AGROLAND: The Agricultural Sciences Journal
Vol. 9, No. 1 June (2022), 52 - 58
P-ISSN : 2407- 7585 & E-ISSN : 2407- 7593, Published by Tadulako University

Original Research **Open Access**

IMMUNE ORGANS AND GROWTH PERFORMANCE OF MALE LAYING HENS WITH USE OF EUGENOL CLOVE LEAF OIL AS A SUBSTITUTE OF ANTIBIOTIC IN FEED

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Submit: 2 June 2022, Revised: 23 June 2022, Accepted: June 2022
DOI : https://doi.org/10.22487/agroland.v9i1.1311

ABSTRACT

The use of antibiotics as growth promoters (AGP) in poultry feed aims to stimulate growth and prevent disease. However, the use of antibiotics in animal feed has been banned spread of resistant bacteria which can be harmful to human health. The use of natural compounds, especially essential oils, as a natural alternative for antibiotics. The aim of the study was to evaluate the inclusion of eugenol clove leaf oil as a feed additive to replace antibiotics in feed on immune organs and growth performance of male laying hens. Experiment was designed in Completely Randomized Design with 5 treatments and 4 replicates. The study Animal used 120 DOC (Day Old Chick) of male laying hens. Treatments consist of: P_1 = Basal feed; P_2 = Basal feed + 0.005% tetracycline ; P_3 = Basal diets + 0.5% eugenol; P_4 = Basal diets + 1.0% eugenol; P_5 = Basal diets + 1.5% eugenol. Variables observed i.e.: immune organs (thymus, bursa fabricus, spleen, liver) and growth performance (weigh gain, feed intake, protein intake, energy intake, feed efficiency). The statistical analyses were carried out using Anova and LSD test. Results were shown high significant ($P<0.01$) effects on thymus (immune organ) and growth performance (weight gain, feed intake, protein intake, energy intake, feed efficiency) of male laying hens. However, no significant ($P>0.05$) effects on bursa fabricus, spleen, and liver (immune organs). Our finding suggest that the use of eugenol clove leaf oil as a substitute for antibiotics in feed, with a level of 0.5%-1.0% of male laying hens resulted similar response to the use of tetracycline 0.005%.

Keywords: Eugenol clove leaf oil, Growth performance, Immune organs, Male laying hens.

INTRODUCTION

In general, the poultry farming business consists of native chickens, laying hens and broilers. The parent stocks of laying hens in breeding farms produce female chicks which are reared to produce eggs while male chicks can be reared for meat production.

The probability of producing either male or female chicks in breeding farm is 50%. Thus, the possibility of laying male

chicks being used as meat-producing birds is quite large. Recently, many poultry farmers have started raising male laying hens for the purpose of producing meat. Due to the taste of male laying hens meats are similar to native chickens. This can meet the demand for native chicken. This meat that is difficult to fulfill. Various efforts have been made to promote growth, improve feed efficiency, chicken health status, and meat quality.

Antibiotic Growth Promotors (AGP) has been effectively used in poultry feed for stimulating growth, preventing disease and treating sickness among the animal (Khaksar, *et.al*., 2012). However, with the increasing use of AGP in animal feed problems of food safety, resistant bacteria and antibiotic residues are harmful to consumers (Phillips *et al*., 2004). Other negative impacts of the use of antibiotics are affecting the balance of intestinal micro flora, accumulation of antibiotic residues, and the development of new strains of drug-resistant pathogenic bacteria (Castanon (2007).

An alternative to reduce the use of AGP in animal feed is the use of plant extracts containing phytobiotics. Cross, *et al*. (2007) reported that the use of natural compounds from plant extracts can function as antibiotics. The use of natural antibiotics (phytobiotics) in animal feed has a positive effect on growth performance and animal health (Windisch, *et.al*. 2008).

Eugenol is one of the components of clove leaf oil which can function as an antibiotic, antimicrobial, and antioxidant. Eugenol content of clove leaf oil is 79.72% and antioxidant is 42.26 ppm. The higher the eugenol content of clove leaf oil, the higher the antioxidant content (Tahir, *et al*. 2019. Razafimamonjison, *et al*.(2015) stated that the smell of clove leaf is caused by the content of eugenol which is the main compound (72-90%) and functions as an antiseptic and anesthetic.

Analysis of clove leaf compounds Bangladesh scientists using the GC-MS method found that eugenol were 74.28%, eucalyptol 5.78%, caryophyllene 3.85%, - cardinol 2.43%, limonene 2.08% (Bhuiyan, *et al*, 2010). Clove essential oil has chemical properties and pharmacological effects that function as anesthetics, antimicrobials, antiseptics, antioxidant and imunomodulator (Dehgani et al, 2012). Clove leaf phenolic compounds also contain antioxidants and flavonoid compounds (Dibazar, *et al*., 2015).

Eugenol clove leaf oil can biologically function as anti-bacterial, anti-fungal, insecticide and antioxidant and has been used traditionally as a flavoring and antimicrobial agent in food (Lee dan Shibamoto, 2001; Huang *et al*, 2002;Velluti *et al*, 2003).

The use of clove leaf eugenol in diets is expected to function as a growth promoter that can replace AGP (Antibiotic Growth Promoter) in poultry feed specifically for male laying hens. The eugenol component can improve digestive enzymes thereby increasing animal growth (Williams and Losa, 2001).

The aim of the study was to evaluate the inclusion of eugenol clove leaf oil as a feed additive to replace antibiotics on immune organs and growth performance of male laying hens.

RESEARCH METHODS

Animal and Feed Experiment

A total of 120 DOC (Day Old Chick) male laying hens were applied in this experiment. The chicks were kept in brooder cages for 2 weeks and the chicks were vaccinated with ND Strain Lasota on day 3. The end of week 2, chicks were weighed and distributed to the cages for feeding the treatment diets. Each cage was marked with treatment code and the 6 chicks were distributed in the cages. Size of each cage 100 x 100 x 75 cm (length x width x height) with a slat cage pad. The chicks were randomly distributed into 20 cage base on the treatment applied.

Feed ingredients used consisted of maize, rice bran, soy bean, fish meal, top mix, methionine, lysine, cooking oil and

mineral mix. Composition of feed ingredients as basal feed and nutrient composition are shown in Table 1.

The chickens and feed were weighed weekly to determine weekly body weight gain. Data collection was carried out 12 weeks. At the end week 12, the chickens were slaughtered by cervical dislocation and the immune organs were observed by removing and weighing each immune organ (thymus, bursa fabricus, spleen, liver).

Research Design and Treatment Diets.

The study was designed using a completely randomized design with 5 treatments and 4 replications. Data were analyzed using Anova. The treatments were:

P_1: Basal diets (Table 1)
P_2: Basal diets + tetracycline 0.005%
P_3: Basal diets + eugenol clove leaf oil 0.5%
P_4: Basal diets + eugenol clove leaf oil 1.0%
P_5: Basal diets + eugenol clove leaf oil 1.5%

Variable Observed

Growth Performance.

Growth performance were observed i.e. weigh gain, feed intake, protein intake, energy intake, and feed efficiency.

Immune organs.

Immune organs measurements were carried out by observing the immune organs. At the end of the experimental period, birds from each treatment were individually weighed and slaughtered. Weight of immune organs (thymus, bursa fabricus, spleen and liver) were individually recorded. Thymus organ is located inside the skin of the neck. Bursa fabricus is located at the bottom of the cloaca in the form of a white bulge. All that organs were expressed as a percentage of live body weight.

Statistical Analysis.

Collected data were subjected to Anova (Steel and Torrie, 1995) and LSD (Least Significant Difference) test for comparison mean test.

Table 1. Feedstufs and basal feed composition, and calculated proportion of nutrient components.

Feedstufs	Basal feed composition (%)
Maize	57.00
Rice bran	7.00
Soybean meal	15.00
Fish meal	18.00
Top Mix	1.00
Methionine	0.22
Lysine	0.18
Cooking oil	2.00
Mineral Mix	0.71
Total	100

Calculated proportion of nutrient components :

Protein (%)	20.41
ME (kcal/kg)	3,035
CF (%)	4.58
Fat (%)	5.86
Ca (%)	0,97
P (%)	0.77
Metionine (%)	0.49
Lysine (%)	1.32

RESULTS AND DICUSSION

Results

Growth Performance

The results of growth performance observed i.e.: weight gain, feed intake, protein intake, energy intake and feed efficiency are shown in Table 2.

Immune Organs

The results of immune organs observed i.e.: thymus, bursa fabricus, spleen and liver are shown in Table 3.

Table 2. Average growth performance of chickens on weeks 12.

Growth Performance	Treatments				
	P_1	P_2	P_3	P_4	P_5
Weigh gain (g)**	566.54[a]	645.46[a]	572.79[a]	415.96[b]	289.48[c]
Feed intake (g)**	3,363.22[a]	3,291.84[a]	3,050.17[a]	2,893.05[b]	2,595.47[b]
Protein intake (g)**	680.38[a]	665.94[a]	646.75[a]	585.26[b]	525.06[b]
Energy intake(kcal)**	10,207.36[a]	9,990.73[a]	9,702.92[a]	8,780.40[b]	7,877.24[b]
Feed efficiency **	0.16[a]	0.19[a]	0.18[a]	0.14[b]	0.11[c]

Noted: ** High significant (P< 0.01) from the treatments [a, b, c] Different superscripts on the same line are significantly different (P<0.05)

Table 3. Average of immune organ on week 12.

Immune Organs	Treatments				
	P_1	P_2	P_3	P_4	P_5
Tymus (%) **	0.23[a]	0.13[b]	0.14[b]	0.12[b]	0.10[c]
B.Fabricus(%) ns	0.06	0.07	0.07	0.09	0.10
Spleen (%) ns	0.25	0.25	0.27	0.28	0.17
Liver (%)ns	2.40	2.30	2.39	2.71	2.54

Noted: ** High significant (P< 0.01); ns = Non significant (P>0.05) [a,b, c,] Different superscrip on the same line are significantly different (P<0.05).

Discussion

Growth Performance

The results proved that there was a high significant (P < 0. 01) effects of treatments of eugenol cloves leaf oil in the feeds on growth performance (weigh gain, feed intake, protein intake, energy intake, and feed efficiency. The LSD test showed that the treatments were significantly different (P<0.05) among the treatments of P_4 and P_5 vs. P_1, P_2 and P_3 on weigh gain, feed intake, protein intake, and energy intake. However, the treatments between P_1 vs P_2 and P_3 , P_2 vs P_3, and P_4 vs P_5 were shown no significant difference (P> 0.05) among the variables observed. These results indicate that the use of 0.5% eughenol of clove leaf oil has a relatively similar effect with the use of 0.005% tetracycline as an antibiotics. Eugenol of clove leaf oil in chicken feed could be function as a natural antibiotic (phytobiotic) that can replace the use of commercial antibiotics which have been prohibited from being used in animal feed.

LSD test of feed efficiency resulted that the treatment shown a significant difference (P < 0.05) between treatment of P_4 vs P_1, P_2, P_3, and between treatments P_4 and P_5. The highest feed efficiency value was found in treatment P_2 (0.19) with the used of 0.005% of antibiotics and similar results with the treatment P_3 (0.18) used of 0.5% of eugenol clove leaf oil and P_1 (0.16) as a control diet.

The increasing use of eugenol of clove leaf oil in the feed of male laying hens resulted in a decrease in feed efficiency. These indicates that the eugenol content of clove leaves oil in the feed affects the absorption of nutrient components, thereby reducing growth performance and feed efficiency.

Scherer *et al*. (2014) reported that eugenol and eucalyptol contain strong antioxidant and antimicrobial activity. The use of eugenol 500 mg/kg feed gave the same response to the growth performance of broilers aged 21 days with antibiotic (avilamycin) 10 mg/kg feed. Several natural compounds used as substitutes for antibiotics in animal feed have a positive effect on growth performance and animal health (Aguilar *et al.* 2013). Supplementation of clove essential oil and cinnamaldehyde in feed can increase feed intake and body weight of broiler chickens (Chalghoumi *et.al*. 2013).

Immune Organs

The results of immune organs variable were shown in a high significant ($P < 0.01$) effects on thymus. However in other variables (bursa fabricus, spleen and liver) were found no significant ($P>0.05$) effects with adding eugenol cloves leaf oil in the feeds. LSD test on thymus showed that the treatments were high significantly different ($P<0.05$) between treatments of P_1 vs P_2 , P_3 , P_4 , P_5, and P_5 vs P_2 , P_3 , P_4. Another treatments P_2 vs P_3 and P_4 , P_3 vs P_4, were shown no significant difference ($P> 0.05$).

The increased use eugenol clove leaf oil in feed resulted a decrease in the thymus organ. This is similar with the decrease in feed intake, which results in reduced nutrient elements entering the digestive organs, cause the chickens become stressed due to the lack of nutrient elements for the body's needs. This finding similar results by Tizard (1988) stated that a rapidly atrophied thymus is a reaction to stress, cause animals that die from prolonged illness have very small thymus. The thymus is a T cell regulator that acts on primitive cells derived from the bone marrow and makes these cells immunologically capable of acting as the body's antibody-forming agent. Ikhsan *et al*., (2019) reported the relative weight of the thymus in super native chickens of 12 weeks old were 0.17-0.22%. This value was similar from the results of this study, which is 0.10-0.23%.

Bursa fabricus, spleen, and liver were not affected by the use of eugenol clove leaf oil. The use of natural antibiotics has a positive effect on the body's metabolism, this is indicated by the relative weight of the liver which is uniform or not significantly different between the treatments. Results of this study range of 2.30 - 2.71% in male laying hens liver, broiler liver with range of 3.03 -4.85% (Hermana *et.al*. 2008) and 4.37 -4.80% on broiler 10 days old (Suminto *et.al*. 2020).

CONCLUSION AND SUGGESTION

Conclusion

Conclusions of this study are as follows:

1. The use of eugenol clove leaf oil as an antibiotic in diets affects growth performance (weigh gain, feed intake, protein intake, energy intake, feed efficiency) and immune organ (thymus)of male laying hens.
2. A level of 0.5%-1.0% of used eugenol clove leaf oil were resulted a similar response to the use of antibiotics (tetracycline) as a commercial antibiotic and control basal diets.

Suggestion

The use of commercial antibiotics such as tetracycline in poultry feed should be avoided and replaced by the use of eugenol clove leaf oil at a dose of 0.5%-1.0%.

Acknowledgement

Authors would like to thank LPPM University of Tadulako and Dean of Faculty of Animal Husbandry and Fishery, who have provided Research Fund.

Me also thank to the students Feni Rahmasari and M.R.Alsahab, who joined the research team.

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