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Evaluation of The Addition of Eugenol of Clove Leave Essential Oil in The Ration on Phenol and Antioxidant Levels of Male Laying Hens Meat

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Abstract

Eugenol is a component of phenol, biologically it can function as an anti-bacterial, anti-fungal, insecticide, and antioxidant. The content of eugenol which is quite large in clove leaf essential oil is used as a bioactive substance to increase antioxidant levels in meat. Experimental by using 100 male laying hens were then placed into 20 cage units, each cage unit filled with 5 experimental chickens. Meat sample collection for analysis of phenol and IC_{50} levels was carried out by slaughtering experimental chickens at 8 weeks of age. Samples of chicken meat were taken from the breast of each experimental animal as much as 100 grams. Determination of the total phenol content of chicken meat using Folin-Ciocalteu with gallic acid as a comparison, while the antioxidant activity testing using the DPPH free radical absorption method.In this experiment, eugenol was added to the basal ration with 5 treatments, namely: E_0 = without eugenol; E_1 = 0.5% eugenol; E_2 = 1% eugenol; E_3 = 1,5% eugenol; E_4 = 2% eugenol. The variables observed were phenol levels and IC_{50} values which can describe the antioxidant power in male laying hens. The results showed that the addition of clove leaf eugenol as an additive in the ratio had a very significant effect ($P<0.01$) on phenol levels and IC_{50} values. male laying hens. Each increase in the use of 0.5% eugenol in the ration can increase the phenol content and decrease the IC_{50} value significantly in the meat produced. The level of eugenol in the ration was positively correlated with the levels of phenol and antioxidants in the meat of laying hens, while the levels of eugenol in the ration and the levels of phenol in the meat had a negative correlation with the IC_{50} value of the meat of laying hens.

Keywords: Laying hens, eugenol, clove leaf, phenol, antioxidant

Introduction

Eugenol is a component of phenol, biologically it can function as an anti-bacterial, anti-fungal, insecticide, and antioxidant and is traditionally used as a flavoring and anti-microbial agent in food (Bhuiyan et al., 2010; Chee & Lee, 2007). One of the bioactive substances produced by plants as secondary metabolite products, namely eugenol, is found in large quantities in the clove plant (*Syzygium aromaticum* L). The advantage of cloves is the fragrance that comes from essential oils in large enough quantities, both in flowers (10-20%), stalks (5-10%), and leaves (1-4%). In addition, clove oil has a large amount of eugenol (70-80%) which has properties as a stimulant, local anesthetic, carminative, antiemetic, antiseptic, and antispasmodic (Sohilait, 2015; Tahir et al., 2020).

The level of eugenol contained in cloves causes clove essential oil to be used as an antioxidant. Phenolic compounds are compounds that are commonly found in plants as a result of secondary metabolites. These compounds have known structures, including flavonoids, phenols, polyphenols (lignin, melanin, and tannins), and phenolic quinones. Phenolic compounds have pharmacological properties, namely as an anti-inflammatory (Balan, et al., 2015), antioxidant (Marjoni et al., 2015), and antibacterial (Buhian et al., 2016).

Eugenol is one of the components of cloves leaf oil which can function as an antibiotic, antimicrobial, and antioxidant. The Eugenol content of cloves leaf oil is 79.72% and the antioxidant is 42.26 ppm. The higher the eugenol content of clove leaf oil, the higher the antioxidant content (Tahir, et al. 2019)

Antioxidants are compounds that can be substances to neutralize free radicals and prevent damage caused by free radicals by complementing the electron deficiency of these free radicals and inhibiting the reaction of free radical formation (Putra et al., 2013).

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The ratio is one of the determinants of the quality of the meat produced in the livestock business. This encourages the use of feed ingredients or additives in preparing rations so that the quality of the meat produced is better. The use of synthetic additives is feared to hurt consumer health. Therefore, it is necessary to use natural additives as anti-oxidants, including cloves leaf essential oil eugenol. The use of additives in rations as a source of antioxidants is expected to increase the antioxidants of meat so that the livestock products produced are not only a source of nutrients but also functional foods rich in antioxidants.

The use of cloves leaf essential oil up to a concentration of 2% in the ration can completely kill Escherichia coli and Salmonella sp bacteria (Tahir et al., 2019) and can increase the digestibility of the ration (Tahir et al., 2022). This became the basis of the treatment in this study to evaluate the use of cloves leaf essential oil eugenol in the ratio of the phenol and antioxidant levels of male laying hens.

Methods

This study was to examine the relationship between the addition of clove leaf essential oil eugenol in the ratio to the phenol content and IC_{50} value of male laying hens which was carried out in the experimental cage in Tondo Village, Palu City, Central Sulawesi Province. Analysis of phenol and IC_{50} levels was carried out at the Chemistry Laboratory of the Faculty of Mathematics and Natural Sciences (MIPA) at Tadulako University Palu

Before use, the cage and equipment for the cage (ration rations and drinking water containers) must be thoroughly cleaned to ensure that they are sterile. Places for rations and drinking water are placed regularly in each unit of the cage. Experimental livestock as many as 100 male laying hens are then placed into 20 cage units, each cage unit is filled with 5 experimental chickens.

Meat sample collection for analysis of phenol and IC_{50} levels was carried out by slaughtering experimental chickens at 8 weeks of age. Samples of chicken meat were taken from the breast of each experimental animal as much as 100 grams. Determination of the total phenol content of the chicken meat using Folin-Ciocalteu with gallic acid as a comparison, while testing the antioxidant activity using the DPPH free radical absorption method.

The basal ration consisted of yellow corn, rice bran, soybean meal, fish meal, DL-methionine, lysine, top mix, palm oil, and mineral mix, with the composition and nutrient content of the feed listed in **Tables 1** and **2**. The treatments that were tried in this study were:

E_0 = Basal ratio without the addition of clove leaf eugenol
E_1 = Basal ration + 0.5% clove leaf eugenol
E_2 = Basal ration + 1.0% clove leaf eugenol
E_3 = Basal ration + 1.5% clove leaf eugenol
E_4 = Basal ration + 2.0% clove leaf eugenol

Table 1. Composition of use of feed ingredients for research basal ration

No	Feed ingredient	Composition (%)
1	Yellow corn	57.00
2	Rice bran	7.00
3	Soybean meal	14.50
4	Fish meal	17.39
5	Top mix	1.00
6	DL-Methionine	0.22
7	Lysine	0.18
8	Palm oil	2.00
9	Mineral mix	0.71
	Total	

Table 2. Nutrient content of the basal ratio used in the study

Nutrien ration	amount
Metabolism energy (kcal/kg)	3035
Crude Protein (%)	20.41
Crude fat (%)	5.86
Crude fiber (%)	4.58
Calcium (%)	0.97
Phosphor (%)	0.77
Methionine (%)	0.49
Lysine (%)	1.32

The experimental method used a completely randomized design (CRD) method which consisted of 5 treatments with 4 replications each. Data on the phenol content and IC_{50} of experimental chicken meat were tabulated and analyzed based on analysis of variance from a completely randomized design. Treatments that had a significant effect on the measured variables were further tested using the BNJ test to see differences between treatments. The relationship between parameters was analyzed by regression to get the correlation value and the regression equation.

Results and Discussion

Phenol content and IC_{50} value of meat

The results of the study on the addition of eugenol as an additive in feed to the phenol content and IC_{50} value of male laying hens are listed in **Table 3.**

Table 3. Phenol content and IC_{50} value of laying chicken meat given clove leaf eugenol as an additive in ration

Treatment	Variable	
	Phenol content (mg/100g)	IC_{50} value (ppm)
E_0	1076.00±20.74^a	175.23±0.41^e
E_1	1262.00±16.43^b	154.14±0.24^d
E_2	1460.00±15.81^c	130.81±0.77^c
E_3	2546.00±20.74^d	120.33±0.29^b
E_4	2760.00±20.00^e	96.95±0.12^a

Note: Numbers followed by different letters in each parameter, indicate a very significant difference (P<0.01)

The results of the analysis of variance showed that the addition of cloves leaf eugenol as an additive

in the ratio had a very significant effect (P<0.01) on the phenol content and IC50 value of male laying hens. Increasing the use of cloves leaf essential oil eugenol in the ration can increase the antioxidant and phenol content of meat. One of the indicators of increasing antioxidants in meat is a decrease in the IC_{50} value of the meat. The eugenol component is the main component in clove leaves and has antioxidant activity (Rorong, 2008).

Table 3 shows that the phenol content of chicken meat between treatments increased with the increasing use of eugenol. Cloves leaf essential oil eugenol is a source of phenol that is added to the diet as a non-nutritive additive. According to (Mu'nisa et al., 2012), cloves contain several phenolic components, including eugenol ($C_{18}H_{12}O_3$). An increase in the use of 0.5% cloves leaf essential oil eugenol for each treatment gave a response to a significant increase in phenol levels and antioxidant levels in male laying hens. Eugenol has the same antioxidant activity as tocopherol in inhibiting peroxidation (Ogata et al., 2000; Rajalakshmi et al., 2000).

In this study, the use of cloves leaf eugenol was only up to 2% in the ration, although it can be seen in Table 3 that increasing the use of cloves leaf eugenol in the ration would increase the phenol content and decrease the IC50 value of chicken meat produced. The use of cloves leaf eugenol above 2% causes the palatability of the ration to decrease so that the consumption of the ration will be low. The decrease in ration consumption with the increasing use of cloves leaf eugenol in the ration was caused by the low palatability of the ration. One of the factors causing the decreased palatability is the spicy eugenol compound. Therefore, the higher use of cloves leaf essential oil eugenol in the ration resulted in lower ration palatability which resulted in a decrease in ration consumption (Tahir et al., 2019). According to Borazjanizadeh et al. (2011), the addition of cloves and oregano as additives in broiler rations can reduce ration consumption. The low consumption of these rations can result in unmet nutrient needs which have implications for decreased growth resulting in low carcass and meat production.

The increase in antioxidant levels of meat can be seen by the decrease in the IC_{50} value of meat in each treatment. The IC_{50} value is the concentration of antioxidants used to reduce 50% of free radicals. Table 3 shows that increasing the use of 0.5% cloves leaf essential oil eugenol after treatment in the ration can significantly reduce the IC_{50} value of male laying hens. Antioxidants are categorized as very strong if the IC_{50} value is less than 50 ppm, antioxidants are categorized as strong if the IC_{50} value is 50-100 ppm, antioxidants are categorized as moderate if the IC_{50} value is 100-150 ppm and antioxidants are categorized as weak if the IC_{50} value is more than 150 ppm, so the smaller the IC_{50} value. the stronger the antioxidant (Molyneux, 2004).

Based on these categories, the addition of 2% eugenol in the ration can produce meat with strong antioxidants (IC_{50} 96.95 ppm), the addition of 1% and 1.5% eugenol in the ration produces meat with moderate antioxidants (IC_{50} 130.81 ppm and 120.33 ppm), while the addition of 0.5% eugenol in the ration produced meat with weak antioxidants (IC_{50} 154.14 ppm).

The strong effectiveness possessed by cloves leaf essential oil as a free radical inhibitor is caused by the secondary metabolites contained in it, as shown in **Table 4**.

Table 4. Chemical Components of Clove Leaf Essential Oil

Component	Amount (%)
Eugenol ($C_{10}H_{12}O_2$)	79.72
Cariophylen ($C_{15}H_{24}$)	0.96
Guaiacol ($C_7H_8O_2$)	4.16
Methylguaiacol ($C_8H_{10}O_2$)	9.79
phenol, 4-ethyl-2-methoxy ($C_9H_{12}O_2$)	1.5
phenol, 2-methoxy-4-propyl ($C_{10}H_{14}O_2$)	3.87

Source: Tahir et al. (2020)

Eugenol and phenol compounds have -OH groups attached to aromatic carbon rings that function as free radical inhibitors because they can donate hydrogen atoms so that free radicals can be reduced to a more stable form (Santoso et al., 2016; Mohandas & Kumaraswamy, 2018).

The effectiveness of flavonoids and phenols in inhibiting DPPH free radicals is influenced by the amount and position of phenolic hydrogen in the molecule. Increasing the number of hydroxyl groups will result in effectiveness in inhibiting free radicals (Wahdaningsih et al., 2011; Sulistyaningtyas & Wilson, 2018). In the structure of terpenoid compounds, the presence of conjugated double bonds serves as a free radical inhibitor, due to its ability to donate electrons so that it can stabilize the reactive charge of free radicals (Young & Lowe, 2018).

The relationship between the use of eugenol in the ration with the phenol content of meat

The relationship between the addition of eugenol in the ratio to the phenol content of laying hens can be seen in **Figure 1**. In **Figure 1**, it can be seen that the addition of cloves leaf essential oil eugenol in the ratio has a positive correlation and a very strong relationship with the phenol content of male laying hens with a regression coefficient (R) = 0.95. The coefficient of determination (R^2) = 0.8992 or 89.92% phenol content of male laying hens is due to the addition of eugenol of cloves leaf essential oil in the ration.

The relationship between eugenol levels in the ration and the phenol content of chicken meat produced can be seen through the regression equation Y= 930.4x + 809.4, meaning that every 1% increase in the use of cloves leaf essential oil eugenol in the ration will increase the phenol content of meat by 930.4 mg/100g sample.

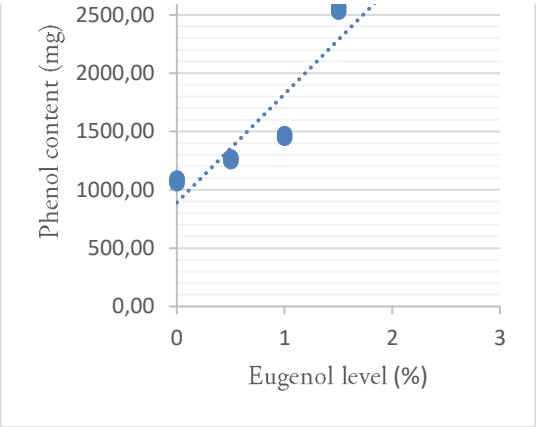

Figure 1. Graph of relationship between ration eugenol levels with a phenol content of laying rooster meat

Eugenol which is widely found in clove leaves and cinnamaldehyde in cinnamon is one of the simple phenolic compounds. Monovalent (simple) phenolic compounds are compounds that have one benzene ring and at least one hydroxyl group (Pasaribu, 2019). This causes an increase in the phenol content of the meat due to the increased use of cloves leaf eugenol in the ration.

The relationship between the use of eugenol in the ration with the IC_{50} value of meat

The relationship between the addition of eugenol in the ratio with the content of the IC_{50} value of laying hens can be seen in **Figure 2**.

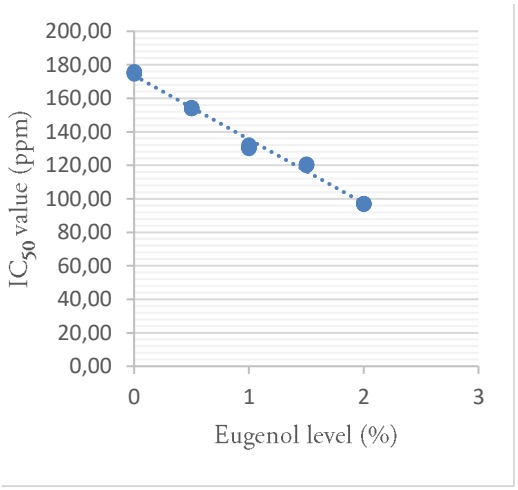

Figure 2. Graph of relationship between ration eugenol levels with IC_{50} value of laying rooster meat

Based on **Figure 2**, it can be seen that the addition of cloves leaf essential oil eugenol in the ratio has a negative correlation and a very strong relationship with the IC50 value of male laying hens with a regression coefficient (R) = 0.99. The coefficient of determination (R2) = 0.9889 or 98.89% IC50 value of male laying hens was due to the addition of cloves leaf essential oil eugenol in the ration. The lower the IC50 value, the higher the antioxidant level, the addition of cloves leaf essential oil eugenol in the ration has a positive correlation with the antioxidant levels in the resulting layer of chicken meat.

The relationship between the levels of eugenol in the ration with the IC_{50} value of chicken meat produced can be seen through the regression equation Y= -38.073x + 173.56, meaning that every 1% increase in the use of eugenol of cloves leaf essential oil in the ration will reduce the IC_{50} value of meat by 38,073 ppm. This indicates that the increased use of eugenol in the diet can increase the antioxidant capacity of meat, which is indicated by a decrease in the IC_{50} value.

Increasing the use of clove leaf eugenol in the ration, which is one of the phenolic compounds, will increase the antioxidants of the meat produced. The increase in antioxidants in meat can be seen through the decrease in the IC_{50} value in meat. The lower the IC_{50} value of a material, the higher the antioxidant content (Molyneux, 2004).

The relationship between phenol content and the IC50 value of meat

The relationship between phenol levels and IC_{50} in laying hens can be seen in **Figure 3**.

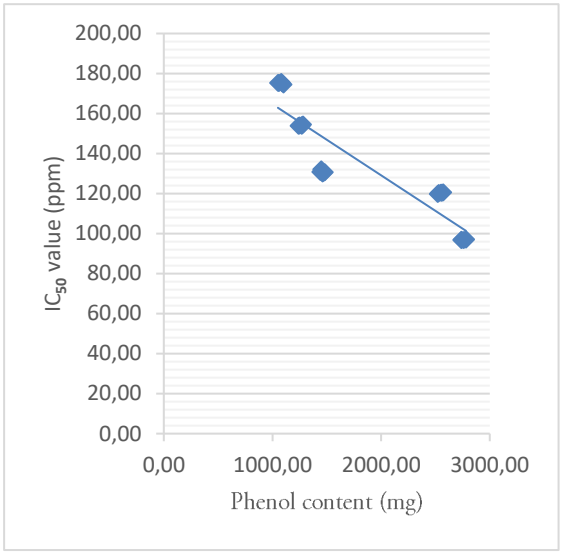

Figure 3. Graph of the relationship of phenol levels with IC_{50} value of laying rooster meat

Based on **Figure 3**, it can be seen that the Phenol content is negatively correlated and has a very strong relationship with the IC_{50} value of male laying hens with a regression coefficient (R) = 0.91. The coefficient of determination (R2) = 0.8293 or 82.93% phenol content affects the IC50 value of the male laying hens. The lower the IC_{50} value, the higher the antioxidant content so the level of antioxidants in the meat is largely determined by the phenol content of the meat.

The relationship between phenol levels and the IC_{50} value of chicken meat produced can be seen through the regression equation Y= -0.0355x + 200.2, meaning that an increase in phenol content of 1 mg can reduce the IC_{50} value of 0.0355 ppm, meaning that IC_{50} levels are largely determined by the phenol content in the meat sample. This shows that increasing the use of phenol content in chicken meat can increase antioxidants, which is marked by a decrease in the IC_{50} value.

An increase in phenol content in meat will increase the antioxidant content of the meat, which is marked by a decrease in the IC_{50} value of the meat. This shows that feeding chickens as a source of phenol will increase the antioxidant content of the meat. The increase in antioxidants in meat can be seen by decreasing the IC_{50} value of the meat (Molyneux, 2004)

Conclusions

Based on the results of research on the evaluation of the addition of eugenol of cloves leaf essential oil in the ratio of the phenol and antioxidant levels of male laying hens, it was concluded that: The addition of eugenol of cloves leaf essential oil in the ratio can increase the phenol and antioxidant levels of a male laying hens produced; The level of eugenol in the ration was positively correlated with the levels of phenols and antioxidants in the meat of laying hens produced; The level of eugenol in the ration and the level of phenol in the meat were negatively correlated with the IC_{50} value of the male laying hens produced.

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