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# Effect of Andaliman (Zanthoxylum acanthopodium DC) Supplementation in Broiler Diets on Meat Fat, Cholesterol, and Malondialdehyde Content

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**ABSTRACT**. This study aimed to evaluate the impact of supplementing broiler diets with andaliman (Zanthoxylum acanthopodium DC) on the fat, cholesterol, and malondialdehyde (MDA) content in broiler meat. The experiment utilized 200 day-old chicks (DOC) of the CP 707 strain, which were fed experimental diets starting at 15 days of age. The average initial body weight was 465±1.50 g per bird, and the birds were reared until 35 days of age. The research design followed a Completely Randomized Design (CRD) with five dietary treatments and four replications per treatment, each containing 10 broilers. The dietary treatments included a basal diet (R1= 0% andaliman) and diets supplemented with 0.25% (R2), 0.5% (R3), 0.75% (R4), and 1% (R5) andaliman. The variables measured were meat fat content, cholesterol content, and MDA levels. The results indicated that andaliman supplementation had no significant effect (P>0.05) on the fat, cholesterol, or MDA content of broiler meat. The observed ranges were 140.61–161.61 mg/100 g for fat content, 140.61–161.61 mg/100 g for cholesterol content, and 8.11–8.56 µg/g for MDA levels. In conclusion, the addition of andaliman to broiler diets did not significantly influence the fat, cholesterol, or MDA content in the meat.

**Keywords**: Andaliman (*Zanthoxylum acanthopodium, DC*), cholesterol, feed additive, fat, *Malondialdehyde* (MDA)

## INTRODUCTION

The growing emphasis on biotechnology in animal feed is crucial for advancing meat production that is safe, healthy, wholesome, and halal. A significant development in this area is the application of biotech feed additives in poultry, aimed at producing meat that meets the rising consumer demand for safer and healthier food options. As public awareness of the health risks associated with high cholesterol, such as heart disease and hypertension, increases, many individuals are reducing their meat consumption or opting for plant-based diets. To address concerns about the consumption of meat high in fat and cholesterol, feed technology innovations, including the incorporation of feed additives, are being explored to reduce cholesterol levels and enhance the antioxidant properties of meat products.

Effective broiler meat production requires careful management, particularly in feeding practices. Animal feed is a critical component of the livestock industry, representing the largest share of production costs. Ensuring that animal feed is of high quality and includes additive compounds that promote growth and safe, healthy meat production is essential. This has led to the application of biotechnology in formulating broiler feed rations, with a focus on feed additives that contain antioxidant compounds and reduce fat and cholesterol, ultimately producing healthier meat products.

Previous research has explored the use of natural feed additives derived from local Indonesian ingredients, demonstrating their potential to enhance broiler performance and immune function. For example, black cumin has been shown to improve liver enzyme activity and maintain normal liver function in broilers (Salam *et al*., 2014), while Curcuma xanthorrhiza supplementation has significantly affected slaughter weight and abdominal fat percentage (Jumiati *et al*., 2017). Other studies have reported positive effects of turmeric as a feed additive in broiler drinking water (Horhoruw *et al*., 2020) and garlic flour in feed to enhance broiler production performance (Nuningtyas, 2014).

The addition of feed additives in broiler diets is an important strategy to mitigate oxidative stress, neutralize free radicals, and prevent damage to body functions caused by environmental factors. Advances in feed biotechnology have increasingly utilized natural resources, particularly spices, rich in antioxidant compounds. Herbal polysaccharides, for instance, have been shown to boost immunity and growth performance in young broilers (Chen *et al*., 2003), while dietary supplements like Lycium barbarum (Liu *et al*., 2017) and Acanthopanax senticosus extract (Long *et al*., 2021) have demonstrated antioxidant properties that enhance growth and immunity in broilers. Antioxidants play a crucial role in mitigating oxidative damage by halting chain reactions and converting free radicals into more stable compounds (Zabolil *et al*., 2013).

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Andaliman (Zanthoxylum acanthopodium DC) is a locally sourced spice from Indonesia, thriving in tropical climates, and is rich in phenolic compounds such as alkaloids, hydroquinones, flavonoids, steroids, glycosides, terpenoids, and essential oils (Wijaya *et al*., 2002). These compounds contribute to its anti-inflammatory (Wijaya *et al*., 2018), antimicrobial (Asbur *et al*., 2018), antioxidant (Tensiska *et al*., 2003), and immunostimulant properties (Kou *et al*., 2016), which collectively inhibit free radical formation (Parhusip, 2006). Andaliman is also known for its aromatic properties, spicy flavor, antipyretic effects, and its ability to stimulate saliva production (Asbur *et al*., 2018), all of which may positively impact broiler performance. Previous studies have shown that incorporating andaliman as a feed additive can enhance broiler ration quality and increase body weight (Anwar *et al*., 2019, 2020). Additionally, supplementation with 0.50% andaliman has been associated with improved broiler immunity, evidenced by increased weights of the bursa of Fabricius and thymus (Faradillah *et al*., 2020), without affecting the size of internal organs (Siregar *et al*., 2020), indicating that andaliman does not interfere with broiler metabolism or cause organ abnormalities.

However, the use of andaliman in livestock feed remains limited, with most studies focusing on its effects on broiler performance rather than meat nutritional content. There is a growing need for research on the application of andaliman as a feed additive to produce functional broiler meat with high antioxidant content, low fat, and low cholesterol levels. This study aims to fill this gap by evaluating the effects of andaliman supplementation in broiler diets on the final quality of meat, thereby providing consumers with healthier meat options.

## MATERIAL AND METHOD

### Location and Time

This research was conducted at the poultry yard of the Animal Husbandry Study Program, Department of Agriculture, Kuantan Singingi University. The analysis of meat quality was carried out in the laboratory of the Faculty of Animal Husbandry, Andalas University, West Sumatra.

### Material

The study used 200 broilers of the CP 707 strain. Experimental rations were provided starting at two weeks of age when the average body weight was 465±1.50 g per bird. The broilers were reared until five weeks of age (35 days). Twenty chickens were selected for slaughter using cervical dislocation. Muscles from the right thigh were isolated and homogenized for the analysis of cholesterol, fat, and malondialdehyde (MDA) content. The rations were formulated to be isocaloric and isoprotein, following the recommendations of Leeson and Summers (2008). The composition of the rations is detailed in Table 1.

### Method

The experiment followed a Completely Randomized Design (CRD) with five treatments and four replications per treatment. Each replication consisted of 10 broilers housed in cages measuring 1.5 x 1.5 meters. The treatments were as follows:

R1 = basal ration (0% andaliman [Zanthoxylum acanthopodium DC])
R2 = basal ration + 0.25% andaliman
R3 = basal ration + 0.5% andaliman
R4 = basal ration + 0.75% andaliman
R5 = basal ration + 1% andaliman

### Variables

The study measured the following variables: meat fat content, meat cholesterol content, and meat malondialdehyde (MDA) content.

**Meat Fat Content**: Fat content in the meat was analyzed using the Soxhlet extraction method as described by AOAC (2005). The procedure involved cutting and weighing filter paper (15x15 cm), weighing 1 gram of the meat sample, and drying it in an oven at 105°C for 6 hours. The sample was then cooled in a desiccator for 15 minutes, placed in a Soxhlet apparatus, and filtered with diethyl ether for 3-5 hours, repeating the process for 8-10 cycles. The sample was then aired to remove the diethyl ether smell, oven-dried at 105°C for 2 hours, and weighed. The fat content was calculated using the formula:

$$\text{Fat Content} = \frac{W_2 - W_1}{W} \times 100$$

Note: where $W_1$ is the weight of the filter paper before drying, $W_2$ is the weight of the filter paper after drying, and W is the weight of the sample.

**Meat Cholesterol Content**: The cholesterol content of the meat was measured using the Liebermann-Burchard method. The Liebermann-Burchard Reagent (LBR) was prepared by mixing 50 ml of anhydrous acetate with concentrated sulfuric acid. Meat cholesterol samples were dissolved in 20 ml of chloroform, transferred to a reaction tube, and mixed with 2 ml of LBR. The mixture was left for 90 minutes until a dark green color developed, after which the absorbance was measured at a wavelength of 200-800 nm using a UV-Vis spectrophotometer (Burke *et al*., 1974).

**Malondialdehyde (MDA) Content of Meat**: The MDA content was assessed by generating an MDA standard curve. MDA stock solutions of varying concentrations (0, 1, 2, 3, 4, 6, 7, and 8 µg/mL) were mixed with distilled water, TCA, HCl, and Na-Thio, homogenized, and centrifuged at 500 rpm for 10 minutes. The supernatant was heated in a water bath at 100°C for 30 minutes, and the absorbance was measured at 523 nm to create the MDA standard curve.

For measuring the MDA content in broiler meat, 1 gram of chicken meat was ground in a cold mortar and homogenized with 500 µl of 0.9% NaCl. The mixture was centrifuged at 8000 rpm for 20 minutes. The supernatant (100 µl) was mixed with distilled water, TCA, HCl, and Na-Thio, centrifuged at 500 rpm for 10 minutes, heated at 100°C for 30 minutes, and the absorbance was measured at 532 nm using a spectrophotometer.

Table 1. The feed composition calculation and nutrition content of experimental ration for the starter and grower feed.

| Feed Composition (%) | Starter Feed (%) | Grower Feed (%) |
|---|---|---|
| Cornmeal | 59 | 59.6 |
| Ricebran | 2.95 | 5.5 |
| *Corn Gluten Meal (CGM)* | 4.5 | 3 |
| Soybean meal | 22 | 19 |
| Fishmeal | 8.5 | 8 |
| Palm Oil | 1.25 | 3 |
| DCP | 0.1 | 0.15 |
| CaCO3 | 0.8 | 0.1 |
| Salt | 0.1 | 0.1 |
| Premix | 0.3 | 0.3 |
| L-Lysine | 0.3 | 0.2 |
| DL-Methionine | 0.2 | 0.15 |
| Total | 100 | 100 |
| Nutrient composition calculation of ration* | | |
| Metabolis Energy (kkal/kg) | 3066.55 | 3128.30 |
| Crude protein (%) | 22.18 | 20.13 |
| Crude kasar (%) | 4.03 | 5.87 |
| Crude fiber (%) | 2.69 | 2.87 |
| Lysin (%) | 1.63 | 1.41 |
| Methionine (%) | 0.71 | 0.62 |
| Sistin (%) | 0.38 | 0.34 |
| Methionine+sistin (%) | 1.09 | 0.95 |
| Calcium (%) | 0.93 | 0.98 |
| Phosphor (%) | 0.54 | 0.54 |

Note: R1= (0% Andaliman (*Zanthoxylum acanthopodium* DC) as control; R2= (0.25% Andaliman (*Zanthoxylum acanthopodium* DC.); R3= (0.5% Andaliman (*Zanthoxylum acanthopodium* DC.); R4= (0.75% Andaliman (*Zanthoxylum acanthopodium* DC.); dan R5= (1% Andaliman (*Zanthoxylum acanthopodium* DC.).

**Statistical Analysis**

The data obtained from the study were analyzed using analysis of variance (ANOVA) using the IBM SPSS V21 program and if they were significantly different they were continued with Duncan's Multiple Range Test (Steel and Torrie, 1995)

The data obtained from the study were analyzed using analysis of variance (ANOVA) with IBM SPSS Version 21. When significant differences were detected, Duncan's Multiple Range Test was applied to determine specific differences among the treatment means (Steel and Torrie, 1995).

## RESULT AND DISCUSSION

The content of fat, cholesterol, and *malondialdehyde* (MDA) in broiler meat aged 35 days with andaliman (*Zantoxylum achantopodium, DC*) as a feed additive in the ration can be seen in Table 2.

The fat, cholesterol, and malondialdehyde (MDA) content in broiler meat at 35 days of age, with Andaliman (*Zanthoxylum acanthopodium* DC) as a feed additive, are presented in Table 2.

Table 2. Fat content, cholesterol, and MDA of broiler meat aged 35 days treatment variables

| Variables | Treatments | | | | |
|---|---|---|---|---|---|
| | R1 | R2 | R3 | R4 | R5 |
| Fat (%) | 06.28±0,56 | 05.65±01,22 | 05.98±01,25 | 05.53±01,17 | 05.95±02,09 |
| Cholesterol(mg/100g) | 140.61±14,52 | 158.31±17,07 | 156.92±38,80 | 153.37±34,01 | 161.61±38,11 |
| MDA (µg/g) | 08.11±01,02 | 08.38±01,67 | 08.45±01,67 | 08.17±01,60 | 08.56±01,34 |

Note: Superscripts in the same column show significant differences (P<0.05) : R1= basal ration (0% Andaliman (*Zanthoxylum acanthopodium* DC.); R2= ration + 0.25% Andaliman (*Zanthoxylum acanthopodium* DC.); R3= ration + 0.5% Andaliman (*Zanthoxylum acanthopodium* DC.); R4= ration + 0.75% Andaliman (*Zanthoxylum acanthopodium* DC.); dan R5= ration + 1% Andaliman (*Zanthoxylum acanthopodium* DC.).

**The Effect of Andaliman as a feed additive in the Meat Fat Content**

The analysis of variance indicated that the inclusion of Andaliman (*Zanthoxylum acanthopodium* DC) as a feed additive at levels up to 1% in broiler rations did not significantly affect the fat content in broiler meat (P>0.05). The average fat content in broiler meat ranged from 5.53% to 6.28%. The addition of Andaliman in the diet primarily influenced the immune system of the lymphoid organs, as it contains flavonoids with immunomodulatory and antioxidant activities. Similar research by Faradillah *et al*. (2020) found that Andaliman supplementation up to 0.50% in rations significantly affected the weight of the bursa fabricius, an indicator of immune response. The use of traditional herbal additives like Acanthopanax senticosus has been shown to enhance immune function (Long *et al*., 2021; Sollehudin *et al*., 2020), and Andaliman has been reported to improve broiler performance (Anwar *et al*., 2020).

Meat fat content is strongly influenced by the balance between feed intake and energy expenditure. While feed additives can impact immunity and performance, they have minimal effects on fat content. Factors such as ration composition, particularly energy and protein levels, play a critical role in body fat accumulation (Fouad and El-Senousey, 2014). Excessive consumption of fatty foods can increase total and LDL cholesterol levels (Maryuni and Wibowo, 2005). The study findings align with other research, such as Mentari *et al*. (2014), which reported no significant effect on fat mass when Chinese key flour was added as a feed additive in broiler rations.

Differences in the percentage of Andaliman as a feed additive in the ration did not significantly affect the fat content in broiler meat, likely due to the isocaloric and isoprotein formulation of the rations. Chickens tend to regulate their feed intake based on energy needs, stopping consumption once those needs are met (Suprijatna, 2005). As Soeparno (2005) noted, energy consumption leads to fat deposition in various parts of the body, including between muscles and under the skin. The amount of feed consumed directly impacts nutrient absorption and, consequently, fat deposition in broilers (Solichedi *et al*., 2003).

Processing technologies can alter fat content in meat, and diets high in fiber can delay the absorption of carbohydrates and fats, leading to reduced fat accumulation (Nashriana *et al*., 2015). For example, using seaweed in rations at a level of 7% has been shown to reduce fat and cholesterol content in broiler meat without affecting body weight (Ngitung, 2020). Krismiyanto *et al*. (2020) reported that increasing the digestibility of crude fat in rations could lead to higher abdominal fat and fat content in meat.

Reducing or increasing fat content in meat can be achieved through feed ingredient processing technologies. A diet high in fiber can delay the absorption of nutrients in the intestine, including carbohydrates (Nashriana *et al*., 2015). This delay, along with inhibited fat absorption, impacts digestion and bile acid fat binding, which in turn stimulates increased cholesterol excretion through feces and urine. Ngitung (2020) reported that incorporating seaweed into rations at a 7% level reduced both fat and cholesterol content in broiler chicken meat without affecting body weight. Additionally, Krismiyanto *et al*. (2020) found that enhancing the digestibility of crude fat in rations could lead to increased abdominal fat and subsequently higher fat content in the meat.

**The Effect of Andaliman as a feed additive in the Meat Cholesterol Content**

The effect of andaliman (Zantoxylum achantopodium, DC) as a feed additive on the cholesterol content in broiler meat was not significant, even at levels up to 1% in the ration (P>0.05). The research indicated that the average cholesterol content in broiler meat ranged from 140.61 mg/100g to 161.61 mg/100g when andaliman was used as a feed additive. This aligns with the reported cholesterol content of broiler meat, which is 158.31 ± 17.08 mg/100g (Santi *et al*., 2015). Cholesterol levels in meat are largely influenced by the fat mass within the body, as cholesterol is a component of fat derived from the energy content of the consumed feed. Given that the nutritional formulation, including protein and energy levels, was consistent across treatments, there was no significant impact on the fat and cholesterol values stored in the meat.

Cholesterol levels in blood or meat are influenced by the consumption of animal feed that contains fat. An increase in cholesterol levels in the blood typically correlates with an increase in cholesterol in meat. Baroroh (2013) demonstrated in experimental research that the total cholesterol levels in the blood serum of rats increased after consuming a high-fat diet, whereas lower cholesterol levels were observed in diets with reduced cholesterol content. Cholesterol levels can be lowered by fermenting bacteria, which produce bile salt hydrolase enzymes from feed ingredients (Sa'diyah *et al*., 2020). Traditional herbal feed, containing active ingredients such as temulawak and black garlic, can also reduce cholesterol deposition, thereby decreasing both meat cholesterol content (Dono, 2010) and blood cholesterol content in broiler chickens (Berliana *et al*., 2021).

The results of giving feed additives in the rations of several studies can reduce the cholesterol content of meat. Marfuah (2016) stated that using garlic flour as a feed additive in rations positively affected meat cholesterol and broiler carcass quality with a content of 58.69 mg/dl. Hasanuddin *et al.* (2013) stated that the administration of lime juice reduced broiler meat cholesterol levels but did not reduce meat fat content in broiler chickens. Santi *et al.* (2015) Furthermore, giving carotenoids as a natural feed additive in broiler rations can reduce cholesterol levels in meat. Betacarotene is one of the carotenoids which is an active component in lowering cholesterol in broiler chicken meat. The role of beta-carotene inhibits the formation of mevalonate, which is responsible for cell growth and affects cholesterogenesis and fatty acid metabolism.

**The Effect of Andaliman as a Feed Additive in the Meat MDA Content**

Malondialdehyde (MDA) is a dialdehyde compound that serves as the end product of lipid peroxidation in the body. High MDA concentration indicates the occurrence of oxidation processes within cell membranes. The analysis of variance revealed that the use of andaliman (Zantoxylum achantopodium, DC) as a feed additive in the ration had no significant effect on MDA content in broiler meat (P>0.05). The average MDA content in broiler meat ranged from 8.11 µg/g to 8.56 µg/g. The inclusion of up to 1% andaliman in the ration did not alter MDA content in broiler meat. The absence of visible damage in the meat peroxidation process across experimental treatments can be attributed to the healthy physiological state of the broiler chickens, which were not afflicted with tissue damage. When the immune response of the body is compromised, it can trigger peroxidation processes in body tissues. Setiyawan (2017) reported that MDA levels in body tissues increase as a secondary effect of E. tenella infection and decrease when feed additives are introduced into the ration. Moreover, Jafari *et al*. (2012) found that chickens supplemented with vitamin E exhibited lower MDA levels compared to infected chickens that did not receive vitamin E supplementation.

One of the damages that can occur due to increased free radicals is damage to the fat membrane (Kregel and Zhang, 2007). Oxidative damage to cells and tissues can be measured using one of the oxidation products, namely malondialdehyde (Sahin *et al*., 2008). Malondialdehyde is a secondary metabolite produced from fat peroxidation (Ayala *et al*., 2014). The results of the study explained that there was no significant effect on the provision of a feed additive in the form of andaliman as an antioxidant. The treatment object gives the same response to the treatment, this indicates that the livestock is healthy and normal. Oxidative damage to cells and tissues is the role of Reactive Oxygen Species (ROS) originating from abnormalities in body tissues so that MDA levels in meat will increase.

## CONCLUSION

The study concluded that the inclusion of andaliman (Zanthoxylum acanthopodium DC) in broiler rations, at levels up to 1%, does not significantly affect the fat content, cholesterol levels, or malondialdehyde (MDA) concentrations in broiler meat.

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