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Effect of organic acid products on growth performance and intestine health of Tam Hoang chicken

Nguyen Thi Thuy*, Nguyen Thi My Phung, Luu Thi Ty, Nguyen Thi Hoang Bich and Thai Viet An

College of Agriculture, Can Tho University, Vietnam

**Correspondence: Nguyen Thi Thuy (email: nthithuycn@ctu.edu.vn)*

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ABSTRACT

*An experiment was conducted to determine the effects of adding to the diet two organic acid products (Menacid and Poulacid) given separately, or as a mixture on growth performance and health status of Tam Hoang chickens (n=720). The experiment was conducted for 12 weeks with 6 treatments in a 6*2 factorial design and 3 replications. The first and second factors were organic acid supplement and gender (male or female). The treatments included the control:basal diet (BD) without any organic acid product, M0.8:BD with 0.8g Menacid/kg feed, M1.0:BD with 1.0gMenacid/kg feed,P1.0:BD with 1g Poulacid acid/kg feed, P1.5:BD with 1.5g Poulacid/kg feed, and MP:BD with 0.5g Menacid and 1g Poulacid acid/kg feed. Results showed that average daily gain of chickens overall 1 to 12 weeks fed M0.8 and MP were better than the control; average feed intake did not differ among the treatments. Feed conversion over the 12-week period was better for the M0.8 treatment compared to the control, but the other treatments did not differ from the control. Lactobacilli and Salmonella spp. were not detected in chicken feces either at 42nd or 70th days of age. Clostridium perfringens and Escherichia coli were reduced in all supplemented treatments, so morbidity (13.3 - 19.1%) and mortality (10-15%) were high. It was concluded that there was an apparent improvement in growth rate (8%), linked with a reduction in pathogenic bacteria (E. coli and C. perfringens) in the feces when chickens were supplemented with a commercial source of organic acids.*

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1 INTRODUCTION

Poultry production has an important role in agriculture in the Mekong Delta, accounting for 28% of total poultry production in the country (GSO, 2016). Besides the development of raising industrial chicken breeds in large farms, there is a strong development of local and improved chicken breeds. Among many kinds of improved chicken breeds, Tam Hoang breed that came to Vietnam from China have become popular in the South of Vietnam. However, due to epidemic and environmental conditions, they are usually supplemented antibiotics in the chicken diet to prevent and treat disease of chickens, otherwise the mortality rate will be quite high. Misuse of antibiotic for animals can result in high residues in meat, which can affect human health. Therefore, the world tendency is that poultry producers should minimize and preferably stop using antibiotics as dietary supplements (Sheikh *et al.,* 2011). In Vietnam, from 2018, antibiotics will be banned to supplement in animal feed as a growth stimulant, and will not be used to prevent animal diseases from 2020 (Department of Livestock Production, 2016). This situation has been pressing researchers and state officials in Vietnam to find alternative solutions, and research in antibiotic substitution has been a top priority, and especially in broiler production. One of the possible substitutes for the antibiotic is organic acid, which does not leave residues and is safe for meat production (Muzaffer*et et. al*., 2003). So, this research had been concentrated on using two kinds of organic acid products (Poulacid and Menacid) with the separate form and combining two products to supplement to Tam Hoang chicken diet in the opening housing system and in small scale farm. The objective of this study was to evaluate the growth, feed efficiency, bacteria density and survival rates of Tam Hoang chicken in the absence of preventive antibiotic use in the process.

2 MATERIALS AND METHODS

2.1 Location, infrastructure and experimental birds

The experiment was conducted during a period of 12 weeks in an experimental farm, in Thoi Lai district, Can Tho city. Tam Hoang chickens (360 male and 360 female) were raised in an open-sided house, in 36 pens (each 2 x 1.3 m) separated by netting (Fig. 1). Feed and water were provided continuously from feeders and automatic drinkers. Prior to starting the experiment, the chickens were vaccinated against common diseases (Gumboro, H_5N_1 and fowl pox).

2.2 Experimental feed

Three basal diets were formulated according to age ranges of 1-4, 5-9 and 10-12 weeks (Table 1).

Table 1: Ingredients (% air-dry) and chemical composition (% in dry matter) of the three basal diets

	1-4 weeks of age	5-9 weeks of age	10 -12 weeks of age
Maize meal	35.52	39.37	42.2
Broken rice	15.00	15.00	15.0
Rice bran	15.28	15.20	16.5
Fish meal	8.00	6.00	5.0
Soya meal	21.50	20.00	17.0
Lysine	0.10	0.03	0
Methionine	0.23	0.10	0
Bone meal	2.0	2.0	2.0
Shell fish meal	1.5	1.5	1.5
Salt	0.17	0.1	0.1
Vitamin- premix[1]	0.70	0.7	0.7
Chemical composition (% in dry matter)			
EE	4.20	4.10	4.01
CP	20.3	19.0	17.3
CF	4.10	4.23	4.46
NFE	65.8	67.5	69.2
Ca	1.59	1.55	1.52
P	0.59	0.52	0.51

[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

Feed ingredients include maize meal, rice bran, broken rice, fish meal, soya meal, bone and shellfish meal, amino acids and vitamin premix. Menacid and Poulacid were provided by Menon Animal Nutrition Technology Co., Ltd. Poulacid is a white powder, of which the main ingredients are fumaric, lactic and phosphoric acids, and calcium formate. The main ingredients of Menacid include fumaric acid, formic acid, propionic acid, benzoic acid, calcium formate and calcium propionate. Menacid is produced in the form of capsules and contains more organic acids than Poulacid. Menacid and Poulacid were mixed into the feeds every day and fed to the chickens continuously for 12 weeks.

2.3 Management and measurements

The chickens were weighed as one group of 20 birds in each pen. This was done at the beginning of the experiment and every two weeks, always in the early morning before feeding. According to the routine immunization program, the chickens were inoculated on days 3 and 18 (the first and second immunization) against infectious bursal disease (Gumboro); on days 7 and 21against Newcastle disease; on days 14 and 42 with H_5N_1; and on day 10 against fowl pox. The parameters included average daily gain (g/head/day), average daily feed intake (g/head/day), feed conversion ratio (kg feed/kg gain), carcass characteristics, *Lactobacillus* (CFU/g), *Salmonella* spp. (+/-), *E. coli* (CFU/g) and *C. perfringens* (CFU/g) in chicken feces, disease incidence and mortality evaluation.

2.4 Experimental design and treatments

The experiment was carried out in a 6*2 factorial design, with 6 treatments and 3 replications; each replication was a pen (20 chickens).The first factor was treatments; the second factor was gender (male and female). A total of 720 one-day-old Tam Hoang chickens was randomly divided into 6 treatment groups. The control group was fed with the basal diet (BD) without any supplementation; the experimental groups were supplemented with different levels and kinds of organic acid products.

Treatments include

1/Treatment 1 (Control): BD without organic acid product supplementation

2/ Treatment 2 (M0.8): BD + 0.8 g Menacid/kg feed

3/ Treatment 3 (M1.0): BD + 1 g Menacid/kg feed

4/ Treatment 4 (P1.0): BD + 1 g Poulacid/kg feed

5/ Treatment 5 (P1.5): BD + 1.5 g Poulacid/kg feed

6/ Treatment 6 (MP): BD + 0.5 g Menacid/kg feed + 1 g Poulacid/kg feed

Fig. 1: Experimental chicken pens

2.5 Sampling and carcass evaluation

Feed samples were collected 3 times at 1, 5 and 10 weeks of age, when BD was changed. The levels of Lactobacillus, Salmonella spp., E. coli and C. perfringens in fecal samples were determined at 42nd and 70th days of age by the colony counting method. Fecal samples were directly collected at 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 for counting the colony. At the end of the experiment (12 weeks of age), two chickens/pen were selected to be slaughtered. The chickens were chosen for a 12-hour fasting (for water only) before surgery. Carcass parameters in chickens include slaughter weight, carcass weight, thigh and breast meat weigh.

2.6 Analysis methods

The chemical composition of feed was determined according to AOAC (1990). Crude protein was determined by the Kjeldahl method. Total ash was the residue after ashing the samples at 550ºC, and the ether extract (EE) was determined by Soxhlet extraction. Bacteria density was tested at the Biology Laboratory according to specific methods: *E. coli* was analyzed according to ISO-16649-2-2001; *C. perfringens* by ISO 7937: 2004; *Salmonella* spp. by ISO-6579-1: 2017; and *Lactobacillus* by TCVN 8737: 2011.

2.7 Data analysis

The data were subjected to analysis of variance (ANOVA) by using the General Linear Model (GLM) and regression analysis of Minitab version16.

3 RESULTS AND DISCUSSION

3.1 Growth performance and feed efficiency

The final weight was highest in chickens in M0.8 and MP to compare with the control, which led to an increase in average daily gain (ADG) of chicken fed with M0.8 and MP diets (Table 2). From 1 to 4 weeks, there were no differences among treatments; from 5 to 12 weeks and for the overall 1 to 12 weeks, M0.8 and MP were better than the control. Feed intake over the 12-week period did not differ among the treatments. Feed conversion over the 12-week period was better for the M0.8 treatment compared with the control, but the other treatments did not differ from the control.

These results are consistent with the research of Sheikh *et al*. (2011), the addition of organic acids in the intestinal tract reduces the intestinal pH, which can inhibit the growth of pathogenic bacteria, improve nutrient utilization, and prevent 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.

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

Items	Treatments						SEM	P
	Control	M0.8	M1.0	P1.0	P1.5	MP		
From 1-4 weeks of age								
Initial weight (g)	32.1	32.5	32.3	32.1	31.9	32.3	0.39	0.90
Weight at 4 weeks of age (g)	253[b]	264[a]	249[b]	256[b]	265[a]	269[a]	2.56	0.01
ADG (g/head/day)	7.9[ab]	8.3[a]	7.7[b]	8.0[ab]	8.3[a]	8.5[a]	0.15	0.01
ADFI (g/head/day)	17.9[a]	17.1[ab]	17.0[b]	16.9[b]	16.7[b]	17.2[ab]	0.18	0.01
FCR (kg feed/kg gain)	2.26[a]	2.06[bc]	2.21[ab]	2.11[bc]	2.01[c]	2.0[c]	0.03	0.01
From 5-12 weeks of age								
Final weight (g)	1453[b]	1577[a]	1500[ab]	1525[ab]	1465[b]	1560[a]	17.1	0.01
ADG (g/head/day)	21.4[b]	23.4[a]	22.4[ab]	22.3[ab]	21.4[b]	23.0[a]	0.31	0.01
ADFI (g/head/day)	69.8	68.4	68.8	75.3	70.0	72.2	2.04	0.19
FCR (kg feed/kg gain)	3.25[ab]	2.93[b]	3.08[ab]	3.32[a]	3.27[ab]	3.14[ab]	0.07	0.02
Overall 1-12 weeks of age								
Duration (day)	84	84	84	84	84	84		
ADG (g/head/day)	16.9[b]	18.3[a]	17.5[ab]	17.8[ab]	17.1[b]	18.2[a]	0.20	0.01
ADFI (g/head/day)	52.2	51.3	51.5	55.8	52.4	53.9	1.35	0.18
FCR (kg feed/kg gain)	3.10[a]	2.80[b]	2.95[ab]	3.14[a]	3.06[ab]	2.97[ab]	0.06	0.02

ADG: average daily gain; ADFI: average daily feed intake; FCR: Feed conversion ratio; Cont: control diet (BD) without supplementation; M0.8: BD + 0.8 g Menacid /kg feed; M1.0: BD + 1g Menacid/kg feed; P1.0: BD + 1.0 g Poulacid/kg feed; P1.5: BD+ 1.5 g Poulacid/kg feed; MP: BD + 0.5g Menacid/kg feed+ 1 g Poulacid/kg feed. a,b,cMeans within a row with different superscripts are significantly different (P<0.05).

Table 3: Growth performance and feed intake of male and female chickens in the experiment

Items	Gender		SEM	P
	Female	Male		
From 1-4 weeks of age				
Initial weight (g)	32.6	31.9	0.36	0.06
Weight at 4 weeks of age (g)	257	261	1.47	0.04
ADG (g/head/day)	8.03	8.21	0.09	0.02
ADFI (g/head/day)	17.0	17.2	0.11	0.23
FCR (kg feed/kg gain)	2.13	2.10	0.02	0.34
From 5- 12 weeks of age				
Final weight (g)	1420	1610	9.87	0.01
ADG (g/head/day)	20.8	24.0	0.18	0.01
ADFI (g/head/day)	66.0	75.5	1.17	0.01
FCR (kg feed/kg gain)	3.18	3.15	0.08	0.67
Overall 1-12 weeks of age				
Duration (day)	84	84		
ADG (g/head/day)	16.51	18.75	0.11	0.01
ADFI (g/head/day)	49.6	56.1	0.78	0.01
FCR (kg feed/kg gain)	3.01	3.00	0.03	0.84

Chickens fed with Menacid diets had higher ADG than those supplemented with Poulacid; this can be explained that Menacid may be better due to the presence of more organic acids in the product and being encapsulated. So, it is possible to avoid these organic acids being metabolized in the crop and gizzard before reaching the intestine. Because in theory, a major limitation in the addition of organic acids to the gastrointestinal tract is that organic acids are rapidly metabolized in the crop and gizzard before reaching the intestine, and this will reduce growth performance (Adil *et al.*, 2011). In addition, Menacid also contains propionic and formic acids, which not only stimulate digestion, increase weight gain but also prevent the growth of pathogenic, destroy harmful bacteria in the intestinal tract. MP diet also has similar weight gain to that in M0.8 diet, combining Menacid and Poulacid in the diet, lactic acid in Poulacid can stabilize the intestinal flora because they reduce the pH in the intestines (Willey *et al.*, 2009). It prevents gastrointestinal disease and increases the absorption of feed nutrients.

The final weight, weight gain and feed intake of male chickens were significantly higher than those of female chickens (Table 3). In fact, male chickens were significantly bigger than female chickens after only a few weeks of age (Leeson and Summers,2001). However, due to the higher feed intake, the FCR is similar to that of female chickens.

3.2 Intestinal microflora

Lactobacillus and *Salmonella* spp. were almost undetectable in chicken feces at 42^{nd} and 70^{th} days of age (Table 4). However, *E. coli* and *C. perfringens* were quite high in chicken feces, especially at 70^{th} day of age higher than that at 42^{nd} day of age. Among these bacteria, *Lactobacillus* is beneficial bacteria, but *Salmonella* spp., *E. coli* and *C. perfringens* are potentially pathogenic bacteria (Yesilbag and Coplan, 2006).

Table 4: Bacteria density in chicken feces

Items	Treatments						SEM	P
	Cont	M0.8	M1.0	P1.0	P1.5	MP		
At 42^{nd} day old								
Lactobacillus (CFU/g)	<10	<10	<10	<10	<10	<10	-	-
Salmonella spp./25g (+/-)	N	N	N	N	N	N	-	-
E. coli (10^6 CFU/g)	8.10^{a}	4.08^{c}	5.15^{b}	4.62^{b}	1.79^{d}	3.67^{c}	0.44	0.00
C. perfringens (10^5 CFU/g)	3.77^{ab}	2.37^{cd}	1.37^{d}	4.23^{a}	2.55^{bc}	3.28^{b}	0.38	0.00
At 70^{th} day old								
Lactobacillus (CFU/g)	<10	<10	<10	26	<10	<10	-	-
Salmonella spp./25g (+/-)	N	N	N	Pos	N	N	-	-
E. coli (10^6 CFU/g)	13.9^{a}	7.73^{b}	7.68^{b}	8.30^{b}	8.2^{b}	6.02^{c}	0.40	0.02
C. perfringens (10^5 CFU/g)	4.46^{ab}	3.82^{b}	4.10^{ab}	4.43^{ab}	5.12^{a}	3.96^{b}	0.21	0.01

^{a,b,c}Means within a row with different superscripts are significantly different ($P<0.05$)

Pos: Positive; N: Non detected

Because bacteria accounts for 90% of the microflora in the intestine, which made stability and balance microflora, and have a great impact on animal health. Normally, the beneficial microflora is around 85%, and harmful microflora is around 15%, if this balance is lost, the rate of harmful bacteria increases, then the increase susceptibility to some diseases, especially gastrointestinal and respiratory diseases. It is possible to change the number of intestinal bacteria, as well as the growth of beneficial bacteria in the gut of the chicken by feeding (Adil and Magray, 2012). Thus, when supplementation with Menacid at 0.8 g/kg feed, and Poulacid at 1.5 g / kg feed, the mixture of both acid preparations reduced *E. coli* and *C. perfringens* in the gastrointestinal tract of the chickens. The addition of organic acids has prevented the development of harmful microorganisms in the intestines, leading to increased digestibility of chickens, reduced morbidity and mortality (Fatufe and Matanmi, 2011).

Table 5: Bacteria density of male and female chickens

Items	Gender		SEM	P
	Female	Male		
At 42 days of age				
Lactobacillus (CFU/g)	<10	<10	-	-
Salmonella spp./25 g (+/-)	N	N	-	-
E. coli (10^6 CFU/g)	4.93	4.88	0.25	0.88
C.perfringens (10^5 CFU/g)	3.11	2.75	0.16	0.14
At 70 days of age				
Lactobacillus (CFU/g)	<10	26	-	-
Salmonella spp./25g (+/-)	N	Pos	-	-
E. coli (10^6 CFU/g)	10.9	10.1	0.12	0.01
C.perfringens (10^5 CFU/g)	4.38	4.25	0.12	0.42
Pos: Positive; N: Non detected				

In terms of gender, the results in Table 5 showed that the bacteria in chicken feces in males and females was not significantly different at 42nd day of age. However, at 70th day of age, the *E. coli* and *C. perfringens* were higher in females than males. In addition, *Salmonella* spp. was found in a small number of males, and *Lactobacillus* was very low in males (26 CFU / g).

The density of *C. perfringens* was highest in the control treatment and lowest in the Menacid supplementation in both levels at 42nd and 70th days of age. This indicates that supplying Menacid at 0.8-1 g/kg feed will inhibit the growth of harmful bacteria, maintain balance of intestinal bacteria. Because intestinal bacteria are usually active in high pH environments e.g. *E. coli* with a pH of 4.3, *Salmonella* spp. at pH of 4.0, and *Staphylococcus* at pH of 4.2. Whereas beneficial microorganisms are active in low pH environments e.g. L*actobacillus* (pH <3.5). Thus, the addition of organic acid to the diet reduced pH to below 3.5, then limited the activity of harmful bacteria and enhanced the activity of beneficial bacteria (Adil *et al*., 2011).

The mortality rate of the experimental chickens was high (Table 6); this mortality corresponds to the number of diseased chickens in the treatments, almost chickens died mainly due to some diseases such as coccidiosis, *E. coli* and respiratory.

Table 6: Morbidity and mortality of chickens in the treatments

Items	Treatments						Gender
	Control	M0.8	M1.0	P1.0	P1.5	MP	Female
Initial chicken number	120	120	120	120	120	120	360
Final chicken number	102	108	106	104	103	107	322
Number of morbidity	23	15	18	20	22	16	52
Morbidity rate (%)	19.1	12.5	15.0	16.6	18.3	13.3	14.4
Number of mortality	18	12	14	16	17	13	38
Death rate (%)	15.0	10.0	11.7	13.3	14.2	10.3	10.5

It may be because chickens were raised in open house, affected by change of weather and no antibiotic supplied in the diets, and when organic acid supplementation in the diet inhibited the pathogenicity of harmful bacteria, enhanced immunity, and reduced poultry mortality.

3.3 Carcass evaluation

The carcass yield of Tam Hoang chickens ranged from 65-68%, which was lower than that of the same chickens raised in industrial house conditions. There was no difference in thigh and breast proportion in the treatments with or without addition of organic acid products in the diet.

Table 7: Carcass characteristic evaluation of chickens in the treatments

Items	Treatments						SEM	P
	Control	M0.8	M1.0	P1.0	P1.5	MP		
Slaughter weight (g)	1466	1516	1458	1483	1450	1533	22.3	0.08
Carcass weight (g)	1000	1040	983	975	981	1035	19.9	0.16
Carcass yield (%)	68.1	68.5	67.5	65.8	67.7	67.5	0.81	0.21
Thigh weight (g)	320.8	322.8	320.0	316.6	320.0	330.3	8.42	0.65
Thigh proportion/carcass (%)	32.0	31.1	32.5	32.4	32.6	32.3	0.68	0.06
Thigh meat weight (g)	225.6	238.5	234.0	227.3	230.5	230.2	6.54	0.74
Thigh meat proportion/carcass (	22.5	23.0	23.8	23.3	23.5	22.3	0.49	0.25
Breast weight (g)	234.2	239.2	226.3	222.0	222.0	245.0	9.19	0.41
Breast proportion/carcass (%)	23.5	23.0	23.0	22.8	22.7	23.7	0.70	0.90
Breast meat weight (g)	187.6	195.8	181.3	176.5	176.5	190.0	5.46	0.06
Breast meat proportion/carcass	18.7	18.8	18.4	18.1	18.0	18.3	0.40	0.16
Liver weight (g)	33.4	34.5	32.1	30.1	32.2	32.6	1.52	0.63

Table 8: Carcass characteristic evaluation of male and female chickens

Items	Gender		SEM	P
	Female	Male		
Live weight (g)	1405	1563	12.8	0.01
Carcass weight (g)	945	1056	11.5	0.01
Carcass yield (%)	67.2	67.6	0.46	0.62
Thigh weight (g)	303	347	4.86	0.01
Thigh proportion/carcass (%)	32.1	32.9	0.39	0.16
Thigh meat weight (g)	218	243	3.77	0.01
Thigh meat proportion/carcass (%)	23.0	23.1	0.28	0.98
Breast weight (g)	220.4	242.8	5.31	0.01
Breast proportion/carcass (%)	23.3	22.9	0.41	0.51
Breast meat weight (g)	174	190	3.15	0.01
Breast meat percentage/carcass (%)	18.4	18.0	0.23	0.27
Liver weight (g)	30.1	33.4	0.88	0.01

Overall observation of the carcass parameters found that the addition of organic acids to the diet of Tam Hoang chicken did not affect carcass proportions, thigh meat and breast both male and female chickens.

4 CONCLUSIONS

Supplementation of Menacid at 0.8 g/kg feed and a mixture of 0.5 g Menacid and 1 g Poulacid/kg feed improved weight gain, FCR, resulting an increase in economic efficiency, decreased density of *E. coli* and *C. perfringens* in the chicken feces.

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