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Effects of Garlic Extract (*Allium sativum* Linn.) Meal on Feed Efficiency and Immunity of Tropical Chickens

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ABSTRACT

Exploring the advantages of medicinal plants encourages research on the effectiveness of medicinal plants as a substitute for antibiotics to stimulate metabolism and immunity and improve the performance and productivity of tropical chickens. This research was conducted to determine the feed efficiency and immunity of tropical chickens fed a basal diet substituted with varying amounts of garlic extract (GE). This study utilized 150 chickens that were grouped into five treatments. Each treatment had three replicates, and each replicate consisted of 10 chickens. The treatment was GE0 = control diet, GE2 = basal diet + GE 2%, GE4 = basal diet + GE 4%, GE6 = basal diet + GE 6%, and GE8 = basal diet + GE 8%. The results showed that adding garlic extract positively affected this research, indicating that garlic extract might improve the feed efficiency and immunity of tropical chickens.

Keywords: feed efficiency, garlic extract, immunity, tropical chickens

INTRODUCTION

Several studies have shown that ambient temperature affects the immune responses of chickens. Indonesia is a tropical country with great potential for developing microorganisms that cause disease, thereby decreasing livestock production. The high temperature of the tropical environment is the cause of oxidative stress when free radicals exceed antioxidants (Arain et al., 2018). Free radicals attack saturated fatty acids on cell membranes and form lipid peroxides. Lipid peroxide formation in the body causes cell damage, such as in immune cells (Yaman and Ayhanci, 2021).

Chickens in the tropics exposed to high temperatures are stressed, which is their response to adapting to the environment. The adaptation process causes the release of stress hormones that require more energy and protein. Stress also increases the levels of corticosterone hormones in chickens (Qaid and Mutassim, 2016), which increases the use of glucose as an energy source (Slimen et al., 2016). The continued effect will inhibit protein synthesis and reduce growth, reproduction, and health (Abdenour et al., 2019)

High environmental temperatures decrease the weight of lymphoid organs such as the thymus, bursa Fabricius, and lymph, reducing the performance of lymphoid organs (Yanai et al., 2018). Leukocytes are mobile or active units of the defence system. Leukocytes form in the bone marrow and lymph organs and are transported by blood to the body (Gasisova et al., 2017). Leukocytes are divided into two classes based on their histology: granulocytes and agranulocytes. The granulocytes consist of heterophile, eosinophils, and basophils; therefore, the agranulocytes consist of lymphocytes and monocytes (Soliman et al., 2023). Furthermore, according to Ebeid and Al-Homidan (2022), stress decreases humoral and cellular immune responses, resistance to infectious diseases, and damage to the immune system of poultry.

High mortality rates and low productivity constrain chickens under tropical conditions due to the influence of high ambient temperatures and humidity. High ambient temperatures suppress the immune response to produce antibodies, affecting non-optimal vaccination. To solve the problem, tropical farmers use antibiotics in animal feed, which becomes problematic due to the adverse effects on chickens and consumers of chicken products through residues on meat and eggs.

Various efforts have been made to find additional ingredients given to feed as a substitute for antibiotics that do not cause harmful effects and do not leave residues on livestock products. Additives to replace antibiotics must be able to increase livestock production without providing side effects for

livestock or consumers. The potential of Indonesian medicinal plants has not been fully utilized. More than 1000 plants in tropical rainforests have medicinal compounds; only a few species are used for the medicine and herbal medicine industries and are intensively cultivated (Utami et al., 2018). Medicinal plants are used mainly for preventive, promotional, and rehabilitative purposes (Sen and Cakraborty, 2017).

Garlic contains alliin and allicin essential oils related to antimicrobial activity (Ezeorba, 2022). Allicin fights amoebas, bacteria, fungi, and viruses. Antimicrobial activity depends on the enzyme alliinase (Alam et al., 2016). The alliinase enzyme kills gram-positive and gram-negative bacteria (Borlinghaus et al., 2021). Another compound found in garlic was allithiamine, which resulted from the reaction of allicin with thiamine and cysteine. Its function is like vitamin B, which helps metabolism (Biró, 2020).

An attempt to reduce the prevalence of disease and heat stress in tropical chickens is to improve the immune system, which can be recognized by lymphoid organs consisting of the bursa Fabricius, thymus, and lymph. The decline of the immune system is indicated by the disruption of immune system function and damage to lymphoid organs (Lewis et al., 2019). Several researchers reported that garlic extract enhances the initial primary immune response in white mice and affects cellular immunity by increasing bacterial killing. Garlic is an antioxidant that scavenges oxygen species and increases the activity of superoxide dismutase enzymes, catalase, and glutathione peroxidase (Yousefi, 2020).

Many researchers have reported the effects of garlic extracts on performance. Still, a lack of information about the effects of garlic extracts is given to tropical chickens subjected to heat stress, and tropical conditions are very conducive to microbial growth. The effect of garlic extract on the metabolism and immunity of chickens in the tropics is not widely known, so this study is necessary to determine the effect of garlic extract with performance parameters to justify the effect of metabolism and parameters of the relative weight of lymphoid organs and the amount of leucocytes and lymphocytes, which are indicators of immunity.

MATERIALS AND METHODS

Garlic extract (*Allium sativum* Linn.) preparation

About 250 grams of mashed garlic were mixed with 96% ethanol to make as much as 500 mL. The garlic solution obtained was filtered and then filtered again with Whatman paper no. 2 to obtain the filtrate (crude extract). The filtrate was collected in an Erlenmeyer tube and then in a vacuum rotary evaporator to evaporate the ethanol and leave the garlic extract (GE) (Utami et al., 2018).

Experimental animal and treatments

A total of 150 chickens were grouped into five treatment groups. Each treatment consisted of three replicates, and each replicate consisted of 10 chickens. The treatment was GE0 = control diet, GE2 = basal diet + GE 2%, GE4 = basal diet + GE 4%, GE6 = basal diet + GE 6%, and GE8 = basal diet + GE 8%. The basal diet of tropical chickens contains metabolic energy of 3100 kcal/kg, as recommended by Phuong and Thiet (2023), and 21% protein. The complete nutrient composition of the experimental feed is shown in Table 1. The diet treatments were fed to one-day-olds for up to 35 days. Furthermore, on day 35, the thymus weight, lymph weight, and bursa of Fabricius weight were carried out, and blood sampling was carried out for the leucocyte test and lymphocyte test, with as many as three blood samples for each replicate.

The observed parameters were feed intake (FI), calculated from the difference between the feed given and the remaining feed daily. Body weight gain (BWG) was calculated by weighing the body weight of chickens starting on the first day of each treatment group.

Table 1. Nutrient composition of the treatments

Nutrient	GE0	GE2	GE4	GE6	GE8
ME, Kcal/Kg	3100	3096	3092	3088	3084
CP, %	21.00	19.04	19.08	19.12	19.16
CF, %	2.65	2.59	2.58	2.52	2.46
EE, %	4.12	3.98	3.88	3.72	3.49

ME: metabolizable energy, CP: crude protein, CF: crude fibre, EE: extract ether

The final weight (FW) was obtained from the difference between the initial and final body weights. Feed conversion (FCR) was obtained from the ratio between the feed consumed and body weight gained. The thymus, the bursa Fabricius, and the lymph were weighed on a scale with a sensitivity of 0.01 gram, and then the relative weight was calculated and compared to body weight. Total leucocyte and lymphocyte cell counting by Seliger et al. (2012) method.

Data Analysis

The data obtained were analyzed using a one-way ANOVA; if the ANOVA indicated significance (P<0.05), a Duncan post hoc test was performed using SPSS version 22.

RESULTS AND DISCUSSION

Growth performance

The growth performance of chickens fed with various substitution levels of garlic extract (GE) is shown in Table 2.

Table 2. Growth performance of chickens affected by garlic extract

Parameter	GE0	GE2	GE4	GE6	GE8	SEM	p
IW (1d), g	42	41	40	41	42	--	--
FW(35d), g	1403.2[a]	1610.2[b]	1557.5[b]	1540.8[b]	1548.5[b]	20.01	0.001
FI, g	3251.0[b]	2991.3[a]	3044.7[a]	3045.0[a]	3062,3[a]	30.87	0.042
EMI, g	287.9[a]	264.6[b]	268.9[b]	268.6[b]	269.8[b]	2.78	0.035
PI, g	18.6[a]	16.3[b]	16.6[b]	16.6[b]	16.8[b]	0.24	0.001
BWG, g	1445.2[a]	1651.2[b]	1597.5[b]	1581.8[b]	1590.5[b]	19.91	0.002
FCR	2.25[a]	1.81[b]	1.91[b]	1.93[b]	1.93[b]	0.04	0.001

[a,b] Means with different superscript letters in the same row are significantly different (P<0.05), IW: initial weight, FW; final weight, FI: feed intake, BWG: body weight gain, FCR: feed conversion ratio, EMI: energy metabolizable intake, PI; protein intake. GE0 = control diet, GE2 = basal diet + GE 2%, GE4 = basal diet + GE 4%, GE6 = basal diet + GE 6%, and GE8 = basal diet + GE 8%

During the growth period, stressed chickens decrease weight gain and feed efficiency. Body weight gain at control (GE0) was the lowest (p<0.05) compared to all garlic extract treatments. Feed intake (FI), body weight gain (BWG), and feed conversion ratio (FCR) were affected (P<0.05). In this study, the feed was given in the amount according to the standard requirements of the NRC, revised by Applegate and Angel (2014).

Antioxidants increase the activity of enzymatic antioxidants such as catalase, superoxide dismutase, and glutathione (Kumbhar et al., 2018). Garlic extract contains diallyl disulfide, a compound that is the most important biological compound (Song et al., 2021). Diallyl disulfide increases the activity of glutathione transferase through sulfur biological activity (Cheng et al., 2016). Glutathione activity prevents liver cell damage due to disease or stress. Preventing liver damage allows the liver to work optimally to secrete enzymes beneficial to the metabolic process (Vairetti et al., 2021). According to Kozeniecki et al. (2020), the liver's primary function is to form and secrete bile into the small intestine and play a role in the metabolism of carbohydrates and proteins.

Without impaired liver performance, the metabolism and absorption of nutrients are optimal and will not be affected. Garlic extract provides metabolic efficiency, evident from the increase in body weight at lower EMI and PI and higher final body weight compared to the control (p<0.05). These showed the efficiency of using protein converted to weight gain in garlic extract treatment compared to controls. Based on weight gain data obtained from Table 3 and the relationship between BWG and FCR (Figure 1).

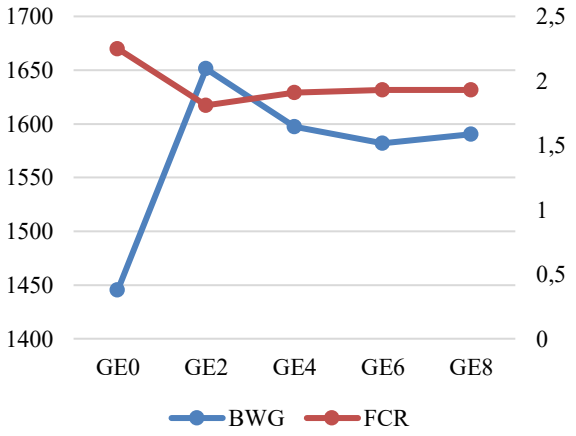

Figure 1. Body weight gain and feed conversion affected by garlic extract

Garlic extract treatment has resulted in almost the exact feed conversion at all levels in the range of 1.81 to 1.93, lower than the control (P<0.05). Low feed conversion indicates high feed efficiency in chickens treated with garlic extract.

Lymphoid Organs

The lymphoid organs that play a role in the immune system are the thymus, the bursa fabricius, and the lymph (Islam et al., 2023). The thymus regulates the immune response to infected cells. The thymus will differentiate into T lymphocytes that regulate the immune system's response. Chickens exposed to high temperatures for a long time will cause the thymus to atrophy, which is a stress reaction, so chickens stressed for a long time have a small thymus size (Ahmad et al., 2022). The relative weight of the thymus in control decreased (P<0.05) compared to the garlic extract treatment; this indicates that garlic extract was able to reduce stress in chickens, which resulted in a decrease in thymus weight.

The fabricius bursa regulates lymphocyte production and differentiation (Taves et al., 2017). Chickens under heat stress will increase the secretion of corticosterone hormones. According to Zhang et al. (2018), the presence of corticosterone hormones strongly influences a decreased weight of the bursa fabricius. El-Kazaz and Havez (2020) and Rebez et al. (2023) reported that corticosterone hormones were proven to reduce the growth of lymphoid organs and cause atrophy of the bursa fabricius. The relative weights of the thymus, the bursa of fabricius, and the lymph are shown in Table 3.

Table 3. The relative weight of lymphoid organs, the total amount of leucocytes, and lymphocytes of chickens affected by garlic extract

Parameter	GE0	GE2	GE4	GE6	GE8	SEM	P
Thymus, %	0.15[a]	0.18[b]	0.18[b]	0.19[b]	0.19[b]	0.001	0.08
Bursa fabricius, %	0.13[a]	0.15[b]	0.15[b]	0.15[b]	0.15[b]	0.003	0.00
Lymph, %	0.14[a]	0.08[b]	0.08[b]	0.08[b]	0.08[b]	0.005	0.00
Leucocytes, /µl	33786[a]	31239[b]	30863[b]	31392[b]	29636[b]	400.83	0.001
Lymphocytes, /µl	32407[a]	28543[b]	29316[b]	28583[b]	28082[b]	446.09	0.000

[a,b] Means with different superscript letters in the same row are significantly different (P<0.05), GE0 = control diet, GE2 = basal diet + GE 2%, GE4 = basal diet + GE 4%, GE6 = basal diet + GE 6%, and GE8 = basal diet + GE 8%

The relative weight of the bursa of fabricius at control was the lowest (P<0.05) compared to treatment using the garlic extract. Garlic extract suppressed the secretion of corticosterone hormones, thereby reducing the decrease in weight of the bursa of fabricius (P<0.05), so there was no weight loss as in control. The relative weight of lymph at control was higher (P<0.05) than the garlic extract treatment. According to Seo et al. (2012), garlic extract protects against the immune system, endothelial function, oxidative stress, and inflammation. In this research, garlic extract reduced the work of the lymph so that the lymph did not enlarge in size compared to the control (P<0.05).

Leukocytes function in the phagocytosis of antigens (Pittman, 2016). Fluctuations in leukocytes depend on stress conditions (Chmielewski and Strzelec, 2018). The highest number of leukocytes in the control was 33786/µL (P<0.05) above the normal range, which is from 6000 to 30000 µL (Bernhard et al., 2016). The increase in leukocytes is due to stress conditions caused by tropical temperatures and humidity (Diaz et al., 2021). The garlic extract treatment had a lower leukocyte count close to normal (P<0.05). Lymphocytes are the most numerous leukocytes, with varying sizes (Mokhtar and Abdelhafez, 2021). Lymphocyte cells are produced by the bursa fabricius, lymph, and thymus (Bölükbaş and Öznurlu, 2022). Lymphocytes act as cellular immunity obtained from the formation of activated lymphocytes (Vazquez et al., 2015). The number of lymphocytes in the treatment with garlic extract was lower than the control (P<0.05); high secretion of leukocytes and lymphocytes indicates severe inflammation (Simpson et al., 2015) due to the garlic extract's anti-inflammatory properties that reduce the secretion of lymphocytes in the blood circulation.

Some points obtained from this study were that garlic extract in feed reduces the effects of heat stress, characterized by a decrease in the secretion of corticosterone hormone, thereby

increasing metabolic efficiency, preventing liver damage, stimulating metabolism, and increasing feed efficiency by reducing feed conversion into body weight. Improvement in garlic extract treatment was characterized by low levels of leukocytes and lymphocytes, which indicate a decrease in stress in tropical chickens.

CONCLUSION

This study concludes that garlic extract in chicken feed positively affects feed efficiency, body weight gain, and performance. The effect of garlic extract on immunity is indicated by the prevention of atrophy in lymphoid organs and the average amount of leukocytes and lymphocyte cells, which indicates the absence of stress in the chicken. However, further research is needed to verify the long-term effect of garlic extract in feed as a substitution for antibiotics in tropical chickens.

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