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Research Article

Effects of Garlic Supplementation and Levels of Supplementation on the Performance of Tre Chickens in the Mekong Delta of Vietnam

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Abstract | A study was carried out in the Mekong Delta of Vietnam to examine how Garlic Supplementation and different levels of supplementation affected the performance of Tre Chickens. The study used experiments (3*2*3 factorial) with 2 factors. The first factor was the level of garlic supplementation, with three levels: 0.5%DM (G0.5), 1%DM (G1.0), and 1.5%DM (G1.5). The second factor was the type of garlic, with two options: Fresh Garlic (FG) and Black Garlic (BG). The study involved 180 Tre chickens, aged 6 weeks and weighing approximately 200±6.5 g/bird. Each experimental unit consisted of 10 birds, with a balanced distribution of sexes. The treatments included FG0.5, FG1.0, FG1.5, BG0.5, BG1.0, and BG1.5, where Fresh Garlic and Black Garlic were added to the basal diet at 0.5%, 1%, and 1.5% (DM) concentrations. The trial lasted for 8 weeks, covering the period from 6 to 13 weeks of age for the Tre chickens. The findings demonstrated that supplementing the diet of Tre chickens with Garlic at a concentration of 1.0 - 1.5% (DM) resulted in weight gain and improved feed conversion ratio (FCR). Notably, the G1.5 treatments yielded the most favorable outcomes. In terms of Garlic type, the BG treatments outperformed the FG treatments. The analysis of chicken manure microorganisms revealed that the G1.5 treatments exhibited the highest levels of Lactobacilli, while the G1.5 treatments demonstrated the most favorable results with regards to lower levels of *E. coli* and Clostridium. Overall, the results indicated that incorporating 1.5% (DM) Black Garlic into the diet of Tre chickens between 6 to 13 weeks of age produced the best outcomes.

Keywords | Tre chickens, Digestibility, *Lactobacilli*, *E. coli*, Clostridium

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INTRODUCTION

Tre chickens, commonly raised in the Mekong Delta of Vietnam, are small in size, with male chickens weighing around 900g-1000g and females weighing approximately 700g-800g after about 100 days of rearing. These chickens are known for their delicious meat qualities and possess a natural ability to forage for food. They also exhibit good resistance to diseases, making them a preferred choice among livestock breeders.

The addition of garlic to chicken diets has been found to enhance the health and meat quality of the birds. Research suggests that supplementing broiler rations with garlic at a 1% level can improve the performance of broilers, serving as a potential alternative to antibiotic growth promoters in broiler feeding (Issa and Omar, 2012).

Black garlic, on the other hand, addresses several drawbacks of fresh garlic and contains valuable biologically active substances. As a result, it is increasingly being utilized in daily practices to promote health and prevent diseases. However, further studies are necessary to fully establish the efficacy of black garlic in preventing and treating diseases in

livestock and poultry. Encouraging additional research on the therapeutic and medicinal properties of black garlic as a complementary food is essential, along with optimizing production conditions to ensure product quality stability and cost reduction.

Black garlic has been found to possess several beneficial effects on cardiovascular health. It can help reduce levels of bad cholesterol while increasing levels of good cholesterol, which aids in removing plaque from blood vessel walls. Additionally, black garlic has been observed to lower blood fat levels, inhibit platelet accumulation, and prevent the formation of blood clots, thereby contributing to the prevention of cardiovascular diseases.

Moreover, black garlic has traditionally been used for its diverse medicinal properties. It is known to have antidotal, expectorant, diuretic, anthelmintic, and digestive-boosting effects. It is also utilized to address vitamin deficiencies, treat respiratory ailments, act as a skin antiseptic, and alleviate symptoms of the common cold.

However, there is currently limited research available specifically on the effects of black garlic supplementation in Tre chickens. The objective of this study is to determine the optimal level of fresh and black garlic supplementation in water, focusing on the growth performance and intestinal microflora of growing Tre chickens raised in the Mekong Delta of Vietnam. The findings of this study aim to provide valuable recommendations to producers in optimizing their practices.

MATERIALS AND METHODS

Location and climate of the study area

The study took place at a private farm in Binh Minh Town, Vinh Long Province, from January to May 2023. The chemical analysis of the feeds was performed at the laboratory of the Faculty of Animal Sciences, Colleges of Agriculture, Can Tho University.

Experimental animals

Tre chickens, which were one day old, were obtained from a farm in Tiền Giang Province. They were fed a concentrate designed for chicks with 20% crude protein (CP) during the rearing and preparation period leading up to the experiment. The chickens were trained to consume the experimental diets for a duration of two weeks prior to the commencement of the experiment. At 36 days old, the chickens were included in the experiment. Before their participation, they received vaccinations against diseases including H5N1, Newcastle, and other relevant illnesses to ensure their health and welfare throughout the experiment.

Experimental design and treatments

Experiments (3*2*3 factorial) with 2 factors. The first factor was the level of garlic supplementation, which included three levels: 0.5% DM (G0.5), 1%DM (G1.0), and 1.5% DM (G1.5). The second factor was the type of garlic, with two options: Fresh Garlic (FG) and Black Garlic (BG). A total of 180 Tre chickens, aged 6 weeks and weighing approximately 200±6.5 g/bird, were utilized for the study. Each experimental unit consisted of 10 birds with a balanced distribution of sexes. The experimental treatments consisted of FG0.5, FG1.0, FG1.5, BG0.5, BG1.0, and BG1.5, with each treatment receiving additions of 0.5%, 1%, and 1.5% (DM) of fresh garlic and black garlic to the basal diet. The trial was conducted over a duration of 8 weeks, covering the age range of 6 to 13 weeks for the Tre chickens. The composition of feed ingredients used in the concentrate basal diet can be found in Table 1.

Table 1: Composition of feed ingredients used in the concentrate basal diet.

S.	Feed	(%)	S.	Feed	(%)
1	Rice bran	5.9	6	Premix vitamin	0.40
2	Maize	29.0	7	Premix mineral	0.50
3	Fish meal	9.3	8	$CaCO_3$	0.49
4	Broken rice	41.2	9	DCP	0.51
5	Soybean extraction	12.7			

Feeds and preparation of fresh and black garlic

Fresh garlic is prepared by peeling and finely slicing it, followed by a drying process under the sun for 4-5 days. Once dried, it is finely ground and stored in refrigeration until it is needed for the experiment.

On the other hand, the production of black garlic involves taking whole cloves of fresh garlic and placing them in a brewing pot set at a temperature of 60 degrees Celsius. The garlic is then aged for 12 days, resulting in its transformation into black garlic.

All the feed ingredients used in the experiment were obtained from a single purchase from a feed store to ensure consistency throughout the study. The basal diet was formulated to contain 12.8 MJ metabolizable energy (ME) per kilogram of dry matter (DM) and 20% crude protein (CP). Black garlic powder was carefully mixed with water according to the experimental design and provided to the chickens as a drink.

Detailed information on the chemical compositions of black garlic, feed ingredients, and the basal diet can be found in Tables 2 and 3.

Housing and management

Each cage was equipped with feeding and drinking troughs

Table 2: Chemical compositions of back garlic and fresh garlic.

Item	Back garlic	Fresh garlic
Sugar dissolved in water	1,88-7,91 x increase	450 mg/g
Total polyphenols	4,19 x increase	13,91 mg/g (Calculated by gallic acid)
Total flavonoids	4,77 x increase	3,22 mg/g (Calculated by rutin)
Compound Amadori/Heyns	40-100 x increase	10 µg/g
Fructan	0,15-0,01 x reduction	580 mg/g
Leucin	1,06 x increase	58,62 mg/100 g
Isoleucin	1,67 x increase	50,04 mg/100 g
Cystein	0,58 x reduction	81,06 mg/100 g
Phenylalanin	2,43 x increase	55,64 mg/100 g
Tyrosin	0,18 x reduction	449,95 mg/100 g
Sugar dissolved in water	1,88-7,91 x increase	450 mg/g
Total polyphenols	4,19 x increase	13,91 mg/g (Calculated by gallic acid)
Total flavonoids	4,77 x increase	3,22 mg/g (Calculated by rutin)
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Isoleucin	1,67 x increase	50,04 mg/100 g
Cystein	0,58 x reduction	81,06 mg/100 g
Phenylalanin	2,43 x increase	55,64 mg/100 g
Tyrosin	0,18 x reduction	449,95 mg/100 g

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Table 3: Chemical compositions of feed ingredients and basal diet (% DM).

Ingredient, %	Concentrated 20 % CP
DM	89.8
OM	92.1
CP	20.0
EE	7.49
CF	4.58
NDF	23.7
Ash	7.9
ME (MJ/kg DM)	12.8
ME (Kcal/kg)	3050

for the birds. The feeding schedule consisted of three times a day, specifically at 7:00, 13:00, and 17:00 hours. The amount of bird feed provided was adjusted on a weekly basis, increasing it gradually from 5% to 10% based on the actual feed intake of the chickens. The Tre chickens had unrestricted access to water throughout the experiment.

The housing for the birds was constructed using bamboo and leaves. The experimental chickens were housed in an area measuring 2.0 m^2, accommodating 10 chickens. The area was enclosed by wooden frames and plastic netting. The floor of the house was covered with a layer of sand, with a 2.5 cm thick layer of rice husk on top, serving as the litter material.

Figure 1: Tre chicken in the experiment. Basal diet was formulated and contained 12.8 MJ ME/kgDM, 20% CP.

Measurements

Daily feed intake and nutrient intake: The amount of feed consumed and the leftovers were measured every morning.

Daily weight gain and feed conversion ratio: The chickens' weights were recorded on a weekly basis and at the end of the experiment to calculate their average daily weight gain. Feed conversion ratio was determined by dividing the total feed intake by the total weight gain.

Carcass evaluation: At the end of the experiment, four chickens (two males and two females) from each experimental unit were selected for slaughter to assess carcass traits. Body measurements of the birds were conducted following the methodology described by Salomon (1996).

Table 4: Daily intakes of feed and nutrient of Tre chicken (g/bird).

Item	Factor garlic level			Factor of garlic type		SE	P
	G0.5	G1.0	G1.5	BG	FG		
DM	46.9 [a]	45.1 [b]	45.3 [b]	45.8	46.1	1.87	0.04/0.06
OM	43.2 [a]	41.5 [b]	41.7 [b]	42.2	42.5	2.22	0.04/0.07
CP	9.37[a]	9.01[b]	9.06[b]	9.15	9.22	0.31	0.01/0.08
EE	2.30[b]	2.29[b]	2.86[a]	2.51	2.17	0.21	0.01/0.06
CF	2.95[b]	2.50[c]	3.40[a]	2.33 [b]	3.20 [a]	0.25	0.01/0.01
NDF	6.01[b]	6.05[b]	7.0[a]	7.09	7.13	1.12	0.01/0.01
Ash	2.3	2.25	2.3	2.46 [a]	2.13 [b]	0.03	0.06/0.01
ME (MJ/kg/DM)	0.60 [a]	0.58 [b]	0.58 [b]	0.59	0.59	0.04	0.04/0.07

Digestive experiment

Accumulated Nitrogen: The calculation of accumulated nitrogen content per 1kg of test diets was performed using the following formula:

Nr = (Nd - Ne x AIAd/AIAe) x 1000/100 (Lammers et al., 2008)

In this formula: Nr represents the mass of accumulated nitrogen (g/kg). Nd corresponds to the nitrogen content in the diet (%). Ne denotes the nitrogen content in the feces (%). AIAd represents the content of acid chlorhydric insoluble ash in the diet (%). AIAe refers to the content of HCl-insoluble minerals in the feces (%).

Determination of nutrient digestibility in diet

The apparent digestibility of EE, dry matter (DM), organic matter (OM), and CF in the diet was calculated using the formula:

DD = (1 - [(ID x AF) / (IF x AD)]) x 100

In the formula: DD represents the full apparent digestibility ratio of nutrients in the diet (%). ID refers to the ash content (AIA) in the diet insoluble in acid (mg/kg). AF denotes the nutrient content in the waste (mg/kg). IF represents the AIA content insoluble in acid of the waste (mg/kg). AD corresponds to the nutrient content in the diet (mg/kg).

Upon completion of the 14-week experiment, fecal samples were collected to assess the levels of Lactobacillus, Salmonella spp., *E. coli*, and Clostridium perfringens. The colony counting method was utilized to quantify the presence of these microorganisms in the stool samples. Approximately 80 grams of stool samples were directly collected from the barn for each treatment group, with a total of 10 Tre chickens per treatment. The collected samples were promptly stored in a cold storage facility to preserve their integrity. Subsequently, the stool samples were homogenized and transferred to the Biology Laboratory at the Center for Analytical Services in Can Tho City for further analysis. In the laboratory, colony counting techniques were employed to determine the population levels of the specific microorganisms in the samples

Chemical analyses

The feeds provided in the experiment were subjected to analysis to determine their chemical compositions, including dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE), crude fiber (CF), and ash content. These analyses were conducted in accordance with the procedures outlined by the Association of Official Analytical Chemists (AOAC, 1990). The NDF (neutral detergent fiber) analysis followed the method described by Van Soest et al. (1991), and the metabolizable energy (ME) content was calculated using the equation developed by Janssen (1989) (Table 4).

Statistical analysis

Data were analyzed by using General Linear Model (GLM) of Minitab program 18.1.0 (Minitab, 2018) and the comparison of significant difference between two treatments was done by Tukey method of Minitab (2018).

RESULTS AND DISCUSSION

Daily intakes of feed and nutrients of growing Tre chickens

The daily intakes of DM, OM, CP, EE, and NDF were found to be significantly lower ($P<0.05$) in Tre chickens receiving G0.5 treatments compared to those receiving G1.5 treatments within the Garlic level factor. However, when considering the additional factor of Garlic type, there were no significant differences ($P>0.05$) in the daily intakes of DM, OM, EE, and NDF among the Tre chickens.

The daily intakes of DM and CP in this study were observed to be lower compared to a previous study on Tau Vang chickens reported by Nha et al. (2021), where the DM intake ranged from 61.6 to 63.4 g/day and the CP intake ranged from 11.1 to 11.5 g/day. Regarding the additional factor of Garlic level, the ME intake was significantly

lower (P<0.05) in the Tre chickens receiving G1.0 and G1.5 treatments compared to those in other treatments. However, within the additional factor of Garlic type, there were no significant differences in the daily intakes of CP and ME among the Tre chickens.

Effects of factor garlic level and garlic type factor on the growth performance of growing Tre chickens

According to Table 5, the daily weight gain (DWG) of Tre chickens was found to be lower in the G0.5 treatments compared to the G1.0 and G1.5 treatments within the Garlic level factor. In terms of the Garlic type factor, Tre chickens with BG treatments exhibited higher DWG than those with FG treatments. The best feed conversion ratio (FCR) was observed in the PM treatments when using fermented feed supplements. Furthermore, in relation to the Garlic type factor, the FCR of the BG treatments outperformed that of the FG treatments, with FCR values of 3.18 and 3.32, respectively. These FCR values fall within the range of 3.1-3.5 reported by Nha (2020), indicating consistency. The final live weights of the chickens in this trial ranged from 970g to 1005g, which were lower than the weights reported in the previous trial conducted by Nha et al. (2021). Additionally, the results reveal that the consumption of crude protein (CP) per unit of weight gain was significantly lower (P<0.05) in the chickens receiving BG treatments (636 g/kg) compared to the FG treatments (664 g/kg) within the Garlic type factor.

The addition of both black and fresh garlic resulted in a decrease in feed conversion ratio (FCR). Among the Garlic level factor, the G1.0 treatments exhibited the lowest FCR in chickens. Regarding the Garlic type factor, the BG treatments had a lower FCR compared to the FG treatments.

Effects of garlic levels and type on carcass quality of Tre chicken

The inclusion of garlic in the diets of Tre chickens had notable effects on carcass values and internal organs. Regarding carcass weight, the G1.0, G1.5, and BG treatments showed significantly higher weights compared to the other treatments (P<0.05), as indicated in Table 6. However, the percentage of carcass did not exhibit significant differences among the treatments (P>0.05), with values falling within the range of 71.0-72.9% reported by Nha et al (2022). The weights of breast meat and thigh meat were significantly higher in the G1.0, G1.5, and BG treatments, while the percentages of breast meat and thigh meat did not show significant variations among the treatments (P>0.05). There were no significant differences observed in the weights of internal organs among the treatments (P>0.05).

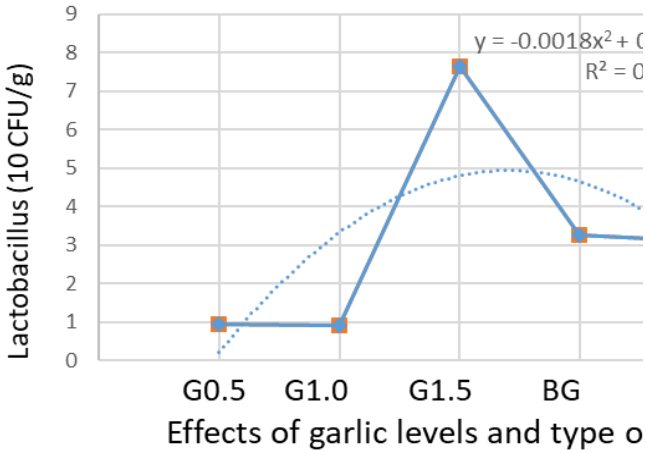

Figure 2: The effect of garlic levels and type of garlic on FCR.

Intestinal microflora

According to Table 6, the presence of *Salmonella* spp. in Tre chicken manure at week 13 was nearly negligible, particularly in the G1.5 treatments. However, higher levels of *Lactobacillus*, *E. coli*, and *Clostridium perfringens* were detected in the manure samples. Among the Garlic supplements, the G1.5 treatments demonstrated the highest abundance of Lactobacillus bacteria, while the levels of *E. coli* and *Clostridium perfringens* were the lowest. This is beneficial for the overall health of the chickens since *Salmonella* spp., *E. coli*, and *Clostridium perfringens* are potentially pathogenic bacteria, whereas *Lactobacillus* is a beneficial bacterium.

Table 5: The growth performance parameters assessed in the study included daily weight gain, final live weight, and feed conversion ratio (FCR) of Tre chickens (g/bird).

Item	Factor garlic level			Factor of garlic type		SE	P
	G0.5	G1.0	G1.5	BG	FG		
Initial live weight	198	201	197	199	202	6.5	0.97/0.95
Final live weight	970[b]	997[a]	990[a]	1005[a]	980[b]	14.5	0.03/0.01
Daily weight gain	13.8[b]	14.2[a]	14.2[a]	14.4[a]	13.9[b]	0.34	0.01/0.01
FCR	3.4[a]	3.17[b]	3.20[b]	3.18[b]	3.32[a]	0.18	0.03/0.01
CP/ weight gain (g/kg)	680[a]	634[b]	640[b]	636[b]	664[a]	2.34	0.01/0.01
ME/weight gain (MJ/kg)	43.5[a]	40.6[b]	41.0[b]	40.7[b]	42.5[a]	1.97	0.01/0.01

Table 6: Carcass measurements and weights of internal organs in Tre chickens fed diets supplemented with garlic (g/bird).

Item	Factor					SE	P
	G0.5	G1.0	G1.5	BG	FG		
Slaughter live weight	970[b]	997[a]	990[a]	1005[a]	980[b]	8.1	0.02/0.05
Carcass weight	689[b]	727[a]	721[a]	724[a]	702[b]	8.4	0.03/0.04
% Carcass	71.0	72.9	72.8	72.0	71.6	0.81	0.23/0.24
Thigh meat weight	124[b]	133[a]	133[a]	135[a]	127[b]	5.75	0.05/0.04
%Thigh meat	18.0	18.3	18.4	18.7	18.1	1.16	0.89/0.86
Breast meat weight	104[b]	116[a]	114[a]	114[a]	105[b]	4.01	0.05/0.03
% Breast meat	15.1	15.9	15.8	15.7	14.9	0.34	0.82/0.89
Heart weight	8.1	8.0	7.9	7.4	7.6	0.85	0.09/0.8
Liver weight	15.0	15.5	16.0	16.7	17.0	4.22	0.44/0.81
Cecal length, cm	14.0	13.5	13.5	13.2	14.6	1.34	0.80/0.79

[a, b, c] Mean values with different superscripts within the same row are different at P<0 05.

Table 7: Bacteria density in Tre chicken at 13 [th] weeks age of the experimental.

Variables	Factor					SEM	p
	G0.5	G1.0	G1.5	BG	FG		
Lactobacillus (10 CFU/g)	1.94[b]	1.99[b]	3.77[a]	4.78	4.08	0.45	0.02/0.18
Salmonella spp./25g (+/-)	Positive	Positive	Non detected	-	-	-	-
E. coli (10^5 CFU/g)	4.00[a]	3.45[a]	2.98[b]	3.05	3.32	0.05	0.02/0.38
Clostridium perfringens (10^4 CFU/g)	6.33[a]	6.09[a]	3.99[b]	4.87	5.87	0.03	0.02/0.18

[a, b, c] Means within a row with different superscripts are significantly different (P<0.05).

Table 8: Percentage of total nutrient digestibility in experiment diet.

Item (%)	Factor					SEM	P
	G0.5	G1.0	G1.5	BG	FG		
DMD	88.1[b]	89.8[a]	89.7[a]	90.6[a]	89.9[b]	0.14	0.03/0.018
OMD	90.1[b]	91.8[a]	91.9[a]	92.7[a]	91.3[b]	0.06	0.02/0.03
Nr (g/kg)	58.9[b]	59.8[b]	60.0[a]	61.4[a]	61.0[a]	0.09	0.01/0.018
EED	79.0[b]	81.8[a]	82.0[a]	82.7[a]	82.0[a]	0.13	0.02/0.07
CFD	33.09[b]	35.03[a]	35.15[a]	36.03[a]	36.06[a]	0.14	0.04/0.08

DMD: Dry matter digestibility, OMD: Organic matter digestibility; EED: Ether extract digestibility; CFD: Crude fiber digestibility;Nr: Accumulated Nitrogen.

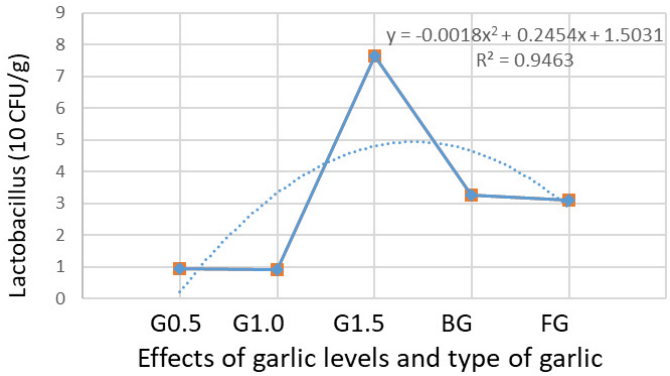

Figure 3: The effect of garlic levels and type of garlic on *Lactobacillus.*

Adding garlic to the Tre chicken diet at 1.5% (DM) in the diet, the content of lactobacilli in the stool also increased. The G1.5 treatments was the highest *Lactobacilli* (Table 7).

In contrast, the incorporation of garlic in the diet led to a reduction in the presence of *E. coli* and clostridium in the gastrointestinal tract of Tre chickens after 13 weeks. This reduction can be attributed to the increased levels of probiotics, which resulted in a decrease in the populations of pathogenic bacteria such as *E. coli* and Clostridium (P<0.05) compared to the group without garlic supplementation. Notably, the G1.5 treatments exhibited the most favorable outcomes, with the lowest levels of *E. coli* and *Clostridium*.

These findings are consistent with a study conducted

by Niem and Nha (2018), which demonstrated that the inclusion of fermented fine bran in the diet of Hoa Lan ducks increased the population of Lactobacillus yeast and reduced the presence of *E. coli* bacteria in duck feces.

Digestive experiment

The results of the study demonstrated that the apparent digestibility coefficients of nutrients in the feeds were notably high. The nutrients present in the test feed ingredients were effectively digested. Among the treatments, the G1.0, G1.5, and BG treatments exhibited the highest digestibility coefficients for DM (89.8%, 89.7%, and 90.6%, respectively) and OM (91.8%, 91.9%, and 92.7%, respectively). The G1.0 and G1.5 treatments showed the highest digestibility coefficients for EE (81.8% and 82.0%, respectively). In terms of CF digestibility coefficient, the BG and FG treatments had higher values compared to the other treatments (Table 8).

CONCLUSIONS AND RECOMMENDATIONS

The findings indicated that the inclusion of 1.5% (DM) Black Garlic in the diet yielded the most favorable outcomes for Tre chickens aged 6 to 13 weeks.

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NOVELTY STATEMENT

Addition of fresh garlic and black garlic to diets of chickens has increased growth achievement, decreased FCR, and increase in beneficial bacteria Lactobacillus and a decrease in harmful bacteria such as *E.coli* and Clostridium in the intestinal tract of growing Tre chickens. At a supplement of 1.5% (DM) black garlic gives the best results.

AUTHOR'S CONTRIBUTION

Both authors Contributed equally.

Conflict of interest

The authors have declared no conflict of interest.

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