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INFLUENCE OF *Zingiber zerumbet* EXTRACTS AS FEED ADDITIVE ON PERFORMANCE, CARCASS CHARACTERISTICS AND INHIBITION OF *Escherichia coli* BACTERIA OF COMMERCIAL BROILER CHICKENS

Rojaur Rafiqi[1], M. Afzalul Ryanda[1], M. Afid Fuadi[1], Nanda Riwa[1], Indra Wahyudi[1], Ilham[1], Allaily Allaily[1], Sitti Wajizah[1], and Samadi[1]*

[1]Animal Husbandry Department, the Faculty of Agriculture, Universitas Syiah Kuala Banda Aceh, Indonesia
***Corresponding author: samadi177@unsyiah.ac.id**

ABSTRACT

The purpose of this study was to evaluate administration of *Zingiber zerumbet (Z. zerumbet)* extracts on performances, carcass characteristics and *Echerichia coli (E. coli)* bacteria of commercial broiler chickens. One hundred day old CP 707 broiler chickens (mixed sex) were randomly distributed into 20 floor pens with four treatments and five replicates. Each group of broilers was treated with diffrent level of *Z. zerumbet* extracts in drinking water (P0= control, P1= 0.025 % in drinking water, P2= 0.050 % in drinking water and P3= 0.075 % in drinking water). Broiler chickens were fed commercial diet based on nutrition requirement for starter and grower. Feed and water were provided *ad libitum.* Body wright of chickens and feed were recorded weekly. All data were recorded to measure for performances, carcass characteristics and the inhibition of *E. coli* bacteria. The data were analyzed by one-way ANOVA. Statistically differences among treatments were stated with a probablity of P<0.05. The results of study indicated that administration of *Z. zerumbet* extract in drinking water with different concentration up to 4 weeks did not significantly influence (P>0.05) on the performances, carcass and giblet characteristics of broiler chickens. The inhibition of *E. coli* bacteria was significantly affected (P<0.05) by inclusion of *Z. zerumbet* at different concentration. In conclusion, administration of *Z. zerumbet* in drinking diet had no detrimental effect on animal performance, carcass and giblet characteristics.

Key words: Broiler, carcass, inhibition bacteria, performances, *Zingiber zerumbet*

ABSTRAK

Tujuan dari penelitian ini adalah untuk megevaluasi penggunakan bahan pakan herbal lempuyang gajah (Z. zerumbet) sebagai imbuhan pakan terhadap performa, karakteristik karkas dan giblet serta daya hambat bakteri E. coli pada ayam broiler. Sebanyak 100 ekor DOC (CP 707) broiler didistribukan secara random pada 20 unit kandang terdiri dari 4 perlakuan dan 5 ulangan. Setiap perlakuan diberikan ekstrak Z. zerumbet dengan level yang berbeda (P0= kontrol, P1= 0,025 % dalam air minum, P2= 0,050 % dalam air minum dan P3= 0,075 % dalam air minum). Pakan yang digunakan dalam penelitian ini adalah pakan komersil untuk masa pertumbuhan dan masa panen. Pertambahan berat badan dan konsumsi pakan dihitung setiap minggu. Semua data dicatat untuk mengetahui performa, karakteristik karkas dan daya hambat terhadap bakteri E. coli dari broiler. Data dianalisis dengan ANOVA satu arah. Perbedaan antar perlakuan dinyatakan apabila P<0.05. Hasil penelitian menunjukkan bahwa pemberian ekstrak Z. zerumbet sebagai imbuhan pakan tidak berpengaruh (P>0.05) terhadap performa, karkas, dan giblet karakteristik dari ayam broiler, tetapi berpengaruh terhadap daya hambat bakteri E.coli. Dari penelitian ini disimpulkan bahwa Z. zerumbet tidak menimbulkan efek negatif terhadap performa dan karkas ayam broiler.

Kata kunci: Broiler, karkas, daya hambat, performa, Zingiber zerumbet

INTRODUCTION

Antibiotic as growth promoters has been commonly used in the poultry diet due to its beneficial effects to improve animal productivity and inhabit the growth of harmful micro-organisms in the animal host. However, the use of antibiotics in animal diet has been banned since last decades due to the accumulation of antibiotic residues in animal origins such as in egg, meat and milk. In addition, it has been also reported that inclusion of antibiotics in animal diet resulted in antibiotic resistance to human and animals (Cosby *et al*. 2015; Medeiros *et al*. 2011). Therefore, the use of non-antibiotic substances such as pre-probiotics, botanical products, organic acids, enzymes as alternative feed additives has been reported (Samadi *et al*. 2019; Samadi *et al*. 2020; Imran *et al*. 2021; Wahyudi *et al*. 2021; Samadi *et al*. 2022). Phytogenic feed additives (PFA's) as one alternative feed additives contain huge variety of biologically active phytochemicals substances such as alkaloids, terpenes, flavonoids and glucosinolates (Upadhyay *et al*. 2014). These biologically active phytochemicals are considered to be able to stimulate blood circulation, inhibit the growth of pathogenic bacteria and improve immune status in host animals (Brenes and Roura 2010, Reisinger *et al*. 2011). Administration of PFA as feed additive in animal feed was able to improve animal performances (Hafeez *et al*. 2016).

Z. zerumbet is one of the potential phytogenic feed additives that can be administrated in animal diet to improve animal production. Dai *et al*. (2013) reported that *Z. zerumbet* contain huge number of bioactive compounds (46 bioactive compounds) that has the potency of beneficial effects to animals by improving animal growth. Suhirman *et al*. (2006) stated that *Z. zerumbet* contain bioactive compound functioned as functional feed such as zerumbon, -pinen, caryophyllene, camphor, cineol 1.8, humulen, caryophyllene oxide, humulent epoxide and cinnamaldehyde with the content of essential oil about 0.82%. Rahayu *et al*. (2020) reported that inclusion of *Z. zerumbet* with the concentration of 0.67% to 1% in animal diet was able to control Salmonellosis in broiler chickens. Furthermore, administration of *Z. zerumbet* as feed additives to improve animal growth has been

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reported (Sutardi *et al*. 2015; Pratama *et al*. 2018). The results of the experiments were not consistence from one to others. Based on these reasons, more researches relating to administration of *Z. zerumbet* extract as feed additives in animal diet are required to reach consistent results and as comparisons from other previous experiments.

The purpose of this study was to evaluate administration of *Z. zerumbet* extracts as alternative feed additives to antibiotics on broilers performances, carcass characteristics and *Echerichia coli (E. coli)* bacteria of commercial broiler chickens. Different from our previous study (Pratama *et al*. 2018), application of *Z. zerumbet* to broiler chickens was in the form of juice. In this study, *Z. zerumbet* was administrated in the form of extract and mixed in drinking water. This study was expected to be able to give more information about administration of biologically active phytochemicals in animals feed to produce a safe animal products for humans as consumers.

MATERIALS AND METHOD

Animal, Feeding, and Data Collection

Chicken growth study was carried out at private enterprise farm in Banda Aceh. The measurement of carcass characteristics was conducted at Animal Nutrition and Feed Technology Laboratory, Animal Husbandry Department, The Faculty of Agriculture, Universitas Syiah Kuala. While, inhibition test for *E. coli* was conducted at the Laboratory of Milk Processing Technology, Animal Husbandry Department, The Faculty of Agriculture, Universitas Syiah Kuala.

Total of 100 mixed-sex broiler chickens (CP 707) was randomly allocated at four different treatments and five replications and placed in 20 wood-shavings litters (1 x 1 m). The number of broilers placed in each cages was five chickens. The initial body weight of the chickens each treatments was 41.4±0.40, 42.0±0.45, 43.2±0.49 and 41.8±0.37 (mean±SE) for P0, P1, P2 and P3 respectively. Each treatment of broiler chickens was administrated different concentration of *Z. zerumbet* extract in drinking water (P0= control, P1= 0.025% of *Z. zerumbet* extract, P2= 0.050 % of *Z. zerumbet* extract, and P3= 0.075% of *Z. zerumbet* extract). The *Z.zerumbet* extract was obtained by maceration using 96% ethanol. A total of 5 kg of *Z. zerumbet* flour was macerated gradually using 96% ethanol with a ratio of 1:2 and allowed to stand for 24 hours, with the aim of breaking down the cell walls and membranes. Then the maceration results were filtered using filter paper and then evaporated using a rotary evaporator at a temperature of 40º C with a rotation speed of 60 rpm until a concentrated extract is obtained. Broiler chickens were fed a commercial broiler diet for starter and grower (511 and 512 Bravo) as requirement for broiler (NRC, 1994) with the content of 24% and 3200 kkal/kg for crude protein and energy respectively. Birds were vaccinated against Newcastle Disease and Infectious Bursal Disease during experimental period. Broiler was provided freely access to feed and water. Temperature of the cages was adapted based on the age of broiler chickens from 35° C at the beginning of study and decreased periodically to reach 24° C up 4 weeks of study. Feed, water and the weight of broiler chickens were recorded weekly to measure for feed intake (FI), water consumption (WTC) and feed conversion ratio (FCR) and body weight gain (BWG).

At the end of the growth study, twenty broilers (one from each pen) were selected to measure for carcass and giblet characteristics. Before selected broilers were slaughtered, feed was drawn and animals were fasted overnight only access for water. All animals were slaughtered by cervical dislocation in Islamic manner to obtain data for dressing percentage, carcass and giblet characteristics. All data carcass and giblet were weighted and recorded to be used for further data analysis.

Kirby-Bauer disk diffusion method to determine the resistence of pathogen bacteria to various antimicrobial compounds. In this method, three gradient doses of extract (250 mg/mL, 500 mg/mL, 750 mg/mL) was applied to test antimicrobial pathogen of *E. coli* bacteria (Biemer, 1973). Aquadest was applied as negative control and chloramphenicol was applied as positive control. *Z. zerumbet* extract was tested based on gradient dose which is placed patogen *E. coli* bacteria and incubated for 24 h. Inhibition zone was measured to determine the effectivity of *Z. zerumbet* as antimicrobial substance by measuring disk edge of paper disk to the end of clear zone in millimeter.

Data Analysis

All data collected in this study was statistically analyzed by employing ANOVA for one way analysis under completely randomized design (CRD). The mathematical model applied for this study was $Yij = \mu + \alpha i + \varepsilon ij$ where Yij is the observed value, μ is the overall mean, αi is the treatment effect and εij is residual error. All data were performed using the SPSS statistical software package (IBM Statistics 25.0). The data were presented as mean values ± SEM. Values were considered statistically different ($P \leq 0.05$) and trends were declared at $P \leq 0.10$. Post hoc test of Duncan's multiple range test (DMRT) were applied to compare different between treatments (Steel and Torrie, 1995).

RESULTS AND DISCUSSION

Broiler Performances

The effect of *Z. zerumbet* extracts administration in drinking water on body weight (a) feed intake (b), feed conversion ratio (c) and water consumption (d) of commercial broiler chickens is presented in the Figure 1. As indicated from the figures, administration of Z. zerumbet extract in drinking water with different concentration up to 4 weeks did not significantly affect the performance of broiler chickens (FI, BWG, FCR, and WTC). The body weight gain of broiler chickens up to 4 weeks was 1381.6g±39.2; 1473.6g±39.4; 1381.0g±82.7 and 1499.4g±59.3 for P0, P1, P2, and P3 respectively. The results of our study was the similar to study conducted by Hafeez *et al*. (2016) in which

broiler Cobb 500 supplemented with different phytogenic feed additives did not influence broiler performance (BW, BWG, FI, and FCR) at the age of 28 d, but in the overall production period (at the age of 1-42 d), administration of phytogenic feed additives (150 mg/kg methol and anethol essential oils in powdered form and 100 mg/kg carvacrol, thymol, and limonene in matrix-encapsulated form) significantly improved BW, BWG and FI. In our study, feed intake for the whole production period (at the age of 1-28) was in the range from 1.41 to 1.47, it was lower compared to Hafeez *et al*. (2016) with the range of feed intake from 1.54 to 1.57 (at the age of 15-28 d).

Study conducted by Fascina *et al*. (2012) found that broilers administrated with phytogenic acid and organic acid improved broiler performance and better carcass characteristics at the age of 42 days. Jamroz *et al*. (2005) stated that phytogenic feed additives such as cinnamon and curcumin stimulated pancreatic and intestinal enzyme secretion. Furthermore, the production of bile in the liver, pancreatic and intestinal lipase produced more enzymes and bile salts to promote the absorption of nutrient in the host animals (Platel and Srinivasan, 2004). El-Ashram and Abdelhafez (2020) found that Indian River broiler chickens supplemented with essential oil of thyme and atar anise improved final body weight gain. However, feed intake, FCR, and mortality ratio was not significantly affected by the supplementation of phytogenic feed additives.

In our study, inclusion of *Z. zerumbet* up to 0.075% in dinking water did not affect all the performance parameters. This is probably due to the low concentration of *Z. zerumbet* in the drinking water or the convenient condition such as environmental cages and temperature for broiler to growth. Others factors may affected for these discrepancies such as the source of microbial, concentration and frequency of phytogenic feed additives supplementation, environmental factors and method of phytogenic feed additives supplementation. As previous studies also indicated that the performances animals supplemented with phytogenic feed additives varied from one study and the others. Several experiments found that phytogenic feed additives improved animal performance by stimulation feed intake, increasing the secretion of digestive enzymes and activation of immune response, while the others were not affected. Botsoglou *et al*. (2002) reported that administration of 50 and 100 mg/kg oregano in the diet did not influence animal performance. Study conducted by Samadi *at al*. (2022) concluded that administration of *S. cumini* extract as phytogenic feed additives in drinking water did not significantly affect on broiler performances.

Carcass and Giblet Characteristics

Carcass characteristics of broiler chickens supplemented with different concentration of Z. zerumbet in drinking water is presented in the Table 1. As shown in the Table 1 inclusion of Z. zerumbet in defferent concentrtion did not significatly affect (P>0.05) carcass characteristics of broiler chickens. However, the weight of carcass characteristics treated with *Z. zerumber* was slightly higher compared to control. The weight of breast administrated with 0.075% Z. zerumber in drinking water was 50 g higher than control. In addition, the weight of drumstick in broiler chickens administrated with 0.025% of *Z.*

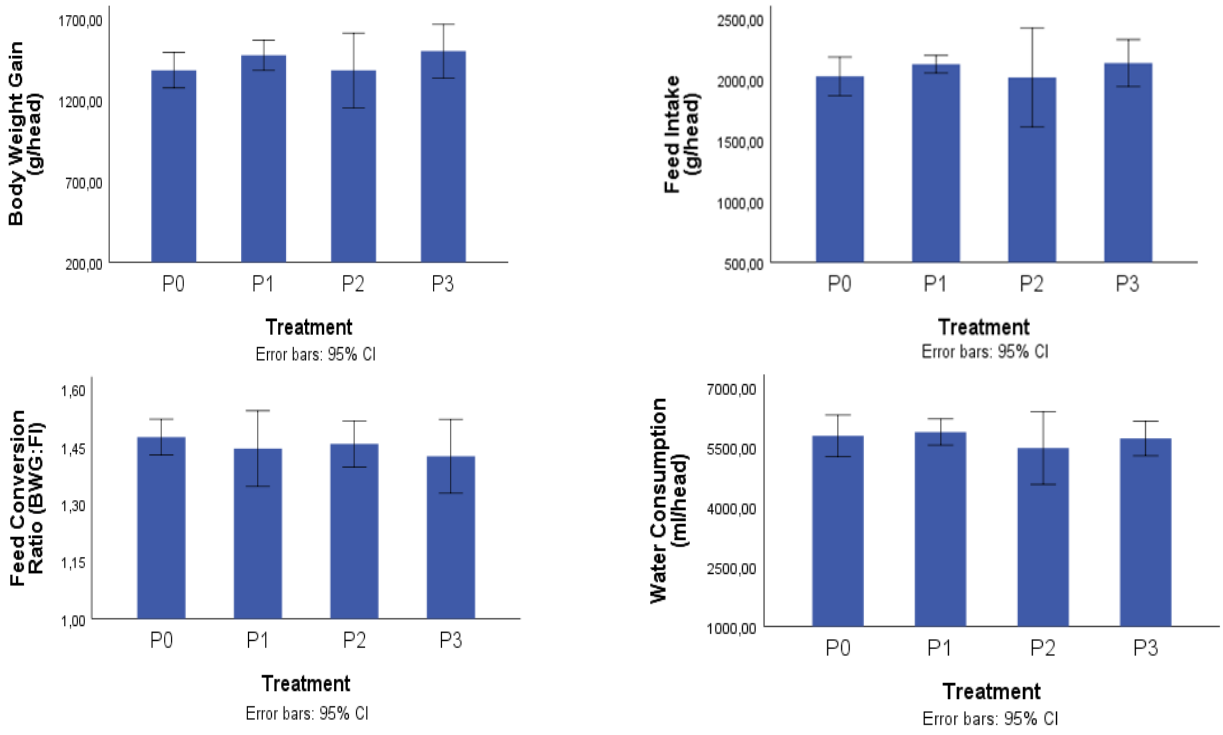

Figure 1. The Effect of *Zingiber zerumbet* extracts administration in drinking water on body weight (a) feed intake (b), feed conversion ratio (c) and water consumption (d) of commercial broiler chickens evaluated for 28 days. P0= control, P1= 0.025 % in drinking water, P2= 0.050 % in drinking water and P3= 0.075 % in drinking water

zerumbet was higher with the weight of 329.00g ±24.08 vs 352.00g±42.81 for P0 and P1 respectively. The higher weight of treated chickens compared to control, it was probably more absorbed nutrients utilized for carcass traits rather than to control. Our study was in line with the study conducted by Köseman *et al*. (2020) administrated phytogenic feed additives *(Alchemilla vulgaris)* in diet with the amount of 1% and 3% had no significant difference on both hot and cold carcass weight. In addition, El-Hady *et al*. (2020) had the similar results in which broilers supplemented with mixed essential oils as phytogenic extract herbs on carcass traits did not affect the relative weight of broiler chickens carcass. Al-Sagan *et al*. (2020) also found that the percentages of carcass dressing and leg weight was not significant influenced with the administration of fennel seed powder as phytogenic feed additives in broiler diet. In this study, the boiler chickens were raised under heat stress conditions.

In the poultry industry, the production of saleable products is very important to reach maximal profit. Therefore, the farmers should feed their animals with the high qualified feed for optimal animal production. In our study, carcass-cut such as breast, wings, drumstick and back of broiler chickens had no significant effect (P>0.05) by administration of *Z. zerumbet* in drinking water. Different from research conducted by Kiyma *et al*. (2017) in which birds fed diets supplemented with 24 and 48 mg/kg essential oil lavender had breast meat with higher brightness compared with birds that did not receive essential oil lavender. Improvement of breast meat by administration of phytogenic feed additive was also reported by El-Hady *et al*. (2020). In addition, supplementation of other feed additives such as various kinds of probiotic and pytobiotic improved significantly carcass weight and dressing percentage of carcass compared to control (Hassan *et al*. 2018). The improvement of carcass characteristics due to phytogenic feed additive administration in broiler chickens resulted in economic benefit for the farmers (Kafi *et al*. 2017).

The effect of *Z. zerumbet* extracts administration in drinking water on giblet characteristics of commercial broiler chickens evaluated for 28 days is presented in the Table 2. The result of the study indicated that giblet weight (gizzard, heart, and liver) was no significantly affected (P>0.05) by administration of *Z. zerumbet* extract in drinking water. No effect of giblet weight by administartion of *Z. zerumbet* indicated that this phytogenic feed additive no detrimental effect on internal organs of treated broilers. The range of giblet in our study was 26.2-30.0 g (1.85-2.03%), 6.3-6.8 g (0.41-0.46%) and 25.4-28.4 g (1.67-1.93%) for gizzard, liver and heart respectively. Our study is in accordance with the study conducted by Al-Sagan *et al*. (2020) the percentage of gizzard, heart, and liver had no significant effect on the boilers fed fennel seed powder

Table 1. The Effect of *Zingiber zerumbet* extracts administration in drinking water on carcass characteristics of commercial broiler chickens (Means±SE) evaluated for 28 days

Carcass Characteristics	Treatment				
	P0	P1	P2	P3	P_{value}
Carcass					
Weight (g)	1109,00±20,35	1121,00±35,62	1064,00±72,01	1158,80±49,82	0,59
Percentage (%)	75,23±0,49	74,53±0,85	74,83±0,99	74,85±0,91	0,95
Breast					
Weight (g)	376,00±14,53	399,00±16,99	379,00±29,77	426,00±24,00	0,38
Percentage (%)	33,87±0,94	35,57±0,81	35,55±1,08	36,78±0,92	0,23
Wings					
Weight (g)	122,00±3,74	124,00±4,85	113,00±3,74	119,00±6,95	0,43
Percentage (%)	11,01±0,37	11,06±0,31	10,72±0,44	11,12±0,81	0,95
Drumstick					
Weight (g)	329,00±10,77	352,00±19,14	382,00±24,63	339,00±13,82	0,76
Percentage (%)	29,56±0,67	31,34±0,93	30,78±0,42	29,27±0,33	0,12
Back					
Weight (g)	267,00±15,94	235,00±5,92	228,00±10,08	286,00±23,37	0,05
Percentage (%)	24,10±1,44	21,03±0,75	22,62±1,61	24,84±2,22	0,37

P0= Control, P1= 0.025 % in drinking water, P2= 0.050 % in drinking water, P3= 0.075 % in drinking water

Table 2. The Effect of *Zingiber zerumbet* extracts administration in drinking water on giblet characteristics of commercial broiler chickens (Means±SE) evaluated for 28 days

Giblet	Treatment				
	P0	P1	P2	P3	P_{value}
Gizzard					
Weight (g)	30,00±2,74	28,00±2,18	26,26±1,66	28,73±2,15	0,69
Percentage (%)	2,03±0,18	1,86±0,14	1,85±0,05	1,85±0,11	0,70
Liver					
Weight (g)	6,28±0,43	6,62±0,39	6,54±0,53	6,84±0,51	0,96
Percentage (%)	0,42±0,03	0,44±0,03	0,46±0,06	0,41±0,04	0,72
Heart					
Weight (g)	26,00±1,87	25,42±1,54	27,13±1,41	28,70±2,72	0,66
Percentage (%)	1,76±0,13	1,67±0,10	1,93±0,13	1,85±0,16	0,57

P0= Control, P1= 0.025 % in drinking water, P2= 0.050 % in drinking water, P3= 0.075 % in drinking water

at 0, 1.6, and 3.2% as a phytogenic feed additive. Similar finding has been reported by Panda *et al*. (2000) with application of other feed additives sources such as probiotic in diet with the amount of 100, 150 or 200 mg/kg diet had no significant influence on the weight of internal organs.

Study conducted by Mehala *et al*. (2021) inclusion of panchagavya as alternative to antibiotic growth promoter with the other phytogenic feed additives such as *Andrographis paniculata*, garlic and turmeric as an in commercial broiler chicken had no significant effect on the percentage of giblet, gizzard, liver and heart. The range percentage of giblet, gizzard, liver, and heart was 4.13-4.41%, 1.79-2.03%, 1.82-1.95% and 0.50-0.59% respectively. Similar response were also observed Umatiya *et al*. (2018) broiler chickens administrated with *Zingiber officinale* and garlic (*Allium sativum)* powder as phytogenic feed additives in diet did not have a significant effect on the percentage of heart, liver, gizzard and spleen of broiler chickens.

Inhibition of *Escherichia coli* Bacteria

Testing for inhibition activities of *Z. zerumbet* extracts as phytogenic feed additives by using *E. coli* at the concentration of 250 mg/mL; 500 mg/mL; and 750 mg/mL is presented in the Figure 2. As indicated in the Figure 2, *Z. zerumbet* extract had a significant effect ($P<0.05$) on the inhibition of *E. coli*. The ability of bioactive substances such as flavonoid in the *Z. zerumbet* extract was able to destroy microbial membrane as stated by Cowan (1999) microbial membrane and bacteria cell was disturbed lipolytic flavonoid. The higher of *Z. zerumbet* concentration was the wider of the formed clear zone. The clear zone each treatment of this study was 0, 2.4±0.25, 3.00± and 4.2±0.37 for P0, P1, P2 and P3 respectively. Davis and Stout (1971) divided antimicrobial activities criteria into four categories according to inhibition zone ≤ 5 mm , 5-10 mm, 10-20 mm, and ≥ 20 mm for weak, middle, strong and very strong respectively. Based on this category, the result of our study indicated that *Z. zerumbet* with the treatment concentration was categories as week antimicrobial activities.

The study conducted by Samadi *et al*. (2020) by using various local phytogenic such as *Vernonia amygdalina* Del., *Calotropis gigantean*, *Syzygium oleana* and *Syzygium cumini*, L as the sources of antimicrobial for non-ruminant animals indicated that *Syzygium cumini*, L was the highest phytogenic feed additives with inhibition zone diameter of 4.80 for *E. coli* and 12.78 for Salmonella with the dose of 750 mg/mL. This finding was in accordance with our study in which the inhibition zone diameter for *E.coli* was about 4.2 at the *Z. zerumbet* extract concentration of 0.075%. The ability of *Z. zerumbet* to kill *Streptococcus mutans* bacteria with the concentration of 500 µg/mL has been reported by da Silva *et al*. (2018). Furthermore, study conducted by Minyi *et al*. (2020) using fresh and dry rhizome essential oil and zerumbone as antimicrobial found that the antimicrobial capacity of *Z. zerumbet* rhizome oil was relating to the content of zerumbon. The results of this indicated that fresh and dray essential oil of *Z. zerumbet* had a significant antimicrobial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *E. coli*, and *Proteus vulgaris* with the concentration of minimum inhibitory about 31.25 to 156.25 µg/mL and the concentration of minimum bactericidal concentration about 62.50 to 625.00 µg/mL. In this study, it was concluded that oven drying of *Z. zerumbet* significantly reduced zerumbone content which resulted in decreasing of the antimicrobial activities of *Z. zerumbet* rhizome essential oil.

CONCLUSION

In conclusion, *Z. zerumbet* extracts as phytogenic feed additives can be applied as alternative to antibiotic growth promoter in broiler chickens. Administration of *Z. zerumbet* in drinking diet of broiler chickens had no significantly effect on animal performance, carcass and giblet characteristics.

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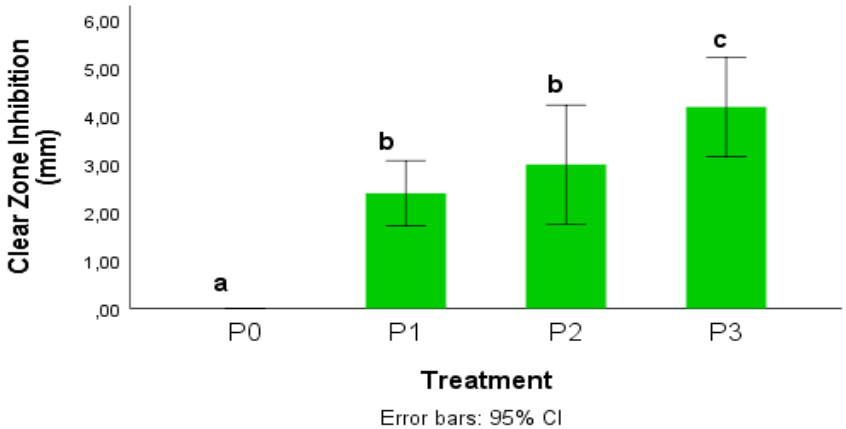

Figure 2. Testing for Inhibition activities of *Zingiber zerumbet* extracts as phytogenic feed additives by using *Escherchia coli* at the concentration of 250 mg/mL; 500 mg/mL; and 750 mg/mL. P0= Control, P1= 0.025 % in drinking water, P2= 0.050 % in drinking water and P3= 0.075 % in drinking water

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