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Research article

Effect of supplementations in drinking water on growth performance, health status, and carcass quality of local Tre chickens

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Abstract

A study was carried out to determine the effect of probiotic, multivitamin, and organic acid (acid butyric) supplementations in drinking water on growth performances, health status, and carcass quality of local chicken (Tre). A total of 480 Tre chickens at 4 weeks of age were randomly distributed in a completely randomized design experiment, with 4 treatments and 4 replicates (2 male and 2 female pens); each replicate consisted of a pen with 30 chickens/pen, the experimental data was collected during 10 weeks. Treatments used: (1) Control (Cont): Basal diet (B) without any supplementation; (2) PRO: B + 1% probiotic; (3) VIT: B+ 1% multivitamin product; (4) OA: B+1% butyric acid, all supplements were supplied in drinking water. The results showed the use of PRO had a significant highest in final weight (1032 g/ chicken) compared with control group (981.6 g/chicken), lead to better daily gain (ADG) and feed conversion ratio (FCR) (13.31 g/head/day and 3.02 g feed/g gain) to compare with the control group (12.5 g/head/day and 3.28 g feed/g gain) ($P<0.05$). Daily feed intake (FI) was not affected by all supplementations ($P>0.05$). VIT and PRO adding in drinking water reduced feather pecking, and there was a reduction of *Salmonella* spp. and *E. coli* in the feces of Tre chickens in PRO and OA compared with control chickens. All treatments did not affect the carcass yield of the birds, but little improvement in the yellow meat color ($b^*=8.48$) of chickens in VIT and PRO compared to the others. In general, adding probiotics at the level of 1% in drinking water could be better for improving ADG and FCR compared with multivitamins, acid butyric, and control treatments; adding probiotic and organic acid reduced *Salmonella* spp. and *E. coli* in the feces, while multivitamin supplementation reduced feather pecking and improved yellow meat color of Tre chickens.

Keywords: Growth performance, Multivitamin, Organic acid, Probiotic, Tre chicken.

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INTRODUCTION

In Vietnam, poultry production has been increasing recently, with chickens occupying around 410 million heads in early 2021 (GSO, 2021). Chicken production is considered a traditional system with a short production cycle and income for householders. Besides the development of raising many local chicken breeds such as Noi, Ac, and Tau chickens, Tre chicken is a small local breed that originated in the Mekong Delta. They are small in size, and their body is covered with multi-color feathers, obtaining very firm and delicious meat. Before, they had been often raised as scenes because of their colorful feather (Giang and Chi, 2022). Recently, Tre chickens have been strongly developed and raised in high numbers by both small and semi-large scale farmers for meat production. They are a low-growth breed and have small body sizes with a weight of 0.9 kg (female) and around 1,1 kg (male) at a mature age (Phuong et al., 2020). In small-scale, opening farming systems and in the condition of reducing using antibiotics in the diets for raising chickens in Vietnam, there is a need to find safer alternative sources to replace antibiotics for chicken production because the use of antibiotics has been banned to supplement in animal feed as a growth stimulant, and not be used to prevent animal diseases. So, vitamins or organic acids and probiotics are priority substitutes for antibiotics, which do not leave residues and safety for meat production. There are many kinds of supplementations in feed or drinking water, but selecting suitable supplements for Tre chickens at open-house farms is a big issue. Therefore, this study was conducted to evaluate the effect of some supplementation products such as probiotics, multivitamins, or organic acid supplementations in drinking water on growth performance, health status, meat quality parameters, and bacterial population in the feces of local Tre chickens during the growing period from 4-13 weeks age.

MATERIALS AND METHODS

Ethics approval

The experimental procedure was approved by the Council of the College of Agriculture, Can Tho University (THC2021-02/KNN). The study was approved for animal care, housing, and sample collection under the Law on Animal Husbandry (No. 32/2018/QH14).

Animals

The experiment was conducted for 10 weeks from October to December 2022 in a chicken farm in Binh Minh district, Vinh Long city. The experiment was conducted on 480 Tre chickens (240 males and 240 females). The chicken house was designed as an opening house, the roof was covered with tole, and bedding was rice husk on the floor about 15 cm thick, and a net was used to separate each pen. A total of 16 pens, corresponding to 4 treatments, each of the experimental pens (2×1.3 m) raising 30 chickens. Drinks are provided through a round drinker for each pen, and a round feeder to provide feed for experimental chickens in each pen. During the experiment, chickens were vaccinated against some diseases such as Newcastle disease, H5N1, pox, etc.

Experimental design and diets

The experiment was carried out in a completely randomized design experiment, with 4 treatments and 4 replications (2 male pens and 2 female pens), each replication was a pen with 30 chickens/pen (male and female separate). A total of 480 (240 males and 240 females) Tre chickens at 4 weeks of age were randomly divided into 4 treatment groups, each group of 2 replications in female chickens and 2 replications in male chickens, a total of 16 experimental units. The control group (Cont) was fed the basal diet (BD) without any supplementation; the

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experimental groups were supplemented with different kinds of supplemental products as follows: probiotics (PRO), multivitamins (VIT), and organic acids (OA).

Feed compositions of basal diet are presented in Table 1. Feed ingredients include maize meal, rice bran, broken rice, fish meal, soya meal, bone and shellfish meal, amino acids, and vitamin premix. Probiotics include *Lactobacillus sporogens* (10^{10} CFU) and *Bacillus subtilis* (10^{10} CFU). Multivitamin products contain Vitamin A, B_1, B_6, B_{12}, D, K, H, C, and D-Calcium pantothenate. Organic acid products contain butyric acid only. All supplemental products were added to drinking water every day continuously for 10 weeks without using antibiotics in feed and water to prevent diseases during the experiment.

Table 1 Chemical composition of the basal diets.

Item	5-9 weeks of age	10 -14 weeks of age
ME (Kcal/kg TA)	2960	2990
EE,%	4.10	4.01
CP,%	19.00	17.30
CF,%	4.23	4.46
NFE,%	67.50	69.20
Ca,%	1.55	1.52
P,%	0.52	0.51

Treatments were:
1/Cont: BD without any product supplementation
2/PRO: BD + 1%probiotic in drinking water
3/VIT: BD + 1% multivitamin in drinking water
4/OA: BD + 1% acid butyric in drinking water

Sampling, measurements, and chemical analysis

During the experimental period, chickens were free to access the water and feed. The remaining food from the previous day was measured before adding a fresh batch in the morning. The chickens were weighed with all 30 birds in each pen as an experimental unit at the beginning of the experiment and every week in the early morning before feeding. Experimental chickens were vaccinated on days 18^{th}, 21^{st}, and 42^{nd} with infectious bursal disease (Gumboro), Newcastle disease, and H5N1.

The experimental data was collected during 10 weeks (from 4-14 weeks of age), and variable observations were feed intake, body weight gain, feed conversion ratio, carcass yield, survival rate, feather pecking, and density of intestinal microflora. Feed samples were collected 2 times at 5 and 10 weeks of age and analyzed for the chemical composition and energy metabolizable of the diets. The content of *E. coli* and *Salmonella* in fecal samples were determined at 10 weeks of age by the colony counting method (Helrich, 1990). Fecal samples were directly collected at the cloaca of 5-6 chickens/pen (about 70g feces/bag) and stored in cold storage. After that, homogenous fecal samples were transferred to the Biology Laboratory of Analysis Service Center in Can Tho City to count the colonies. At the end of the experiment (14 weeks of age), four chickens in each pen were selected to be slaughtered. Chickens were chosen for a 12-hour fasting before surgery. Carcass parameters in chickens include slaughter weight, carcass weight, thigh and breast weights, and meat according to the method of Doan et al. (2011). Meat samples of thigh and breast were collected for color measurement.

The parameters included average daily gain (g/head/day), average daily feed intake (g/head/day), feed conversion ratio (kg feed/kg gain), carcass quality; *Salmonella* (+/-), *E. coli* (CFU/g) in chicken feces; mortality, and feather pecking

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rate also were evaluated by counting the chickens that were pecking divided by the total of whole chickens in each pen.

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 $P \leq 0.05$. The statistical model used is as follows:

$$Y_{ij} = \mu + \alpha_i + e_{ij}$$

Where Y_{ij}: Growth performances, 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

The growth performance and fed efficiency of the experimental chickens between 4 and 14 weeks of age are shown in Table 2. The initial live weight was from 100 g/head to 107 g/head, but there was a higher tendency in the PRO treatment compared with the others at the final weight. 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 PRO (13.31 g/head/day) than in the Cont (12.55 g/head/day) chickens. Lead to a better feed conversion ratio in PRO (3.02 kg feed/kg gain) and VIT (3.04 kg feed/kg gain) than that in OA (3.27 kg feed/kg gain) and Cont (3.28 kg feed/kg gain).

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

Items	Treatments				SEM	*P*
	Cont	PRO	VIT	OA		
Initial weight (4 weeks age), g	103.30	100.80	101.70	107.50	2.65	0.33
Final weight (14 weeks age), g	981.60^{b}	1032.00^{a}	1000.00^{b}	987.70^{b}	10.41	0.04
ADG, g/head/day	12.55^{b}	13.31^{a}	12.83^{ab}	12.57^{ab}	0.24	0.04
ADFI, g/head/day	41.17	40.31	39.04	41.16	2.01	0.75
FCR, kg feed/kg gain	3.28^{a}	3.02^{b}	3.04^{b}	3.27^{a}	0.06	0.02

ADG: Average daily gain; ADFI: Average daily feed intake; FCR: Feed conversion ratio;
^{a,b,c} Means within a row with different superscripts are significantly different ($P \leq 0.05$)

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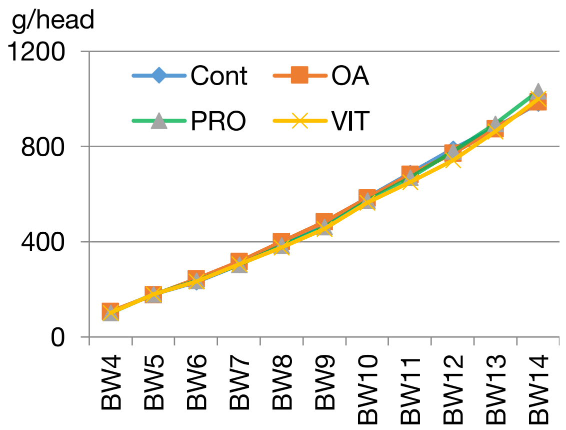

Figure 1 Live weight of Tre chickens from 4 to 14 weeks age

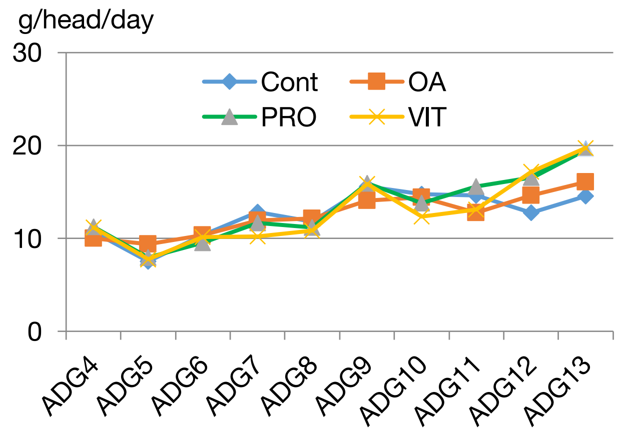

Figure 2 Average daily gain of Tre chickens from 4 to 14 weeks age

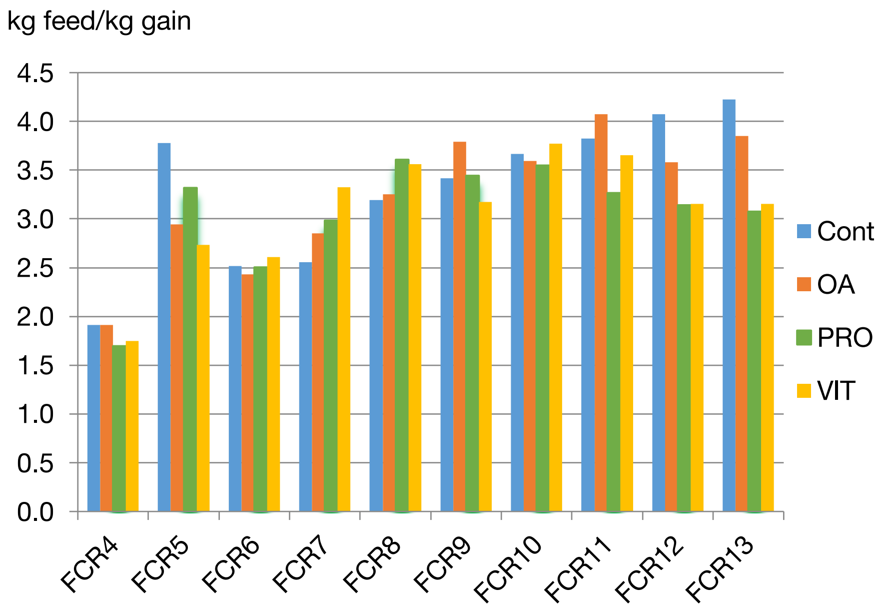

Figure 3 Feed conversion ratio of Tre chickens from 4 to 14 weeks age

Intestinal microflora

Supplementation of VIT and PRO reduced the feather pecking of chickens more than other treatments, and also chickens in PRO treatment reduced the death proportion more than others. *Salmonella* spp. were undetectable in chicken feces in PRO (N) and OA (N) treatments but not in VIT (Pos) and Cont (Pos) chickens at 10th weeks of age (Table 3). However, *E. coli* content is quite high in chicken feces in all treatments, in which the higher *E. coli* content was in Cont (6.90×10^6 CFU/g) and VIT (6.68×10^6 CFU/g) than in PRO (5.73×10^6 CFU/g) and OA (5.30×10^6 CFU/g) chickens. Both *Salmonella* spp. and *E. coli* are potentially pathogenic bacteria.

Table 3 Health status of experimental chickens from 4-14 weeks age

Items	Treatments				SEM	*P*
	Cont	PRO	VIT	OA		
Initial chicken number, head	120	120	120	120	-	-

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Death proportion,%	3.33	0.83	2.50	2.50	-	-
Feather pecking proportion,%	9.16	5.83	3.33	8.33	-	-
Salmonella/25g (+/-)	Pos	N	Pos	N	-	-
E.coli (10^6 CFU/g)	6.90^a	5.73^b	6.68^a	5.30^b	0.42	0.03

^{a,b}: Means within a row with different superscripts are significantly different ($P \leq 0.05$)
Pos: Positive; N: Non detected

Carcass evaluation

Supplementation of VIT and PRO reduced the feather pecking of chickens The carcass yield of Tre chickens ranged from 65.10-66.50%, there was no difference in thigh and breast meat proportion in the treatments with or without supplementations in drinking water. But there was little improvement of yellow color (b) in breast meat of chickens in VIT than the others.

Table 4 Carcass characteristic evaluation of the experimental chickens

Items		Treatments			SEM	*P*
	Cont	PRO	VIT	OA		
Slaughter weight (g)	980.00	1010.00	1000.00	990.00	15.32	0.07
Carcass weight (g)	637.00	672.00	662.00	652.00	14.52	0.06
Carcass yield (%)	65.10	66.50	66.20	65.90	0.92	0.12
Thigh weight (g)	194.00	202.00	203.00	204.00	5.67	0.23
Thigh proportion/carcass (%)	30.50	30.10	30.80	31.30	0.81	0.11
Thigh meat weight (g)	143.00	155.00	150.00	145.00	5.13	0.26
Thigh meat proportion/carcass (%)	22.50	23.10	22.80	22.30	0.45	0.51
Breast weight (g)	156.00	155.00	163.00	162.00	5.62	0.12
Breast proportion/carcass (%)	24.50	24.10	24.70	24.90	0.72	0.51
Breast meat weight (g)	119.00	127.00	119.00	117.00	7.12	0.32
Breast meat proportion/carcass (%)	18.70	18.90	18.10	18.00	0.34	0.57
Color of the breast meat						
L*	43.40	43.20	43.87	43.45	0.23	0.16
a*	15.47^b	15.56^a	15.50^{ab}	15.46^b	0.02	0.02
b*	8.32^b	8.40^{ab}	8.48^a	8.30^b	0.05	0.02
Color of the thigh meat						
L*	37.80	37.30	38.10	37.30	0.35	0.07
a*	9.89^a	9.38^{ab}	9.12^b	9.45^{ab}	0.21	0.04
b*	8.34	8.40	8.41	8.31	0.08	0.06

^{a,b,c} Means within a row with different superscripts are significantly different ($P \leq 0.05$)
**Lightness (L), redness (a), and yellow-ness (b)*

DISCUSSION

There was a small improvement in the final weight of chickens fed the PRO diet compared with chickens in other groups, which led to a slightly higher ADG of chickens fed the PRO diet than that in the Cont diet, even though the difference was too small. It can be explained that when probiotics are added to the diet into the digestive tract, they produce organic acids that change the pH environment, suppress pathogenic bacteria, and allow nutrient absorption; they also enhance the activity of beneficial microbes in the gastrointestinal tract, increases the

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performance of digestive enzymes, stimulates the humoral and cell-mediated immunity (Krysiak et al., 2021). Research by Poberezhets et al. (2021) and Mohsin et al. (2022) also showed the probiotic supplement in the chicken diet improves the digestibility of feed nutrients and increases the absorption of amino acids and minerals in chickens. The results are in agreement with the study by Cengiz et al. (2015), who showed the effective action of a probiotic on live weight and the growth of broiler chickens. Similarly, the decreased FCR may be one of the reasons for the promoted growth in groups fed probiotic and vitamin diets. It may be associated with improved intestinal status and internal villus function, whereas the blind end allows for an increased retention time for dietary content within the digestive tract (Reynolds et al., 2020). These results are in agreement with the research of Ravangard et al. (2017), who found an improvement in growth and nutrient utilization with the addition of probiotics under optimal or suboptimal nutritional levels. So, probiotics can be linked to the improved intestine at the structural or microbial levels that induce nutrient digestion and absorption; also, intestinal enzyme activity showed improvement, which explains the improvement in FCR and growth.

In addition, chickens in the VIT diet also showed better FCR than OA and Cont chickens, and it can be seen that it is necessary to provide enough nutrients and vitamins in the diet for the improvement of growth performance. According to Jang et al. (2014), all vitamin deficiencies lead to metabolic disorders because these vitamins may stimulate appetite, increase feed intake, and grow quickly. In addition, vitamins are important components of the coenzyme-A function, which is important in fat synthesis and results in higher weight gain. In fact, Hoseein et al. (2013) found that supplementing vitamins to chicken diets improved the weight gain of chickens more than control because their presence in the intestinal tract helps increase the absorption of the intestinal parenchyma, participates in the coenzymes to promote the breakdown and metabolism of substances in the body. Research by Moravej et al. (2012) found that vitamins participating in the structure of many enzymes in the system catalyze biological reactions to maintain all normal life activities and growth.

However, supplementation of organic acid in the diets had no positive effect on growth and feed efficiency in the present study. This is in contrast with research from Sheikh et al. (2011) who showed that 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. Research from Thuy et al. (2018) found that butyric acid could reduce intestinal pH, and increase enzyme activity, so it should improve the digestion and absorption of protein and change the pH of the intestinal environment, allowing absorb nutrients and prevent disease too.

Raising chickens in the open house creates a high risk of disease infection due to the effects of weather and the environment. Lampang et al. (2014) indicated that the resistance to *Salmonella* contamination is a problem in chicken farms. However, in this experiment, the morbidity number of chickens was quite low, even though they were raised in an open house, which is affected by changing weather and no antibiotics supplied in the diets. It can be explained that the addition of probiotics or organic acids has prevented the development of harmful microorganisms in the intestines, leading to increased digestibility of chicken and reduced morbidity and mortality. In the present study, the amount of *E. coli* in chicken feces in the PRO and OA treatments was reduced compared with control and VIT treatments, and it may be because probiotics produce organic acid, which causes a reduction in intestinal pH, but disease bacteria are often active at high pH, the pH is suitable for the activity of E. coli (4.3) while beneficial bacteria such as Lactobacillus are active at low pH (<3.5). Thus, it will limit the activity of harmful bacteria and enhance the activity of beneficial bacteria (Thuy et al., 2018). Therefore, it is possible to change the number of intestinal bacteria, as well as the growth of beneficial bacteria in the gut of the chicken (Adil and Magray, 2012), so

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this is the reason why supplemented PRO and OA chickens reduced number of *E. coli* and *Salmonella* in the intestinal tract and lead to lower morbidity and mortality.

The weight of Tre chicken that the consumer prefers is around 1000-1200 g (male) and 800-1000 g (female); this is higher than that in research of Giang and Chi (2022), and the carcass percentage of Tre chickens was between 65-66.5% which is similar with Thai native chicken in research of Promket and Ruangwittayanusorn (2021). This small native chicken breed had quite a lower body weight than the other native chicken breeds in Vietnam, such as Tau Vang, Noi, and Ta Vang. Funaro et al. (2014) explained that carcass composition mainly reflected the genetic difference in breeds. Overall observation of the carcass parameters found that the addition of probiotics, vitamins, or acid butyric into the diets of Tre chickens had almost no effect on carcass proportions, thigh meat, and breasts of male and female chickens compared to control chickens. However, there is a trend of increasing yellow color (b) of breast meat in VIT and PRO chickens compared with the other treatments. It may be explained that the probiotic played a role in converting muscle fat to favorable fat and improved visual appearance through increased xanthophyll accumulation in the soft tissues of broilers' thigh muscles (Yang et al., 2010). Similarly, several studies have revealed that dietary probiotic supplements improved broiler meat quality and sensory properties after cooling for 5 days. In contrast, research from Kamil and Kokoszyński (2021) showed that the probiotic preparations did not differ significantly in carcass weight and the content of carcass components. Also, the results of Pe˘cjak et al. (2022) showed a difference when the addition of higher levels of vitamin E, vitamin C, and Se to broiler diets had no adverse effects on carcass traits, oxidative stability, and meat quality parameters when supplemented either alone or in combination.

CONCLUSIONS

Supplementation of probiotics at the level of 1% in drinking water could be better for improving ADG and FCR compared with multivitamin, acid butyric, and control treatments; adding probiotic and organic acid reducing *Salmonella spp. and E. coli* in the feces, while multivitamin supplementation reduced feather pecking, and supply multivitamin and probiotic improved yellow breast meat color of Tre chickens.

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AUTHOR CONTRIBUTIONS

Details of each author with his/her contribution to this paper are mentioned as follows; **Nguyen Thi Thuy**: Design, literature search, sampling, experimental studies, and manuscript editing. **Nguyen Thi Ngoc Linh**: Design, sampling, and manuscript preparation. **Nguyen Cong Ha:** Prepare the supplements, Provide material and reagents, data analysis, and manuscript editing.

CONFLICT OF INTEREST

We have no conflict of interest.

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