Veterinary Integrative Sciences 2023; 21(2): 557 - 565 DOI; 10.12982/VIS.2023.039

Vet Integr Sci

Veterinary Integrative Sciences

ISSN; 2629-9968 (online)

Website; www.vet.cmu.ac.th/cmvj

Research article

Effects of organic acid and garlic powder supplementations in the diet on growth and intestinal microflora of the exotic Tam Hoang chickens

Nguyen Thi Thuy[1*] Le Thanh Phuong[2] and Nguyen Cong Ha[1]

[1]Department of Animal Science, College of Agriculture, CanTho University, CanTho 900000, Vietnam
[2]Vietswan Poultry Production Joint Stock Company, Binh Duong 820000, Vietnam

Abstract

A study was carried out to evaluate the effect of diet's organic acid (OG) and garlic powder (GP) on the growth, carcass quality, and intestinal microflora of Tam Hoang chickens. The experiment consisted of three treatments and four replications in a completely randomized design; each replication was a pen (20 chickens/pen), and males and females were raised separately. The treatmentswere:1/Cont: Basal diet (BD) no supplementation; 2/OG: BD + 1g Poulacid /kg feed; 3/GP: BD + 1 g garlic powder/kg feed. Results showed that chickens fed Cont (1451 g /head) had lower final live weight than those having OG (1568 g/head) and GP (1521 g/head/day). So, the average daily gain (ADG) of chickens in Cont (16.8 g/head/day) and GP (17.7 g/head/day) were lower than in OG (18.2 g/head/day). Average daily feed intake (ADFI) was similar in all treatments. Therefore, the feed conversion ratio (FCR) of chickens fed OG (3.04 kg feed/kg gain) was better than that in Cont (3.32 kg feed/kg gain). The density of *Salmonella* bacteria was not detectable in chicken feces at the 4th week age but became positive in Cont and GP treatments at the 10th week age. Supplementation of OG and GP reduced *E. coli* and *Clostridium perfringens* in the feces compared to control chickens at the 4th and 10th week age. In conclusion, adding OG or GP at the same dose (1g/kg feed) in Tam Hoang chicken diets improved ADG, FCR, and reduced *E. coli* and *Clostridium perfringens* densities in the feces compared to control chickens

Keywords: *Clostridium perfringens, E. coli,* Garlic powder, Organic acid, Salmonella, Tam Hoang chicken

Corresponding author: Nguyen Thi Thuy, Department of Animal Science, College of Agriculture, Can Tho University, Can Tho 900000, Vietnam. Tel.: +84 989019578, Email: nthithuycn@ctu.edu.vn

Article history; *received manuscript: 3 June 2022,*
revised manuscript: 29 December 2022,
accepted manuscript: 10 January 2022,
published online: 25 April 2023
Academic editor; *Nguyen Trong Ngu*

INTRODUCTION

Chicken production has been developed rapidly in Vietnam recently, and registered approximately 409 million heads in year 2021, and increased by nearly 8% compared to 2020 (GSO, 2021). Not only are industrial chicken breeds being raised, but local and exotic chicken breeds are also developing in high numbers. Among many improved chicken breeds, the Tam Hoang breed has become popular in the South of Vietnam. However, due to epidemics and environmental conditions, antibiotics have been added to the chicken diet to prevent diseases, which otherwise would cause high mortality rates. However, antibiotics have been limited to supplements in animal feed in Vietnam since 2018. Therefore, other safer alternative solutions in order to improve chicken health should be explored. Herbal powder is considered a safer alternative source that can improve chicken health. Research from Panpatil et al. (2013) indicates that turmeric, ginger, and garlic have antimicrobial and antioxidant activities. Using organic acid supplementation in chicken diets has been recommended as one of the possible substitutes for the antibiotic because organic acid does not leave residues and is safe for meat production (Muzaffer et al., 2003). In Vietnam, organic acids have been applied for many years but are mainly used to preserve food, and the direct delivery of organic acids into animal feed has not been widespread.For that reason, this research concentrated on using organic acid and garlic powder to supplement the Tam Hoang chickens' diet in the opening housing system and small-scale farms and aimed to evaluate the growth, feed efficiency, bacteria density and carcass quality characteristics of Tam Hoang chicken in the absence of preventive antibiotic use in the process.

MATERIALS AND METHODS

Materials

The experimental procedure was approved by the Council of College of Agriculture, Can Tho University (THC2021-02/KNN). The chickens were cared for and handled in accordance with Vietnam's Animal Husbandry Law (32/2018/QH14). The study was carried out on an experimental farm belonging to Can Tho city. The experiment was conducted on 240 Tam Hoang chickens (120 males and 120 females). The chicken house was designed as an opening house with a net to separate each pen, and the bedding was rice husk on the floor about 15 cm thick. There were 12 pens (2m x 1.3m) corresponding to 3 treatments, with each of the experimental pens containing 20 chickens. Drinks are provided through an automatic drinking system that provides drinking water and a large feeder to provide feed for experiments. The experimental chickens were vaccinated with Gumboro, $H_5N_{1,}$ and pox during the experiment.

Experimental feed

Diet formulas and feed compositions of basal diet are presented in Table 1. Main feed ingredients included broken rice, rice bran, maize meal, soya bean meal, and fish meal; supplemental ingredients included bone and shellfish meal, amino acids, and vitamin premix. Organic acid (Poulacid) is a white mixture powder with the main ingredients of fumaric, lactic, phosphoric acid, and calcium formate. Garlic powder was produced before starting the

experiment. The whole origin garlic was cleaned, dried sufficiently in the oven, then proceeded with fine grinding and storage for the whole experiment. These supplemented products were mixed into the feeds every week and fed continuously for experimental chickens.

Table 1 Ingredient (%) and chemical composition of the basal diets

Ingredients, %	Basal diet (BD)		
	1-4 weeks of age	5-8 weeks of age	9 -12 weeks of age
Maize meal	35.52	40.0	46.0
Rice bran	14.28	14.2	16.3
Broken rice	16.0	15.37	11.4
Soya been meal	21.50	20.0	17.0
Fish meal	8.00	6.00	5.0
Bone meal	2.0	2.0	2.0
Shellfish meal	1.5	1.5	1.5
Lysine	0.10	0.03	0.0
Methionine	0.23	0.10	0.0
Salt	0.17	0.1	0.1
Vitamin- premix[1]	0.70	0.7	0.7
Chemical composition and energy metabolizable of the diets, %			
ME (Kcal/kg feed)	2940	2960	2990
EE	4.20	4.15	4.30
CP	20.2	19.0	17.5
CF	4.20	4.25	4.48
NFE	65.2	66.5	67.2
Ca	1.58	1.55	1.57
P	0.58	0.52	0.54

[1]Per kg of Vitamin- premix[1]: Vitamins: A 48 x 10^5 IU; D 48 x 10^4 IU; E 44 x 10^2; K_3 280 mg; B_1 600 mg; B_2 200 mg; B_6 320 mg; B_{12} 6 x 10^3 mcg; Biotin 10^4 mcg; Minerals: Fe 475 x10^2 mg; Cu 315 x 10^2 mg; Zn 475 x 10^2; I 350 mg; Co 47 mg; Mn 195 x 10^2; Se 39 mg.

Management

The experimental chickens were free to access the water and feed during the experimental period, and feed residues were weighed before adding new feed the following morning. The chickens were weighed with all 20 birds in each pen as an experimental unit at the start of the experiment and every two weeks. The feeding experiment was divided into two stages (1- 4 and 5-12 weeks of age), and variables were body weight gain, feed intake, feed conversion ratio, carcass yield, and density of intestinal microflora.

Experimental design

The experiment was carried out in a completely randomized design, with three treatments and four replications. Each replication was a pen (20 chickens/pen) with males and females separated, and every treatment included two male and two female pens. A total of 240 1-day-old Tam Hoang chickens were randomly divided into three treatment groups, each group with 4 replications (pen), a total of 12 experimental units. The control group was fed the basal diet (BD) without supplementation, and the experimental group was supplemented with Poulacid or garlic powder. The treatments were as followed:

1/ Cont: Basal diet (BD) no supplementation (control)

2/ OG: BD + 1g Poulacid/kg feed

3/ GP: BD + 1g Garlic powder/kg feed

Sampling and carcass evaluation

Feed samples were collected and analyzed at 1, 5, and 9 weeks of age when the period diets were changed following the chicken nutrient requirements. The colony counting method determined the density of *Salmonella*, *E. coli,* and *Clostridium perfringens* in feces at the 4th and 10th weeks. Fecal samples from 5 chickens/pen (about 50-70g feces/bag) were directly collected at the cloaca and stored in cold storage. Then, homogenous fecal samples were transferred to Laboratory for counting the colony. Carcass parameters of experimental chickens were evaluated by slaughtering. At the end of the experiment, four chickens/pen were selected to be slaughtered, and chosen chickens were kept for 12 hours fasting before surgery. Slaughter weight, carcass weight, and thigh and breast meat weight were evaluated.

Analysis methods

Experimental feed determined the chemical compositions according to AOAC (1990). Ether extract (EE) and crude protein (CP) were determined by Soxhlet extraction and the Kjeldahl methods. Bacterial densities were tested according to specific methods: *E. coli* was analyzed according to ISO-16649-2-2001 (TCVN 7924. 2008); *Clostridium perfringens* by ISO 7937: 2004 (TCVN 4991.2005); *Salmonella* by ISO-6579-1: 2017 (TCVN 10780. 2017).

Measurement

The parameters included: Average daily feed intake (g/head/day), average daily gain (g/head/day), feed conversion ratio (kg feed/kg gain), carcass characteristics; *E. coli* (CFU/g feces); *Salmonella* (+/-), and *Clostridium perfringens* (CFU/g feces) in chicken feces.

Statistical analysis

Data were analyzed using the General Linear Model (GLM) of Minitab Statistical Software Version 16. Tukey pair-wise comparisons were used to determine differences between treatment means at $P<0.05$. The statistical model used was as follows: $Y_{ij} = \mu + \alpha_i + e_{ij;}$ where Y_{ij}: Growth performance, carcass quality, and intestinal microflora; μ is the overall mean averaged over all treatments; α_i is the treatment effect; e_{ij} is a random error associated with treatment and replicated within the treatment.

RESULTS

Growth performance and feed efficiency

Results in Table 2, Figure 1 and Figure 2 showed the growth performance and fed efficiency between 0-12 weeks of age. Initially, the initial live weight was from 34.7 g/head to 35.3 g/head, but there was a higher tendency in the supplemented treatments compared with control at four weeks of age and similarly at the final weight. The final weight of chickens in OG and GP treatments was higher than that in control chickens. Average daily feed intake was not significantly different in supplemented diets compared with the control diet. Hence, the average daily gain was higher in chickens fed OG than in the Cont treatment chickens.

Table 2 Growth performance and feed intake of chickens in the experiment

	Treatments			SEM	P
	Cont	OG	GP		
From 1-4 weeks of age					
Weight at four weeks of age, g	264[b]	271[a]	275[a]	2.56	0.03
AGD, g/head/day	8.15	8.43	8.57	0.35	0.11
ADFI, g/head/day	18.2	18.8	19.4	0.58	0.42
FCR, kg feed/kg gain	2.24	2.23	2.26	0.15	0.21
From 5-12 weeks of age					
Final weight, g	1451[b]	1568[a]	1521[a]	16.3	0.02
ADG, g/head/day	21.3[b]	23.1[a]	22.2[ab]	0.30	0.03
ADFI, g/head/day	74.7	73.4	73.8	3.14	0.19
FCR, kg feed/kg gain	3.51[a]	3.17[b]	3.32[ab]	0.08	0.02
Overall 1-12 weeks of age					
Duration, day	84	84	84	-	-
ADG, g/head/day	16.8[b]	18.2[a]	17.7[ab]	0.40	0.01
ADFI, g/head/day	55.9	55.3	55.7	1.35	0.23
FCR, kg feed/kg gain	3.32[a]	3.04[b]	3.15[ab]	0.07	0.02

ADG: Average daily gain; ADFI: Average daily feed intake; FCR: Feed conversion ratio; Cont: control diet (BD) without supplementation; OG: BD + 1g Poulacid/kg feed; GP: BD + 1g Garlic powder/kg feed.[a,b]Means within a row with different superscripts are significantly different (P<0.05)

Intestinal microflora

The density of bacteria in the feces of experimental chickens is presented in Table 3. Garlic powder and organic acids added to the diets had reduced (P<0.05) *E. coli* density in feces compared to the control chickens. *Clostridium perfringens* and *E. coli* densities in chicken feces at the 4th week age were lower than that at the 10th week age. *Salmonella* was almost undetectable in chicken feces at the 4th week age, but there was a positive effect of *Salmonella* in the feces of chickens in GP and control treatments at the 10th week age.

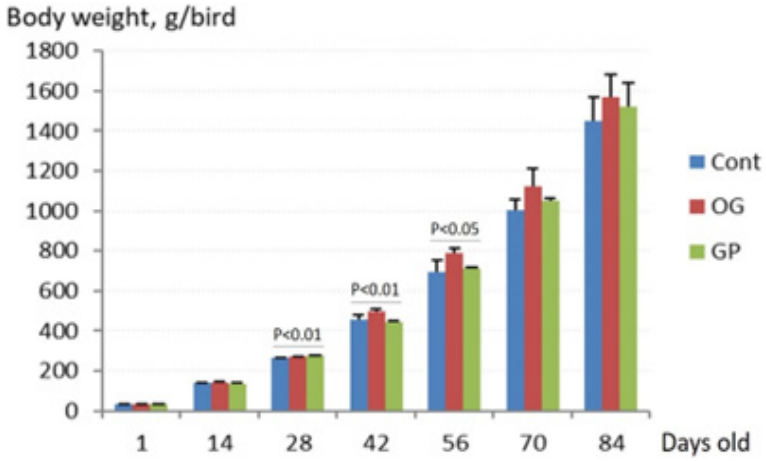

Figure 1 Body live weight (BW) of experimental chickens from 1-84 days old (Error bars: Standard Deviation).

Table 3 Density of bacteria in the feces of chickens.

	Treatments			SEM	P
	Cont	OG	GP		
In the 4th week age					
Salmonella/25g (+/-)	N	N	N	-	-
E.coli (10^6 CFU/g)	4.71[a]	3.01[c]	3.80[b]	0.28	0.01
Clostridium. P (10^5 CFU/g)	2.77[a]	1.37[b]	1.65[b]	0.18	0.01
At the 10th week age					
Salmonella ((+/-)25g)	P	N	P	-	-
E.coli (10^6 CFU/g)	6.91[a]	4.73[b]	4.68[b]	0.40	0.03
Clostridium. P (10^5 CFU/g)	3.46[a]	2.60[b]	2.82[b]	0.24	0.01

Cont: Control diet (BD) without supplementations; OG: BD + 1g Poulacid/kg feed; GP: BD + 1g Garlic powder/kg feed; [a,b,c] Means within a row with different superscripts are significantly different (P<0.05); N: Negative; P: Positive.

Carcass evaluation

There were no statistically significant differences between the carcasses of chickens in all treatments. The carcass yield of Tam Hoang chickens ranged from 68.0-68.4%, which was lower than that of the same chickens raised in industrial house conditions. There was no difference in the thigh and breast proportion in the treatments with or without adding organic acid or garlic powder supplementations to the diet.

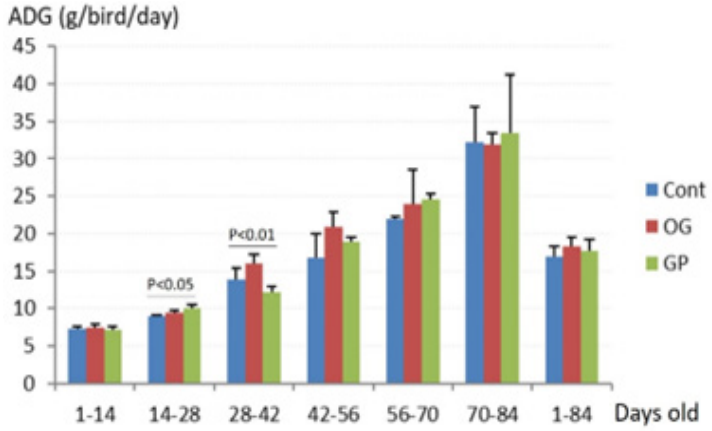

Figure 2 Average daily gain (ADG) of experimental chickens (Error bars: Standard Deviation).

DISCUSSION

Growth performance and feed conversion ratio of chickens fed OG improved compared to chickens in the control group. These results are consistent with the research of Adil et al. (2011b), who suggested that adding organic acids to the intestinal tract would reduce intestinal pH, inhibit pathogenic bacteria's growth, improve nutrient utilization, and preventing disease. The organic acid changes the structure of the small intestine as it increases the velocity of the villi in all segments of the small intestine, especially in the ileum, thus improving absorption and feed efficiency. The lactic acid Poulacid can stabilize the intestinal flora because it reduces the intestines' pH (Willey et al., 2009), prevents gastrointestinal disease and increases the absorption of feed nutrients.

In addition, garlic powder is a medicinal plant that is generally used as a

growth promoter, and the sulfur compound is garlic's most important chemical compound (Ali and Zahran, 2010). In addition, garlic also had antimicrobials, denoted to bioactive components present in garlic (Amagase et al., 2001); the sulfur-containing organic compound may be responsible for promoting growth. Moreover, Jamroz et al. (2003) reported that the chemical and physiological functions of the digestive tract might be affected by the plant metabolites and effecting intestinal microflora associated with intermediate nutrient metabolism. Consequently, it improves broilers' total tract digestibility and feeds efficiency. Furthermore, garlic and some compound in garlic may be similar to antibiotic, and this compound can reduce the growth of pathogens in the gastrointestinal tract of chickens (Goodarzi et al., 2014), resulting in better feed efficiency and leading to better feed conversion ratio (Bedford, 2000).

Fatufe and Matanmi (2011) found that adding organic acids has limited the growth of harmful microorganisms in the intestines and reduced morbidity and mortality of chickens. The density of *Clostridium perfringens* was highest in the control treatment, and there was no significant difference in *Clostridium perfringens* in OG and GP treatments at the 4th and the 10th weeks of age. This indicates that supplying organic acid at 1g/kg feed will inhibit the growth of harmful bacteria, maintaining the balance of intestinal bacteria. Harmful intestinal bacteria are usually active in high pH environments, such as *E. coli* with a pH of 4.3, *Samonella* at a pH of 4.0, and *Staphylococcus* at a pH of 4.2, while beneficial microorganisms are active in low pH environments (pH <3.5). Therefore, the supplementation of organic acid to the diet reduced pH to below 3.5, limiting the activity of harmful bacteria and enhancing the activity of beneficial bacteria (Adil et al., 2011a). This is similar to the study of Moutzouris et al. (2010), who found that adding organic acid to the diet may improve digestion, limit the growth of harmful bacteria, enhance immunity, and decrease poultry mortality.

Garlic powder and organic acids added to the diets had reduced *E. coli* density in feces ($P<0.05$) compared to that in control chicken groups. It may be due to garlic content allicin (the main phytogenic compound); this compound is metabolized from the natural amino acid allicin, which is transformed into allicin by the enzyme alliinase. These natural additives have inhibited the growth of harmful bacteria in the chicken intestines (Olobatoke and Mulugeta, 2011). In addition, Diaz-Sanchez et al. (2015) also reported the antibacterial effect of garlic as probiotics to be used as potential feed additives. Gull et al. (2012) also found that the antimicrobial activity of herbs may limit the growth of pathogens in the intestinal tract of chickens.

CONCLUSIONS

Supplementation of both organic acid (Poulacid) and garlic powder into the diets of Tam Hoang chickens showed better weight gain, feed conversion ratio, and decreased density of *E. coli* and *Clostridium perfringens* in the chicken feces better than in control.

ACKNOWLEDGEMENTS

We would like to thank the experimental farm managers and staff for their assistance with the experimental procedures. Many thanks to Nguyen Thi My Phung and Bui An Khuong for their support in collecting data and samplings.

AUTHOR CONTRIBUTIONS

Nguyen Thi Thuy; Investigation, methodology, design the experiment, supervision, editing, and finalization.
Le Thanh Phuong; Investigation, methodology, manuscript preparation.
Nguyen Cong Ha; Investigation, prepare experimental supplementation products, formal analysis, manuscript preparation.

CONFLICT OF INTEREST

We have no conflict of interest.

REFERENCES

Adil, S., Banday, M., Bhat, G., Qureshi, S., Wani, S., 2011a. Effect of supplemental organic acids on growth performance and gut microbial population of broiler chicken. Livest. Res. Rur. Dev. 23(1), 1-8.

Adil, S., Banday, T., Ahad Bhat, G., Salahuddin, M., Raquib, M., Shanaz, S., 2011b. Response of broiler chicken to dietary supplementation of organic acids. J. Cent. Eur. Agric. 12(3), 498-508.

Ali, F., Zahran, D., 2010. Effect of growth enhancers on quality of chicken meat during cold storage. Adv. J. Food. Sci. Technol. 2(4), 219-226.

Amagase, H., Petesch, B.L., Matsuura, H., Kasuga, S., Itakura, Y., 2001. Intake of garlic and its bioactive components. J. Nutr. 131(3), 955S-962S.

Association of Official Analytical Chemists, 1990. Official methods of analysis, 5th edition. AOAC, Arlington, Virginia.

Bedford, M., 2000. Removal of antibiotic growth promoters from poultry diets: Implications and strategies to minimise subsequent problems. Worlds Poult Sci J. 56(4), 347-365.

Diaz-Sanchez, S., D'Souza, D., Biswas, D., Hanning I., 2015. Botanical alternatives to antibiotics for use in organic poultry production. Poult. Sci. 94(6), 1419-1430.

Fatufe, A., Matanmi, I., 2011. Effect of probiotics, organic acids or their mix on the growth performance of starting cockerels. Arch. Zootec. 60(229), 149-152.

Goodarzi, M., Nanekarani, S., Landy, N., 2014. Effect of dietary supplementation with onion (Allium cepa L.) on performance, carcass traits and intestinal microflora composition in broiler chickens. Asian. Pac. J. Trop. Dis. 4, S297-S301.

General Statistics Office of Vietnam, 2021. Statistic of livestock heads and production of Viet Nam Animal husbandry. GSO, Vietnam.

Gull, I., Saeed, M., Shaukat, H., Aslam, S.M., Samra, Z.Q., Athar, A.M., 2012. Inhibitory effect of Allium sativum and Zingiber officinale extracts on clinically important drug resistant pathogenic bacteria. Ann. Clin. Microbiol. Antimicrob. 11(1), 1-6.

Jamroz, D., Orda, J., Kamel, C., Wiliczkiewicz, A., Wertelecki, T., Skorupińska, J., 2003. The influence of phytogenic extracts on performance, nutrient digestibility, carcass characteristics, and gut microbial status in broiler chickens. **J. Anim. Feed Sci**. 12(3), 583-596.

Moutzouris, J.P., Che, W., Ramsay, E.E., Manetsch, M., Alkhouri, H., Bjorkman, A.M., Schuster, F., Ge, Q., Ammit, A.J., 2010. Proteasomal inhibition upregulates the endogenous MAPK deactivator MKP-1 in human airway smooth muscle: mechanism of action and effect on cytokine secretion. Biochim. Biophys. Acta. Mol. Cell Res. 1803(3), 416-423.

Muzaffer, D., Ferda, O., Kemal, C., 2003. Effect of dietary probiotic, organic acid and antibiotic supplementation to diets on broiler performance and carcass yield. Pak. J. Nutr. 2(2), 89-91.

Olobatoke, R., Mulugeta, S., 2011. Effect of dietary garlic powder on layer performance, fecal bacterial load, and egg quality. Poult. Sci. 90(3), 665-670.

Panpatil, V.V., Tattari, S., Kota, N., Nimgulkar, C., Polasa, K., 2013. In vitro evaluation on antioxidant and antimicrobial activity of spice extracts of ginger, turmeric and garlic. J. Pharmacogn. Phytochem. 2(3), 143-148.

TCVN 7924, 2008. Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of β-glucuronidase-positive Escherichia coli - Part 2: Colony-count technique at 44 °C using 5-bromo-4-chloro-3-indolyl β-D-glucuronide (ISO 16649-2 : 2001). Vietnam Standard and Quality Institute, Vietnam.

TCVN 4991, 2005. Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of Clostridium perfringens - Colony count technique (ISO 7937 : 2004). Vietnam Standard and Quality Institute, Vietnam.

TCVN 10780, 2017. Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of Salmonella - Part 1: Detection of Salmonella spp (ISO 6579-1:2017). Vietnam Standard and Quality Institute, Vietnam.

Willey, J.M., Sherwood, L., Woolverton, C.J., 2009. Prescott's principles of microbiology. McGraw-Hill, New York.

How to cite this article;

Nguyen Thi Thuy, Le Thanh Phuong and Nguyen Cong Ha. Effects of organic acid and garlic powder supplementations in the diet on growth and intestinal microflora of the exotic Tam Hoang chickens. Veterinary Integrative Sciences. 2023; 21(2): 557- 565.