value, water holding capacity, cooking loss, and meat tenderness, all of which were assessed in vitro. The test results for each of these physical quality variables of the meat are presented in Table 3 as follows.

Table 3. Physical quality of broiler chicken meat given lime leaf extract drinking water

Variable	Treatment				SEM	P Value
	T0	T1	T2	T3		
Ph value	6.07 ^b	6.01 ^b	6.00 ^b	5.86 ^a	0.11	0.028
Water holding capacity (%)	25.48 ^a	36.89 ^b	39.18 ^b	40.56 ^b	2.1	0.026
Cooking loss ^{ns} (%)	29.34	28.27	28.97	29.614	0.52	0.849
Tenderness ^{ns} (kg/cm ²)	2.09	2.57	2.68	2.9	0.56	0.220

(T0; negative control) : without adding additives, (T1; positive control) : 50 ppm antibiotics Tetracycline, (T2) : 15ml/liter EDJ (T2), dan (T3) : 30ml/liter EDJ. ^{abc} Different superscripts in the same column show significant differences (P<0,05). ^{ns}: non significant

3.2.1. pH value

Statistical analysis of the data on pH values (Table 3) revealed that the addition of lime leaf extract through drinking water, specifically at a concentration of 30 mL/L, led to a significant decrease in the pH value of chicken meat (P<0.05). The pH value of chicken meat in the T3 treatment group was 5.86, while it was 6.07 for T0, 6.01 for T1, and 6.00 for T2. It

is worth noting that the typical pH range for broiler meat is approximately 5.4 to 5.8 (Van Laack et al., 2000) and 5.96 to 6.07 (Soeparno, 2015).

The lower pH observed in chicken meat treated with a high dose (30 mL/L) of lime leaf extract is likely a result of the active compounds in the extract. These compounds have the potential to increase carbohydrate digestibility, impacting muscle glycogen levels. High carbohydrate absorption can lead to excess energy being stored as glycogen in the muscles, causing a decrease in meat pH after cutting (Legawa, 2014). Lime leaf extract's active compounds, including saponins and sulfur, may influence carbohydrate digestibility. Saponins are known to have a toxic effect on protozoa, increasing cellulolytic microbial populations, which in turn enhances crude fiber breakdown in broiler chicken caeca (Yanuartono et al., 2017). Consequently, the addition of 30 mL/L lime leaf extract can affect the pH value of broiler chicken meat.

3.2.2. Water Holding Capacity

The results of the research data on the water-holding capacity (DIA) of meat (Table 3) showed that the addition of lime leaf extract through drinking water increased the value of the water-holding capacity of chicken meat ($P < 0.05$). Control treatment (T0) yielded meat with 25.48% DIA, while the positive control (T1), the 15 mL/L extract addition (T2), and the 30 mL/L extract addition (T3) produced meat with DIA levels of 36.89%, 39.18%, and 40.56%, respectively. The normal range for water-holding capacity in chicken meat typically falls between 20% and 60% (Soeparno, 2015), and the results of this study were within this range.

The significant increase in water-holding capacity observed in meat treated with lime leaf extract is attributed to the antioxidant compounds in the extract. These antioxidants can mitigate heat stress in livestock, reducing the likelihood of muscle protein denaturation. Lower protein denaturation results in higher water-holding capacity in the meat. The supplementation of lime leaf extract as a phytobiotic appears to enhance the DIA value of the meat, which is consistent with the findings of (Lan et al., 2020) that phytobiotic supplementation increased the DIA value of broiler chicken meat.

3.2.3. Shrink Cooking

The analysis of variance data in Table 3 indicates that adding lime leaf extract through drinking water does not significantly affect the cooking shrinkage value of chicken meat. The results fall within the normal range, as cooking loss for broiler chicken typically ranges from 18.87% to 30.93% (Prayitno et al., 2010). Cooking loss can be influenced by various factors unrelated to water-holding capacity, such as pH values, muscle fiber length, myofibril contraction status, meat size and weight, and cross-section (Bouton et al., 1971).

3.2.4. Tenderness

The analysis of tenderness values (Table 3) revealed that the addition of lime leaf extract through drinking water did not significantly affect the tenderness of Lohmann strain broiler chicken meat. These values were within the normal range, falling between 2.47 and 2.91 kg/cm². The lack of significant change in tenderness suggests that lime leaf extract did not enhance the micronutrient digestibility of the broiler chickens. Tenderness is typically dependent on factors like connective tissue condition and marbling in the meat, which were not significantly influenced by the lime leaf extract supplementation (Prayitno et al., 2010). Variations in tenderness can be related to factors like fat percentage, water content, fat distribution, protein's water-holding capacity, and actual meat juice percentage (Soeparno, 2015).

Therefore, the addition of lime leaf extract through drinking water appears to positively affect the water-holding capacity and pH value of chicken meat, contributing to improved meat quality without adversely impacting tenderness or cooking shrinkage.

4. Conclusions

The study on the effect of lime leaf extract (*Citrus aurantifolia*) added to drinking water on the chemical and physical quality of broiler meat yielded several key findings:

1. **Chemical Quality:** Lime leaf extract supplementation did not significantly affect the chemical quality of the broiler meat, including moisture, ash, protein, and fat content. This suggests that the addition of lime leaf extract had no adverse impact on these important chemical attributes of the meat.
2. **Physical Quality:** Lime leaf extract supplementation had a positive effect on the physical quality of broiler meat. It increased the meat's water-holding capacity, which is a desirable trait as it indicates improved juiciness and tenderness in the meat. Additionally, at a dosage of 30 mL/L, it reduced the pH value of the meat. This reduction in pH can also contribute to improved meat tenderness.

In summary, the study concludes that adding lime leaf extract to broiler chickens' drinking water does not negatively affect the chemical quality of the meat. Instead, it enhances the physical quality of the meat by increasing water holding capacity and reducing pH, which can lead to juicier and more tender broiler meat. This suggests that lime leaf extract has the potential to be a beneficial phytobiotic in broiler chicken production, offering advantages in meat quality without compromising its chemical composition.

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