Veterinary Integrative Sciences2025; 23(2): e2025040-1-9. DOI: 10.12982/VIS.2025.040

Veterinary Integrative Sciences
ISSN: 2629-9968 (online)

Research article

Effect of adding *Houttuynia cordata* and *Perilla frutescens* leaves powders in diets on growth, intestinal microflora and meat quality of local chickens in Vietnam

Le Thanh Phuong[1], Nguyen Cong Ha[2] and Nguyen Thi Thuy[3,*]

[1]*Vietswan Poultry Production Joint Stock Company, Binh Duong 820000, Vietnam.*
[2] *Institute of Food and Biotechnology, Can Tho University, Can Tho City 900000, Vietnam.*
[3]*Faculty of Animal Sciences, College of Agriculture, Can Tho University, Can Tho City 900000, Vietnam.*

Abstract

A study was carried out to determine the effect of *Houttuynia cordata (*HC) and *Perilla frutescens* (PF) leaves powders supplementation in diet on growth performance, *intestinal microflora* and meat quality of local chickens (Noi). A total of 240 Noi chickens at 4 weeks of age were randomly distributed in a completely randomized design experiment, with 3 treatments and 4 replicates (2 male and 2 female pens), each replicate consisted of a pen with 20 chickens/pen. Control treatment (Cont): Basal diet (B) no supplementation; HCP: B + 2% HC leaves powder; PFP: B+ 2% PF leaves powder in the diet. The results showed that, average daily feed intake (ADFI) was not affected by all supplementations (P>0.05), and average daily gain (ADG) slightly improved in HCP (17.26 g/day) and PFP (16.94 g/day) to compared with Cont (15.65 g/day) (P>0.05). Feed conversion ratio (FCR) was improved (P<0.05) in HCP (3.42 kg feed/kg gain), and PFP (3.54 kg feed/kg gain) compared to control (3.61kg feed/kg gain). And also adding HC and PF leaves powders reduced *Salmonella.spp* and *E. coli* in the feces of Noi chickens (P<0.05). Supplementation of HC and PF leaves powders reduced fat content in breast meat (P<0.05), and slightly improved yellow color (b value) of breast meat (P<0.05) to compare to the control. In conclusion, HC and PF leaves powders supplemented in the diet at 2% improved FCR and color of meat, reduced fat content of breast meat and also reduced *intestinal microflora* in the feces of Noi chickens.

Keywords:Growth performance, Houttuynia cordata, Intestinal microflora, Meat quality, Noi chicken, Perilla frutescens.

* **Corresponding author**:Nguyen Thi Thuy,Faculty of Animal Sciences, College of Agriculture, Can Tho University, Can Tho City 900000, Vietnam.E-mail: nthithuycn@ctu.edu.vn.

Article history; *received manuscript: 24May2024,*
revised manuscript: 25June2024,
accepted manuscript: 31July 2024,
published online:6 August 2024,
Academic editor; *Korakot Nganvongpanit*

INTRODUCTION

Poultry production has an important role in Vietnam's agriculture, accounting for 544.5 million heads of total poultry production in the country (GSO, 2023), in which local chicken breeds have been raised in semi-large and small farms. In the Mekong Delta, many small farms have raised local chickens such as Noi and Tre chickens for meat production recently. And some commercial feed additives have been used as antibiotic replacements to prevent the disease. So, many researchers are looking for a natural product as a reducing classical antibiotic and feed additive used in poultry nutrition. Herbal plants are considered a possible alternative to antibiotics for growth promotion and improvement of feed efficiency in poultry (Thuy, 2019). The use of herbs in chicken diet is not important only for the health of the animals, but also for the improvement of meat quality. Because, herbal plants are used to improve health conditions by the establishment of an ideal microbial population in animal's digestive tract, dietary feeding of essential oil from herbs improves the secretion of digestive enzymes, therefore improving the digestibility of the feeds (Eltazi, 2014). *Perilla frutescens* and*Houttuynia cordata* leaves powders are uncommon feed supplements in chicken feed up to now, they are usually used for humans. So, there are very few studies have been conducted on *Perilla* meal as a protein supplement for monogastric animals (Montha et al., 2021). As *perilla frutescens* and *Houttuynia cordata* leaves meals have not yet been investigated too. So, this study was conducted to evaluate the effect of *Houttuynia cordata* and *Perilla frutescens* leaves powders supplementations in the diets on growth, meat quality parameters and bacterial population in the feces of local Noi chickens.

MATERIALS AND METHODS

Animals

The experiment was conducted during a period of 10 weeks (from 4 to 14 week old of chickens) in an experimental farm, Thoi Lai district, Can Tho city. A total of 240 Noi chickens (120 males and 120 females) were raised in an open-sided house, in 12 pens (each 2m x 1.3m) separated by netting, each pen contained 20 chickens (male and female separate pens). Feed and water were provided continuously by feeders and round drinkers. Prior to starting the experiment, the chickens were vaccinated against common diseases (Gumboro, H_5N_1 and fowl pox).

Ethics approval

The procedure of the experiment 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).

Experimental design and diets

The experiment was designed in a completely randomized design with 3 treatments and 4 replicates (2 male and 2 female pens), each replicate consisted of a pen with 20 chickens/pen. The chickens were weighed as one group of 20 birds in each pen, and this was done at the beginning of the experiment and every week, always in the early morning before feeding. The data collections were ADG (g/head/day), ADFI (g/head/day), FCR (kg feed/kg gain) by the week. Carcass characteristics, *intestinal microflora* in feces and meat quality were evaluated. At the end of the experiment (14 weeks of age), four 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 were slaughter weight, carcass weight, thigh and breast meat weight. Breast meat samples were collected and measured for color and meat chemical compositions.

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Treatments were:
1/ Cont: Basal diet (B) without any supplementation in the diet
2/ HCP: B + 2% of *Houttuynia cordata* leaves powder in the diet
3/ PFP: B + 2% of *Perilla frutescens* leaves powder in the diet

Sampling, measurements and chemical analysis

Fecal samples were collected at the 10th week of age, the levels of *E. coli* and *Salmonella.spp* in feces were determined by the colony counting method. Fecal samples were directly collected at cloaca of 5-6 chickens/treatment (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.

The chemical composition of basal feed and meat was determined following Association of Official Analytical Chemists methods (AOAC, 1990). Meat sample color was recorded using a colorimeter (Chromameter Minolta, CR-400 Head, DP-400/ Japan), which indicated degrees of lightness of meat sample (L), red-ness (a) and yellow-ness (b). Bacteria density in feces was tested at the Biology Laboratory according to specific methods: *E. coli* (Quantitative) was analyzed according to ISO-16649-2- 2001; *Salmonella.spp* (Qualitative) by ISO-6579-1: 2017.

Table 1 Chemical composition of basal diets

		4-8 weeks age	9-14 weeks age
Ingredients, %	Maize meal	40.4	43.2
	Broken rice	15.0	15.0
	Rice bran	14.2	15.5
	Fish meal	7.00	5.0
	Soya meal	19.0	17.0
	Lysine	0.03	0
	Methionine	0.10	0
	Bone meal	2.0	2.0
	Shellfishmeal	1.5	1.5
	Premix	0.77	0.8
Chemical composition and Metabolisable energy, %	ME, kcal/kg feed	3000	3050
	EE	4.10	4.01
	CP	18.5	17.1
	CF	4.23	4.45
	NFE	67.5	69.3
	Ca	1.55	1.52
	P	0.52	0.51

Statistical analysis

The data of the study was analyzed by ANOVA using the General Liner 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 is as follows: $Y_{ij} = \mu + \alpha_i + e_{ij}$. Where Y_{ij} is growth performances; μ is the overall mean averaged over all treatments; $\alpha_i$is effect of treatment; eij is the random error associated with treatment and replicated within the treatment.

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RESULTS

The data in Table 2 showed that, the average initial weight of the experimental chickens was not significantly different in all treatments, but the average final weight was higher in HCP (1490.1 g/head) and PFP (1472.5 g/head) to compared with Cont (1381.2 g/head) (P<0.05). Average daily feed intake was also not significantly different in all treatments. The ADG was highest in chickens fed HCP (17.25 g/day), and lowest in Cont (15.65 g/day) (Figure 1). Also, the FCR overall 10 weeks was improved in HCP (3.42 kg feed/kg gain) and PFP (3.54 kg feed/kg gain) compared with Cont (3.61 kg feed/kg gain) (P<0.05).

There were no statistically significant differences between the carcasses of chickens in all treatments. The carcass yield of the experimental chickens ranged from 65-67%. There was no difference in the thigh and breast proportion in the treatments with or without adding *Houttuynia cordata* and *Perilla frutescens* leaves powders supplementation to the diets. But, there was an increasing yellow color (b value) of breast meat of chickens fed HCP (9.48) and PFP (9.24) compared with cont (8.11). And also the EE content of breast meat was reduced in HCP (1.87 %) and PFP (1.91%) compared with Cont (2.45 %) (Table 3).

The density of bacteria in the feces of experimental chickens is presented in Table 4. *Houttuynia cordata* and *Perilla frutescens* leaves powders added to the diets had reduced (P<0.05) *E. coli* density in feces compared to the control chickens. *Salmonella* was almost undetectable in chicken feces of supplemented treatments, but there was a positive effect of *Salmonella* in the feces of chickens in control treatments at the 10th week age. And the mortalities of the experimental chickens were 1.25% (HCP) and 2.5% (PFP) compared to that in control (6.25%).

Table 2 Growth performance of experimental chickens from 4-14 weeks age (70 days)

Weeks of age	Treatments			SEM	P
	Cont	HCP	PFP		
Initial weight (4 weeks age)	288.32	281.23	286.45	4.77	0.15
Final weight (14 weeks age)	1381.2^{b}	1490.1^{a}	1472.5^{a}	21.2	0.03
ADG (g/day)	15.62	17.26	16.94	0.61	0.23
ADFI (g/head/day)	56.45	59.01	59.96	5.15	0.46
FCR (kg feed/kg gain)	3.61^{a}	3.42^{b}	3.54^{b}	0.09	0.04

Cont: Control diet (B) without supplementation in feed; HCP: B+ 2% *Houttuynia cordata* leaves powder in feed; PFP: B+2% *Perilla frutescens* leaves powder in feed. ADG: Average daily gain; ADFI: Average daily feed intake; FCR: Feed conversion ratio.

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

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Table 3 Carcass characteristic evaluation and breast meat color of chickens in the treatments

	Treatments			SEM	P
	Cont	HCP	PFP		
Slaughter weight (g)	1400	1452	1450	21.2	0.07
Carcass weight (g)	918	967	972	22.1	0.48
Carcass yield (%)	65.6	66.6	67.1	0.68	0.44
Thigh weight (g)	326.8	348.0	347.2	12.1	0.08
Thigh percentage/carcass,%	35.6	36.0	35.7	1.61	0.18
Thigh meat weight, (g)	192	215	210	5.88	0.56
Thigh meat percentage/carcass, %	21.1	22.2	21.6	0.66	0.61
Breast weigh (g)	218	232	236	9.20	0.33
Breast percentage/carcass,%	23.8	24.0	24.3	0.97	0.72
Breast meat weight (g)	166	183	185	4.57	0.21
Breast meat percentage/carcass,%	18.0	19.0	19.1	0.51	0.34
Abdominal fat, %	2.10	1.85	1.65	0.15	0.14
Color of breast meat					
L*	49.12	49.41	48.25	1.12	0.12
a*	2.62	2.74	2.70	0.30	0.27
b*	8.11^{b}	9.48^{a}	9.24^{a}	0.34	0.03
Chemical compositions of breast meat,%					
DM	32.6	33.1	32.1	0.89	0.08
CP	29.1	29.4	29.5	0.78	0.16
EE	2.45^{a}	1.87^{b}	1.91^{b}	0.11	0.02

Lightness (L), red-ness (a) and yellow-ness (b).Cont: Control diet (B) without supplementation in feed;* HCP: B+ 2% *Houttuynia cordata* leaves powder in feed; PFP: B+2% *Perilla frutescens* leaves powder in feed.

Table 4 Bacteria density in chicken feces at 10th week age and mortality of the experimental local chickens

Weeks of age	Treatments			SEM	P
	Cont	HCP	PFP		
Salmonella. spp/25g (+/-)	Pos	N	N	-	-
E. coli (10^{6} CFU/g)	2.32^{a}	2.11^{b}	2.12^{b}	0.03	0.02
Mortality (%)	6.25	1.25	2.50	-	-

^{a,b,c}Means within a row with different superscripts are significantly different (P<0.05); Pos: Positive; N: Non detected
Cont: Control diet (B) without supplementation in feed; HCP: B+ 2% *Houttuynia cordata* leaves powder in feed; PFP: B+2% *Perilla frutescens* leaves powder in feed.

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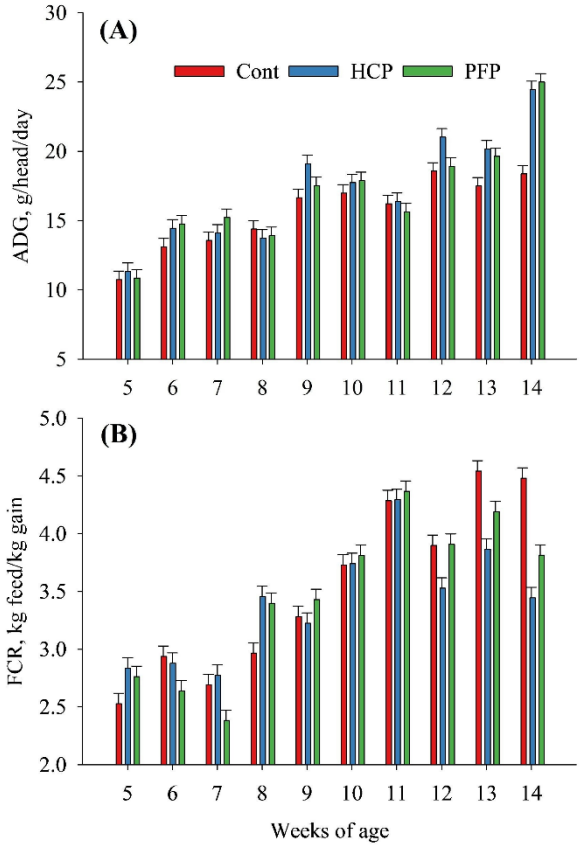

Figure 1(A) Average daily gain (ADG) and (B) feed conversion ratio (FCR) of the experimental chickens

DISCUSSION

In the present research, HC and PF leaves powders added in the diets have not affected the ADFI of the experimental chickens. However, there was a small improvement in the final weight of chickens fed supplemented diets to compare with chickens in the control group, resulting in a little higher ADG and have positive effect on FCR. These may be because of the effect of plant biologically active components in herbs, these compounds are also called phytobiotics or botanicals which help to improve the productivity of livestock (Windisch et al., 2008). In fact, PF leaves powder is rich in terpenes and flavonoids, which are the main bioactive components with antibacterial and anti-inflammatory, and PF leaves powder also contains abundant of carotenoids, vitamins, crude proteins, crude fibers and carbohydrates (Wu et al., 2023). In addition, Michael and Sanchai (2021) concluded that the highest proportion of PF meal enhanced the growth performance of broilers, supplementation of PF meal into the diet in an iso-nitrogenous way increased feed intake and metabolisability of nitrogen. Howerver, few studies have been conducted on PF meal as a protein supplement for monogastric animals until now.

In this research, HC supplementation in the diet also has improved feed efficiency. It may be because the chemical constituents present in the HC are houttuynum, decanoyl acetaldehyde, acetic acid and pyridinamine (Xu et al., 2006; Ch et al., 2007), which increase the activities of endogenous digestive enzymes and antioxidant status, and reduce the secretion of intestinal satiety hormone (Xiao-Fang et al., 2022). But Xiao-Fanget al. (2022) also reported the supplementation of HC has no obvious effect on the growth performance and slaughter performance

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of Hyline Brown chicks. Howerver, the research of Jang et al. (2019) concluded that adding 1% HC in pellets and coated-pellet form with fermented red koji to duck diets improved growth performance.

Dietary supplementation had no significant negative impact on carcass weight, dressing percentage and relative organ weight, but abdominal fat was substantially reduced in HC and PF leaves powders supplemented chickens relative to control. These are in agreement with research of Montha et al. (2021), who shows the proportion of abdominal fat declined with increasing levels of PF meal in the diet of chickens. Research from Sarker and Yang (2011) found no difference in internal organs among the supplemented groups except large intestine weight. And Michael and Sanchai (2021) reported the proportion of abdominal fat declined with increasing levels of PF meals. Breast muscle crude protein content was also similar in all treatments, but crude fat content was lower in HC and PF leaves powders supplemented groups in comparison to control. These are in agreement with research from Bostami et al. (2017), who reported that the dietary inclusion of HC and multi-microbe probiotics did not show any significant negative impact on the growth performance and relative organ weight of broilers, but immunity was significantly improved, mortality and abdominal fat content was significantly reduced, and broiler crude fat content in meat was significantly lower in the supplemented groups compared to control.

The color (yellowness) of meat is another important quality attribute of broiler meat as it influences the acceptance of the product by consumers in Vietnam. Supplementation of HC and PF leave powders increased the yellow color (b) of the breast meat. It can be explained that dietary antioxidants can modify the meat color, the oxidative status of meat muscle is directly related to meat quality and has negative effects on meat color (Zhang et al., 2017). Moreover, Wu et al. (2023) showed the PF content flavonoids, carotenoids and vitamins., which would be a potential strategy to improve the meat color in broilers, because of the pigmenting ability of these leaves seemed to increase the dietary content of xanthophylls, thereby imparting the yellowish color to the broiler skin and meat.

Supplementation with HC and PFleaves powders in the diet reduced *E. coli* and *Salmonela* in the gastrointestinal tract of the chickens. So, HC and PF leaves powders could be an alternative to antibiotics for the prevention of E*. coli* infection. It may be because the main bioactive components of PF leaves powder are terpenes and flavonoids that have antibacterial and anti-inflammatory functions (Wu et al., 2023). Also HC and PF leaves powders are more beneficial in increasing overall gut health and increasing the population of beneficial bacteria in the intestine. Lee and Kim. (2022) show that HC extract had significant antiprotozoal activity against *Eimeria tenella,* these findings may have implications for the development of anticoccidial drugs. Moreover, Wu et al. (2023) reported the PF is high flavonoid biosyntheticand the abundant chemical compositions of PF are the material basis of its homology as medicine. And HC also possesses a variety of pharmacological functions including anti-bacterial, anti-microbial, anti-inflammatory and immunomodulatory activities (Meng et al., 2009). Similarly with research from Thuy et al. (2023) when supplementation of another herb powder such as garlic in the diet reduced *E. coli* and *Clostridium perfringens* densities in the feces of chickens. And research from Sarker and Yang (2011) found the addition of 1.0% of HC with probiotics can be replaceable to antibiotics for broiler production, these results are in agreement with present results. In addition, the recorded mortalities of the present study chickens were 1.25% (HCP) and 2.5% (PFP) on average compared to that in control (6.25%), these findings are in agreement with above previous research where herbal supplementation reduced harmful intestinal *microflora* activities. This reduction in chickens' mortalities could be explained by improvement in birds general performance and better build-up of the immunity caused by the supplement.

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CONCLUSIONS

Houttuynia cordata and *Perilla frutescens* leaves powders supplemented in the diet at 2 % improved the growth and color of meat, reduced the fat content of breast meat and also reduced *Salmonella.spp*and *E. coli* in the feces of Noi chickens.

ACKNOWLEDGEMENTS

The author would like to thank to the manager of the experimental farm for supplying all materials of the experiment. And also sincere gratitude thanks to Mr. Nguyen Tan Tai and Mr. Tran Thanh Son for taking care the experiment.

AUTHOR CONTRIBUTIONS

Detail of each author with his/her contribution in this paper are mentioned as following; Le Thanh Phuong: Design, prepare experimental materials, collect data, manuscript preparation. Nguyen Cong Ha: Prepare the supplements, provided material and reagents, sampling, data analysis, manuscript editing. Nguyen Thi Thuy: Design, experimental studies, sampling, data collection management, manuscript editing.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial, personal, or other relationships with other people or organizations related to the material discussed in the manuscript.

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How to cite this article;

Le Thanh Phuong, Nguyen Cong Ha and Nguyen Thi Thuy. Effect of adding *Houttuynia cordata* and *Perilla frutescens* leaves powders in diets on growth, intestinal microflora and meat quality of local chickens in Vietnam. Veterinary Integrative Sciences. 2025; 23(2): e2025040-1-9

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