Journal of Animal Husbandry Sciences and Technics (JAHST)

No. 311 (Aug, 2025)

Permission: Ministry of Information and Communications of the Socialist Republic of Vietnam 119/GP-BTTTT date 26/1/2010

ISSN 1859–476X Publish: Monthly

Office: Room 902, 9th Floor, VUSTA Building Lot D20, Alley 19, Duy Tan, Dich Vong Hau, Cau Giay, Hanoi.

Tel / Fax: +84.2466898488

Hotline: +84.986422026 / +84.913340186

E.mail: tapchikhktchannuoi@gmail.com

Website: www.hoichannuoi.vn

Account:

Name: Hội Chăn nuôi Việt Nam (AHAV)

Number: 10050034744 in Vietcombank

Print 1,000 copies, Size 19x27cm at Hoang Quoc Viet Technology and Science.,JSC.

ANIMAL GENETICS AND BREEDING

ANIMAL FEEDS AND NUTRITION

ANIMAL PRODUCTION

SCIENTIFIC NEWS

EGG PRODUCTION AND EGG QUALITY OF NOI AND LUONG PHUONG HENS UNDER THE FARM CONDITION

Phan Ba Huu[1*], Nguyen Thi Thuy[2] and Nguyen Thiet[2]

Submitted: 30-Jun-2025 – Revised: 27-Jul-2025

Accepted: 31-Jul-2025

ABSTRACT

A study was carried out to evaluate egg production and egg quality of Noi Ben Tre (NBT), Noi Binh Dinh (NBD) and Luong Phuong (LP) hens under the farm condition. The experiment was arranged in a randomized complete block design with 11,580 hens/breed, raised in floor pens at 34-46 weeks old. There were three treatments corresponding to the three breeds, each with three replications, each replication was a commercial house raised 3,860 hens. The treatments included NBT, NBD, and LP hens. The results showed that LP hens have highest egg performance in terms of laying rate (68.0%), egg mass (38.0g of egg/hen/day), and feed conversion ratio (4.49g feed/g egg) compared to the other two breeds (P<0.05). Additionally, LP eggs had significantly higher values in egg weight (56.8 g/egg), albumen index (0.068), albumen ratio (56.9%), and yolk weight (18.0 g/egg) (P<0.05). Besides, NBT chickens had higher yolk index (0.384), eggshell thickness (0.37mm), and eggshell ratio (12.6%), whereas NBD hens tended to show intermediate values across many traits. These results suggest that LP hens have outstanding potential for commercial egg production in terms of productivity and quality, while NBT hens exhibit advantages in eggshell strength and structure. This provides a basis for breed selection and improvement of egg quality according to different production goals.

Keywords: *Noi hens, Luong Phuong hens, egg productivity, egg quality.*

1. INTRODUCTION

In the past 5 years, the Vietnamese poultry industry has witnessed a significant development in native colored chicken breeds, along with the development of mass and mass enterprises specializing in raising colored chickens. While some hybrid colored chicken breeds introduced by foreign companies are raised on an industrial scale, the meat quality has not been highly appreciated. On the contrary, many native colored chicken breeds are still raised in the form of small households, low density, semi-free range, so the meat quality is considered better. However, due to the free range form, these chickens are more susceptible to external pathogens.

Recently, some enterprises have started raising native colored chickens or domesticated chicken breeds: Luong Phuong, Sasso,… These activities are being carried out on a large scale using industrial farming methods to improve productivity and strengthen disease control. In addition to meat production, the demand for eggs is also increasing, prompting domestic and foreign enterprises to focus on developing commercial layer chicken flocks. However, to date, there has been no published scientific research on the productivity and egg quality of these colored feather chicken breeds raised in industrial farming systems. To address this issue, the study "Productivity and egg quality of Noi and Luong Phuong hens under industrial farm condition" was conducted.

2. MATERIAL AND METHODS

2.1. Time and location

The study was conducted from March 2025 to June 2025 at the breeding chicken farm of Vietswan Poultry Production Joint Stock Company, Gia Bien hamlet, Tam Lap commune, Phu Giao district, Binh Duong province. Egg quality analysis was carried

[1] Vietswan Poultry Breeding Joint Stock Company
[2] Can Tho University
[*] Corresponding author: Phan Ba Huu, Vietswan Poultry Breeding Joint Stock Company. Phone: 0084 918180185; Email: pbhuu@vietswan.com.

out at the Faculty of Animal science, College of Agriculture, Can Tho University.

2.2. Animals and experimental design

The experiment was arranged in a completely randomized block design with three treatments of chicken hens: Ben Tre Noi breed (NBT), Binh Dinh Noi breed (NBD), and Luong Phuong (LP) breeds. Each breed corresponds to one treatment, with 3 replications, each replication is a cage (456m^2) raising 3,860 hens at 22 week of age as the experimental unit, with a density of 8.5 hens/m^2.

2.3. Experimental treatments and feed

All hens were fed with experimental feed (V8214-T) this feed was produced by Vietswan Animal Nutrition Joint Stock Company (Tabele 1). Feeding was conducted twice a day, at 7:00 and 15:00. The chickens were raised and managed according to the company's standard procedures. Eggs were collected during the period at 34-46 weeks old, with five daily collections at 7:00, 9:00, 10:00, 13:00, and 16:00. Defective eggs (broken, cracked, misshapen, thin-shelled, undersized, or deformed) were discarded. Eggs were stored in a warehouse at a temperature of 20-21°C and relative humidity of 77-85%. All hens were vaccinated and cared according to the process of Vietswan Poultry Breeding Joint Stock Company.

Table 1. Chemical composition of diet

Composition	%	Composition	%
Crude Fat	3.2	Ca	3.3
Crude Fiber	2.7	Lysine	0.77
Crude Protein	15	Total P	0.6
ME (kcal/kg feed)	2,850	Na	0.17
Methionine	0.52	Cl	0.19

2.4. Management and measurements

2.4.1. Egg production

During 12 weeks of data collection, the daily egg production was recorded to calculate the hen-day egg production rate (%). All eggs were weighed, and their shape index measured immediately after collection. Egg weight was determined using a digital balance for all eggs produced per experimental unit. Daily feed intake (FI) was recorded, while egg mass and feed conversion ratio were calculated weekly. The reproductive performance indicators were assessed according to Bui Huu Doan *et al.* (2011).

Body weight (kg) of hens was randomly weighed 50 hens/pen at 34-46 weeks old; Feed intake (g of feed/hen/day) was amount of feed per day; Laying rate (%) was total eggs produced/total hens day)×100; Egg mass (g of egg/hen/day): Laying rate×Egg weight; Feed conversion ratio (FCR) (g of feed/g of egg) was feed intake/Egg mass.

2.4.2. Egg quality

A total of 2,600 eggs from NBT, NBD and LP hens at 34-46 weeks old were used to evaluate egg quality in the experiment. Every week, 60 eggs were randomly collected for each experimental unit for egg quality analysis. The eggs were broken and the egg contents were gently poured onto a horizontal glass surface. The yolk, albumen and eggshell of each egg were carefully separated and weighed separately using a digital balance. The egg quality parameters analyzed according to Bui Huu Doan *et al.* (2011).

Shape index (%): (Egg width/Egg length) ×100

Albumen index: [Albumen height/(Albumen length + Albumen width)/2]

Yolk index: (Yolk height/Yolk diameter)

Albumen ratio (%): (Albumen weight/Egg weight)×100

Yolk ratio (%): (Yolk weight/Egg weight) ×100

Yolk/Albumen ratio (%): (Yolk weight/Albumen weight)×100

Eggshell ratio (%): (Eggshell weight/Egg weight)×100

Eggshell thickness (mm): Using a micrometer to measure at three points (air cell, equator, and pointed end). Mean of triplicate measurement from three points.

Haugh unit (HU): HU=100×log10 (AH-1.7×EW $^{0.37}$+7.57)

AH (mm): height of albumen and EW (g): egg weight

Egg yolk color score: Using a Roche yolk color fan on a scale of 1-15.

2.5. Statistical analyses

The data were recorded by using Excel software and using generalized linear regression model (GLM) of Minitab 16.0 software (a data analysis software package used for data analysis) to analyse the variance. The significant differences between mean values within and between treatments were determined according to Tukey with P<0.05.

3. RESULTS AND DISCUSSION

3.1. Egg production

As shown in table 2, significant differences were observed in production performance among the NBT, NBD, and LP hens at 34-46 weeks old (P<0.05). The number of hens at 46 weeks old did not differ significantly among treatments (P>0.05), indicating similar survival rates (14%) across groups. However, there were marked differences in hen body weight (HW) at 46 weeks old with NBT hens had the lowest average weight (1.93 kg/hen), while NBD and LP hens were significantly heavier (2.78 and 2.75 kg/hen, respectively). At 34-46 weeks old, LP hens produced the heaviest eggs (56.0 g/egg), followed by NBD (53.6 g/egg) and NBT (49.0 g/egg), with significant differences among all groups (P=0.001). Feed intake (FI) also varied significantly among treatments, with LP hens consuming the most feed (169 g/hen/day), followed by NBD (147 g/hen/day) and NBT (130 g/hen/day). In addition, the laying rate was significantly higher in LP hens (68.0%) compared to NBD (50.9%) and NBT (41.0%). Correspondingly, egg mass (EM) was greatest in the LP group (38.0g egg/hen/day), followed by NBD (27.2g egg/hen/day) and NBT (20.0g egg/hen/day). Moreover, LP hens had the most FCR (4.49), significantly lower than NBT (6.59) and moderately lower than NBD (5.45). In summary, LP chickens exhibited superior production performance in terms of laying rate, egg mass, and FCR, while NBD hens demonstrated intermediate characteristics between the two extremes.

Table 2. Egg production of hens 34-46 weeks old

Items	Treatments			SEM	P
	NBT	NBD	LP		
Number of hens 22w	3,860	3,860	3,860	-	-
Number of hens 34w	3,411	3,476	3,387	144	0.91
Number of hens 46w	3,290	3,321	3,306	121	0.98
HW 46w (kg/hen)	1.93^{b}	2.78^{a}	2.75^{a}	0.03	0.001
FI (g feed/hen/day)	130^{c}	147^{b}	169^{a}	3.39	0.003
Laying rate (%)	41.0^{b}	50.9^{b}	68.0^{a}	2.05	0.002
Egg weight (g/egg)	49.0^{c}	53.6^{b}	56.0^{a}	0.31	0.001
EM (g egg/hen/day)	20.0^{b}	27.2^{b}	38.0^{a}	1.10	0.001
FCR (g feed/g egg)	6.59^{a}	5.45^{ab}	4.49^{b}	0.18	0.003

Where: Means with different letters in the same row differ significantly (P<0.05)

In addition, the average egg weight of the three chicken breeds at 34-46 weeks of age in this study was significantly higher than that of some Vietnamese native chicken breeds. LP chickens also laid heavier eggs than F_1(Dong Tao×Luong Phuong) hens, averaging 52.5g at 38 weeks of age (Nguyen Van Duy *et al.*, 2020). The egg weight of NBT chickens (49.9-51.3g) reported by Le Thanh Phuong and Nguyen Thiet (2022) was comparable to the values observed in this study. Raziq *et al.* (2024) reported a positive association between hen body weight (HW) and egg weight (EW), consistent with the present findings that LP and NBD hens (2.75-2.78kg) laid heavier eggs than NBT hens (1.93kg) at 34-46 weeks of age. The laying rate of hens in this study was higher than that of some other indigenous chicken breeds such as Bang Troi chickens at 37-40 weeks of age (34.1%), Ri Lac Son chickens (31.4%) at 40 weeks of age (Nguyen Hoang Thinh *et al.*, 2021). H'Mong chickens require 0.66kg of feed to produce 10 eggs with an average weight of 38.1 g/egg (Nguyen Thi Phuong *et al*., 2017). However, the current study showed that the FCR was higher than the results of Nguyen Van Duy *et al*. (2020) on Ho chickens (4.36) and Dong Tao chickens (4.06). These variations in results may be explained by differences in the genetic background of the studied chicken breeds, observation time, and management and rearing conditions.

3.2. Egg quality

The average egg weights of the three breeds collected at 34, 38, 42 and 46 weeks of age are presented in table 2. Hens on the LP diet produced the heaviest egg weight (56.8 g/egg), which was significantly greater than those on the NBT diet (50.1 g/egg). The hens in the present study produced eggs with higher weights than reported indigenous chickens. Sani et al. (2024) found that exotic chickens in Nigeria produced eggs weighing 47.9 g/egg, while indigenous chickens raised under improved feed conditions and omnivorous diets had egg weights of 42.3 and 40.3 g/egg, respectively.

Table 3. Egg quality of hens at 34-46 weeks old

Items	Treatments			SEM	P
	NBT	NBD	LP		
EW, g	50.1^{c}	53.2^{b}	56.8^{a}	0.61	0.004
ESI, %	75.4^{b}	75.4^{b}	75.5^{a}	0.03	0.030
Albumen index	0.065^{b}	0.065^{b}	0.068^{a}	0.002	0.001
Yolk index	0.384^{a}	0.369^{b}	0.376^{ab}	0.002	0.010
Albumen ratio, %	54.9^{c}	56.1^{b}	56.9^{a}	0.06	0.001
Yolk weight, g	16.5^{c}	17.7^{b}	18.0^{a}	0.02	0.001
Yolk ratio, %	32.6^{a}	32.2^{b}	31.8^{c}	0.04	0.001
Yolk/Albumen, %	59.7^{a}	57.7^{b}	56.1^{c}	0.15	0.001
Eggshell weight, g	6.39	6.46	6.37	0.03	0.160
Eggshell ratio, %	12.75^{a}	12.14^{a}	11.21^{b}	0.16	0.001
Eggshell thickness, mm	0.37^{a}	0.33^{b}	0.33^{b}	0.001	0.001
Haugh unit	75.4	75.5	75.5	0.06	0.940
Egg yolk color score	6.26	6.13	6.13	0.04	0.100

In addition, the egg shape index (ESI) of the three chicken breeds ranged from 75.4 to 75.5%. According to Duman *et al*. (2016), eggs are classified as flat (<72%), standard (72-76%), and round (>76%). Therefore, the ESI observed in this study were within the standard range, indicating a well-proportioned egg shape. LP chickens had the highest albumen percentage (56.9%), followed by NBD (56.1%) and NBT (54.9%). A higher albumen percentage indicates a thicker, more viscous albumen, reflecting freshness and better protein quality (Sun *et al*., 2019; Altunatmaz *et al*., 2020). The albumen index can be influenced by factors such as egg age, storage conditions, breed, and diet (Krisnaningsih *et al*., 2023).

The eggshell weight did not differ significantly among NBT, NBD and LP chickens (6.39, 6.46 and 6.37 g/egg, respectively; *P*=0.160). However, NBT eggs had the highest eggshell ratio (12.75a), followed by NBD (12.14a) and LP (11.21b). These values exceeded those reported by Kumar *et al.* (2022), who found eggshell ratios of 11.2% in Aseel and 10.6% in Kadaknath hens, with corresponding shell weights of 4.65 and 4.28 g/egg under backyard farming conditions in India. Eggshell thickness differed significantly among the three chicken breeds (P=0.001). NBT chickens had the thickest shells (0.37mm), significantly higher than NBD and LP (both 0.33 mm). According to Ketta and Tůmová (2018), shell thicknesses of 0.28-0.30mm are considered thin, 0.33-0.36mm medium, and 0.39-0.41mm thick. Thus, all values in the present study fell within the medium range, indicating acceptable egg quality. These results were comparable to findings of Sun *et al.* (2019) reported 0.34 mm in White Leghorns, Kumar *et al.* (2022) found 0.35mm in Aseel and 0.34mm in Kadaknath hens, while Milkias and Molla (2022) recorded a higher average of 0.54 mm in indigenous chickens from the Dawro highlands.

The eggshell weight did not differ significantly among NBT, NBD and LP chickens (6.39, 6.46 and 6.37 g/egg, respectively; P=0.16). However, NBT eggs had the highest eggshell ratio (12.75a), followed by NBD (12.14a) and LP (11.21b). These values exceeded those reported by Kumar *et al.* (2022), who found eggshell ratios of 11.2% in Aseel and 10.6% in Kadaknath hens, with corresponding shell weights of 4.65 and 4.28 g/egg under backyard farming conditions in India. Eggshell thickness differed significantly among the three chicken breeds (P=0.001). NBT chickens had the thickest shells (0.37mm), significantly higher than NBD and LP (both 0.33mm). According to Ketta and Tůmová (2018), shell thicknesses of 0.28-0.30mm are considered thin, 0.33-0.36mm medium, and 0.39-0.41mm thick. Thus, all values in the present study fell within the medium range, indicating acceptable egg quality.

In addition, yolk index is an important parameter to evaluate the internal egg quality, which showed significant differences among the three chicken breeds studied with NBT chicken showing the highest value (0.384). Table 3 shows that LP chickens had the highest yolk weight (18.0 g/egg), followed by NBD (17.7 g/egg), while NBT chickens had the lowest (16.5 g/egg). In contrast, NBT chickens had the highest yolk ratio (32.6%), while LP recorded the lowest (31.8%). Nguyen Nhut Xuan Dung and Luu Huu Manh (2016) found that yolk ratio in Noi chickens was higher at the onset of laying (34.5%) than in later stages (33.7%). Furthermore, the yolk to albumen ratio was significantly higher in NBT chickens (59.7%) than in NBD (57.7%) and LP (56.1%). Based on the classification by Kuchida *et al.* (1999), which defines five levels (1: ≤35%, 2: 35-45%, 3: 45-55%, 4: 55-65%, 5: ≥65%), these values fall into Level 4, indicating relatively good egg quality. The ratios observed in this study were higher than those reported by Sun *et al.* (2019), who found a yolk to albumen ratio of 43.9% in White Leghorn layers. These differences likely reflect variations in genetic background and management practices.

A higher HU indicates a higher quality egg with thicker, more intact whites. The HU values are classified into four quality grades: AA (≥72), A (60-71), B (30-59), C (≤29) (UNECE, 2010). Although HU were not significantly different among treatments (P>0.05), values remained above 75 in all treatments, in which the refrigerated eggs were classified as AA that reflecting good freshness. The current result was lower than the findings reported by Kumar *et al.* (2022), who observed HU of 82.9 in Aseel chickens and 79.82 in Kadaknath chickens. The study by Sani *et al.* (2024) on exotic and indigenous chickens in Nigeria demonstrated that yolk index was influenced by both breed and rearing conditions. The yolk index was 78.5 in exotic breeds, 70.9 in indigenous chickens reared under improved feeding conditions, and 65.7 in indigenous chickens reared under scavenging feeding conditions.

Finally, the egg yolk color score did not differ significantly among the three chicken breeds (P>0.05), though NBT chickens had a slightly higher mean (6.26) than NBD and LP (both at 6.13). This value was lower than those reported by Sözcü *et al.* (2023) in commercial layers (11.7-12.3), and Seid and Tesfa (2025), who observed the highest score (13.13) in indigenous chickens. Yolk color was mainly affected by dietary pigments like xanthophylls, carotenoids, and cryptoxanthin and consumers often prefer yellow to orange yolks, which are associated with better health and higher satisfaction (Kartikasari *et al.*, 2021).

4. CONCLUSIONS

In conclusion, Luong Phuong hens have outstanding potential for egg performance, while Ben Tre hens exhibit advantages in eggshell strength and structure. The egg quality of the Noi Ben Tre hens was superior compared to that of Noi Binh Dinh and Luong Phuong breeds. However, the egg quality of all three breeds met the required standards.

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GROWTH PERFORMANCE OF LANDRACE PIGS WITH GENETIC RESOURCES FROM FRANCE

Do Duc Luc[1], Pham Duy Pham[2], Trinh Hong Son[2], Nguyen Thi Hong Nhung[3] and Pham Doan Lan[4]*

Submitted: 30-Jun-2025 – Revised: 27-Jul-2025

Accepted: 31-Jul-2025

ABSTRACT

The experiment was carried out at the Ky Son Station on Research and Development of nucleus pigs belong to Thuy Phuong Pig Research Centre, National Institute of Animal Science from September 2015 to July 2019. A total of 1125 Landrace pigs (365 intact males and 760 females) originated from Gene+ (France). The data of productive performance were collected from 4 generations, including (1) the initial (5 intact males and 40 females), the first (120 intact males and 240 females), the second (120 intact males and 240 females) and the third (120 intact males and 240 females). The productive traits were initial body weight (IBW, kg), final body weight (FBW, kg), average daily gain (ADG, g), backfat thickness (BFT, mm), *longissimus* muscle depth (LDD, mm), lean meat percentage (LMP, %), and intramuscular fat content (IMF, %). Generation, season and sex affected all study traits (P<0.05) except LMP between generations (P=0.2801), season (P=0.6532) and IBW between sexes (P=0.1654). FBW, ADG, LDD and LMP were lowest in the initial generation, increased in the first generation and reached highest at the second and third generations. Inversely, BFT decreased gradually from the initial to the third generation (P<0.05). Whereas IMF did not significantly difference between generation (P>0.05). ADG was improved through generation while LMP was stable.

Keywords: *Swine, production performance, carcass traits, intramuscular fat.*

1. INTRODUCTION

According to FAO (2024), Vietnam is one of the leading countries in pig production. National pig production had been ranked sixth worldwide in terms of both herd size and pork output. In 2023, 3.12 million sows were recorded, in which 27.5% were exotic pigs (MARD, 2025). A total of 48,806 breeding pigs were imported from different countries mainly in France, Denmark, Canada (Livestock Department, 2022) in order to to improve the productive performance of the national pig population. Currently, Landrace and Yorkshire pigs are mainly used in Vietnam due to high economic efficiency. Gene+ was a genetic company who produced pigs breeding breeds, including Landrace and Yorkshire pigs. In Vietnam, productive performance of these two imported breeds from Gene+ were compared in previous studies (Nguyen Thi Hong Nhung *et al.*, 2020, Nguyễn Thị Hồng Nhung *et al.*, 2020a,b). The adaptation of imported Landrace and Yorkshire pigs was mentioned in different research (Hà Xuân Bộ and Đỗ Đức Lực, 2020; Do Duc Luc *et al.*, 2024a, Do Duc Luc *et al.*, 2024b). Landrace pigs from French genetic resources were multiplied and created a nucleus herd through generations at Thuy Phuong Pig Research Centre, National Institute of Animal Science. The objective of this study was to evaluate factors affected productive performance of Landrace pigs sourced from Gene+ genetic company (France).

[1] Vietnam National University of Agriculture
[2] Thuy Phuong Pig Research Centre
[3] Hanoi Department of Animal Husbandry and Veterinary Medicine
[4] National Institute of Animal Science
* *Corresponding author, Assoc. Prof. Dr. Do Duc Luc, Faculty of Animal Science, Vietnam National University of Agriculture, Hanoi, Vietnam. Phone: 0084 912370193; Email: ddluc@vnua.edu.vn.*

2. MATERIALS AND METHODS

2.1. Experimental design

The experiment was carried out at the Ky Son Station on Research and Development of nucleus pigs belong to Thuy Phuong Pig Research Centre, National Institute of Animal

Science from September 2015 to July 2019. A total of 1,125 Landrace pigs (365 intact males and 760 females) originated from Gene+ (France). The data of productive performance were collected from 4 generations, including (1) the initial (5 intact males and 40 females), the first (120 intact males and 240 females), the second (120 intact males and 240 females) and the third (120 intact males and 240 females). The productive traits were initial body weight (IBW, kg), final body weight (FBW, kg), average daily gain (ADG, g), backfat thickness (BFT, mm), *longissimus* muscle depth (LDD, mm), lean meat percentage (LMP, %), and intramuscular fat content (IMF, %).

IBW and FBW of pigs were weighted by the electronic scale Kelba (Australia) at age of 74.08±1.19 and 152.44±3.32 days, respectively. At the same time of FBW measurement, ultrasound images were taken 6.5cm from the dorsal midline at the last ribs using Agroscan AL ultrasound device with ALAL 350 probe (ECM, France) for BFT and LDD measurement and Exago ultrasound device with L3130B probe (ECM) for taking images for IMF. IMF was predicted by using Biosoft Toolbox II for Swine from ultrasound images. (LMP) was predicted from BFT and LDD using a regression equation recommended by Ministère des Classes Moyennes et de l'Agriculture (1999): $Y=59.902386-1.060750X_1+0.2229324X_2$; *where: Y=LMP(%),* X_1*=BFT (including skin),* X_2*=LDD.* ADG was calculated by IBW, FBW, and the duration of experiment.

2.2. Data analysis

Data were analyzed using SAS 9.1 software (2002) with the statistical model $Y_{ijkl}=\mu+TH_i+MV_j+TB_k+\varepsilon_{ijkl}$; where, y_{ijkl}*=productive traits,* μ*=overall mean,* TH_i*=effect of generation i (i=4: initial, first, second and third),* MV_j *=effect of season j (j=4: Spring, Summer, Autumn and Winter),* TB_k*=effect of sex k (k=2: intact male and female),* ε_{ijkl}*=random error*. The statistical parameters presented in the tables are number of observations (n), least square mean (LSM), and standard error (SE). Pairwise comparisons between LSMs were performed using Tukey's test.

3. RESULTS AND DISCUSSION

3.1. Effect of the fixed factors on productive performance

The significant levels (p-values) of fixed factors on growth performance and carcass traits are presented in table 1. The fixed factors (generation, season and sex) affected all study traits (P<0.05) except LMP between generations (P=0.2801), season (P=0.6532) and IBW between sexes (P=0,1654). The coefficient of determination (R^2) varied from 8.57 for FBW 83.31 for LMP.

Table 1. Effect of generation, season and gender

Variable	Generation	Season	Sex	R^2 (%)
IBW (kg)	0,0119	0,0168	0,1654	21,14
FBW (kg)	<0,0001	<0,0001	<0,0001	8,57
ADG (g)	<0,0001	<0,0001	<0,0001	82,71
BFT (mm)	<0,0001	<0,0001	<0,0001	80,02
LDD (mm)	<0,0001	<0,0001	<0,0001	39,68
LMP (%)	<0,0001	<0,0001	<0,0001	83,31
IMF (%)	0,2801	0,6532	<0,0001	9,15

3.2. Productive performance of Landrace by generations

The productive traits of Landrace according to generation are presented in table 2. FBW, ADG, LDD and LMP were lowest in the initial generation, increased in the first generation and reached highest at the second and third generations. Inversely, BFT decreased gradually from the initial to the third generation (P<0.05). Whereas IMF did not significantly difference between generation (P>0.05). Productive performance was mentioned in the studies of Nguyễn Thi Hương *et al.* (2018) and Pham Thi Minh Nu (2022). Pham Thi Minh Nu (2022) found that ADG, LDD and LMP of DVN1 and DVN2 pigs improved through 3 generations, while BFT was decreased.

Table 2. Productive performance of Landrace by generations

Variable	Initial		Generation 1		Generation 2		Generation 3	
	n	LSM±SE	n	LSM	n	LSM	n	LSM
IBW (kg)	45	30,65^{a}±0,16	360	30,20^{b}±0,05	360	30,13^{b}±0,05	360	30,25^{ab}±0,05
FBW (kg)	45	99,13^{c}±0,28	360	100,29^{b}±0,07	360	101,05^{a}±0,08	360	101,27^{a}±0,09
ADG (g)	45	840,82^{d}±3,55	360	889,20^{c}±0,90	360	912,12^{b}±0,99	360	918,92^{a}±1,20
BFT (mm)	45	12,67^{a}±0,12	360	12,62^{a}±0,04	360	11,98^{b}±0,04	360	11,70^{c}±0,04
LDD (mm)	45	56,70^{c}±0,13	360	57,76^{b}±0,04	360	58,46^{a}±0,04	360	58,59^{a}±0,04
LMP (%)	45	59,47^{c}±0,12	360	59,76^{c}±0,04	360	60,59^{b}±0,04	360	60,93^{a}±0,04
IMF (%)	15	2712±0,046	80	2768±0,018	80	2792±0,018	80	2798±0,018

Notes: In the same row, LSMs with different letters are significantly different (P<0,05)

3.3. Productive performance of Landrace by seasons

The productive traits according to seasons (table 3). FBW (100.78kg), ADG (898.57g) were highest in Spring (P<0.05). However, the season did not affect IMF (P>0.05). The effect of season on ADG and LMP on Landrace pigs was found in the study of Luu Van Trang (2021).

Table 3. Productive performance of Landrace by seasons

Variable	Spring		Summer		Autumn		Winter	
	n	LSM±SE	n	LSM±SE	n	LSM±SE	n	LSM±SE
IBW (kg)	270	30,16^{b}±0,07	270	30,34^{ab}±0,07	315	30,31^{ab}±0,06	270	30,43^{a}±0,07
FBW (kg)	270	100,78^{a}±0,09	270	100,31b^{c}±0,10	315	100,50^{b}±0,08	270	100,16^{c}±0,10
ADG (g)	270	898,57^{a}±1,17	270	887,62^{c}±1,21	315	892,32^{b}±0,98	270	882,56^{d}±1,25
BFT (mm)	270	12,20^{bc}±0,06	270	12,04^{c}±0,06	315	12,21^{b}±0,05	270	12,52^{a}±0,06
LDD (mm)	270	58,03^{a}±0,06	270	57,90^{a}±0,06	315	57,99^{a}±0,05	270	57,58^{b}±0,06
LMP (%)	270	60,27^{a}±0,06	270	60,41^{a}±0,06	315	60,25^{a}±0,05	270	59,82^{b}±0,06
IMF (%)	60	2784±0,024	60	2748±0,024	75	2776±0,019	60	2761±0,024

3.4. Productive performance of Landrace by sexes

The productive traits females and intact males are in the Table 4. IBW was not different between sexes (P>0.05). FBW, ADG, LDD and LMP of males were higher than those of female (P<0.05). Inversely BFT and IMF of females were higher than those of males (P<0.05).

Results of our study are consistent to studies on the growth performance and carcass traits of different pig breeds. Lowell et al. (2019) found that sex significantly affects ADG in purebred Piétrain and Duroc pigs raised in the US. Trinh Hong Son *et al.* (2019) found that sex factors affected ADG, BFT, LDD and LMP however did not affect IMF of LVN1 and LVN2 pigs. This trend was also observed in the study of Pham Thi Minh Nu (2022). The study of Lê Văn Sáng *et al.* (2019) on Landrace shown that ADG, LDD and LMP of males were higher than that of females, while BFT were lower. The effect of sex on growth performance and carcass traits was reported by Do Duc Luc *et al.* (2023).

Table 4. Productive performance of Landrace by sexes

Variable	Female		Intact male	
	n	LSM±SE	n	LSM±SE
IBW (kg)	760	30,27±0,05	365	30,35±0,06
FBW (kg)	760	100,16^{b}±0,10	365	100,71^{a}±0,08
ADG (g)	760	875,10^{b}±1,29	365	905,43^{a}±1,04
BFT (mm)	760	13,75^{a}±0,04	365	10,73^{b}±0,05
LDD (mm)	760	57,40^{b}±0,04	365	58,35^{a}±0,05
LMP (%)	760	58,48^{b}±0,04	365	61,90^{a}±0,05
IMF (%)	130	2,814^{a}±0,017	125	2,720^{b}±0,018

4. CONCLUSIONS

Generation, season and sex affected all growth traits (P<0.05) except LMP between generations (P=0.2801), season (P=0.6532) and IBW between sexes (P=0,1654). FBW, ADG, LDD and LMP were lowest in the initial generation, increased in the first generation and reached highest at the second and third generations. Inversely, BFT decreased gradually from the initial to the third generation (P<0.05). Whereas IMF did not significantly difference between generation

(P>0.05). ADG was improved through generation while LMP was stable.

ACKNOWLEDGMENTS

This study was carried out with the support of the directorate and staff of Ky Son Station on Research and Development of nucleus pigs belong to Thuy Phuong Pig Research Centre.

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MORPHOLOGICAL CHARACTERIZATION OF RACING BULLS IN TINH BIEN DISTRICT, AN GIANG PROVINCE

Pham Thi Kim Phuong[1], Nguyen Ba Trung[1], Trang Quang Vinh[1] and Nguyen Thi Bao Tran[1]*

Submitted 29-Jun-2025 – Revised: 28-Jul-2025

Accepted: 31-Jul-2025

ABSTRACT

This study aimed to characterize the morphological traits of racing bulls in Tinh Bien district, An Giang province to the traditional bull racing festival celebrated by the Khmer ethnic community in Southern Vietnam. A total of 30 bulls aged 3-8 years from specialized households were assessed. Twelve morphometric traits were measured, including estimated body weight (via heart girth), five height-related indices (withers, back, hip, sacrum, ischial tuberosity), four length and width traits (straight body and rump length and width, cannon bone circumference), and chest dimensions. The results showed that the average body weight was 595.4kg, chest girth 195.2cm, withers height 155.1cm, straight body length 148.8cm, and cannon bone circumference 20.0cm. These values indicate that Tinh Bien bulls are physically superior to several Southeast Asian indigenous breeds, such as Kedah-Kelantan cattle (Malaysia) and Madura bulls (Indonesia), likely due to selective breeding and intensive training. Comparison with prior studies (Suprapto, Kuswati, and Islam) suggests that the unique morphology of these bulls may result from both genetic and phenotypic selection. The phenotypic data provide a scientific basis for establishing standardized selection criteria, facilitating genetic improvement, and conserving cultural heritage. Furthermore, this foundational work could demonstrate the advantages of integrating biometric and genetic tools to enhance traditional livestock management systems.

Keywords: *Morphological characterization*, *racing bulls, cultural heritage.*

1. INTRODUCTION

Bull racing in the Bay Nui region of An Giang province, especially in Tinh Bien , is not merely a traditional sport but a deeply rooted cultural practice of the Khmer ethnic minority in Southern Vietnam. The annual bull racing event, held during the Dolta festival, is a unique expression of spiritual life, community pride, and local identity. For centuries, this tradition has been maintained through the efforts of local farmers, who breed and train bulls not only for agricultural purposes but also for competitive performance in culturally significant races (Trung and Tham, 2025). Training and raising racing bulls involves a combination of traditional experience and folk practices. Trung and Phuong (2025) reported, rearing practices focus heavily on nutritional enrichment to enhance muscle mass, physical strength, and endurance. Additionally, bulls are subjected to rigorous exercise routines, mental conditioning, and the use of herbal folk remedies to optimize their health and performance on the racetrack. Despite the intensity and precision involved in these practices, most selection and management decisions are still based on subjective evaluation, lacking scientifical frameworks for classification and improvement.

Comparable traditional bull-racing systems exist in other Southeast Asian cultures. For instance, the Kerapan Sapi festival in Madura, Indonesia, is a prominent example of bull racing rooted in local wisdom. In his studies on ethnophysics and cultural preservation (Ainol *et al*., 2019), Nadi *et al.* (2022, 2023) introduces the concept of "glocalization," referring to the integration of global scientific tools with indigenous practices to sustain and enhance traditional sports. These studies underscore the value of applying biometric and statistical models to optimize animal performance while preserving cultural identity.

[1] An Giang University, VNU-HCM, Vietnam.
* Corresponding Author: MsC. Pham Thi Kim Phuong, An Giang University, Vietnam National University, HoChiMinh City (VNUHCM): 18 Ung Van Khiem street, Long Xuyen Ward, An Giang Province, Vietnam. Phone: 0084 918589914. Email: ptkphuong@agu.edu.vn.

Beyond cultural perspectives, scientific research has also demonstrated the biological and genetic basis of desirable phenotypes in cattle used for performance and work. Kuswati *et al.* (2023) identified a significant association between single nucleotide polymorphisms in the leptin gene and morphometric traits such as BW and skeletal development in Madura cattle. Similarly, Islam *et al.* (2022) studied on Malaysian Kedah-Kelantan cattle has contributed to the development of non-invasive methods to estimate BW based on external measurements.

In Tinh Bien, current selection criteria for racing bulls are mainly based on physical appearance—such as shoulder height, chest girth, and body length-but lack empirical data to support these standards (Trung and Phuong, 2025). As a result, the development of a systematic morphometric profile for racing bulls is essential to support scientific breeding, selection, and performance prediction. Therefore, the objective of this study is to characterize the external morphological traits of racing bulls in Tinh Bien, An Giang. By quantifying key body measurements and analyzing their variation, this research results could contribute to standardizing the selection criteria for racing bulls, creating the premise for further research on genetics and breeding in the future.

2. MATERIALS AND METHODS

2.1. Study Population

Antotal of 30 racing bulls that are being trained and used in bull racing festivals in localities such as Vinh Trung, An Cu, An Nong, Nui Voi, Van Giao, An Giang province. The selected racing bulls are between 3 and 8 years old, in the stage of vigorous competition, and selected from households that have specialized in raising racing bulls for many years. The criteria for selecting bulls are healthy, disease-free; have an appearance that meets the standards of racing bulls: balanced shoulder height, deep and broad chest, and firm muscles; and have experience participating in at least one racing season or are being trained to participate in competition.

2.2. Morphometric measurements

External morphological traits were measured using standard veterinary and animal husbandry tools, including a tape measure, measuring stick, and steel tape measure (Bosch 1600A016BH). Determine the mass of the bull by measuring the chest circumference (CC); measure 12 indicators related to physical size, including CC is the circumference around the cow's body, measured at the position right behind the shoulder blade. Chest depth (CD) is measured from the shoulder blade to the middle of the beak bone. Diagonal body length (DBL) is the length from the front tip of the shoulder blade to the tip of the ischial tuberosity behind. Wither height (WH) is the distance from the ground to the highest point of the shoulder blade. Back height (BH), from the ground to the last thoracic vertebrae, equivalent to the lowest point of the back. Hip height (HH), from the ground to the top point of the vertical plane in contact with the head of the hip bone. Arch height (AH), from the ground to the highest point of the ischial tuberosity. Ischial tuberosity (IT), from the ground to the last ischial tuberosity. Straight body length (SBL), from the rearmost ischial tuberosity stretched straight parallel to the ground to the middle of the shoulder blade. Buttock length (BL), from the rearmost ischial tuberosity stretched straight parallel to the ground to the vertical plane in contact with the head of the hip bone. Buttock width (BW), the distance between the two outermost points of the acetabular joint. Tube circumference (TC), the circumference of the upper third of the left forefoot bone (Astika and Hidayat, 2020); Islam *et al*., 2022).

2.3. Statistical processing

Initial data on mass and physical dimensions were entered into Microsoft Excel 2010 software for descriptive statistical analysis. Values Mean, Standard Error (SE), minimum (MIN), maximum (MAX) values were calculated to describe the distribution characteristics of each variable in the study sample. Descriptive statistics help identify the uniformity and variation in the data, thereby preliminarily assessing the representativeness and quality of the study sample.

3. RESULTS AND DISCUSSION

3.1. Chest size, body length and body weight

Results from table 1 show that racing bulls in Tinh Bien have an average body weight (BW) of 595.4±49.0kg, chest girth (CG) 195.2±5.3cm, chest depth (CD) 81.6±4.7cm, and diagonal body length (DBL) 160.6±2.6cm. These measurements indicate that the animals possess well-developed physiques, suitable for the traction strength and endurance required in traditional racing practices.

Compared to the findings of Islam *et al*. (2022) on Kedah-Kelantan cattle in Malaysia, where BW ranged 350-450kg, CG 160-175cm, the racing bulls in Tinh Bien exhibit significantly larger frames. This difference is likely due to both genetic and intensive training practices aimed at competition performance. Similarly, Nadi *et al*. (2022, 2023) reported that Madurese racing bulls in the Kerapan Sapi festival had lower BW (380-500kg) and BL (145-155cm), indicating a smaller morphology suited for sprint-style racing, in contrast to the pair-pulling races in Vietnam, which require greater mass and stability. Moreover, Kuswati *et al.* (2023) identified a significant association between the leptin gene polymorphism c.73TC and BW and CG in Madura cattle, suggesting that genetic markers can be explored to link morphometric traits. This opens a future direction for applying molecular genetics to the selection of racing bulls in Tinh Bien.

Table 1. Chest size, body length and weight

Variable	Mean±SE	MIN	MAX
BW (kg)	595.4±8.9	478	689
CG (cm)	195.2±0.9	182	205
CD (cm)	81.6±0.8	74	90
DBL (cm)	160.6±0.5	155	165

3.2. Dimensions of heights

Table 2 show the height measurements of racing bulls in Tinh Bien, showing average values of 155.1cm at the withers (WH), 150.9cm at the back (BH), 153.5cm at the hips (HH), 158.7cm at the ilium (IH), and 140.6cm at the ischial tuberosity (IBH).

Table 2. Height values

Variable	Mean±SE	MIN	MAX
WH (cm)	155.1±1.0	144	172
BH (cm)	150.9±1.1	143	170
HH (cm)	153.5±1.3	143	176
IH (cm)	158.7±1.4	151	185
IBH (cm)	140.6±1.7	129	168

These relatively uniform and well-developed skeletal dimensions suggest a robust body frame, consistent with the physical demands of traditional Khmer-style double-yoke bull racing. When compared to findings by Islam *et al.* (2022) on Kedah-Kelantan in Malaysia, which reported WH ranging of 115-135cm, the Tinh Bien bulls are significantly taller. This difference is likely attributed to different breeding purposes-Kedah-Kelantan cattle are mainly meat producers, while Tinh Bien racing bulls are selected for pulling strength and endurance in competition. Similarly, Madura bulls used in the Kerapan Sapi festival exhibit WH of approximately 130-145cm (Nadi *et al*., 2022, 2023), smaller in stature than Tinh Bien bulls. The difference reflects adaptation to different racing styles: sprint racing in Madura versus strength-based racing in Vietnam. Moreover, Kuswati *et al.* (2023) identified significant associations between leptin gene polymorphisms c.73TC and morphometric traits, including BW and WH, in Madura cattle. These findings support the hypothesis that the impressive height characteristics observed in Tinh Bien bulls may result from both morphological selection and underlying genetic potential.

3.3. Body length and width dimensions

The average BL of racing bulls in Tinh Bien is 148.8cm, rump length (RL) is 58.8cm, rump width (RW) is 44.3cm, and the cannon bone circumference (CBC) is 20.0cm. These indicate a well-proportioned and muscular body, particularly with a broad rump and sturdy forelimbs-key traits for balance and propulsion during paired traction races.

In comparison, Madura racing bulls used in Kerapan Sapi events typically have shorter BL (around 140-145cm) and narrower RL (Nadi *et al*., 2022, 2023), reflecting a lighter build suited for sprint-style racing rather than strength-based competitions like in Vietnam. Furthermore, Kuswati *et al.* (2023) reported that BL and RW are significantly associated with leptin gene polymorphisms (c.73TC), which influence muscle development and locomotive efficiency. This suggests that such morphometric traits in Tinh Bien bulls may also be partly genetically determined. Islam *et al.* (2022) emphasized the correlation between BL and BW in Kedah-Kelantan cattle, supporting the use of these traits as predictors of overall body development. The well-developed body dimensions of Tinh Bien bulls thus reflect both functional adaptation and potential as a foundation for genetic selection.

Table 3. Body length and width index

Variable	Mean±SE	MIN	MAX
BL (cm)	148.8±1.19	139	162
RL (cm)	58.8±0.7	51	68
RW (cm)	44.3±0.6	38	53
CBC (cm)	20.0±0.3	17	23

4. CONCLUSION

The morphological characterization conducted in this study reveals that Tinh Bien racing bulls possess superior physical attributes, likely reflecting a combination of selective breeding, environmental adaptation, and training practices. These traits, including high body weight, tall stature, and muscular development, align with the functional demands of traditional bull racing.

The systematic collection and analysis of morphometric data provide a scientific foundation for establishing standardized criteria in breeding and selection programs. Such efforts will facilitate genetic improvement, contribute to the conservation of culturally significant livestock, and promote sustainable development of local communities.

Future work should focus on integrating biometric data with genomic analyses to identify genetic markers linked to performance traits, thereby advancing precision breeding in traditional livestock systems.

ACKNOWLEDGEMENTS

This research is funded by Vietnam National University HoChiMinh City (VNU-HCM) under grant number C2024-16-10.

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EFFECTS OF VITAMIN C, VITAMIN E AND LICORICE ON GROWTH PERFORMANCE AND CARCASS YIELD OF COBB500 CHICKENS

Nguyen Thi Kim Khang[1], Duong Tuan Kiet[1], Huynh Canh Van[1] and Le Thanh Phuong[2]*

Submitted: 17-Jun-2025 – Revised: 17-Jul-2025

Accepted: 23-Jul-2025

ABSTRACT

The experiment was conducted to evaluate the effects of vitamin C, vitamin E and licorice powder (CT) as feed additives on growth performance and carcass yield of Cobb500 chickens. 360 broilers at 14 days of age were arranged in a completely randomized design with 4 treatments, each treatment was repeated 3 times and each replication was 30 chickens. The NTs were as follows: control, chickens were fed a basal diet (KPCS) without supplemented vitamin C, E and CT powder; C125CT including KPCS containing 125mg of vitamin C and 1g of CT powder per kg feed; E250CT including KPCS containing 250mg of vitamin E and 1g of CT powder per kg feed; and C125E2CT including KPCS containing 125mg of vitamin C, 250mg of vitamin E, and 1g of CT powder per kg feed. The results showed that chickens in the supplemented experimental groups had a lower incidence of chronic respiratory disease (CRD) in broilers compared to the control group. In addition, the supplemented experimental groups had higher weight, absolute weight gain, cumulative weight gain ($P<0.05$) and lower FCR over the entire period than the control group ($P>0.05$). The results of the autopsy showed that C125E2CT had the highest live weight, carcass weight, dressing percentage, breast weight, percentage of breast weight, thigh weight and thigh meat, which were statistically significant ($P<0.05$) compared to the control group. Breast meat exudation at 12, 24 and 36hrs after slaughter was significantly different among the experimental groups, lowest in C125CT and C125E2CT, highest in E250CT. From the above results, it is recommended that vitamin C or/and vitamin E supplementation combined with CT powder, especially C125E2CT, helps improve health, growth ability and meat productivity in Cobb500 chickens.

Từ khóa: *Broiler, body weight, FCR, vitamin, licorice powder.*

1. INTRODUCTION

Vitamin E is a biological antioxidant that could contribute to improved growth, physiological, and immunological performance in broiler chickens because of its ability to neutralize free radicals and reduce lipid peroxidation in both the plasma and skeletal muscle (Gao *et al*., 2010, Selim *et al*., 2013). Vitamin E supplementation has been investigated because of the reported benefits in laying hens during heat stress, and reduced body vitamin E levels during heat stress (Sahin *et al*., 2001). The antioxidant activity of vitamin E is increased by vitamin C, which reduces tocopheroxyl radicals back to the active form of vitamin E. According to Sahin *et al.* (2003) and Panda *et al.* (2011), broilers had the highest performance when supplemented with 250mg of vitamin E in the diet, while Khang (2014) concluded that diets supplemented with 70 and 250mg of vitamin E had the highest performance. Khang *et al.* (2020) showed that supplementation of 250mg vitamin E to dietary feed improved FCR and egg production of crossbred Noi pullet hens. Khang *et al*. (2014) reported that dietary supplementation with 250mg vitamin E, or/and plus 250mg vitamin C were more positively enhance the Isa Brown chicks' growth performance.

[1] CanTho University

[2] Emivest Feedmill Company, Vietnam

[*] Corresponding author: Assoc. Prof. Dr. Nguyen Thi Kim Khang, Can Tho University, Campus II, 3/2 street, Ninh Kieu district, Can Tho city, Viet Nam, Phone 0084 939205355, Email: ntkkhang@ctu.edu.vn.

Recent studies have shown that herbs and plant extracts such as licorice, turmeric powder, ginger powder, etc. have been proven to be a solution that can replace antibiotics to stimulate growth in animal diets. Licorice herb offers some of bioactive molecules as glycyrrhizin and flavonoids (Tiwari *et al*., 2018). Licorice root contains 1-9% glycyrrhizin which has several nutritional roles such as its role in enhancing growth rate and pharmacological actions like immunomodulatory, antioxidant, antiviral, and anti-inflammatory properties (Alagawany *et al*., 2019a, 2019b). According to phytochemical analysis, the extract of licorice consists of flavonoids (e.g., liquiritin, formononetin and isoflavonoids), triterpene saponins (e.g., glycyrrhetinic acid, glycyrrhizin and licorice acid) and starch, sugars, amino acids, tannins, ascorbic acid, coumarins, choline, phytosterols, and other molecules (Shalaby *et al*., 2004). Recent studies by Jagadeeswaran and Selvasubramanian (2014) showed that adding licorice powder to the diet at 0.1% improved immunity in chickens. In addition, Reda *et al*. (2021) found that Japanese quail fed licorice at 750 mg/kg for 5wks had significantly higher final BW, DBW, and lower FCR compared to the control group. However, the study of Sedghi *et al.* (2011) showed that the addition of licorice at 0.5, 1 and 2g to the diet of Ross 308 broilers could reduce cholesterol content and did not have any negative effects on body weight or FCR of chickens. Alagawany *et al*. (2019a) suggested that dietary liquorice supplementation at level of 2.5 g/kg showed the best results in broiler chicken, and demonstrated it can be safely used in poultry diets. Several studies have used this herb with varying levels. However, the combined use of this herb with vitamin E or vitamin C is very limited. From the above facts, the study was conducted to evaluate the effects of vitamin C, vitamin E and licorice powder on growth performance and carcass productivity of Cobb500 chickens.

2. MATERIALS AND METHODS

2.1. Materials

The experiment was conducted on 360 Cobb500 broilers from 14 to 41 days old at the Sau Mung chicken farm of Emivet company Ltd., Thuy Thuan hamlet, An Phuoc commune, Mang Thit district, Vinh Long province. The raw materials used for the experiment were pelleted mixed feed provided by Emivest Company. Of which, mixed feeds were 8201 (1-7 days old), 8202 (8-21 days old), 9203 (22-35 days old), and 9204 (35 days old-market). Corresponding to the exchangeable energy and CP, respectively, were 2,900kcal and 22% CP; 3,000kcal and 20% CP; 3,100kcal and 19% CP; 3,100kcal and 19.8% CP. Licorice powder was purchased at Duc Tho Sanh pharmacy, 57, 1/5 Street, ward 1, Vinh Long city, Vinh Long province. The licorice in powder form was light yellow, smooth, and has the aroma of essential oils. Vitamin C and E were purchased at Intech Aquatic Veterinary company Ltd., 194 Huynh Cuong, Ninh Kieu district, Can Tho City.

2.2. Methods

A total of 360 broilers at 14 days of age were arranged in a completely randomized design with 4 treatments, the treatments were as follows: control (ĐC): chickens were fed a basic diet (KPCS) without supplementing vitamin C, E and licorice powder; C125CT including KPCS containing 125mg of vitamin C and 1g of licorice powder in 1kg of feed; E250CT including KPCS containing 250mg of vitamin E and 1g of licorice powder in 1kg of feed; C125E2CT including KPCS containing 125mg of vitamin C, 250mg of vitamin E and 1g of licorice powder in 1kg of feed. The experiment was repeated 3 times with 12 experimental units, each experimental unit was arranged with 30 chickens at 14 days of age with equivalent body weight and equal male-female ratio.

All experimental chickens were reared and cared for under the same conditions, except for the diets. Chickens were fed 3 times a day (8:00, 14:00, 17:00) at feeding rates of 30, 20, 50% of the daily feed intake, respectively. Chickens were given clean water and free-flowing drinking water through automatic nipples. Feed intake and feed residues were recorded by daily weighing, health status of chickens was observed and any abnormal signs were also recorded every day as such chickens infected with coccidiosis, CRD, Gumboro disease. The weight of chickens was weighed in each experimental unit and recorded at the ages of 14, 21, 28, 35, 41 days.

A total of 32 broiler chickens at 41 days old were selected for slaughter evaluation with weight equivalent to the average of each treatment in the feeding experiment and the chickens were kept for 12 hours before slaughter, during which time the chickens were not fed but only given water, then the chickens were weighed. The chickens were re-weighed after each stage after bleeding, plucking, carcass, weight of parts such as heart, liver, gizzard, abdominal fat... The thighs and breasts were separated to each part such as meat, skin and bones.

2.3. Statistical analysis

Raw data were preliminarily processed using Excel 2010 software and statistically processed using Minitab 16 software with GLM, to determine the significance of the difference between treatments using Tukey's method at 95% confidence level. The incidence of CRD, coccidiosis and mortality of broilers were processed using Chi-Square test.

3. RESULTS AND DISCUSSION

3.1. Health status of experimental chickens

The health status of experimental chickens was monitored and recorded daily, some diseases such as CRD and coccidiosis as well as mortality rate were recorded in table 1 showing that the control had the highest CRD infection rate (31.11%) and C125E2CT had the lowest CRD infection rate (10%) (P<0.05). The coccidiosis infection rate in experimental chickens also showed similar results, but there was no statistically significant difference (P>0.05). The mortality rate was recorded in E250CT, C125E2CT and there was also no statistically significant difference between treatments (P>0.05). Infection with CRD, a chronic respiratory disease in chickens, depends on the microclimate of the house and the weather outside the house. It was observed that during the experiment, the house temperature fluctuated up and down, combined with continuous heavy rain in the evening and early morning, causing the humidity in the house to increase, leading to an increase in respiratory infections in the experimental chickens. Relative humidity records showed that this index was very high at 80-90%, Nayak *et al.* (2015) stated that relative humidity higher than 75% tends to cause problems with wet litter, ammonia emissions and some related diseases.

Table 1. Infection status and mortality rate

Parameters (%)	Treatments				P
	ĐC	C125CT	E250CT	C125E2CT	
CRD	31.11^{a}	22.22^{ab}	17.78^{ab}	10.0^{b}	0.02
Coccidiosis	31.11	22.22	17.78	15.56	0.12
Mortality rate	0	0	1.11	1.11	0.57

Akbarian *et al.* (2016) reported that the immunity and well-being of poultry are positively correlated to the animal's oxidative status. Previously researches illustrated the antioxidant capacity of licorice components enhanced the antioxidant status of Japanese quail (Rahal *et al*., 2014; Vlaisavljević *et al*., 2018; Yatoo *et al*., 2018; Reda *et al*., 2021). Although the present results showed that all experimental chickens were infected with CRD, however, the diets supplemented with licorice powder had a lower infection rate. It may be because of the bioactive component of licorice (18b-glycyrrhetic acid, glucuronic acid, glycyrrhizic acid, flavonoids, saponins, sugars, coumarins, amino acids, starch, tannins, phytosterols, choline, and vitamins) can improve in the immune and oxidation status of bird (Kataria *et al.*, 2013; Karahan *et al.*, 2016; Pastorino *et al.*, 2018). Min *et al.* (2018) suggested that addition of 300 mg/kg vitamin C, 200 mg/kg vitamin E or their combination could improve antioxidant ability and immune performance. In addition, the supplementation of vitamin C and vitamin E in the diet, a part of vitamin E in the body is converted into vitamin C precursor linked to the bioactive compound of licorice, lead to boots the immunity of broilers, effective in preventing diseases.

3.2. Growth performance and carcass yield

The results in table 2 show that the initial weight of experimental chickens ranged of 439.4-441.1 g/bird and there was no statistically significant difference between treatments (P>0.05). The body weight of chickens at 21 days old in the supplemented treatments ranged from 964.5-975 g/bird, higher than the control with 962.8 g/bird (P>0.05). However, at the stages of 28, 35 and 41 days of age, the body weight of chickens between the treatments was statistically different (P<0.05), specifically at 41 days of age, the body weight of chickens in C125E2CT was the highest (2512.95 g/bird), followed by C125CT (2424.5 g/bird), control (2395.6 g/bird), and the lowest was E250CT (2384.3 g/bird). The results of Table 2 also show that at the stage of 14-21 and 35-41 days old, the average daily weight gain (ADG) of chickens between the treatments was different but not statistically significant (P<0.05). The ADG of chickens at the stages of 21-28, 28-35 and 14-41 days old had statistically significant differences between the treatments (P<0.05). The ADG at 14-41 days old was highest in C125E2CT (76.8 g/bird/day), followed by C125CT (73.46 g/bird/day), the control (72.43 g/bird/day) and the lowest was E250CT (72.03 g/bird/day).

Table 2. Growth performance of Cobb500 chickens

Paramet	Treatments				SEM	P
	ĐC	C125CT	E250CT	C125E2CT		
Body weight, g/bird						
14	440	441	439	439	2.040	0.928
21	962.8	964.5	975.0	965.0	8.957	0.768
28	1448.9[c]	1486.7[b]	1473.3[b]	1528.9[a]	4.156	0.001
35	1957.8[b]	1991.1[b]	1955.6[b]	2088.9[a]	8.677	0.001
41	2395.6[bc]	2424.5[b]	2384.3[c]	2512.95[a]	7.612	0.001
ADG, g weight gain/bird/day						
14-21	74.68	74.76	76.51	75.08	1.098	0.632
2-28	69.44[c]	74.60[b]	71.19[bc]	80.56[a]	1.091	0.001
29-35	72.70[b]	72.06[b]	68.89[b]	80.00[a]	1.065	0.001
36-41	72.96	72.22	71.46	70.68	0.797	0.281
14-41	72.43[bc]	73.46[b]	72.03[c]	76.80[a]	0.268	0.001
FCR, g feed/g weight gain						
14-21	1.16	1.15	1.12	1.15	0.012	0.153
22-28	1.42	1.46	1.56	1.46	0.030	0.061
29-35	1.96[a]	1.94[ab]	2.04[a]	1.84[b]	0.024	0.004
36-41	2.23	2.22	2.21	2.25	0.037	0.910
14-41	1.71	1.67	1.70	1.66	0.012	0.045
Feed intake, g feed/bird/day						
14-21	86.86	85.81	85.62	86.67	1.087	0.811
22-28	103.14[b]	105.20[b]	107.11[b]	116.57[a]	1.229	0.001
29-35	142.35	139.84	140.47	147.40	1.737	0.052
36-41	162.70	160.00	158.05	158.81	1.883	0.379
14-41	123.76[b]	122.71[b]	122.81[b]	127.39[a]	0.749	0.007

Feed conversion ratio (FCR) of chickens at the stages of 14-21, 22-28, and 36-41 days of age were not statistically different between treatments (P<0.05). FCR was statistically different between treatments at the stages of 29-35 and 14-41 days of age (P<0.05). The results recorded feed conversion ratio at 14-41 days of age showed that the supplemented treatments were lower than the control. Feed intake of chickens was not statistically different between treatments at the 14-21, 28-35, and 35-41 day old stages. At the 22-28 and 14-41 day old stages, feed intake between treatments had statistically significant differences (P<0.05).

The results of carcass performance of experimental chickens in Table 1 showed that there were statistically significant differences between treatments in terms of live weight, live weight after 12 hours, carcass weight and its percentage (P<0.05), in which the highest was in C125E2CT, followed by C125CT, control and the lowest was in E250CT. The results of breast and breast meat weight, thigh meat weight and their percentages also showed statistically significant differences between the treatments (P<0.05), C250CT and control had the lowest breast weight and its percentage, and the highest in C125E2CT, while C125CT and control had the lowest thigh percentage, thigh meat weight and its percentage. The weight of internal organs, liver, gizzard, abdominal fat weight and its percentage were statistically significantly different among the treatments (P<0.05). C125E2CT had the highest weight of abdominal, liver, and gizzard and the lowest abdominal fat percentage, while control had the highest abdominal fat percentage. Similarly, the control had the highest intestinal length compared to the supplemented treatments (P<0.05).

These present findings indicate that 1g of licorice combined with vitamin C or/and vitamin E can influence performance and carcass characteristics. These effects are positive and improve FCR, ADG and body weight of broiler chickens. The lower FCR may partially explain the faster growth of groups fed on licorice diets. These findings are in line with other relevant research reports (Jagadeeswaran and Selvasubramanian, 2014; Alagawany *et al.,* 2019b, Reda *et al.,* 2021). Jagadeeswaran and Selvasubramanian (2014) reported that a diet with 1% licorice resulted in higher BW and lower FCR in 42-day-old broiler chickens compared to the control group. Similarly, up to 0.5% licorice supplementation during the pullet growing period resulted in enhanced performance in laying hens (Alagawany *et al*., 2019b). Reda *et al.* (2021) found that Japanese quail fed licorice at 750 mg/kg for 5wks had significantly higher final BW, DBW, and lower FCR. Despite differences in dietary licorice supplementation levels and poultry species, results consistently demonstrated beneficial effects of licorice on growth performance parameters. Moreover, Min *et al.* (2018) suggested that addition of 300 mg/kg vitamin C, 200 mg/kg vitamin E or their combination could improve antioxidant ability and immune performance, and notably increased body weight under oxidative stress of the 45-week-old Lveyang black-boned breeder roosters. Khang *et al.* (2014) reported that dietary supplementation with 250mg vitamin E, or/and plus 250mg vitamin C positively enhanced the Isa Brown chicks' growth performance. The above results suggest that the combination of licorice powder and vitamin C or both vitamin E and C in the present study could improve digestion and palatability in chickens, as well as enhance antioxidant and immune functions, which helped chickens gain weight better and have better FCR.

The results in table 3 showed that licorice combined with vitamin C or a combination of vitamin C and E had positive effects on the carcass performance parameters of Cobb500 broilers. The present carcass yield results are consistent with other relevant research reports (Kalantar *et al.,* 2017; Alagawany *et al*., 2019a). The increasing carcass part weight and reducing abdominal fat weight accompanied by a reduction in liver weight percentage, which in itself indicates a reduced rate of lipogenesis in the liver and fat absorption by this organ (Kalantar *et al.,* 2017). Similarly, abdominal fat in broiler chickens was decreased when liquorice was included at 2 g/kg in feed or 0.3 g/l in drinking water. Broiler chickens with access to drinking water containing 450 mg/l of liquorice increased the dressing percentage, with or without giblets. Carcass organoleptics were improved in birds receiving drinking water with liquorice at levels of either 1, 2 and 4 mg/kg body weight (Alagawany *et al.,* 2019a). In addition, the addition of vitamin C or vitamin C and vitamin E, part of the vitamin E in the body is converted into vitamin C precursor, combined with the bioactive compound of licorice, helps to enhance the antioxidant capacity in the muscles of broiler chickens, which has a positive effect on the chicken carcass. In contrast, licorice powder was added at levels of 250, 500, 750 and 1000mg per kg diet had no affect quail carcass characteristics, including percentages of carcass, liver, gizzard, heart, giblets, and dressing (Reda *et al*., 2021).

Table 3. Carcass performance of Cobb500 chickens

Parameters	Treatments				SEM	P
	ĐC	C125CT	E250CT	C125E2CT		
Live weight, g	2330.0[b]	2422.5[b]	2230.0[c]	2545.0[a]	24.65	0.000
Pre-slaughter LW12h, g	2262.5[b]	2335.0[b]	2165.0[c]	2475.0[a]	21.64	0.000
Carcass weight, g	1710.0[c]	1812.5[b]	1605.0[d]	2060.0[a]	22.42	0.000
Dressing percentage, %	75.51[c]	77.50[b]	74.09[c]	83.00[a]	0.43	0.000
Breast weight, g	608.8[c]	670.0[b]	573.75[c]	777.5[a]	10.61	0.000
Breast percentage, %	35.65[b]	36.92[ab]	35.79[b]	37.82[a]	0.42	0.004
Breast meat weight, g	498.8[c]	537.5[b]	475.0[c]	618.8[a]	9.35	0.000
Thigh percentage, %	31.68[a]	29.29[ab]	33.66[a]	26.22[b]	1.22	0.002
Thigh meat weight, g	401.3[c]	386.25[c]	432.5[b]	503.8[a]	7.44	0.000
Thigh meat percentage, %	23.40[b]	21.47[c]	26.91[a]	24.42[b]	0.47	0.000
Internal organ weight, g	235.0[c]	264.4[b]	234.4[c]	281.9[a]	3.92	0.000
Liver weight, g	53.75[ab]	55.00[ab]	49.38[b]	57.50[a]	1.62	0.014
Gizzard weight, g	25.63[b]	28.75[b]	28.13[b]	33.13[a]	1.00	0.000
Gizzard percentage, %	1.51[b]	1.59[ab]	1.76[a]	1.60[ab]	0.06	0.040
Abdominal fat weight, g	55.63[a]	48.75[b]	49.38[b]	50.63[b]	0.97	0.000
Abdominal fat percentage, %	3.27[a]	2.70[b]	3.09[a]	2.46[c]	0.05	0.000
Intestine length, cm	224.38[a]	189.38[b]	199.88[b]	196.38[b]	5.98	0.002

4. CONCLUSION

Supplementing vitamin C, or/and vitamin E combined with licorice powder, especially C125E2CT, into chicken diets has a beneficial effect on growth as well as carcass performance of Cobb500 chickens.

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EFFECTS OF ORGANIC ACID MEGACID-L SUPPLEMENTATION ON GROWTH PERFORMANCE AND INTESTINAL MICROBIOTA OF NOI CHICKENS RAISED FOR MEAT

Pham Tan Nha[1] and Le Thu Thuy[1]*

Submitted: 17-Jan-2025 – Revised: 17-Feb-2025

Accepted: 03-Mar-2025

ABSTRACT

A study was conducted to assess the effects of Megacid-L supplementation on the growth performance and intestinal microbiota of Noi chickens aged 6-13 weeks. The experiment followed a completely randomized design with five treatments representing different levels of Megacid-L (0, 0.1, 0.2, 0.3 and 0.4%) mixed into the drinking water, labeled as Me0, Me0.1, Me0.2, Me0.3 and Me0.4, respectively. Each treatment was replicated four times with 10 chickens per replicate. The results showed significant improvements (P<0.05) in daily weight gain (DWG) and reductions in feed conversion ratio (FCR) in the Me0.3 and Me0.4 groups compared to the control (Me0), indicating enhanced feed efficiency. Additionally, carcass traits such as breast and thigh meat weights were significantly increased (P<0.05) in the supplemented groups. The findings suggest that including Megacid-L at levels between 0.3 and 0.4% in drinking water during the growth phase can improve the growth performance of Noi chickens. Moreover, Megacid-L supplementation positively influenced the intestinal microbiota, resulting in higher levels of *Lactobacillus* and lower levels of *E. coli* and *Clostridium perfringens* in chicken manure.

Keywords: *Megacid-L, Noi chicken, Lactobacillus, E.coli.*

1. INTRODUCTION

In addition to commonly raised chicken breeds such as Tau Vang, Tre, and Ac chickens, which have been successfully farmed, Noi chickens have become increasingly popular in the Mekong Delta due to their delicious meat, ease of rearing, and suitability to local climatic conditions (Nha *et al.*, 2021). Megacid-L improve chicken health and digestive function, as organic acids enhance nutrient absorption, promote rapid growth and support overall development. It also helps prevent and reduce diarrhea and respiratory diseases, dries chicken manure, and minimizes unpleasant odors, thereby maintaining environmental hygiene in poultry housing.

The aim of this research was to determine the optimal level of Megacid-L, an organic acid supplement, in drinking water to enhance the growth performance and intestinal microbiota of Noi chickens raised in the Mekong Delta region of Vietnam. The goal is to provide valuable recommendations for poultry producers based on the study's findings.

2. MATERIALS AND METHODS

2.1. Location, animals and climate of the study area

The experiment was conducted on a private farm in Vinh Long Province, Vietnam, during the dry season (January to May 2024). Average temperatures during the study ranged from 25°C to 32°C, with humidity levels between 50-80%. Chemical analyses of the experimental diets were performed at the Laboratory of the Faculty of Animal Sciences, School of Agriculture, Can Tho University. One-day-old Noi chickens were obtained from a breeding farm in Ben Tre Province, Vietnam. From days 2 to 28 post-hatch, the chicks were fed exclusively with a commercially prepared concentrated pellet containing 20% crude protein. From days 29 to 35, this base diet was gradually supplemented with increasing amounts of

[1] CanTho University

* Corresponding author: Dr. Pham Tan Nha, Can Tho University, Can Tho City, Vietnam. Phone: 0084 985512504, Email: ptnha@ctu.edu.vn.

the experimental diets. At 36 days of age, all chickens were vaccinated against H5N1, Newcastle disease, and other common poultry diseases prior to the start of the formal trial.

2.2. Experimental design and treatments

A completely randomized design was used for the study, involving 200 six-week-old Noi chickens with an average body weight of 359±10.1g. The chickens were randomly assigned to five treatments, each comprising four replicate units of ten birds with a balanced sex ratio. The treatments consisted of different levels of Megacid-L (an organic acid) added to the drinking water at concentrations of 0, 0.1, 0.2, 0.3 and 0.4%, labeled as Me0, Me0.1, Me0.2, Me0.3 and Me0.4, respectively. The trial lasted 8 weeks, covering the chickens' growth from 6 to 13 weeks of age. A detailed composition of the feed ingredients used in the diets is presented in table 1.

Table 1. Feed ingredient of concentrate diet

Feed	%	Feed	%
Rice bran	5.1	Premix vitamin	0.40
Maize	34.8	Premix mineral	0.50
Fish meal	10.1	$CaCO_3$	0.49
Broken rice	36.3	DCP	0.51
Soybean extraction	11.8		

2.3. Feeds and preparation of Megacid-L

Megacid-L organic acid was purchased once at the beginning of the experiment and stored in cold conditions to preserve its quality. The chickens were provided with free access to water daily, with Megacid-L added according to the prescribed concentrations. It is important to note that all feed ingredients were procured at the same time from the feed store for use throughout the experiment. The basal diet (BD) was specifically formulated to contain 12.9MJ of metabolizable energy per kilogram of dry matter (DM) and 18% crude protein (CP). Before feeding, the spring onions were thoroughly mixed with the feed. The compositions of Megacid-L, the feed ingredients, and the basal diet are presented in tables 2 and 3.

Table 2. Chemical compositions of Megacid-L

Item	Unit
Water	23%
Formic	35%
Lactic Acid	7%
Citric Acid	20%
Phosphoric Acid	10%
Other acidic salts	5%

Nguyen Hieu Hoc anh Pham Tan Nha (2019)

Table 3. Chemical compositions and BD (%DM)

Feed Item	Corn	Broken rice	Rice bran	Soybean extraction	Fish meal	Basal diet
DM	88.6	86.7	86.0	89.5	91.9	89.1
OM	98.6	99.5	89.6	94.8	78.1	92.7
CP	8.08	9.29	12.5	43.4	60.4	18.1
EE	4.85	0.82	18.1	1.22	12.7	4.00
CF	2.12	0.59	6.59	5.44	0.19	3.60
NDF	28.5	7.35	32.1	12.3	11.0	17.0
Ash	1.40	0.51	10.4	6.82	21.9	7.30
ME	13.9	13.5	13.0	10.3	12.6	12.9

Janssen et al. (1989); DM: dry matter, OM: orgarnic matter, CP: crude protein, EE: ether extraction, CF: crude fibre, NDF: neutral detergent fibre, ME: metablolizable ernergy (MJ/kgDM).

2.4. Housing and management

The experimental Noi chickens were housed in custom-built enclosures constructed from a combination of wood and metal sheets. Each pen provided 2.5m² of floor space for every 10 birds, ensuring adequate comfort and freedom of movement. The walls were made using a combination of wood and plastic netting to allow for proper ventilation and natural light. To provide bedding and encourage natural foraging behavior, each pen floor was layered with 20cm of sand and rice straw. Feeders and waterers were strategically placed in front of each pen and cleaned every morning to maintain hygiene. The birds were fed a balanced diet three times daily (at 7:00,13:00 and 17:00). Feed quantities were adjusted weekly based on actual intake, gradually increasing from 5% to 10% of body weight to meet nutritional requirements. The treatments consisted of different levels of

Megacid-L organic acid supplementation in the drinking water 0, 0.1, 0.2, 0.3 and 0.4%.

2.5. Measurements

Daily feed intake and nutrient consumption were carefully monitored by collecting and weighing both the offered feed and the refusals every morning. Weekly measurements were also conducted to track individual body weight gain and to calculate the feed conversion ratio (FCR), which reflects feed efficiency. At the end of the trial, four birds (two males and two females) from each experimental unit were humanely euthanized for comprehensive carcass trait evaluation. This assessment followed the procedures described by Salomon (1996) and included various body measurements. In addition, blood samples were collected at this time for subsequent laboratory analysis of key biochemical parameters

2.6. Chemical analyses

A thorough chemical analysis of the provided feed was conducted to determine its exact composition. The analysis included measurements of dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE), crude fiber (CF), and ash content. All procedures followed the methods outlined by AOAC (1990). Neutral detergent fiber (NDF) content was determined using the method described by Van Soest *et al.* (1991). Metabolizable energy (ME) was calculated based on the approach developed by Janssen (1989).

2.7. Statistical analysis

Statistical analysis of the collected data was performed using the General Linear Model (GLM) in Minitab version 18.1.0 (Minitab, 2018). To identify statistically significant differences among treatment groups, post-hoc analysis was conducted using Tukey's Honestly Significant Difference (HSD) test available within the Minitab software.

3. RESULTS AND DISCUSSION

3.1. Daily intake and nutrients for Noi chicken

Daily consumption of DM, OM, CP, EE and NDF was significantly reduced (P<0.05) in birds receiving Megacid-L at 0.3-0.4% in drinking water compared to other dietary groups. Conversely, the group receiving Megacid-L at 0.1% in water demonstrated the highest intake levels of these nutrients. Interestingly, these findings differ from those reported by Nguyen Hieu Hoc (2019) study on Tau Vang chickens, which recorded lower DM and CP intake levels, ranging of 45.9-49.4 g/day and 9.17-9.59 g/day, respectively. Furthermore, metabolizable energy (ME) intake was significantly higher (P<0.05) in birds from the Me0, Me0.1, and Me0.2 groups compared to those in the Me0.3 and Me0.4 groups, likely due to their higher DM consumption.

Table 4. DI and nutrient of Noi chicken (g/bird)

Item	Me0	Me0.1	Me0.2	Me0.3	Me0.4	SE	P
DM	55.9^{b}	57.6^{a}	56.1^{b}	55.1^{b}	54.2^{c}	0.35	0.017
OM	51.8^{b}	53.4^{a}	51.9^{b}	51.3^{bc}	50.2^{c}	1.85	0.020
CP	10.3^{ab}	10.4^{a}	10.2^{ab}	10.1^{ab}	9.79^{b}	0.06	0.021
EE	2.26^{ab}	2.31^{a}	2.25^{ab}	2.23^{b}	2.15^{b}	0.04	0.018
CF	2.03	2.07	2.02	1.99	1.94	0.03	-
NDF	9.47^{ab}	9.75^{a}	9.55^{ab}	9.38^{b}	9.21^{c}	0.05	0.016
Ash	4.09^{b}	4.18^{a}	4.10^{b}	4.06^{c}	3.91^{d}	0.00	0.015
ME	0.73^{b}	0.75^{a}	0.73^{b}	0.72^{c}	0.69^{c}	0.03	0.027

Notes: Mean values with different superscripts within the same row are different at P<0 05.

3.2. Effects of Megacid-L on the growth

Table 5 illustrates that DWG was notably higher in birds supplemented with Megacid-L (Me0.3 and Me0.4 treatments) compared to those without supplementation (Me0 treatment). These findings align with a study by Hoc (2019), which reported improved weight gain in Tau Vang chickens supplemented with 0.3% Megacid-L in their diet. The DWG achieved in the present study ranged from 15.6 to 17.3 g per bird, consistent with values of 15.3-16.8g per bird observed in previous trials with Tau Vang chickens (Hoc, 2019; Nhan, 2019) and Noi chickens (Nha *et al.*, 2021).

Chickens supplemented with Megacid-L also exhibited significantly higher final live weights compared to those in the Me0 treatment (P<0.05), which can be attributed to their greater daily weight gain. These final weights were within the range of 1,242-1,333g, as reported in a previous study involving Tau Vang chickens (Nhan, 2019).

Additionally, the results revealed a significantly lower crude protein (CP) consumption per unit of weight gain among chickens in the Me0.3 and Me0.4 treatments (P<0.05). The feed conversion ratio (FCR) of Noi chickens was also significantly improved in the Me0.3 and Me0.4 groups (P<0.05), likely due to the higher DWG observed in these treatments. The FCR values in this study ranged from 3.24 to 3.53, which were comparatively lower than those reported by Nha (2019) in similar research on Noi chickens.

Table 5. DWG, W and FCR of Noi chicken (g/bird)

Item	Me0	Me0.1	Me0.2	Me0.3	Me0.4	SE	P
ILW	360	358	362	363	360	7.6	-
FLW	1242^{d}	1271^{c}	1293^{b}	1330^{a}	1333^{a}	4.01	0.003
DWG	15.6^{c}	16.2^{b}	16.6^{b}	17.2^{a}	17.3^{a}	0.23	0.005
FCR	3.51^{a}	3.54^{a}	3.38^{b}	3.21^{c}	3.14^{c}	0.4	0.004
CP/WG	647.0^{a}	642.1^{ab}	613.2^{b}	582.3^{c}	570.0^{d}	6.14	0.001

3.3. Effects of Megacid-L on carcass quality

Consistent with the final live weights, slaughter weights did not exhibit significant differences across treatment groups. Notably, carcass weight showed a significant increase (P<0.05) in birds whose diets were supplemented with 0.3% and 0.4% Megacid-L (Me0.3 and Me0.4, respectively), as detailed in table 6. However, the percentage of carcass yield remained comparable across all treatments, ranging of 70.0-72.2%, aligning with observations reported by Hoc (2019). Both breast and thigh meat weights significantly increased (P<0.05) in the Me0.3 and Me0.4 groups, while the ratio of breast to thigh meat was unaffected by dietary treatment (P>0.05). Furthermore, no significant differences were observed in the weights of internal organs among the treatment groups (P>0.05).

Table 6. Caracass and internal organs (g/bird)

Item	Me0	Me0.1	Me0.2	Me0.3	Me0.4	SE	P
SW	1242^{d}	1271^{c}	1293^{b}	1330^{a}	1333^{a}	4.01	0.003
CW	869^{d}	896^{c}	918^{b}	956^{a}	962^{a}	28.0	0.033
% CW	70.0	70.5	71.0	71.9	72.2	1.97	-
BMW	165.2^{d}	171.1^{c}	182.7^{b}	197.0^{a}	205.0^{a}	8.10	0.001
% BM	19.0	19.1	19.9	20.6	21.3	0.80	-
TMW	132.1^{d}	138.0^{c}	145.0^{b}	158.7^{a}	161.7^{a}	3.11	0.044
%TM	15.2	15.4	15.8	16.6	16.8	0.46	-

3.4. Intestinal microflora

At week 13, *Salmonella spp*. was almost undetectable in the manure of Noi chickens in the Me0.3 and Me0.4 treatment groups (Table 7). However, *Lactobacillus*, *E. coli*, and *Clostridium perfringens* were found in relatively high concentrations. Among the Megacid-L supplemented groups, Me0.3 and Me0.4 treatments exhibited the highest counts of *Lactobacillus bacteria* and the lowest counts of *E. coli* and *Clostridium perfringens*. This microbial profile is highly beneficial for chicken health. It's important to note that *Salmonella spp*., *E. coli*, and *Clostridium perfringens* are potentially pathogenic bacteria, whereas *Lactobacillus* is a beneficial bacterium. The addition of Megacid-L at 0.3 and 0.4% to the drinking water of Noi chickens led to an increase in fecal *Lactobacilli* content. Specifically, the Me0.3 and Me0.4 treatments resulted in the highest *Lactobacilli* counts.

Table 7. Bacteria density in Noi chicken at 13 th weeks age of the experimental

Variables	Me0	Me0.1	Me0.2	Me0.3	Me0.4	SE	P
Lactobacillus (10 CFU/g)	1.94^{c}	1.99^{c}	3.88^{b}	4.05^{a}	4.22^{a}	0.438	0.03
Salmonella spp./25g (+/-)	Positive	Positive	Non detected	-	-	-	-
E. coli (10^5 CFU/g)	4.22^{a}	3.96^{a}	3.07^{b}	2.82^{c}	2.32^{c}	0.04	0.01
Clostridium perfringens (10^4 CFU/g)	6.55^{a}	6.11^{b}	5.09^{bc}	4.45^{c}	4.05^{c}	0.05	0.02

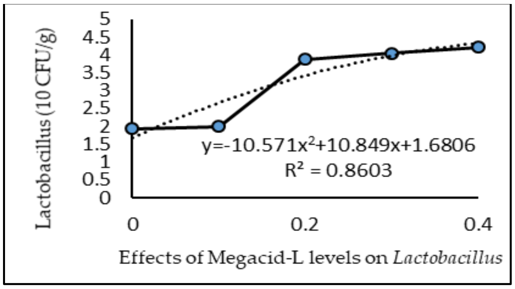

Figure 1. Effect of Megacid-L on *Lactobacillus*

In contrast, supplementing Megacid-L in the water reduced the levels of *E. coli* and *Clostridium* in the gastrointestinal tract after 13 weeks. This reduction can be attributed to the increase in beneficial probiotics, which consequently suppressed pathogenic bacteria such as *E. coli* and *Clostridium* ($P<0.05$) compared to groups not receiving Megacid-L supplementation. The Me0.3 and Me0.4 treatments yielded the most favorable results, demonstrating the lowest *E. coli* and *Clostridium* counts. This finding aligns with the study by Niem and Nha (2018), which reported that adding fermented fine bran to the diet of Hoa Lan ducks increased *Lactobacillus* yeast and reduced *E. coli* bacteria in duck feces.

4. CONCLUSIONS

In conclusion, supplementing the drinking water of growing Noi chickens with Megacid-L at levels of 0.3-0.4% (w/w) significantly improved their growth performance. Furthermore, Megacid-L supplementation led to a beneficial shift in intestinal microflora, characterized by the highest counts of *Lactobacillus bacteria* and the lowest counts of *E. coli* and *Clostridium perfringen*s in chicken manure.

ACKNOWLEDGMENTS

I extend my sincere gratitude to the Faculty of Animal Husbandry, School of Agriculture, Can Tho University, for providing the invaluable opportunity to conduct this experiment.

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EFFECTS OF GARLIC SUPPLEMENTATION ON GROWTH PERFORMANCE AND BLOOD BIOCHEMISTRY PARAMETERS OF TRE CHICKENS FROM 6 TO 12 WEEKS OLD

Le Thanh Phuong[1] and Pham Tan Nha[2]*

Submitted: 17-Jun-2025 – Revised: 17-Jul-2025

Accepted: 23-Jul-2025

ABSTRACT

The study carried out the effect of garlic supplementation in the diet on growth performance and some blood biochemistry indicactors of Tre chickens. The experiment was arranged completely randomized on 150 Tre chickens raised in floor cages in the period of 6-12 weeks old with 5 treatments, each treatment had 3 replications, 10 bird/replication. The treatments corresponding to the diets were T1 (Control): basic diet (BD); T2: BD+0.5% garlic; T3: BD+1.0% garlic; T4: BD+1.5% garlic; T5: BD+2.0% garlic. The results showed the highest average daily weight gain was in T4 (14.4 g/bird/day) and the lowest feed conversion ratio was in T2 (3.17). In addition, dietary garlic supplementation also reduced the total cholesterol and triglyceride levels in Tre chickens. In conclusion, supplementing a diet with 0.5% garlic have a positive impact on body weight gain, feed conversion ratio and blood lipids (cholesterol and triglycerides) in Tre chickens.

Keywords: *Feed conversion ratio, garlic, growth, Tre native chicken.*

1. INTRODUCTION

Nowadays, the human needs of animal source foods in the context of population growth is a challenge for the livestock industry. The poultry industry has significant potential for strong growth due to the increasing demand for healthy, low-fat meat and the growing recognition of poultry as a sustainable protein source (Castro *et al*., 2023). In Vietnam, the Tre chickens is a native breed that was commonly raised in the provinces of the Southwest. The Tre chickens has the potential to develop strongly in the coming time, not only serving the domestic market but also having export potential. The Tre chickens have offer several advantages, including a short growth period, easy management, low disease susceptibility, and low housing costs (Nguyen *et al*., 2020), so they are becoming a popular animal breeding for many households and farms.

In addition, the poultry industry is facing the antibiotic abuse problem in disease prevention and treatment that lead to increasing serious antibiotic resistance, treatment costs in livestock products (Abrue *et al*., 2023). In this situation, garlic (*Allium sativum*) was used as a natural antibiotic with the active ingredient allicin, which possessed strong antibacterial properties, improved the immune system and supported poultry digestion (El-Ghany, 2024). Incorporating garlic into chicken diets throught powder, juice or mixed with feed is widely recognized a safe, effective, and environmentally friendly solution. Presently, there are not many studies on adding garlic to the feed of Tre chickens. Therefore, this study was conducted to evaluate the effects of fresh garlic supplement on growth performance and some blood biochemical indicators of Tre chickens.

2. MATERIALS AND METHODS

2.1. Materials

This experiment was conducted from August 2024 to October 2024 at a chicken farm in Phong Dien district, Can Tho City. An opened housing system for chickens was made by wood and tole, which were surrounded by plastic net and its floor was overlaid with 20cm of sand and rice straw layer. Feeders and drinkers were cleaned

[1] Emivest Feedmill Company, Vietnam
[2] Can Tho University
[*] Corresponding Author: Dr. Le Thanh Phuong, Emivest Feedmill Company, Vietnam. Phone: 0084 0967551973; Email: ngongtroi13052002@yahoo.com.

daily every morning. Randomly arranged 5 cocks and 5 hens into each pen with a density of 3 m^2/10 bird. Each pen corresponded to one repetition, so there were a total of 3 repetitions.

2.2. Methods

Total 150 Tre chickens at 5 weeks old was randomized design in 5 treatments: T1 (Control): basic diet (BD); T2: BD+0.5%garlic; T3: BD+1.0%garlic; T4: BD+1.5%garlic; T5: BD+2.0%garlic, 3 replications.

Table 1. Feed ingredients in this study

Ingredient	Content
Crude protein (Min) (%)	15.0
Crude fiber (Max) (%)	8.0
Metabolisable energy (Min) (kcal/kg)	2,600
Calcium (Min-Max) (%)	0.4-1.4
Phosphorus (Min-Max) (%)	0.4-1.4
Lysine (Min) (%)	0.3
Methionine and Cysteine (Min) (%)	0.4
Moisture (Max) (%)	14.0

Source: Anova Feed Joint Stock Company, Vietnam.

Chickens were fed 3 times daily at 7.00AM, 13.00PM and 17.00PM with feed of Anova Feed Joint Stock Company (Table 1), amount: 50 g/bird/day and drank water freely using automatic nipples. The lighting regime ensured 16 h/day at 40-60 lux. All chickens are vaccinated and cared according to the process of Emivest Feedmill Company (Vietnam).

The body weight (BW) of chickens was recorded weekly from the 6-12 weeks old. The average daily weight gain (ADWG), average daily feed intake (ADFI) and feed conversion ratio (FCR) was calculated according to Oluwafemi *et al.* (2021).

At the end of the experiment, blood samples were randomly collected (4 chickens/treatment) at 6AM., before feeding the chickens. From each individual, 2ml of wing vein blood was collected with a syringe and placed in a vacutainer (containing EDTA solution to prevent blood clotting). All blood samples were centrifuged at 3,000g for 15 minute and plasma was collected in vials and kept at -20°C until analysis (Abdulla *et al.*, 2019). The plasma samples were analyzed to determine triglycerides, total cholesterol, low density lipoprotein cholesterol (LDL-C), high DL-C (HDL-C) and albumin.

2.3. Statistical analyses

The data were recorded by using Excel software and using GLM of Minitab 16.0 software to analyse the variance. The significant differences between mean values within and between treatments were determined according to Tukey with P<0.05. Experimental model according to the formula: $Y_{ij}=\mu+G_i+\xi_{ij}$. *Where, Y_{ij}: traits observed; μ: general mean, G_i: influence of treatments; ξ_{ij}: random error).*

3. RESULTS AND DISCUSSION

3.1. Effect of garlic on the growth performance

Table 2 showed the GW of Tre chickens in all treatments increased during the study period. At 6 weeks old ranged from 197 to 201 g/bird. At 12 weeks old, the lowest BW was 968 g/bird (T5) and the highest was 1,005 g/bird (T4). During the period of 6-12 weeks old, chickens in T4 exhibited superior weight compare to other treatments, especially from the 7th week old onwards. At 10-12 weeks old, the rate of WG tended to slow down but remained stable that indicated the chickens were entering a stable growth phase.

Table 2. Effects of garlic on BW (g/bird)

Weeks old	Treatments				
	T1	T2	T3	T4	T5
6	198	201	197	199	197
7	278	280	285	305	279
8	420	424	432	470	434
9	578	581	593	618	579
10	707	713	724	764	711
11	823	830	840	865	827
12	970	997	990	1,005	968

In general, the BW increased gradually over time in the treatments. Notably, at 10-12 weeks old, the treatments showed statistically significant differences (P<0.05). The ADWG in T4 reached the highest value (15.5 g/bird/day) at 11-12 weeks old; 6-12 weeks old, T2, T3 and T4 had higher than T1 and T5, in which T4 had the highest (14.4 g/bird/day) (P<0.001). These results expressed garlic supplementation in feed at levels of 0.5, 1.0

and 1.5% could be used for raising Tre chickens for meat production.

Table 3. Effects of garlic on BWG (g/bird/day)

Weeks old	Treatments					SEM	P
	T1	T2	T3	T4	T5		
6-7	12.9	13.1	14.2	14.4	13.4	1.95	0.082
7-8	13.7	13.2	13.2	13.7	13.4	1.92	0.080
8-9	13.1	14.1	14.7	13.7	13.7	2.41	0.074
9-10	13.6	15.2	14.2	14.5	13.6	2.10	0.063
10-11	14.6^{ab}	14.2^{b}	14.2^{b}	14.6^{ab}	15.0^{a}	2.17	0.001
11-12	15.0^{ab}	15.3^{a}	14.7^{b}	15.5^{a}	15.1^{ab}	0.83	0.001
6-12	13.8^{b}	14.2^{a}	14.2^{a}	14.4^{a}	14.0^{ab}	1.50	0.001

Table 4 presented the initial body weight (IW) of Tre chickens in treatments (197-201 g/bird) was not significantly different (P>0.05). However, the final body weight (FW) was significantly different (P=0.001), in which the highest in T4 (1,005g) and the lowest in T5 (968g). The result of the highest ADWG was in T4 (14.4 g/bird/day) and the lowest was in T1 (13.8 g/bird/day). In addition, ADFI was also statistically different (P>0.05), ranging of 45.0-47.5 g/bird/day, in which the lowest was in T2 (45.0 g/bird/day). In addition, the lowest FCR in T2 (3.17) demonstrated better feed efficiency than the others. These results showed that adding 0.5% garlic into the feed of Tre chickens (T2 treatment) can be used in raising for growth production. The study indicated this level of garlic supplementation can improve FCR, potentially leading to better weight gain.

Table 4. Effects on the growth performance

Item	Treatments					SEM	P
	T1	T2	T3	T4	T5		
IW, g/bird	198	201	197	199	197	1.95	0.082
FW, g/bird	970^{b}	997^{a}	990^{ab}	$1,005^{a}$	968^{b}	0.83	0.001
ADG, g/b/d	13.8^{b}	14.2^{a}	14.2^{a}	14.4^{a}	14.0^{ab}	1.50	0.001
ADFI, g/b/d	46.9^{a}	45.0^{b}	45.4^{b}	45.8^{b}	47.5^{a}	0.45	0.001
FCR	3.40^{a}	3.17^{b}	3.20^{b}	3.18^{b}	3.39^{a}	0.09	0.001

In Vietnam, there have been some studies on the effects of garlic on the growth performance in some native chicken breeds with promising results. In a study on Choi chickens (1-15 weeks old), adding garlic powder in their diet at a 1.5% level resulted in the highest BW (2,285g) and ADWG (21.4 g/bird/day) (Hoang and Nguyen, 2020). Besides, supplementing Noi broiler diets with 1% garlic powder had been shown to results in the highest BW (1,111g) at 4-13 weeks old (Nguyen and Nguyen, 2020).

Many worldwide studies proclaimed the positive effects of garlic on the growth performance of commercial broiler. The research of Oluwafemi *et al.* (2021) signified that adding a 0.4% ginger and garlic oil mixture to Ross-308 broiler's feed (1-56 days old) obtained the highest BW (2,677 g/bird) and the lowest FCR (1.5). Supplementing 0.75g of garlic powder/kg of feed to Cobb-500 broiler's feed (1-42 days old) increased the ADWG (55.4 g/bird/day) (Ismail *et al.*, 2021). Adding the diet of Cobb-400 broilers with 1% garlic powder during the period of 120-148 days old resulted in an ABW of 1,462 g/bird (Faruk *et al.*, 2023). In addition, garlic juice supplemented in drinking water was also investigated to evaluate the effect on broiler performance. Using 10ml of garlic juice/litter of drinking water for Ross-308 broilers (1-35 days old) resulted in a BW of 2,187 g/bird (Sallam *et al.*, 2024). The improvement in broiler performance was due to the antibacterial and antioxidant substances in garlic (Rusli *et al.*, 2022; Ashour *et al.*, 2025). Taufik and Maruddin (2019) suggested that garlic contains active compounds that can replace synthetic antibiotics used in poultry production. Thus, the differences between the above research results are due to differences of dosage, type, study period, nutritional regimen and experimental conditions. Moreover, the content of bioactive substances in garlic also varied depending on geographical, climatic and storage conditions (Issa and Omar, 2012), thus also affecting the experimental results.

In addition, the results of the present study exhibited garlic had a positive effect on FI and FCR of Tre chickens. The provided statement indicates that Choi native chickens fed a diet with 1.5% garlic powder exhibited the lowest values for both ADFI (90.2 g/bird/day) and FCR (2.76) (Hoang and Nguyen, 2020). The study of Nguyen (2022) indicated that adding 2% garlic powder to the turkey diets (5-14 weeks old) and chickens resulted in the lowest FCR of 2.7. Furthermore, some published recently in the world also stated the positive role of garlic in commercial broiler farming. Sallam *et al.* (2024) have found that adding 10ml of garlic juice/liter of drinking water resulted in a FCR of 1.16 in Ross-308 broilers (1-35 days old). Besides, garlic contained many organosulfur compounds (allicin, alliin, ajoee, diallylsulfide, dithiin and S-allylcysteine) (Ziarlarimi *et al.*, 2011). Garlic improved the villi height in the small intestine of chickens, thereby increasing the efficiency of nutrient absorption (Taufik and Maruddin, 2019; Chitra, 2020). Moreover, the positive impact of garlic on the broiler growth were due to the bioactive compounds with antibacterial, antifungal and immunomodulatory properties (Ismail *et al.*, 2021). Therefore, garlic could be used as a natural feed additive for broiler chickens to improve growth and survival rate (Puvača *et al.*, 2019; Aarti and Khusro, 2020).

3.2. Effects of garlic on some blood biochemistry parameters of Tre chickens

LDL-C has been demonstrated to play an essential role in the progression of atherosclerotic coronary artery disease (Shin *et al.*, 2017). HDL-C removes LDL cholesterol from the arteries, acts as a scavenger that carrying LDL-C cholesterol away from the arteries and back to the liver (Madsen *et al.*, 2017). Triglycerides are composed of glycerol and three fatty acid chains. High levels of triglycerides in the blood are associated with cardiovascular disease risk, pancreatitis, metabolic syndrome (Hinou *et al.*, 2024). Therefore, high levels of triglycerides and LDL-C in chicken blood can indirectly affect the health of consumers, especially if they consume a lot of chicken products containing high levels of blood fat.

Table 5. Effect on blood biochemistry parameter

Item	Treatments				
	T1	**T2**	**T3**	**T4**	**T5**
Triglycerides, mmol/l	1.81	1.77	1.72	1.67	1.55
Total cholesterol, mmol/l	4.64	3.59	3.52	3.48	3.41
HDL-C, mmol/l	2.45	2.01	1.54	2.20	1.55
LDL-C, mmol/l	1.63	1.22	1.00	0.80	0.75
Albumin, g/l	15.5	16.4	13.5	15.0	12.4

Table 5 indicated the level of triglycerides, total cholesterol, HDL-C, LDL-C and albumin index varied significantly across the different treatments. The provided data indicates a trend of reduced blood lipids among the treatments, as the triglyceride index decreased from T1 (1.81 mmol/l) to T5 (1.55 mmol/l). Similarly, total cholesterol levels decreased, with the highest level in T1 (4.64 mmol/l) and the lowest in T5 (3.41 mmol/l). The provided LDL-C values indicate T1 has the highest concentration at 1.63 mmol/l, while T5 has the lowest at 0.75 mmol/l. This decrease suggested the treatments were effective in lowering blood lipids levels. Additionally, the highest HDL-C index was recorded in T1 (2.45 mmol/l), while the lowest was found in T3 (1.54 mmol/l). Albumin is the most abundant circulating protein found in plasma and playing an essential role in many physiological functions (Yuwen *et al.*, 2017). Albumin index assesses general health status, especially liver, kidney and nutritional status (Duque *et al.*, 2018). Albumin levels in T2 was the highest at 16.4 g/l, while the level in T5 was the lowest at 12.4 g/l. These results expressed a positive impact of garlic on lipid and protein metabolism in Tre chickens.

The present study were similar to the report of Ismail *et al.* (2021) stated that adding 0.25-0.75g of garlic powder/kg of feed that reduced triglyceride, total cholesterol, and LDL levels. Similarly, adding garlic powder at 0.1-0.3% to the diet of Cobb-500 broilers lead to a decrease in triglycerides, total cholesterol, and LDL levels, while increasing HDL levels (Kairalla *et al.*, 2022). The research of Sallam *et al.* (2024) indicated that dietary supplementation of garlic can lower triglyceride levels in Ross 308 broilers. These results exibitied garlic contains organosulfur compounds such as diallyl thiosulfonate, diallyl sulfide, diallyl disulfide, diallyl trisulfide, E/Z-ajoene, S-allyl-cysteine and S-allyl-cysteine sulfoxide (alliin) (Kodera *et al.*, 2017; Mansingh *et al.*, 2018). These active ingredients reduced total cholesterol, LDL and triglycerides in blood by reducing the activity of cholesterol and fat-producing enzymes in the liver (Rehman and Munir, 2015). The dietary supplementation of broilers with garlic improved in the production parameters, carcass quality, and intestinal integrity. The beneficial effects of garlic on blood lipids and other aspects of chicken health involved the ability to modulate oxidative stress, reduce free-radical production, and improve gut health (El-Ghany, 2024).

4. CONCLUSIONS

In summary, adding 0.5-1.5% crushed garlic to the diet improved the ADWG, ADFI and FCR of Tre chickens at 6-12 weeks old. In addition, garlic also reduced the LDL-C and triglyceride levels in the chickens blood. It could be concluded that adding 0.5% garlic reduce production costs by improving growth performance and enhancing health of Tre chickens.

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EFFECT OF MORINGA LEAF POWDER IN THE DIET ON THE PRODUCTIVITY AND EGG QUALITY OF HUBBARD HENS

Phan Ba Huu[1] and Nguyen Hong Nhung[2]*

Submitted: 17-Jun-2025 – Revised: 17-Jul-2025

Accepted: 23-Jul-2025

ABSTRACT

The study was conducted in My Tho city, Tien Giang province from March 2024 to May 2024 to evaluate the possibility of using Moringa leaves (*Moringa oleifera*) powder in the form of grass powder in the diet of Hubbard laying hens. The experiment was arranged in a completely randomized design on 360 hens at 22-28 weeks old with three treatments corresponding to three levels of Moringa leaf powder supplementation. Control treatment (M0): supplemented with 0% Moringa leaf powder, M2: supplemented with 2% Moringa leaf powder and M5: supplemented with 5% Moringa leaf powder. The results showed that the productivity and egg quality of Hubbard hens in the experimental treatments were similar.

Keywords: *Moringa oleifera, Hubbard chickens, egg productivity, egg quality*.

1. INTRODUCTION

Nowadays, meeting the demands of a growing population and the increasing need for human protein intake poses a significant challenge to the livestock industry. Chicken eggs are among the most widely consumed animal products globally due to their low cost and high nutritional value. According to statistics from the Department of Livestock Production, Vietnam's poultry egg output increased from 16 billion eggs in 2020 to 18.3 billion eggs in 2022. To meet consumer demand for poultry eggs, Vietnam has imported, adapted, and introduced into production several high yielding commercial laying breeds such as Leghorn, ISA Brown, and HyLine. However, poultry production in developing countries faces increasing feed costs due to the rising prices of protein and energy sources. As a solution, some leguminous plants and tropical vegetation have been incorporated into poultry diets as alternative protein sources to reduce feed expenses. *Moringa oleifera*, a tree commonly found in tropical regions, has multiple uses and considerable economic value. Nutritionists have included Moringa leaves in poultry diets to investigate their effects on the performance of both broilers and layers. Currently, many studies have focused on supplementing poultry diets with Moringa leaf powder (MLP) to improve productivity in both meat and egg laying breeds. However, research on the impact of MLP on the performance and egg quality of Hubbard laying hens remains limited. Therefore, this study was conducted to evaluate the effects of dietary MLP supplementation on the productivity and egg quality of Hubbard hens.

2. MATERIALS AND METHODS

2.1. Materials

The experiment was carried out on 360 Hubbard laying hens at 22-28 weeks old, from March 2024 to May 2024, at a household poultry farm located in My Tho City, Tien Giang Province. An opened housing system for chickens was made by wood and tole, which were surrounded by plastic net and its floor was overlaid with 20cm of sand. Feed was offered twice daily (07 AM and 02 PM) at 110 g/bird, according to the formulation presented in table 1. The hens had free access to drinking water via plastic drinkers. Feeders were placed inside the house at an appropriate stocking density. The chickens were supplemented daily with Super layer, Nutrilaczyme, and Vitamin

[1] Vietswan Poultry Breeding Joint Stock Company
[2] Tien Giang University
[*] Corresponding author: Phan Ba Huu, Vietswan Poultry Breeding Joint Stock Company. Phone: 0084 918180185; Email: pbhuu@vietswan.com.

C. Lighting was provided for an average of 16 hrs/day using incandescent bulbs, with a density of 18 m^2/bulb and a power of 1.4 W/m^2. Disease prevention and treatment were carried out according to the health management protocol of Emivest Feedmill Vietnam Company Limited.

2.2. Methods

The experiment was arranged in a completely randomized design with 360 Hubbard laying hens, including three dietary treatments and 04 replications/treatment. A total of 12 experimental units were used, with each unit consisting of 30 hens of similar age, body weight, and laying rate. During the experimental period, the feed formulations remained unchanged as presented in table 1. Randomly arranged 30 chickens (15 males, 15 females) into each pen with a density of 4 m^2/10 birds (area: 2mx2m=4m^2). Each pen corresponded to one repetition, so there were a total of 4 repetitions. The MLP experimental treatments were as follows: M0: basal diet (BD) without MLP; M2: BD supplemented with 2% MLP; M5: BD supplemented with 5% MLP.

Table 1. Feed formulations for Hubbard hens

Ingredient (%)	Treatments		
	M0	M2	M5
Regular rice bran	8	8	8
Fine rice bran	11	9	6
Broken rice	20	37	54
Corn	34	17	-
Fish meal	10	10	10
Soybean meal	10	10	10
Coconut oil cake	4	4	4
Bone meal	1	1	1
Shell powder	1.9	1.9	1.9
Premix	0.1	0.1	0.1
MLP	-	2	5
Total	100	100	100
Crude protein (%/DM)	18.49	18.49	18.76

Experimental parameters: The reproductive performance and egg quality paramaters were assessed according to Bui Huu Doan *et al.* (2011). During the experimental period, laying rate was recorded daily and expressed as the percentage of eggs produced per hen. Eggs were collected twice daily at 09AM and 03PM during the laying period of 22-28 weeks old. All eggs were weighed, and shape index was measured immediately after collection. Daily feed intake (FI) was also recorded. Each week, 60 eggs per experimental unit were randomly sampled for quality assessment. Eggs were broken onto a flat glass surface, and yolk, albumen, and shell were carefully separated and individually weighed using a digital scale.

Laying rate (%): (∑eggs produced/∑hens day)x100

Feed intake (g of feed/hen/day): (∑feed per day-∑excess feed)/Hens number presented

Feed intake (g of feed/egg): ∑FI per treatment /∑eggs number per treatment

Egg weight (g/egg): Weighed by a digital balance immediately after collecting.

Yolk ratio (%): (Yolk weight/Egg weight)x100

Eggshell ratio (%): (Eggshell weight/Egg weight)x100

Egg shape index (%): (Egg width/Egg length)x100

Eggshell thickness (mm): Using a micrometer to measure at three points (air cell, equator, and pointed end). Mean of triplicate measurement from three points.

Egg yolk color score: Using a Roche yolk color fan on a scale of 1-15.

2.3. Statistical analysis

The data were recorded by using Excel software and using generalized linear regression model (GLM) of Minitab 16.0 software (a data analysis software package used for data analysis) to analyse the variance. The significant differences between mean values within and between treatments were determined according to Tukey with $P<0.05$. Experimental model according to the formula: $Y_{ij}=\mu+G_i+\xi_{ij}$. *where, Yij: traits observed; μ: general mean, Gi: influence of treatments; ξij: random error*. A probability value of less than 0.05 was considered to be significant.

3. RESULTS AND DISCUSSION

3.1. Moringa powder production

To make a nutritionally balanced compound feed, green forages must be processed into dried powder form, commonly referred to as grass powder. Therefore, this study carried out the production of grass powder at a laboratory scale. The results showed that *Moringa oleifera* leaves can be processed into grass powder by either sun drying or oven drying. Chopped leaves, when sun dried under good sunlight conditions, became crisp within a single morning. Oven drying at 60°C took about 1-2hrs after partial wilting. To prevent moisture buildup, which could cause leaf darkening, the oven door was opened every 15-20 minutes to release heat. After drying, the leaves were ground using a 2mm mesh sieve. The resulting powders from both drying methods retained a pleasant aroma and green color. On average, 4.6kg of fresh leaves yielded 1kg of grass powder. During harvesting, overly mature and very young leaves were excluded.

3.2. Egg production and feed intake

Table 2 presented the highest laying rate was highest in M5 (76.6%), followed by M2 (75.4%), and the lowest in M0 (74.5%). When taking the laying rate of the control as 100%, M2 was higher by 1.14% and M5 by 2.87%. Compared to the laying rate before the experiment, the differences in laying performance among the experimental groups were not significant. Thus, partial or complete substitution of corn with broken rice, supplemented with grass powder, did not adversely affect egg production performance among the diets. Additionally, the average daily feed intake (FI) tended to decrease across the treatments, with the highest value recorded in M0 (107.6 g/hen/day), followed by M2 (106.3 g/hen/day), and the lowest in M5 (101.0 g/hen/day). Despite this downward trend, the differences among the treatment groups were not statistically significant (P>0.05), indicating that the dietary modifications applied in M2 and M5 did not markedly affect the FI of laying hens compared to the control (M0).

Table 2. Egg production and feed intake

Items	Treatments			P
	M0	M2	M5	
Laying rate before expt. (%)	74.2	75.9	75.1	ns
Laying rate during expt. (%)	74.5	75.4	76.6	ns
Compared %	100.0	101.2	102.8	ns
FI (g of feed/hen/day)	107.6	106.3	101.0	ns
FI (g of feed/egg)	138.0	137.7	121.0	ns

Furthermore, table 2 also indicated that FI was highest in M0 (138.0g of feed/egg), followed by M2 (137.7g of feed/egg), and lowest in M5 (121.0 g of feed/egg). Melesse *et al.* (2011) reported that incorporating MLP into the diet of Rhode Island Red chicks significantly (P<0.05) improved FI, ADG, and FCR compared to the control group. Similarly, Abbas (2013) found that supplementing 20% MLP in the diet of laying hens improved FCR and significantly increased egg weight (P<0.05). A study by Ebenebe *et al.* (2013) demonstrated that dietary inclusion of MLP at levels of 2.5 and 5% enhanced egg production, egg weight, yolk height, and yolk percentage in laying hens compared to the control.

Besides, the use of natural plant based on feed additives to improve egg quality is a growing trend with great potential, as many herbal plants contain bioactive compounds beneficial to animal health. In Vietnam, several recent studies have explored the positive effects of herbal supplements in poultry diets. Nguyen Thi Thuy (2019) evaluated the effects of Turmeric powder supplementation on egg production and quality in 240 Hisex Brown laying hens at 30 weeks old. The results indicated that supplementation with 0.2 and 0.3% turmeric powder slightly improved egg production, egg weight, and eggshell color. Similarly, Nguyen Thi Thuy and Nguyen Cong Ha (2022) investigated the effects of Moringa

(*Moringa oleifera*) and Turmeric (*Curcuma longa*) powder supplementation on egg production, quality, and hatchability in black-boned chickens (Ac chickens) from 23 to 37 weeks old. The results exhibited the improvement of egg production in hens diets supplemented with Moringa powder (54.1 and 61.6%) compared to Turmeric powder (53.5 and 59.7%) and the control group (52.3 and 58.1%).

3.3. Egg quality

Egg weight is an important economic trait, as larger commercial eggs tend to have higher market value due to their greater internal content. In the present study, the highest egg weight was observed in M0 (56.9 g/egg), followed by M2 (56.5 g/egg), and the lowest was in M5 (56.2 g/egg). The average egg weight in present study were higher than other local chickens in Vietnam such as Ri chickens (41.7 g/egg) and Mia chickens (44.7 g/egg) (Moula *et al.*, 2012); black Noi hens (48.3 g/egg) and dark brown Noi hens (49.7 g/egg) (Dang Vu Hoa *et al.*, 2021); Ac hens at 23-37 weeks old (31.2-36.2 g/egg) (Nguyen Thi Thuy and Nguyen Cong Ha, 2022); Ac hens at 16-40 weeks old (35.8-36.2 g/egg) (Le Thanh Phuong and Nguyen Thi Thuy, 2025).

Table 3. Egg quality in different treatments

Items	Treatments			P
	M0	M2	M5	
Egg weight (g)	56,9	56,5	56,2	ns
Egg shape index (%)	75,4	74,8	75,9	ns
Yolk ratio (%)	26,3	26,8	26,4	ns
Eggshell ratio (%)	11,5	11,3	11,5	ns
Eggshell thickness (mm)	0,37	0,38	0,39	ns
Egg yolk color score	8,89^{a}	8,42^{a}	8,19^{b}	<0,01

Note: Means with different letters in the same row differ significantly (P<0.05)

In addition, table 3 showed the egg shape index was highest in M5 (75.9%), followed by M0 (75.4%), and lowest in M2 (74.8%). Egg shape is influenced by the oviduct structure of hens, internal organ distribution, and pelvic bone shape. Segura *et al.* (2024) investigated the effects of egg shape index, egg weight, and surface area to volume ratio in Lohmann Brown eggs. Their findings indicated that eggs with a shape index ranging from 75.1-79% and a surface area to volume ratio of 1.01-1.04 cm^2/cm^3 had lower embryonic mortality rates. Eggs were categorized based on shape index as flat (<72%), normal (72-76%), and round (>76%) (Duman *et al.*, 2016). Therefore, the egg shape indiced in all treatments of the present study fall within the normal range, indicating well-formed eggs suitable for hatching. Supplementation with MLP did not affect egg shape index or egg quality (Ebenebe *et al.*, 2013). However, a study by Mabusels *et al.* (2018) found that adding 10% Moringa seed powder to the diet of laying hens increased eggshell thickness compared to the control group.

Also, the yolk ratio was highest in M2 (26.8%), followed by M5 (26.4%), and lowest in M0 (26.3%). Compared to the control (M0), M2 exhibited an increase of 1.86%, while M5 was higher by 0.38%. Nguyen Nhut Xuan Dung and Luu Huu Manh (2016) reported that in Noi chickens, the yolk ratio was higher during the early laying period (34.5%) compared to the later stage (33.7%). Similarly, Sun *et al.* (2019) found that White Leghorn layers had a yolk proportion of 27.5%. These variations may be attributed to differences in chicken breeds, age, rearing conditions, and the duration of the study.

Moreover, table 3 exhibited the eggshell ratio was relatively similar among the treatments. This result indicated that supplementing MLP in diets with reduced corn or complete corn replacement by broken rice did not affect mineral digestion or absorption in laying hens. Eggshell strength was determined by shell thickness; eggs with weak shells are prone to breakage, which complicates storage and transportation. Table 3 presented that eggshell thickness was highest in M5 (0.39mm), followed by M2 (0.38mm), and lowest in M0 (0.37mm). According to Yamak *et al.* (2015), eggshell thickness can be classified into three groups:

thin (≤0.34mm), medium (0.35-0.38mm), and thick (≥0.39mm). Thus, the eggshell thickness observed in the present study falls within the medium to thick range, meeting quality standards for egg durability. Eggshell thickness is a critical factor influencing both egg quality and viability. Thicker shells offer better protection against microbial contamination and environmental stress, thereby improving hatchability in breeding programs. Additionally, consumers often associate thicker shells with superior freshness and quality (Seid and Tesfa, 2025). The present findings were comparable to those of Sun *et al.* (2019), who reported a thickness of 0.34mm in White Leghorn layers, and Kumar *et al.* (2022), who recorded 0.35mm in Aseel and 0.34mm in Kadaknath chickens. Notably, Milkias and Molla (2022) observed significantly thicker shells (0.54mm) in indigenous chickens from the highlands of the Dawro Zone. Krisnaningsih *et al.* (2023) further demonstrated that storage duration significantly (P<0.01) affected eggshell thickness in Isa Brown hens, which declined from 0.51mm at 0 days to 0.19mm after 21 days of storage.

Furthermore, yolk color is a key indicator of feed quality and consumer preference, with higher scores reflecting diets rich in vitamin A and carotenoids. As shown in table 3, yolk color was highest in M0 (8.89), followed by M2 (8.67) and lowest in M5 (8.19). The reduction in yolk color in M2 and M5 compared to the control may be attributed to variations in carotene retention during manual sun drying of MLP. These values were lower than those reported by Hrnčár *et al.* (2016) for traditional breeds (9.49-9.74), Sözcü *et al.* (2023) for commercial layers (11.7-12.3), and Seid and Tesfa (2025), who recorded a peak score of 13.13 in indigenous chickens. Similarly, yolk color scores were lower than those reported by Milkias and Molla (2023) (8.15-8.65 across ecological zones) and Krisnaningsih *et al.* (2023), who observed a decline from 9.56 to 7.25 over 21 days of storage. Yolk pigmentation is primarily influenced by dietary pigments such as xanthophylls, carotenoids, and cryptoxanthin (Ortiz *et al.*, 2021), with yellow to orange yolks generally preferred by consumers due to their perceived health benefits and visual appeal (Kartikasari *et al.*, 2021).

Finally, the study also noted that during the off-season, the price of corn increases significantly compared to broken rice. Moreover, stored corn is often contaminated, resulting in reduced quality. Therefore, replacing corn with broken rice and supplementing the diet with MLP may provide economic benefits. However, during the corn harvest season, the price difference between corn and broken rice is minimal, and corn remains the best source of carotene.

4. CONCLUSIONS

In conclusion, supplementing MLP in the diet of Hubbard laying hens did not affect egg production. However, complete replacement of corn with broken rice combined with MLP resulted in pale yolk color, which may not meet consumer preferences. Therefore, supplementing 2% MLP in the diet while replacing 50% of the corn content is considered acceptable. In addition, the inclusion of MLP in diets with reduced or completely replaced corn by broken rice had no negative impact on egg production or quality, offering an economic advantage during corn scarce seasons.

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USING SWEET POTATO LEAVES AS A FEED SOURCE FOR MUSCOVY DUCK

Le Thanh Phuong[1] and Nguyen Hong Nhung[2]*

Submitted: 17-Jun-2025 – Revised: 17-Jul-2025

Accepted: 23-Jul-2025

ABSTRACT

The study was conducted in Chau Thanh district, Tien Giang province from March 2024 to May 2024 to evaluate the possibility of using fresh sweet potato leaves (*Ipomoea batatas*) as a dietary supplement for Muscovy ducks (*Cairina moschata*). The experiment was arranged in a completely randomized design on 120 ducks from 4 to 12 weeks old, with 3 treatments corresponding to 3 levels of sweet potato leaves supplementation. Control treatment: basic diet; treatment 1 (T1): replace 10% of soybean meal with broken rice and bran, ducks were fed ad libitum with sweet potato leaves; and treatment 2 (T2): replace 10% of fish meal with broken rice and bran, ducks were fed ad libitum with sweet potato leaves. The results indicated that growth performance and meat yield were comparable among the treatments. Therefore, the use of fresh sweet potato leaves supplemented in low protein diets still met the normal growth of Muscovy ducks and brought economic efficiency.

Keywords: *FCR, Muscovy duck, growth performance, sweet potato leaves.*

1. INTRODUCTION

Nowadays, increasing the global demand for animal-based foods for humans is a challenge for the livestock industry. Muscovy duck (*Cairina moschata*) is one of the most popular animals consumed in the world due to its delicious meat quality, rich in nutrients and easy to process into many attractive dishes. Muscovy ducks originate from South America and are commonly raised in Vietnam, especially in rural areas and the Southwest. Muscovy ducks are not only popular in Asian countries such as Vietnam, China, Thailand but also present in many European and American markets (Kovitvadhi *et al*., 2019). Muscovy ducks exhibit a short growth period, ease of management, lower susceptibility to diseases, and require relatively low housing and investment costs (Nguyen *et al*., 2020), so they are becoming a popular breeding subject for many households and farms. Muscovy duck meat is highly nutritious, rich in protein, vitamin B, iron, and zinc, and notably low in fat, making it highly favored by consumers (Slobodyanik *et al*., 2021). With the growing demand for healthy foods and the trend toward consuming low-fat meat, Muscovy ducks possess strong development potential in the near future, not only to meet domestic market needs but also for export opportunities.

In addition, the poultry industry in developing countries is facing the problem of increasing feed costs, so some plant species are added to the poultry diet to reduce feed costs. Sweet potato (*Ipomoea batatas*) is not only known for its starchy tubers but also has high nutritional value with many proteins, vitamins and minerals that are good for health (Ahmed *et al*., 2021). Consequently, sweet potato leaves are utilized as a supplementary feed source for poultry, contributing to enhanced immunity, improved digestive function, and growth performance. As an affordable and easily cultivated green feed, they also play a role in reducing overall livestock production costs. Currently, there are not many studies on adding sweet potato leaves to the diet of Muscovy ducks to partially replace industrial feed. Therefore, the study was conducted to evaluate the impact of adding fresh sweet potato leaves to the diet of Muscovy ducks on meat yield and reducing livestock costs.

[1] Emivest Feedmill Company, Vietnam
[2] Tien Giang University
* Corresponding author: Dr. Le Thanh Phuong, Emivest Feedmill Company Limited, Vietnam. Phone: 0084 0967551973. Email: ngongtroi13052002@yahoo.com.

2. MATERIALS AND METHODS

2.1. Materials

The study was conducted on 120 Muscovy ducks (*Cairina moschata*) at 4 weeks old from March 2024 to May 2024 at a farm in Chau Thanh district, Tien Giang province. Ducks were raised at a stocking density of 3 birds/m^2 in an open cages with a cold corrugated iron roof. The cage floors were covered with a 10 cm thick layer of sand, and the surrounding area were enclosed with nylon net. Duck feed was mixed according to the ration in table 1 with the amount of feed according to age. Ducks were prevented and treated for diseases according to the process of Emivest Feedmill Vietnam Company.

2.2. Methods

The experiment was arranged in a completely randomized design on 120 at 4 weeks old Muscovy ducks with 3 treatments and 4 replications. There were 12 experimental units, each unit had 10 ducks (5 males, 5 females) of the same age and similar body weight (0.63-0.65 kg/female duck and 0.74-0.78 kg/male duck).

Control (C): basic diet.

Treatment 1 (T1): 10% of soybean meal was replaced with broken rice and bran, and ducks were fed *ad libitum* with fresh sweet potato leaves.

Treatment 2 (T2): 10% of fish meal was replaced with broken rice and bran, and ducks were fed *ad libitum* with fresh sweet potato leaves.

Table 1. Feed formulation of diets for ducks

Ingredients	C	T1	T2
Broken rice (%)	39.5	44.5	44.5
Broken bran (%)	39.5	44.5	44.5
Fish meal (%)	10.0	10.0	-
Soybean meal (%)	10.0	-	10.0
Bone meal (%)	0.5	0.5	0.5
Seeshell powder (%)	0.5	0.5	0.5
Total (%)	100	100	100
CP (%/dry matter)	18.9	16.1	14.1
ME (kcal/kg feed)	2,684	2,608	2,655

Cares: Each experimental group was provided with a feeding trough and a drinking trough. Sweet potato leaves were chopped and offered *ad libitum* to the ducks. Feeding was conducted twice daily, at 8:00 AM and 3:00 PM.

Performance paramater measurements: The body weight of ducks was recorded weekly from the 4-12 weeks old. The average daily weight gain (ADWG), average daily feed intake (ADFI) and feed conversion ratio (FCR) was calculated according to Oluwafemi *et al.* (2021).

ADWG (g/bird/day) = [Final weight (g) - Initial weight (g)]/Total days of the experiment (day)

ADFI (g/bird/day) = [Amount feed provide per day (g) - Amount feed remain per day (g)]/Number of ducks in a treatment

FCR = ADFI/ADWG

Carcass traits: At the end of the experiment (12 weeks old), all ducks were fasted for 8hrs prior to slaughter. Four ducks (two males and two females) were randomly selected from each experimental unit for slaughtering. The individual live body weight (pre-slaughter weight) was recorded. The slaughter weight was recorded, followed by scalding and plucking to remove feathers. Subsequently, the carcass was opened, and the dressing percentage was calculated based on the live body weight (Sallam *et al.*, 2024).

2.3. Statistical analyses

The data were recorded by using Excel software and using generalized linear regression model (GLM) of Minitab 16.0 software (a data analysis software package used for data analysis) to analyse the variance. The significant differences between mean values within and between treatments were determined according to Tukey with $P<0.05$. Experimental model according to the formula: $Y_{ij}=\mu+G_i+\xi_{ij}$. *Where, Yij: traits observed; μ: general mean, Gi: influence of treatments; ξij: random error*. A probability value of less than 0.05 was considered to be significant.

3. RESULTS AND DISCUSSION

3.1. Body weight, weight gain and FCR of Muscovy ducks

The results in table 2 showed the average weight of Muscovy ducks at the beginning of the experiment between the treatments had no statistical significance (P>0.05). This is explained by the ducks selected for distribution into the treatments had relatively equal weight. In addition, Table 1 indicated the crude protein content in the diet in T1 and T2 was lower than the control. However, the ducks weight at the end of the experiment had no statistical significance between the treatments. This presented the growth of the ducks weight in the treatments was almost the same. The reason could be explained by the fact that sweet potato leaves compensated for the low protein content in the diets of T1 and T2 for ducks.

Table 2. Muscovy duck weight in experiment

Iterms	Treatments			P
	C	T1	T2	
Initial body weight, kg/bird				
Female	0.63	0.66	0.65	0.73
Male	0.78	0.74	0.74	0.61
Average	0.71	0.70	0.70	0.96
Final body weight, kg/bird				
Female	1.72	1.72	1.70	0.95
Male	2.79	2.55	2.60	0.45
Average	2.27	2.14	2.15	0.53
Female ADWG, g/bird/day	18.1	17.7	17.4	0.88
Male ADWG, g/bird/day	33.6	30.2	31.1	0.48
ADWG, g/bird/day	25.8	24.0	24.2	0.45
FCR, kg feed/kg BWG	4.47	4.52	4.51	0.97

Table 2 exhibited the body weight of male ducks at 12 weeks old was highest in the control group (2.79 kg/bird), followed by T2 (2.60 kg/ bird) and lowest in T1 (2.55 kg/bird). This result was lower than the findings of Castillo *et al.* (2020), which reported body weights of 3.7 kg/bird in male Muscovy ducks aged from 6 to 12 weeks, fed a diet supplemented with 1.5-2.5% Quebracho tannin (bioactive substance). The average body weight of Muscovy ducks at 12 weeks old observed in the present study was consistent with the results reported by Dang *et al.* (2012) with a body weight ranging from 2.2 to 2.5 kg/bird. In addition, Li *et al.* (2024) reported that Muscovy ducks fed a diet enriched with lactic acid-producing bacteria (Probiotics) achieved an average body weight of 2.2 kg/bird at 11 weeks old.

On the other hand, this result was lower than that reported by Nguyen *et al.* (2019), in which Muscovy ducks fed diets supplemented with 0.1-0.2% turmeric powder during weeks 9-12 achieved an average weight of 2.3-2.4 kg/bird. The positive effect of turmeric powder on duck weight can be attributed to its bioactive compound, curcumin, which enhances the digestive and immune systems, thereby promoting improved growth performance in ducks. In addition, the study of Abdel-Hamid and Abdelfattah (2020) also expressed crude protein levels in the diet affect the weight of Muscovy ducks during the 6-12 week old period. The results proclaimed the average weight of ducks was highest at 22% CP (3.32 kg/bird) and lowest at 14% CP (2.09 kg/bird). Dao (2023) reported that supplementing the diet with 30% banana stem silage enabled Muscovy ducks at 5-12 weeks old to reach an average body weight of 2.3 to 2.5 kg/bird. Thus, the differences in the above research results were due to differences in study time, feeding regime and care conditions.

In addition, the average weight gain of ducks was highest in the control (25.8 g/bird/day) and lowest in T1 (24.0 g/bird/day), but the difference between these treatments was not statistically significant (P>0.05). This result was higher than the study of Nguyen *et al.* (2020) on Muscovy ducks (9-12 weeks old) with a diet containing 13.3 MJ/kg and 17% CP (increased 15.1-17.7 g/bird/day). The impact of dietary crude protein levels on weight gain in Muscovy ducks (6-12 weeks od) was examined in the study by Abdel-Hamid and Abdelfattah (2020). The results indicated the average weight gain of ducks was highest at 18% CP (34.9 g/bird/day) and lowest at 14% CP (9.5 g/bird/day). Moreover, the inclusion of 30% silage banana stem in the diet was reported by Dao (2023), with results indicating that ducks aged 5 to 12 weeks achieved an average daily weight gain of 29.6 to 33.0 g/bird.

At the same time, the lowest FCR was in the control group (4.47), followed by T2 (4.51) and the highest in T1 (4.52), but this difference was not statistically significant (P>0.05). Although the dietary ingredients varied between groups, the energy levels remained statistically similar, resulting in no significant differences in FCR among the groups. On the other hand, the dietary fibers in sweet potato leaves may contribute to prolonged retention of concentrated feed in the small intestine of ducks, thereby enhancing nutrient digestion and absorption (Nurrofingah *et al.*, 2019; Cao *et al.*, 2022). This contributed to the insignificant difference in FCR values between the groups. The FCR observed in the present study was comparable to that reported by Nguyen *et al.* (2019) with values ranging from 5.94 to 6.35 in Muscovy ducks at 9-12 weeks old, and to the findings of Abdel-Hamid and Abdelfattah (2020), which reported an FCR of 4.78 in ducks aged 11 to 12 weeks fed a diet containing 22% CP. However, this result was lower than the study by Dao (2023) adding banana stems silage at 30% to the diet of Muscovy ducks (5-12 weeks old) and the results signified the FCR was 5.87-6.99. Thus, the difference in these results was due to differences in study time, nutrition and care conditions.

3.2. The carcass characteristics of Muscovy duck

The results of the post-mortem of Muscovy ducks at the end of the experiment were presented in table 3. The average values of the carcass traits between the treatments did not differ statistically (P>0.05). This expressed the addition of fresh sweet potato leaves to the diet of Muscovy ducks did not affect the quality of the carcass. In addition, the pre-slaughter revealed that ducks in treatments T1 and T2 exhibited a more yellow skin coloration compared to the control group. This physical characteristic is essential to satisfy consumer preferences and demands. Furthermore, it represents an important aspect warranting further detailed investigation in future studies.

Table 3. Some carcass traits of Muscovy duck

Iterms	Treatments			P
	C	T1	T2	
Live BW (g)	2,172	2,110	2,055	0.68
Slaughter BW (g)	1,946	1,829	1,846	0.90
Carcass weight (g)	1,474	1,398	1,396	0.92
Breast weight (g)	411.7	366.3	374.2	0.40
Thigh weight (g)	161.3	153.8	154.8	0.12
Carcass ratio (%)	67.9	66.3	67.9	0.36
Breast ratio (%)	27.9	26.2	26.8	0.46
Thigh ratio (%)	10.9	11.0	11.1	0.44

The results of table 3 exhibited the highest carcass weight was in the control (1,474 g/bird) and the lowest in T2 (1,396 g/bird). The carcass ratio between the treatments was in the range of 66.3-67.9% and the difference was not statistically significant (P>0.05). This result was similar to that reported by Nguyen *et al.* (2020), observing a carcass ratio of 66.1-67.2% in Muscovy ducks (9-12 weeks old) fed diets containing 14-17% CP. The highest breast meat was in the control (441.7 g/bird) and the lowest in T1 (366.3 g/bird). Simultaneously, the breast ratio showed no statistically significant difference between treatments (P>0.05), fluctuating between 26.2 and 27.9%. This result was higher than the study of Nguyen *et al.* (2020) on 12-week-old Muscovy ducks at 21.2-23.3%. The highest thigh meat was in the control group (161.3 g/bird) and the lowest in the T1 (153.8 g/bird). In addition, the thigh ratio did not differ significantly between groups (P>0.05), ranging from 10.9 to 11.1%. This result was lower than that reported by Nguyen *et al.* (2019), in which Muscovy ducks (9-12 weeks old) fed diets supplemented with 0.1-0.2% turmeric powder exhibited thigh ratios of 15.4 to 16.3%.

4. CONCLUSIONS

Using fresh sweet potato leaves to supplement the low protein diet still meets the normal growth of Muscovy ducks and brings economic efficiency. Therefore, the use of fresh sweet potato leaves can be used in rural areas where protein supplementary feed is scarce.

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EFFECTS OF JACKFRUIT LEAF SILAGE ON *IN VITRO* DIGESTIBILITY AND METHANE PRODUCTION USING INOCULUM FROM MEAT GOATS

*Tran Thi Thuy Hang[1], Vo Thi Phuong Tien[1] and Lam Phuoc Thanh[1]**

Submitted: 30/6/2025 - Revised: 27/7/2025

Accepted: 31/7/2025

ABSTRACT

This *in vitro* study was conducted to elucidate the effects of jackfruit leaf silage (JLS) replacing Napier grass silage (NGS) on nutrient digestibility, ruminal fermentation and methane emission using ruminal fluid obtained from 4 crossbred Boer goats. The study was assigned as a completely randomized design with 5 treatments and 4 replicates. Five experimental treatments were developed by substituting JLS for NGS in the diet at 0, 25, 50, 75, and 100% (DM), corresponding to JLS0, JLS25, JLS50, JLS75, and JLS100, respectively. After 72 h incubation, it was observed that the digestibility of DM and neutral detergent fiber was gradually decreased (P<0.001) when the JLS proportion increased. Crude protein digestibility and NH_3-N concentration were not affected (P>0.05) by the replacement of jackfruit leaf silage for Napier grass silage. Total VFA production in JLS0 was higher (P<0.05) by 26.5% compared to JLS100. Acetic acid proportion was increased (P<0.01), the highest value was recorded in JLS100 (87.4%), while propionic acid proportion was decreased (P<0.01), the lowest value was noted in JLS100 (9.15%). As a consequence, the acetic acid:propionic acid ratio in JLS0, JLS25, and JLS50 was lower (P<0.001) by 1.54, 1.66, and 1.68 times, respectively, compared to JLS100. Iso-butyric acid, butyric acid, and valeric acid proportions were highest (P<0.05) in JLS50, then gradually reduced in JLS75 and JLS100. Methane production in JLS75 and JLS100 was lower by 28.2 and 38.0%, compared to JLS0. Combined results suggest that higher jackfruit leaf silage inclusion as a replacement for Napier grass silage in the diet can alleviate methane production; however, it also reduces nutrient digestibility and total VFA production.

Keywords: *Digestibility, jackfruit leave silage, meat goat, methane, Napier grass silage.*

1. INTRODUCTION

In recent years, in addition to the development of cattle and buffalo farming, the Vietnamese government has gradually paid more attention to goat farming. This is because goats are gentle animals, easy to care for, require relatively low investment in breeding stock and housing, yet bring high economic efficiency to farmers. According to the General Statistics Office (2021), the total goat population nationwide was 2,675,188, with 434,032 goats in the Mekong Delta, increasing of 35,597 compared to 2020.

Nowadays, alongside climate change, one of the biggest challenges facing the ruminant livestock sector in Vietnam is ensuring a sufficient supply of high-quality roughage resources. Napier grass (also known as elephant grass) is known as one of the most commonly chosen forage crops by goat farmers. Napier grass thrives in hot, humid conditions, making the southern region's climate suitable for year-round cultivation. However, with the rapid increase in population and urbanization, land for growing forage for ruminants has been increasingly reduced. Therefore, to meet the demand for roughage, it is necessary to efficiently utilize agricultural by-products.

Previous studies indicate that agricultural by-products in Vietnam are increasing and offer a plentiful source for livestock feed, helping to reduce feed costs. Among these, jackfruit is an easy-to-grow tree that is rapidly expanding in Vietnam due to its high economic returns. The Mekong Delta has the largest jackfruit cultivation area in the country, with 10,105 hectares, accounting for 38.6% of the total national area and 37.1% of total production. Thus, jackfruit

[1] Can Tho University
* Corresponding author: Assoc. Prof. Dr. Lam Phuoc Thanh, Can Tho University, Campus II, 3/2 street, Ninh Kieu ward, Can Tho city, Viet Nam, Phone: 0084 975763555, Email: phuocthanh@ctu.edu.vn.

by-products are abundant and can be used as a potential feed for livestock. In particular, jackfruit leaves (JL) are considered a favorite feed for goats (Van *et al*., 2005). However, most agricultural by-products in Vietnam are low-quality roughage and highly seasonal. Therefore, to sustainably develop grass-fed livestock, it is essential to study processing and preservation methods for agricultural by-products to ensure nutritional adequacy for livestock. As a result, the method of ensiling feeds, specifically JL and Napier grass (NG), has been developed as a solution to a major problem in the livestock industry.

In reality, both JL and NG have great potential and abundant yields for use as goat feed. However, JL contain 33.2% total tannins (Mui *et al*., 2002), of which 17.9% are condensed tannins (Malik *et al*., 2017). Condensed tannins have been found to reduce methane (CH_4) production in ruminants (Malik *et al*., 2017). To date, there are few studies on the use of JL silage in ruminant diets in Vietnam. Therefore, this study was conducted to evaluate the impact of replacing NG silage with JL silage on *in vitro* ruminal fermentation parameters, digestibility, and methane production using rumen fluid from meat goats.

2. MATERIALS AND METHODS

The study was performed at the Laboratory of Ruminant Production Techniques, Can Tho Uni. All procedures were performed according to the ethical standards in the Helsinki Declaration of 1975, as revised in 2000, as well as the national law.

2.1. Experimental design and diets

The experiment was divided into two sub-expts conducted under *in vitro* conditions: Experiment 1 evaluated nutrient digestibility and ruminal fermentation, while Experiment 2 assessed total gas and methane production.

***Expt 1*:** The experiment used 100 and 50ml glass bottles to assess nutrient digestibility and ruminal fermentation patterns. It was arranged in a completely randomized design with five treatments: JLS0, JLS25, JLS50, JLS75, JLS100- corresponding to the increasing replacement (DM basis) levels of jackfruit leaf silage for Napier grass silage at 0, 25, 50, 75, 100%. Each treatment was replicated four times using individual rumen fluid samples collected from four crossbred Boer male goats.

Table 1. Feed ingredients, diet chemical composition

Items	JLS0	JLS25	JLS50	JLS75	JLS100
Feed ingredients, %DM					
Napier grass silage	0	25	50	75	100
Jackfruit leaf silage	100	75	50	25	0
Total	100	100	100	100	100
Chemical composition, %DM (unless otherwise noted)					
DM	22.5	26.1	29.7	33.3	36.9
Ash	13.5	14.5	15.6	16.6	17.7
OM	86.5	85.5	84.4	83.4	82.3
CP	8.68	10.2	11.8	13.3	14.9
EE	1.29	1.33	1.38	1.42	1.47
CF	43.4	38.6	33.9	29.1	24.3
NDF	65.1	57.5	49.8	42.1	34.4
NFE	33.1	35.3	37.4	39.5	41.7
ME*	7.41	7.63	7.85	8.06	8.28

JLS0, JLS25, JLS50, JLS75, JLS100: replacement of jackfruit leaf silage for Napier grass silage at 0, 25, 50, 75, 100% (DM basis). OM: organic matter, Ash: total minerals, NDF: neutral detergent fiber, CF: crude fiber, EE: ether extract; ME: metabolizable energy (MJ/kg DM).

***Expt 2*:** The gas production technique was applied using 500ml glass bottles under *in vitro* conditions to evaluate total gas and methane production. The experiment was also arranged in a completely randomized design with the same five treatments as in Experiment 1. Each treatment was replicated three times using individual rumen fluid samples from three crossbred Boer male goats.

After harvesting, JL and NG were brought to the laboratory and chopped into small pieces of 0.5-1cm. Each kg of NG and JL was ensiled with 30g of sugarcane molasses. After 3 weeks, the quality of the ensiled samples was assessed, and the bag with the best quality, with a pH in the range of 3.8-4.2, was selected for *in vitro* experiments.

2.2. Inoculum and medium solution

The medium solution was prepared according to the method of Menke and Steingass (1988), with some modifications to the mineral content: KH_2PO_4 was reduced from 6.2 to 6.0g (macromineral solution), and $FeCl_2 \cdot 6H_2O$ was increased from 0.8 to 8.0g (micromineral solution). In addition, the medium was supplemented with L-cysteine hydrochloride and sodium sulfide. The medium solution was prepared and used on the same day *in vitro* experiment was conducted. Rumen fluid was collected in the morning from four 3-month-old crossbred Boer goats (♂Boer×♀Bach Thao) before feeding. A volume of 300ml of rumen fluid was collected from each goat. After collection, the rumen fluid was filtered at the farm and placed in a thermos flask, then immediately transported to the laboratory at the College of Agriculture, Can Tho University. Upon arrival, the rumen fluid was filtered a second time through a 1mm metal sieve, and the pH was measured using a digital pH meter (HI5522, Hanna Instruments, USA) before experimenting. Donor fluid from each goat was kept separately and served as statistical replicates during *in vitro* experiments.

2.3. *In vitro* incubation

Expt 1: The determination of digestibility and rumen fermentation parameters was carried out as follows: After weighing the samples (0.625 and 0.313g) into 100 and 50ml glass incubation bottles, respectively, the bottles were sealed and vacuumed to create an anaerobic condition. Thereafter, 25ml of the mixture, comprised of ruminal fluid:medium solution (1:4 vol/vol), was added. The experimental bottles were incubated in a shaking incubator (ISF-7200R, Jeiotech, Korea) at a speed of 90rpm and a temperature of 39°C for 72h. At 72h post-incubation, the fermentation reaction was stopped by placing bottles in the ice and pH was measured using a digital pH meter (HI5522, Hanna Instruments, USA). To determine NH_3-N and total volatile fatty acids (VFA) concentrations, the incubation fluid was acidified with 1M H_2SO_4 at a 10:1 ratio, centrifuged at 10,000rpm for 10min, and the supernatant was stored at −20°C until further analysis. Digestibility of DM, crude protein (CP), and neutral detergent fiber (NDF) was also determined after 72h of incubation.

***Experiment 2*:** 5.0g of sample was weighed into 500ml glass incubation bottles, followed by adding 400 mL of incubation solution composed of medium and rumen fluid in a 4:1 ratio. The bottles were sealed with rubber stoppers, and air was removed using a vacuum pump (Rocker 300, Rocker, Taiwan). The bottles were then connected to specialized gas collection bags (22952, Restek, USA) and incubated in a shaking incubator (ISF-7200R, Jeiotech, Korea) at 90rpm and 39°C. Total gas production was recorded after 72h of incubation.

2.4. Chemical analysis and calculations

Feed samples were analyzed for DM, OM, CP, CF and EE following the methods outlined by AOAC (1990), whereas NDF was analyzed according to the method of Van Soest *et al*. (1991). Chemical composition was calculated and presented on a DM basis. NH_3-N content in ruminal fluid was analyzed using the Kjeldahl method (AOAC, 1990). Concentrations of individual volatile fatty acids (VFA) were determined by gas chromatography (Trace 13010, Thermo Scientific, USA). Concentrations of produced gases were measured using a gas analyzer (GeoTech GA5000, Queensway, UK). The metabolized energy (ME) of feed ingredients and diets was calculated according to Abate and Mayer (1997): ME=20.27-0.1431CF-0.111NFE-0.22Ash. The digestibility of DM, CP, and NDF was calculated using the formula 100×(nutrient content of the substrates before incubation–nutrient content of the substrate after incubation)/nutrient content of the substrates before incubation.

2.5. Statistical analysis

Data were statistically analyzed with the General Linear Model procedure using Minitab 22.2. The statistical model was $Y_{ij}=\mu+D_i+\varepsilon_{ij}$, *where* Y_{ij}*=the dependent variable,* μ*=the overall mean,* D_i*=the diet effect, and* ε_{ij}*=the random residual error*. Significant differences among diet means were statistically compared using Tukey test at P<0.05.

3. RESULTS AND DISCUSSION

3.1. Chemical composition of feed ingredients

The DM content of fresh Napier grass after cutting was relatively low, specifically 15.4%. According to Li *et al.* (2023), Napier grass achieved higher silage quality at 25% DM than 15% DM. Therefore, before ensiling, Napier grass was sun-dried for half of day to reduce the moisture content. After ensiling, the DM content of Napier grass reached 22.5% (Table 2). Jackfruit leaves used in the experiment had a DM content of 36.9% after ensiling, which is consistent with the findings of Thanh *et al.* (2022), who reported a DM content of fresh jackfruit leaf silage was 38.5%. The crude protein content of jackfruit leaf silage was 14.9%, which was 1.72 times higher than that of Napier grass silage. This result aligns relatively well with Keir *et al.* (1997), who reported a crude protein content of 15.1% in fresh jackfruit leaves. Napier grass silage had a NDF content of 65.1%, which was 1.89 times higher than that of jackfruit leaf silage. Regarding Ash content, ensiled jackfruit leaves had 4.2% more than ensiled Napier grass. Metabolized energy (ME) value of Napier grass silage and jackfruit leaf silage was similar to each other.

Table 2. Chemical composition of feed ingredients

Item	**Chemical composition** (%DM, except other noted)								
	DM	Ash	OM	CP	EE	CF	NDF	NFE	ME
Napier grass silage	22.5	13.5	86.5	8.68	1.29	43.4	65.1	33.1	7.41
Jackfruit leaf silage	36.9	17.7	82.3	14.9	1.47	24.3	34.4	41.7	8.28

3.2. Nutrient digestibility

Table 3. Nutrient digestibility (%) **72h incubation**

Item	JLS0	JLS25	JLS50	JLS75	JLS100	SEM	P
DMD	56.5^a	43.4^{ab}	41.7^{bc}	34.3b^c	28.4^c	3.05	<0.001
CPD	73.4	68.3	73.3	72.3	73.7	4.22	0.882
NDFD	69.8^a	65.3^a	58.7^b	52.2^c	45.3^d	1.30	<0.001

Notes: DMD: dry matter digestibility, CPD: crude protein digestibility, NDFD: neutral detergent fiber digestibility

The substitution of JLS for NGS dramatically influenced *in vitro* nutrient digestibility after 72h incubation (Table 3). The digestibility of DM was gradually decreased (P<0.001) as the inclusion proportion of JLS increased. When JLS totally replaced NGS (JLS100), DMD was only one-half, compared to JLS0. A similar pattern was recognized in the digestibility of NDF. Compared to JLS0, its digestibility was reduced (P<0.001) by 11.1, 17.6, 24.5% in JLS50, JLS75, JLS100, respectively. The effects of jackfruit leaf silage on *in vitro* nutrient digestibility in this study were in accordance with the previous study when it replaced fresh Napier grass using ruminal fluid from dairy goats (Thanh *et al.*, 2024). These observations might be related to the presence of tannins, a well-known anti-nutrient compound which creates a complex binding with dietary nutrients and inhibits the activities of ruminant microbiota, leading to a reduction in nutrient digestibility. A meta-analysis by Al Rharad *et al.* (2025) reported that dietary tannins in small ruminants reduced the digestibility of various nutrients, including DM and CP.

3.3. Ruminal fermentation

The utilization of JLS did not affect NH_3-N concentration at 72 hours after incubation (P>0.05, Table 3). The NH_3-N concentration at 72 hours ranged from 4.03 to 5.11 mg/dL. The ruminal pH in goats ranged from 6.80 to 7.30 (P<0.01), which is generally within the normal range for the activity of rumen microorganisms. The VFA concentration after 72h showed a significant difference (P<0.05) among treatments, with the lowest value in

JLS100 (75.0mM) and the highest in JLS0 (102mM). Replacing NGS with JLS at levels of 25-75% resulted in VFA concentrations comparable to the treatment containing only Napier grass silage.

Table 4. Ruminal fermentation pattern at 72h incubation

Item	JLS0	JLS25	JLS50	JLS75	JLS100	SEM	P
pH	6.80^{b}	7.09^{ab}	7.30^{a}	7.24^{a}	7.28^{a}	0.09	0.006
NH_3-N,mg/dl	4.03	4.45	5.11	4.83	4.41	0.53	0.649
VFA, mM	102^{a}	81.5^{ab}	83.3^{ab}	78.8^{ab}	75.0^{b}	5.59	0.041
C2, %	82.0^{ab}	81.0^{b}	79.5^{b}	84.7^{ab}	87.4^{a}	1.24	0.003
C3, %	13.2^{a}	14.2^{a}	14.2^{a}	11.4^{ab}	9.15^{b}	0.79	0.002
Iso-C4, %	0.68^{ab}	0.72^{ab}	1.06^{a}	0.63^{b}	0.61^{b}	0.09	0.015
C4, %	1.89^{ab}	1.99^{ab}	2.59^{a}	1.56^{b}	1.38^{b}	0.22	0.014
Iso-C5, %	0.69	0.72	0.98	0.60	0.55	0.11	0.127
C5, %	1.50^{ab}	1.43^{ab}	1.76^{a}	1.11^{ab}	0.96^{b}	0.18	0.045
C2/C3	6.24^{b}	5.76^{b}	5.72^{b}	7.68^{ab}	9.61^{a}	0.62	<0.001

Acetic acid proportion in JLS100 was higher (P<0.01) that those in JLS25 and JLS50 by 6.40 and 7.90%, respectively. In contrast, when the JLS inclusion at 25 and 50%, the propionic acid proportion in these treatments was greater (P<0.01) compared to that in the treatment containing 100% JLS. Consequently, the C2/C3 ratio was increased (P<0.001) when the dietary replacement of JLS for NGS was gradually increased. Compared to JLS0, JLS25, JLS50, this ratio in JLS100 was 1.54, 1.67, 1.68 times higher, respectively. According to Bettelli *et al.* (2023), supplementation of neither condensed tannin or hydrolysable tannin from 2-8% DM did not alter the acetic acid:propionic acid ratio after 48h incubation. Using condensed tannin extract from *Cistus ladanifer L.* at 2.5, 5.0, 7.5, 10% DM in Merino Branco rams' diet, Guerreiro *et al*. (2021) investigated that concentrations of acetic acid and propionic acid reduced after 24h incubation. Iso-butyric acid proportion was slightly increased in JLS50, compared to JLS0 and JLS25, then decreased (P<0.05) in JLS75 and JLS100. A similar tendency was also noted in iso-valeric acid proportion; however, there was no difference (P=0.127) among treatments. These results possibly indicate the negative impact of high-dose tannins on dietary protein degradation, despite the similarity in CP digestibility and NH_3-N concentration (Roca-Fernández *et al.*, 2020; Menci *et al.*, 2021).

3.4. Total gas and methane production

Total gas production linearly decreased (P<0.01) across treatments as the level of JLS increased. Compared to JLS0, total gas production in JLS100 was lower by up to 32.5%. The likely reason for the lower total gas production in treatments containing JLS is the reduction of VFA production. CH_4 concentration showed no differences (P>0.05) among treatments. However, due to the reduction in total gas production, CH_4 production in JLS75 and JLS100 after 72h incubation was 28.2 and 38.0% lower (P<0.01) compared to JLS0. Consistent with CH_4 concentrate, CO_2 concentration didn't show a difference (P>0.05) between treatments. Nevertheless, CO_2 production was linearly decreased when JLS inclusion was increased. At 100% of the replacement, CO_2 production was reduced by 36.7%, compared with that contains no JLS.

So far, tannins are listed as one of the comprehensive methane mitigators utilized in ruminant livestock (Beauchemin et al., 2022; Berca et al., 2023; Martins et al., 2024). The inclusion of Leucaena leucocephala at 20% DM in a diet containing alfalfa hay and concentrate (30:50%, DM) using ruminal fluid from steers showed a reduced CH_4 production by 25.5% after 96h incubation (Araiza Ponce et al., 2023). A meta-analysis reported that tannin interventions can reduce CH_4 production by more than 20% (Martins et al., 2024). However, the influences of tannin on methane mitigation depend on several factors such as chemical structure, type, inclusion dose, basal diet, and animal species. There are some major mechanisms responsible for the methane mitigation effect of tannin, including: 1) the indirect effect on reducing microbial degradation, 2) the direct inhibition of methanogenic archaea activity, and 3) the reduction in H_2 availability for methanogenesis (Tedeschi *et al.*, 2021). In this study, although microbial degradation was reduced, higher acetic acid but lower propionic acid proportion indicated that there was more H_2 available for the formation of CH_4 via the hydrogenotrophic pathway. However, the lower CH_4 production revealed in this study suggests that it might be due to the direct effect of tannins present in the JLS on the archaea population and therefore inhibiting the methanogenesis.

Table 5. Total gas production after 72h incubation

Item	JLS0	JLS25	JLS50	JLS75	JLS100	SEM	P
Total gas,ml	923[a]	870[a]	710[b]	682[b]	623[b]	32.2	0.001
CH_4, %	13.6	11.0	14.0	13.2	12.3	0.70	0.073
CO_2, %	54.2	49.1	54.0	53.6	50.5	1.95	0.299
CH_4, ml	125[a]	95.7[ab]	99.1[ab]	89.8[b]	77.5[b]	6.80	0.007
CO_2, ml	499[a]	428[ab]	384[bc]	365[bc]	316[c]	23.0	0.002

4. CONCLUSION

Methane production was mitigated by 38.0% when Napier grass silage was totally replaced by jackfruit leaf silage. However, high inclusion of jackfruit leaf silage can cause an adverse effect on nutrient digestibility and total VFA production.

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EFFECTS OF BACTERIOPHAGES AND PROBIOTICS ON BLOOD BIOCHEMICAL PROFILES OF NOI CHICKENS CHALLENGED WITH *SALMONELLA* TYPHIMURIUM OR *ESCHERICHIA COLI*

*Le Trung Kien[1], Huynh Tan Loc[1], Le Minh Thanh[1], Luu Huynh Anh[1], Nguyen Trung Truc[2], Tran Hoang Diep[3], Trinh Thi Hong Mo[4], Nguyen Thiet[1] and Nguyen Trong Ngu[1]**

Submitted: 26-May-2025 – Revised: 22-Jun-2025

Accepted: 25-Jun-2025

ABSTRACT

Alternative therapeutic approaches like probiotics and bacteriophages are receiving a lot of attention due to the rising incidence of antibiotic resistance in poultry production, especially in native breeds. This study evaluated the effects of orally administered bacteriophages and probiotics on the serum biochemical parameters of one-day-old Noi chicks challenged with *Salmonella* Typhimurium (*S*. Typhimurium) or *Escherichia coli* (*E. coli*). Three independent trials totaling 1,260 chicks were used. The findings showed that the metabolic and hepatic health of the birds treated with bacteriophages had significantly improved. In particular, uric acid concentrations dropped by 32.3% (319-216 mmol/l) and alanine aminotransferase (ALT) levels dropped by up to 57.9% (f9.85-4.15U/l) in the *S*. Typhimurium-challenged group treated with bacteriophage B1, B2, or their combination, compared to the positive control. A similar trend was also found in the *E. coli*-challenged group treated with bacteriophages. There was also a significant decrease (P<0.05) in uric acid and aspartate aminotransferase levels in both bacteriophage-treated groups challenged with *S*. Typhimurium or *E. coli*. Similarly, taking probiotic supplementation in the *S*. Typhimurium-challenged group resulted in positive changes, such as higher albumin and lower levels of ALT, uric acid, and serum cholesterol. These results demonstrate the potential of bacteriophages and probiotics as potent substitutes for antibiotics in the management of bacterial infections and enhancement of blood biochemical profiles in Noi chickens.

Keywords: *Bacteriophage, biochemical blood parameters, E. coli, probiotic, S. Typhimurium, Noi chicken.*

1. INTRODUCTION

Noi chicken is a kind of Vietnamese native chicken that is believed to be the most qualified source of chicken meat among Vietnamese people. The health condition of chickens is supported by the existence of a balanced physiological condition of the blood, considering the critical function of blood, such as adsorbing and conveying nutrients, hormones, and enzymes. Blood also plays an essential role in water turnover, metabolic regulation, and homeostatic control. To maintain the balance of blood physiological and biochemical conditions, feed additives seem to be considered as a control solution. Feed additives include substances, microorganisms, or preparations that are used to improve the poultry's performance and health (Gigante and Atterbury, 2019). Remarkably, in the case of prevention or control of chickens infected with pathogenic bacteria such as *Escherichia coli* (*E. coli*), and *Salmonella*, some feed additives, including bacteriophage cocktails and probiotics, are considered as prevention and treatment methods resulting in positive effects on growth performance, reducing the morbidity and mortality of infected chickens.

Antimicrobial resistance is a global issue and consequently drives the alternative approach for novel treatment in the multidrug-resistant control of pathogenic bacteria. The infections caused by pathogenic bacteria significantly burden healthcare systems and economic productivity and cause mortality. Antimicrobial resistance is a

[1] Can Tho University
[2] Nam Bo Agriculture College
[3] Tien Giang University
[4] Tay Do University
* Correspondence author: Prof. Dr. Nguyen Trong Ngu, College of Agriculture, Can Tho University; Phone: 0084 989828295; Email: ntngu@ctu.edu.vn.

global issue and consequently drives the alternative approach for novel treatment in the multidrug-resistant control of pathogenic bacteria. The infections caused by pathogenic bacteria significantly burden healthcare systems and economic productivity and cause mortality. In addition, the use of antibiotics in livestock and agriculture was considered in the One Health approach, especially the increase of multidrug-resistant pathogenic bacteria in developed and developing countries. However, integrating these approaches is challenging as antibiotic use in agriculture is generally widespread (Manyi-Loh *et al.*, 2018). Therefore, alternative antibiotic treatment measures need to be approached and tested to be put into practice.

Bacteriophages, also called phages, are viruses that play an essential role in regulating bacterial populations by triggering bacterial lysis. Recently, phages have been implemented as antibacterial agents in the food industry to increase food safety and in food animal production and crop protection to prevent bacterial animal and plant diseases, respectively. Several studies indicate that phages may be a useful tool for controlling bacterial pathogens (Wójcik *et al.*, 2020). In addition, a positive impact of probiotic supplementation in poultry has been well reported on production performance, feed intake, weight gain and feed conversion efficiency, immune responses, and the body's resistance to infectious diseases and helps to lower chick mortality (Alkhalf *et al.*, 2010). The advantages of probiotics are based on improving the microbial environment of a bird's intestinal tract by displacing harmful bacteria. Thus, the use of defined probiotic cultures in the poultry industry has recently become more common.

Serum biochemical parameters are valuable indicators for evaluating both pathological conditions and overall health status in individual animals. Accordingly, assessing the effects of therapeutic interventions on these parameters provides important insights into their potential efficacy and safety in poultry. This study was conducted to investigate the influence of bacteriophages and probiotics on the blood biochemical profile of native Vietnamese chickens experimentally challenged with bacterial infections. The results help clarify the therapeutic potential of these alternatives in managing bacterial diseases in poultry.

2. MATERIALS AND METHODS

2.1. Location and animal ethics

The animal experiment was conducted at Can Tho University's experimental farm, located on Campus IV in Phung Hiep district of Hau Giang province. The animal care and handling were conducted in accordance with the Animal Husbandry Law of Vietnam (32/2018/QH14).

2.2. Experimental design

All birds were vaccinated as instructed by the chicken breeding company. During the finishing stage, they were housed in confinement houses with a density of 10 birds/m². A commercial broiler diet was provided ad libitum to the chickens, of which the starter diet contained 16% crude protein and metabolite energy of 2,800 kcal/kg while the grower and finisher diet contained 14% crude protein and metabolite energy of 2,800 kcal/kg. Drinking water was available at all times.

A total of 1,260 1-day-old Noi broiler chicks were used in the 98-day experiment. Three independent experiments were conducted, including (i) bacteriophage treatment in *Salmonella* Typhimurium (*S.* Typhimurium) infection; (ii) bacteriophage treatment in *E. coli* infection; (iii) probiotic prevention and treatment in *S.* Typhimurium infection. Chicks were randomly assigned to seven experimental groups on each independent experiment, each with four replicates and 15 birds per replicate.

2.3. Selected bacteria and bacteriophages

In this study, the virulent *S.* Typhimurium strain ATCC®14028™ (American Type Culture Collection) and *E. coli* strain ATCC® 25922™, serotype O6, purchased from ATCC, were used for the experimental infection in Noi chickens. Moreover, two *Salmonella* bacteriophages, namely B1 and B2, and two *E. coli* bacteriophages, namely BP1 and BP2, were isolated and screened from chicken intestines and environmental sources at various poultry farms in the Mekong Delta of Vietnam. In addition, the antibiotic (enrofloxacin) and commercial probiotics (*Bacillus subtilis*, 10^9-10^{10} cfu/g; *Lactobacillus* spp., 10^6-10^9 cfu/g and *Saccharomyces cerevisiae*, 10^6-10^9 cfu/g) were supplied by a veterinary medicine company. Moreover, two probiotic strains, *Lactobacillus plantarum* and *Bacillus subtilis*, were isolated and characterized for probiotic properties from fermented food and intestines of chicken raised at different poultry farms in the Mekong Delta.

2.4. Serum biochemical profiling

Blood samples were drawn from the brachial vein using a 5ml disposable syringe tube. Plasma was collected by centrifugation at 3,000g for 15min, and blood parameters (AST: aspartate aminotransferase; ALT: alanine aminotransferase; T.PRO: total protein; ALB: albumin; GLO: globulin; UACID: uric acid; CRE: creatinine; CHOL: cholesterol; HDL: high-density lipoprotein; LDL: low-density lipoprotein; TRIGLI: triglycerides) were determined using the BA-88A Semi-auto chemistry analyzer (Mindray).

2.5. Statistical analysis

The data were subjected to analysis of variance using the General Linear Model procedure of the Minitab version 17 software (State College, PA, USA). Tukey's comparison test was used to determine the difference in means between treatments with $P \leq 0.05$.

3. RESULTS

3.1. Bacteriophage therapy for *Salmonella* infection

The effect of the bacteriophage-treated therapy in *S.* Typhimurium infection on the biochemical blood parameters of the experimental chicks is presented in table 1. The AST and ALT levels were significantly decreased ($P<0.05$) in the *S.* Typhimurium-challenged groups treated with bacteriophage B1, B2, or their combination, whereas the other criteria remained unaltered. Moreover, in our study, the negative treatment had the highest concentration of cholesterol, HDL, and LDL (4.01, 2.52, and 1.43 mmol/l, respectively). Regarding albumin level, most treatments approached significance showing similar patterns whereas the NC+B1 treatment showed a higher level (12.7 mmol/l) than that of the PC (10.4 mmol/l) ($P=0.003$).

There was no statistically significant difference in globulin levels which showed a consistent pattern across most treatments compared to the PC. The level of uric acid was found to be remarkably higher ($P<0.001$) in the PC (319 mmol/l) compared to the PC+B1 (206 mmol/l) and PC+B1B2 (216 mmol/l) treatments. In addition, all bacteriophage treatments showed no considerable effect on creatinine and cholesterol levels compared to the PC. Although the triglycerides in NC+B2 showed the highest concentration (0.35 mmol/l), no significant difference was observed in triglyceride levels among bacteriophage treatments compared to PC.

Table 1. Blood biochemical parameters of Noi chickens treated with bacteriophages in *S.* Typhimurium infection

Parameters**	Treatments*							SEM	P
	NC	PC	NC+B1	NC+B2	PC+B1	PC+B2	PC+B1B2		
AST (U/l)	243[a]	230[ab]	202[cd]	212[bc]	184[d]	212[bc]	207[bcd]	5.48	<0.001
ALT (U/l)	11.5[a]	9.85[a]	8.10[ab]	10.3[a]	7.75[abc]	4.15[c]	4.75[bc]	0.81	<0.001
T. PRO (mmol/l)	36.2	29.4	34.7	31.3	30.8	31.7	35.7	1.47	0.031
ALB (mmol/l)	12.3[ab]	10.4[bc]	12.7[a]	12.4[ab]	9.95[c]	12.6[ab]	12.4[ab]	0.47	0.003
GLO (mmol/l)	23.9[a]	19.1[b]	22.0[ab]	18.9[b]	20.8[ab]	19.2[b]	23.4[ab]	0.96	0.007
UACID (mmol/l)	119[cd]	319[a]	201[bc]	101[d]	206[b]	315[a]	216[b]	17.0	<0.001
CRE (mmol/l)	4.00	6.50	5.50	6.67	8.00	4.50	6.00	0.93	0.112
CHOL (mmol/l)	4.01[a]	2.38[b]	2.68[ab]	3.05[ab]	2.87[ab]	3.24[ab]	2.57[b]	0.29	0.028
HDL (mmol/l)	2.52[a]	1.70[bc]	1.73[bc]	2.14[abc]	1.65[c]	2.31[ab]	1.81[bc]	0.13	0.001
LDL (mmol/l)	1.43[a]	0.61[b]	0.63[b]	0.78[ab]	1.14[ab]	0.81[ab]	0.65[ab]	0.16	0.021
TRIGLI (mmol/l)	0.12[b]	0.15[b]	0.12[b]	0.35[a]	0.18[ab]	0.23[ab]	0.14[b]	0.04	0.010

NC: negative control, without S. Typhimurium, without bacteriophage; PC: positive control, S. Typhimurium challenged, without bacteriophages; NC+B1, NC+B2: negative control plus B1 or B2 bacteriophage, respectively; PC+B1, PC+B2: positive control plus B1 or B2 bacteriophage, respectively; PC+B1B2: positive control plus both B1 and B2 bacteriophages. AST: aspartate aminotransferase; ALT: alanine aminotransferase; T.PRO: Total protein; ALB: Albumin; GLO: Globulin; UACID: Uric acid; CRE: creatinine; CHOL: cholesterol; HDL: high-density lipoprotein; LDL: low-density lipoprotein; TRIGLI: Triglycerides. Within a row, values with different superscripts differ statistically at P≤0.05.

3.2. Bacteriophage therapy for in *E. coli* infection

Table 2. Blood biochemical parameters of Noi chickens treated with bacteriophages in *E. coli* infection

Parameters**	Treatments*							SE	P
	E.NC	E.PC	E.NC+B3	E.NC+B4	E.PC+B3	E.PC+B4	E.PC+B3B4		
AST (U/l)	209[ab]	187[b]	227[a]	204[ab]	201[ab]	235[a]	224[ab]	8.35	0.008
ALT (U/l)	6.32[a]	3.05[b]	6.15[a]	5.75[a]	4.28[ab]	4.75[ab]	5.58[a]	0.49	0.001
T. PRO (mmol/l)	34.6	30.6	34.5	33.3	26.9	31.8	32.5	2.09	0.186
ALB (mmol/l)	12.6	10.9	12.9	12.1	9.25	12.3	12.4	0.93	0.128
GLO (mmol/l)	22.3	19.9	21.8	21.5	17.9	19.2	20.4	1.47	0.385
UACID (mmol/l)	234[c]	304[ab]	236[c]	321[a]	135[d]	132[d]	255[bc]	12.2	<0.001
CRE (mmol/l)	10.8[a]	4.25[d]	8.58[ab]	9.25[ab]	5.25[cd]	2.50[d]	7.25[bc]	0.62	<0.001
CHOL (mmol/l)	3.97[a]	2.56[b]	2.57[b]	2.49[b]	2.42[b]	3.19[ab]	3.29[ab]	0.24	0.001
HDL (mmol/l)	2.52[a]	1.87[ab]	1.24[b]	1.81[ab]	1.76[ab]	2.34[a]	1.88[ab]	0.21	0.006
LDL (mmol/l)	0.94[b]	0.60[c]	0.79[bc]	0.60[c]	0.59[c]	0.61[c]	1.68[a]	0.05	<0.001
TRIGLI (mmol/l)	0.58[a]	0.12[d]	0.31[bc]	0.47[ab]	0.31[bc]	0.54[a]	0.18[cd]	0.04	<0.001

[*]E.NC: negative control, without E.coli, without bacteriophage; E.PC: positive control, E.coli challenged, without bacteriophages; E.NC+B3, E.NC+B4: negative control B3 or B4 bacteriophage, respectively; E.PC+B3, E.PC+B4: positive control plus B3 or B4 bacteriophage, respectively; E.PC+B3B4: positive control plus both B3 and B4 bacteriophages.

[**]AST: aspartate aminotransferase; ALT: alanine aminotransferase; T.PRO: total protein; ALB: albumin; GLO: globulin; UACID: uric acid; CRE: creatinine; CHOL: cholesterol; HDL: high-density lipoprotein; LDL: low-density lipoprotein; TRIGLI: triglycerides.

The biochemical profile of chicken under *E. coli* infection and bacteriophage treatment is presented in table 2. The parameters assessed include enzymes, metabolites, electrolytes, and plasma lipid levels. Significantly lower values were observed for AST and ALT in the E.PC compared with the E.PC+B4 and E.PC+B3B4, respectively. In contrast to the bacteriophage treatment under *Salmonella* infection, the total protein, albumin, and globulin were not influenced by different treatments under *E. coli* infection in this study whereas the level of uric acid was found to be remarkably higher in E.PC (304 mmol/l) compared to E.PC+B3 (135 mmol/l) and E.PC+B4 (132 mmol/l) treatments (P<0.001). Compared with the E.PC, the E.PC+B3B4 treatment had the highest values for triglyceride, total cholesterol, low-density lipoprotein, and high-density lipoprotein. In contrast, the E.PC+B3 treatment had the lowest values for total cholesterol, low-density lipoprotein.

3.3. Probiotic therapy for *Salmonella* infection

Using the probiotic in the treatment, the biochemical profile of chicks under the experimental infection of *S.* Typhimurium is shown in table 3. Among the treated groups, CProbi treatment reduced the AST concentration compared to other therapies; however, there was no significant difference. The ALT concentration in the LP+BS-treated group showed the highest concentration (15.2 U/l) compared to the other treated groups (P<0.001). Despite there being no significant difference in the levels of AST and ALT in CProbi compared to S.PC, a lower level could be seen in the CProbi-treated group. The uric acid and cholesterol levels in the CProbi-treated group displayed a lower concentration than the others. In comparison with the S.PC, the CProbi and LP+BS group had significantly lowest values for TRIGLI, LDL, and HDL, whereas the LP and CProbi group had the highest concentration for HDL and TRIGLI, respectively.

Table 3. Serum biochemical parameters of Noi chickens treated with probiotics in *Salmonella* infection

Parameters	Treatments							SEM	P
	S.NC	S.PC	LP	BS	LP+BS	CProbi	Enro		
AST (U/l)	207^{c}	240^{bc}	311^{a}	254^{b}	246^{bc}	223^{bc}	239^{bc}	8.76	<0.001
ALT (U/l)	10.8^{bc}	9.38^{cd}	13.9^{ab}	5.40^{d}	15.2^{a}	7.10^{cd}	8.46^{cd}	0.98	<0.001
T. PRO (mmol/l)	22.1^{d}	44.5^{a}	38.1^{b}	27.1^{cd}	26.9^{cd}	31.0^{c}	25.0^{d}	1.27	<0.001
ALB (mmol/l)	6.42^{d}	14.2^{a}	13.1^{ab}	10.6^{bc}	9.50^{c}	9.65^{c}	8.50^{cd}	0.68	<0.001
GLO (mmol/l)	15.8^{c}	29.2^{a}	24.2^{ab}	16.9^{c}	17.7^{bc}	17.9^{bc}	17.1^{c}	1.47	<0.001
UACID (mmol/l)	376^{a}	280^{bcd}	312^{b}	303^{b}	243^{cd}	223^{d}	299^{bc}	13.2	<0.001
CRE (mmol/l)	7.74^{de}	12.6^{a}	11.4^{ab}	8.80^{cd}	10.0^{bc}	2.40^{f}	6.20^{e}	0.45	<0.001
CHOL (mmol/l)	2.64^{bc}	3.04^{ab}	3.24^{a}	2.83^{abc}	2.63^{bc}	1.97^{d}	2.39^{cd}	0.13	<0.001
HDL (mmol/l)	1.47^{c}	1.56^{c}	2.14^{a}	2.01^{ab}	1.99^{ab}	1.52^{c}	1.70^{bc}	0.08	<0.001
LDL (mmol/l)	1.21^{ab}	1.44^{a}	0.88^{bc}	0.68^{cd}	0.61^{cd}	0.46^{d}	0.68^{cd}	0.08	<0.001
TRIGLI (mmol/l)	0.25^{cde}	0.23^{de}	0.34^{bc}	0.41^{b}	0.21^{e}	0.66^{a}	0.33^{bcd}	0.03	<0.001

Note: S.NC: Negative control, S.PC: Positive control, LP: Lactobacillus plantarum, BS: Bacillus subtilis, Enro: Enrofloxacin, CProbi: Commercial probiotics.

4. DISCUSSIONS

It is commonly acknowledged that serum biochemical parameters are accurate markers of the physiological and pathological conditions of poultry. Changes in a number of parameters, including triglycerides, cholesterol, uric acid, ALT, and AST, can indicate both metabolic balance and organ function, particularly hepatic and renal. Both bacteriophage and probiotic treatments in this study significantly altered these markers, indicating possible health advantages for broiler chickens infected with *S.* Typhimurium or *E. coli*.

The ALT and AST levels were significantly lower in the bacteriophage-treated groups, particularly in the chickens with *Salmonella*. Hepatocellular damage brought on by infectious or toxic agents is frequently linked to elevated serum transaminases, such as ALT and AST (Wójcik *et al.*, 2020). The decrease in these enzymes in our investigation raises the possibility that bacteriophage therapy may have lessened liver stress by preventing either direct bacterial infection-induced hepatic damage or the systemic inflammatory response. Wójcik *et al.* (2020) reported similar results, showing that anti-Salmonella bacteriophage cocktails decreased the levels of liver enzymes in poultry.

Our findings did not show a consistent dose-dependent response across a range of bacteriophage concentrations, in contrast to some earlier reports. Kim *et al.* (2013), who found no discernible dose effect of anti-Salmonella Enteritidis bacteriophage on cholesterol profiles, are consistent with this. However, in our investigation, bacteriophage treatment significantly reduced levels of triglycerides, LDL, and total cholesterol are key parameters closely associated with liver function and lipid metabolism. These results could be an indirect advantage of lowering the bacterial load or altering the gut-liver axis. Bacteriophage therapy also improved lipid, ALT, and uric acid profiles in chickens challenged with *E. coli*. Interestingly, bacteriophage-treated groups showed a significant decrease in uric acid, a crucial nitrogenous waste product in birds and a sign of protein catabolism. Increased protein turnover during infection and kidney stress are commonly linked to elevated uric acid (Piotrowska *et al.*, 2011). According to our research, bacteriophage therapy may help reduce the systemic stress brought on by bacterial infections.

In a similar trend, probiotic supplementation produced positive changes in serum biochemistry. In particular, treatments with *Bacillus subtilis* and *Lactobacillus plantarum* increased albumin levels while lowering ALT, LDL, and cholesterol levels. These findings are consistent with those of Abudabos *et al.* (2017), who found that broilers supplemented with *Bacillus subtilis* under *S.* Typhimurium challenge had stable serum enzyme levels and improved lipid profiles. Probiotics have been linked to a number of mechanisms that lower cholesterol, such as decreased intestinal absorption of cholesterol, deconjugation of bile salts, and inhibition of hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase (Fukushima and Nakano, 1995). Furthermore, prior research has indicated that probiotics may improve immunological responses and gut health, which may indirectly support systemic metabolic stability (Pourakbari *et al.*, 2016; Reuben *et al.*, 2021). Although this study did not examine gut microbiota composition or hematological indicators, the observed biochemical improvements still provide meaningful support for this broader physiological perspective.

It should be mentioned that growth performance metrics and hematological evaluations were not included in the current study. The safety, effectiveness, and practical ramifications of employing bacteriophages or probiotics as antibiotic substitutes in poultry production would be further clarified by these extra measurements. Long-term impacts and possible synergies between bacteriophages and probiotics should be further investigated.

5. CONCLUSIONS

This study shows that the challenge experiment in Noi chickens with *S.* Typhimurium or *E. coli*, along with supplementation of bacteriophages and probiotics effectively enhanced blood biochemical parameters, resulting in favorable changes. In particular, significant drops in lipid profiles, uric acid, and liver enzymes may contribute to improved metabolic and hepatic health. These results support the hypothesis that bacteriophages and probiotics could serve as effective alternatives to antibiotics in the treatment of bacterial infection in poultry. Their long-term impacts on gut health, immunity, and field performance should be assessed in future studies.

ACKNOWLEDGMENTS

This study was funded by the Technical Cooperation Project of the Japan International Cooperation Agency (JICA).

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DETERMINATION OF HEMATOLOGICAL PROFILES OF HOLSTEIN FRIESIAN YOUNG CALVES AND HEIFERS UNDER SMALLHOLDER FARMS CONDITION

Thuc Anh Bui[1], Kim Ly Phan[2], Thi My Huyen Dinh[2], Gia Han Ly[2], Duc Danh Nguyen[3] and Ngoc Tan Nguyen[2]*

Submitted: 05-Jul-2025 – Revised: 27-Jul-2025

Accepted: 31-Jul-2025

ABSTRACT

The objective of this study was to initially determine some hematological indices of Holstein Friesian (HF) young calves and heifers that are raised under smallholder farms condition. A total of 79 individual blood samples from 22 calves within 1-6 months of age (HF1-6), 12 young calves within 7-12 months of age (HF7-12) and 44 heifers within 13-18 months of age (HF13-18) were taken at smallholder dairy farms in Don Duong commune-Lam Dong province. The samples were then analyzed for blood hematological parameters using the Mindray BC-2800 Vet analyzer. The results showed that the difference between three basic blood physiological indices among three groups of HF calves and heifers was identified. The WBC (10^9/l) value of HF1-6 group was lower than those in the HF13-18 group (7.66 vs 11.31, $P<0.05$). The RBC($\times 10^{12}$/l) value was 9,28 in HF1-6, 7.72 in HF7-12 and 7.09 in HF13-18, the significant difference was found between groups of HF1-6 and HF13-18 ($P<0.05$). The PLT($\times 10^9$/l) value was higher ($P<0.05$) in HF1-6 group (505.00) as compared to in HF7-12 (238.60) or in HF13-18 group (339.10). No significant difference of three basic hematologic parameters in pregnant and non-pregnant heifers was found yet. In conclusion, the hematological profiles of HF young calves and heifers with different physiological stages are determined and the variation of some critical parameter among groups was found. Further research is needed to better understand the variability of hematological profiles for further applications in diagnostic, health and production performance assessment.

Keywords: *Calf, heifer, hematology, platelet cell, red blood cell, white blood cell.*

1. INTRODUCTION

As like human, hematologic parameters are of great importance for clinicians and researchers when assessing the health status of animals (Zhang *et al*., 2022; Gonzalez-Garduno *et al*., 2025). Up to date, the measurement of hematological parameters in domestic animals in general or special in cattle was rarely performed as compared to pet animals. There are several reasons for this, such as the costs associated with labors and laboratory testing, especially due to the low economic value of an individual animals and the limited availability of reference intervals for different age categories in animals required for correct interpretation of laboratory results (Perri *et al*., 2017; Jezek *et al*., 2018; Oliveira *et al*., 2019). Since the ranges of most hematological parameters are quite wide, they vary depending on many factors, including diet, age, gender, physiological appearance, different husbandry techniques, biosecurity, season, restraint, sample collection techniques, time of sample transportation or preparation, and the type of the analyzer used for hematological analysis (Jezek *et al*., 2018; Thorn *et al*., 2022; Mekroud *et al*., 2023, Fanta *et al*., 2024). In addition, assessment of hematological parameters can contribute to early identification of diseases or poor growth performances (Perri *et al*., 2017; Sanchez *et al*., 2019) and may be highly valuable in the treatment or prognosis of many diseases (Eze *et al*., 2010; Gonzalez-Garduno *et al*., 2025). Based on the assumption that many factors such as breed,

[1] Diamond Bar High School, 21400 E. Pathfinder Road, Diamond Bar, California 91765, USA
[2] Nong Lam University in Ho Chi Minh City, Vietnam
[3] Cowcare Vietnam Company, Vietnam
* Corresponding Author: Assoc. Prof. Dr. Ngoc Tan Nguyen, Advance Lecturer. Faculty of Biological Sciences – Nong lam University in HCMc; Phone: 0084 948993338; Email: nntan@hcmuaf.edu.vn.

age, nutrition, physiological or disease can impact on the variation of hematologic parameters.

In Vietnam, for the last decade, several studies have been directed to the field of veterinary hematology in goat or sheep (Bui *et al.*, 2015; Nguyen *et al.*, 2023), native pig (Dao *et al.*, 2023) and cattle (Nguyen *et al.*, 2023, Dau *et al.*, 2024), however, less information of works in calves and heifers of Holstein Friesian cattle under normal physiological conditions, especial in growing HF cattle under field condition. So, the aim of this study was to present the differences between basic hematological parameters of HF young calves and heifers under field condition.

2. MATERIALS AND METHODS

2.1. Animals and feeding

The growing calves and heifers that are raised at smalholfer farms of Don Duong commune, Lam Dong province were used in this study. A total of 79 individual samples were collected, including 23 samples from calves at 1-6 months of age (HF1-6), 12 samples from young calves at 7-12 months of age (HF7-12) and 44 samples from heifers at 13-18 months of age (HF13-18). The animals were fed twice daily based on the basic ration of farms and without any clinical signs of diseases at the time of sample collcetion.

2.2. Samples and hematological analysis

2.2.1. Blood sample collection

Blood samples were collected from coccygeal vein, in tube containing ethylenediamine tetra-acetic acid as anticoagulant after animal was carefully restrained. Samples were performed about 2hrs after morning feeding, collected samples stored at 4°C on ice, and transported to the laboratory. The analysis was run immediately after the blood samples delivery to Lab.

2.2.2. Hematological analysis

An automated hematology analyzer (Mindray BC-2800 Vet; Mindray Bio-Medical Electronics Co., Ltd., Shenzhen, China) was used to perform complete blood cell count: WBC: White Blood cell (10^9/l), Lym# (×10^9/l), Mon# (×10^9/l), Gran# (×10^9/l), Lym (%), Mon (%), Gran (%); RBC: Red Blood Cell (10^{12}/l), HGB: Hemoglobin (g/l), HCT: Hematocrit (%), MCV: Mean corpuscular volume (fl), MCH: Mean Corpuscular Hemoglobin (pG), MCHC: Mean Corpuscular Hemoglobin Concentration (g/l), RDW: Red Cell Distribution Width (%); PLT: Platelet Count (10^9/l), PDW: Platelet Distribution Width (%), MPV: Mean Platelet Volume (%), and PCT: Procalcitonin (%). Results were compared with calibrated Mindray references for cattle, according to Walczak *et al*. (2021).

2.3. Data analysis

The descriptive statistic was applied to obtain the mean and standard error of the mean. All obtained data were subjected to one way ANOVA followed by Tukey´s test (Minitab) for multiple comparation, percentage data were transformed to arsine before subjecting to analysis. Data are presented as Mean±SEM, and significance level was set at $P<0.05$.

3. RESULTS AND DISCUSSION

3.1. The hematological indices of HF young calves and heifers at different stage of age

3.1.1. The White Blood Cell count and other related parameters

An automatic measurement analyzer of individual samples was performed, the data of WBC and other related WBC parameters were analysed and presented in table 1 showed that the WBC (10^9/l) value of HF1-6 group was lower than the HF13-18 group (7.66 vs 11.31, $P<0.05$), and no significant difference between HF7-12 compared to other groups. The value of Lym#(×10^9/l) was low in group of HF1-6 and HF7-12 compared to group of HF13-18 (3.27 and 3.19 vs. 5,81, $P<0.05$). The value of Mon# (×10^9/l) was 0.09 in HF1-6, 1.38 in HF7-13 and 1.46 in HF13-18 group, the significant difference was found between HF1-6 and HF13-18 groups. The

Gran#(×10^9/l) value was ranged 3.49 to 4.43 among groups without significant difference. The value of Lym (%) was 43.66 in HF1-6, 37.88 in HF7-12 and 50.45 in HF13-18 group, the difference only found between HF7-12 and HF13-17 groups. The value of Mon (%) was higher in HF7-12 (14.88, P<0.05) as compared to 11.87 in HF1-7 or 12.96 in HF13-18 group. The value of Gran (%) was 44.47 in HF1-6, 47.24 in HF7-12 and 36.58 in HF13-18, the significance was found in HF7-17 and HF13-18 groups.

Table 1. The WBC count and other related WBC parameters in young HF females

Parameters	Stage of age (month)			Reference
	HF1-6 (n=23)	**HF7-12** (n=12)	**HF13-18** (n=44)	
WBC (×10^9/l)	7.66^b±0.70	9.00^{ab}±1.35	11.31^a±0.75	5.00-16.00
Lym# (×10^9/l)	3.27^b±0.36	3.19^b±0.43	5.81^a±0.52	1.50-9.00
Mon#(×10^9/l)	0.90^b±0.11	1.38^{ab}±0.28	1.46^a±0.11	0.30-1.60
Gran#(×10^9/l)	3.49±0.422	4.43±0.79	4.04±0.34	2.30-9.10
Lym (%)	43.66^{ab}±2.55	37.88^b±3.66	50.45^a±2.31	20.00-60.30
Mon (%)	11.87^b±0.62	14.88^a±0.90	12.96^b±0.28	4.00-12.10
Gran (%)	44.47^{ab}±2.55	47.24^a±3.43	36.58^b±2.17	30.00-65.00

Where: Within the same row (excluding Ref.) the value with different superscript letter differs (P<0.05).

In the current study, the results indicate that the variation in WBC count and related parameters was found at different stages of age in calves and heifers examined, the trend of the data was similar to previous studies in calves with or without sepsis (Unal and Uslu, 2024), in cattle infected or noninfected parasite disease (Nguyen *et al.*, 2023; Kim *et al.*, 2024; Samabu *et al.*, 2024), in normal physiological condition of beef cattle (Dau *et al.*, 2025).

3.1.2. The Red Blood Cell count and other related parameters

Table 2. The RBC count and other related RBC parameters in young HF females

Parameters	Stage of age (month)			Reference
	HF1-6 (n=23)	**HF7-12** (n=12)	**HF13-18** (n=44)	
RBC (×10^{12}/l)	9.28^a±0.31	7.72^{ab}±0.62	7.09^b±0.29	5.00-10.10
HGB (g/l)	108.09±3.88	102.92±6.57	105.70±3.80	90.00-139.00
HCT (%)	30.75±1.13	30.13±1.85	30.32±1.03	28.00-46.00
MCV (fl)	33.17^b±0.42	39.81^a±1.23	43.95^a±1.04	38.00-53.00
MCH (pg)	11.59^c±0.14	13.50^b±0.42	15.16^a±0.31	13.00-19.00
MCHC (g/l)	351.39^a±1.72	340.83^b±1.85	347.32^{ab}±1.53	300.00- 370.00
RDW (%)	19.86^a±0.64	18.53^{ab}±0.27	17.71^b±0.16	14.00-19.00

Based on the data from table 2, the RBC(×10^{12}/l) value was 9.28 in HF1-6, 7.72 in HF7-12 and 7.09 in HF13-18, the significant difference was found between groups of HF1-6 and HF13-18 (P<0.05). The HGB (g/l) was ranged from 102.92 to 108.09 among groups (P>0.05). Similarly, the value of HCT (%) was ranged from 30.13 to 30.75 and no difference among groups of animals was found (P>0.05). The MCV (fl) was lower in HF1-7 as compared to HF7-12 or HF13-18 (33.17 vs 39.81 or 43.95, P<0.05). The value of MCH (pg) was lowest in HF1-6, higher in HF7-12 and highest in HF13-18 (11.59, 13.50 and 15.16, respectively, P<0.05). The MCHC (g/l) value was 351.39 in HF1-6, 340.83 in HF7-12 and 347.32 in HF13-18, however, the significant difference was only found between HF1-6 and HF7-12 groups. Similar, the RDW(%) value was 19.86 in HF1-6, 18.53 in HF7-12 and 17.71 in HF13-18 group. Our results in present study are similar to the results that are obtained by Dau *et al.* (2025) in beef cattle. Besides, several studies reported the confidence intervals for hematological parameters indicative of anemia in calves as follows: HCT <20.1%, HGB <6.1 g/dl, and RBC count <5.9×10^6 cells/µl (Jones *et al.*, 2007; Katsogiannou *et al.*, 2018; Gonzalez-Garduno *et al.*, 2023, 2025).

3.1.3. The Platelet Cell count and other related parameters

According to the presented data in table 3, the PLT(×10^9/l) value was higher (P<0.05) in HF1-6 group (505.00) as compared to in HF7-12 (238.60) or in HF13-18 group (339.10). The MPV (fl) was lower (P<0.05) in HF1-6 group (5.16) compared to those in HF7-12 (5.65) or in HF13-18 group (6.02). The value of PDW (%) was 15.62 in HF1-6, 15.99 in HF7-12 and 16.28 in HF13-18 group, the difference was only found in HF1-7 and HF13-18 groups. The PCT (%) value was 0.25 in HF1-6, 0.13 in HF7-12 and 0.21 in HF13-18, the difference was found between HF1-6 and HF7-12 groups. The present results were lower than the results that reported by Ramabu *et al.*, (2024) in beef and dairy cattle, however, it is similar to the PLT value in HF dairy cattle (Yang *et al.*, 2024) or beef cattle (Motta *et al.*, 2023; Dau *et al.*, 2025).

Table 3. The PLT count and other related PLT parameters in young HF females

Parameters	**Stage of age** (month)			Reference
	HF1-6 (n=23)	**HF7-12** (n=12)	**HF13-18** (n=44)	
PLT (×10^9/l)	505.00^a±71.50	238.60^b±63.20	339.10^b±26.0	120.00-820.00
MPV (fl)	5.16^b±0.09	5.65^a±0.14	6.02^a±0.08	3.80-7.00
PDW (%)	15.62^b±0.05	15.99^{ab}±0.08	16.28^a±0.10	-
PCT (%)	0.25^a±0.03	0.13^b±0.04	0.21^{ab}±0.02	-

3.2. The hematological parameters of pregnant and non pregnant HF heifers

Classified data of hematological parameters between pregnant and non pregenant herfers from group of HF13-18, the value of WBC, RBC and PLT count was calculated and presented in table 4 indicated that no significant variation in blood parameters according to pregnant or non-pregnant heifers was found. From this result can be implied that it could be due to the early stage of gestation of heifers examined, it can be considered for further studies.

Table 4. The effect of pregnacy status of heifers on main values of blood cell count

Parameters	**Non pregnant** (n=24)	**Pregnant** (n=20)
WBC (×10^9/l)	10.44±1.22	12.36±0.75
RBC (×10^{12}/l)	7.34±0.51	6.79±0.23
PLT (×10^9/l)	326.8±38.8	353.90±34.20

4. CONCLUSION

Under smalholder farms condition, for the first time, hematological parameters of HF young calves and heifers were determinated and variations of some critical parameters at different stages of age were found. More studies are required to better understand the variability of HF cattle hematological profiles for further applications in diagnostic, health as well as productivity performance assessment.

ACKNOWLEDGEMENT

The authors offer their sincere appreciation to all owners of dairy farms at Don Duong commune of Lam Dong province for donating the blood samples for this research.

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SOME BIOLOGICAL CHARACTERISTICS OF *APIS LABORIOSA* IN NORTHERN VIETNAM

Chinh H. Phung[1], Lam D. Nguyen[2] and Hanh D. Pham[3]*

Submitted: 17-Jan-2025 – Revised: 17-Feb-2025

Accepted: 03-Mar-2025

ABSTRACT

In Vietnam, the giant bee *Apis laboriosa* is distributed in some mountainous provinces in the North and North Central region, where the altitude is >900m above sea level. Because it nests in difficult terrain, *A. laboriosa* is rarely studied. This study aims to determine some biological characteristics, nesting characteristics of this bee species and sustainable exploitation methods to contribute to their conservation. In addition to nesting on cliffs, *A. laboriosa* also nest on tree branches like *Apis dorsata*, even nesting more commonly on trees than on cliffs in some places. *A. laboriosa* bees build a comb with sizes ranging from 0.8x0.6m to 1.6x1.5m. The width of worker cell of *Apis laboriosa* is 5.9mm, the largest among honey bees. The percentage of capped cells infested by *Tropilaelaps* sp. mites is 0.66-1.33%. Unlike *A. dorsata* and other honey bee species that usually cap cells containing ripe honey, most *Apis laboriosa* colonies do not cap the honey cells. In some regions these two species of giant bees co-occur. Harvesting honey by spreading a tarpaulin to collect honey under the honeycomb and then using a pole to poke the honey part reduces the rate of absconding after harvest.

Keywords: *Giant honey bee, Apis laboriosa, biological characteristics, nesting characteristics, uncapped honey cells.*

1. INTRODUCTION

The giant honeybee *Apis laboriosa* Smith 1871 is one of five native honey bee species in Vietnam along with *Apis cerana* Fabricius 1793, *Apis dorsata* Fabricius 1793, *Apis florea* Fabricius 1787 and *Apis andreniformis* Smith 1858 (Pham, 2014). *Apis laboriosa* has the largest body size among honeybee species (Joshi *et al*., 2004). They typically nest on cliffs at an elevation of 1,000-3,000m above sea level, along the Himalayas and neighboring mountain ranges of Asia in India, Nepal, Bhutan, Myanmar, Thailand, China, northern Vietnam, Laos… (Kitnya *et al*., 2020; Voraphab *et al*., in press). In Vietnam, *Apis laboriosa* colonies were first discovered in 1996 in mountainous areas of Hoa Binh and Son La provinces (Trung *et al*., 1996). Later they were found in Lao Cai, Lai Chau, Yen Bai, and Dien Bien provinces (Pham, 2014), and were recently discovered in the Northeastern provinces of Tuyen Quang, Ha Giang, Cao Bang, Nghe An (Otis *et al*., 2024).

In addition to providing humans with honey and wax, *Apis laboriosa* plays an important role in pollinating plant species, contributing to maintaining the biodiversity of ecosystems. Due to its distribution in rugged terrain in high mountains, difficulty of access and small number of colonies, there is not much research on this bee species. Therefore, we have focused our research on some of the nesting characteristics of *A. laboriosa* that distinguish it from *A. dorsata* and sustainable harvesting methods to conserve this bee species.

2. MATERIALS AND METHODS

2.1. Materials

Apis laboriosa colonies, during 2018-2023, in mountainous provinces of Northern Vietnam.

2.2. Methods

Determine some biological characteristics of *Apis laboriosa*, the "cliff bee"; the worker cell size; and parasitism rates by *Tropilaelaps* mites.

[1] Mountain Bee Development JSC
[2] Center for Bee Research and Animal Technology Transfer
[3] National Institute of Animal Science
* Corresponding Authors: Dr. Chinh H. Phung, Mountain Bee Development JSC, 54/211 Alley, Khuong Trung St., Thanh Xuan District, Hanoi, Vietnam; Phone: 0084 912351178; Email: phunghchinh7@gmail.com.

Nest characteristics: the size of *Apis laboriosa* comb and differences compared to *Apis dorsata*.

Method for determining nest location and nest size: observe directly, using a phone with GPS to determine the coordinates and altitude where the *Apis laboriosa* nests.

Document the cliff bee nesting season and nesting behavior, through direct observation and interviews with honey hunters.

Determine the average size of brood cells, using Mitutoyo digital calipers (Model No. CD-6" ASX) to measure 6 adjacent cells in all 3 orientations, then calculate the average value.

Determine the rate of parasitism by *Tropilaelaps* sp. mites in capped brood cells, using magnifying glass and forceps to examine about 600 capped brood cells per 2 combs and about 300 cells in each comb.

We watched videos and images of *A. laboriosa* and *A. dorsata* bees and bee colonies shared by honey hunters on YouTube and Facebook. When we see worker bees with black and white stripes, we know they are *Apis laboriosa* bees, then contacted the author and visited the sites in person to confirm. Because *A. laboriosa* worker bees are black, honey hunters call them "black giant bees." They distinguish them from "yellow giant bees," *Apis dorsata*, in which the anterior gastral tergites of the worker bees are orange-yellow in color.

3. RESULTS AND DISCUSSION

3.1. *Apis laboriosa* nests on both cliffs and tree branches

In previous studies, *Apis laboriosa* colonies nest on overhanging cliffs in aggregations of a few nests to dozens or even hundreds of nests, at elevations between 1000–3000m. Joshi *et al.* (2004),) and Kitnya *et al.* (2020). For comparisoin, in In Vietnam, *A. laboriosa* was also found building a few nests on cliffs in the Northwest provinces at altitudes above 1000m Trung *et al*. (1996), Pham (2014). Recently we discovered *A. laboriosa* nesting at altitudes below 1000m such as in Na Hang district, Tuyen Quang province at 970m (10 nests), there are 2 cliffs with bees, one with 30 colonies and the other with 22 colonies at Quang Thanh commune, Nguyen Binh district, Cao Bang province at 890m and Especially in Liem Phu commune, Van Ban district, Lao Cai province, there is a colony of bees building nests at the lowest altitude of 470m. Surprisingly, in Van Ban, Bat Xat (Lao Cai), Tram Tau, Mu Cang Chai (Yen Bai), Quan Ba (Ha Giang) *A. laboriosa* colonies constructed nests on cliffs and on tree branches like *A. dorsata*.

However, unlike *A. dorsata*, often nest in large aggregations from 20-80 colonies on one largest tree in (Robinson, 2012) and 80 colonies on largest tree in Muong Ang district, Dien Bien province (Vietnam), *A. laboriosa* has only been observed to nest singly on trees. They often choose sturdy tree branches with slopes from 0° to about 45° to build their nests. The number of *A. laboriosa* colonies on cliffs and trees is shown in table 1.

Table 1. ***Apis laboriosa*** **colonies in Northern**

Location	On cliffs	In trees	Note
Van Ban, Lao Cai	14	25	2020-22
Mu Cang Chai, Yen Bai	6	9	2021
Tram Tau, Yen Bai	2	6	2022
Na Hang, Tuyen Quang	10	-	2023
Nguyen Binh, Cao Bang	52	-	2021
Tuan Giao, Dien Bien	>30	-	2022

Thus, in Vietnam, the phenomenon of *A. laboriosa* building nests in trees is quite common. In Van Ban - Lao Cai and Tram Tau, Mu Cang Chai - Yen Bai, we observed more nests in trees than on cliffs. Nesting on trees has not been observed previously. All other authors have indicated that this bee species builds nests under cliffs without exceptions (Trung *et al*., 1996; Woyke *et al*., 2001; Joshi *et al*., 2004). *A. laboriosa* nest construction on tree branches may be due to a lack of large cliffs in those regions.

According to honey hunters in Van Ban, in warm years, bees make more nests in trees. Alternatively, in colder years, bees build more nests on cliffs because trees become snow-covered, forcing the bees to nest the cliffs to avoid the cold.

3.2. Nesting characteristics of *Apis laboriosa*

Like *A. dosata*, *A. laboriosa* builds a large single comb perpendicular to the ground. In Nguyen Binh, Cao Bang, bees build up to 30 nests on a cliff, the nests are 0.5-5m apart and not oriented in a specific compass direction. The direction of the honeycomb depends on the cliff surface where it attaches and its shape changes according to the location of the nest. If built on a cliff, the height and width of the honeycomb are approximately equal. Comb size varies from about 1.6m wide x 1.5m long to 0.8x0.6m (Pham *et al*., 2020). On steep cliffs, comb width is less than the comb length. Nests on trees are often wider than long. Such a shape allows the comb to be firmly attached to the tree, perhaps serving to prevent strong winds from causing nests to fall.

A. laboriosa and *A. dorsata* combs have essentially the same structure. The upper part of the comb contains honey, followed by the pollen-containing part, then the part that contains brood.

The width of worker cell: Like other honeybee species, the comb of *Apis laboriosa* bees is built with beeswax the worker bees shape into six-sided cells; the comb midrib is shared by cells on both sides of the comb. The width of worker cell of this species is measured to be 5.9±0.02mm (Pham *et al*., 2020). Thus, it is significantly larger than that of *Apis dorsata* 5.65±0,003 (Phung *et al*., 1996) so *A. laboriosa* bees are the largest honey bees (Trung *et al*., 1996; Cao *et al*., 2012).

Table 2. The average size of worker cells of honey

Species	Worker cell (mm, Mean±SD)	Note
Apis florea	3.0	Pham *et al*. (2020)
Apis cerana	4.6	Phung and Vu (1999)
Apis mellifera	5.2	Pham *et al*. (2020)
Apis dorsata	5.65±0.003	Phung *et al*. (1996)
Apis laboriosa	5.9±0.02	Pham *et al*. (2020)

Parasitism: Careful inspection of ~500 adult bees collected in Na Hang detected no bee lice (*Megabraula* sp.) or mites. The prevalence of *Tropilaelaps* sp. in capped brood cells of *Apis laboriosa* was low 1.33% (n=600 capped brood cells) (Pham *et al*., 2020) and in Van Ban, 0.66% (n=300 cells).

3.3. *Apis laboriosa* do not cap honey cells

Honey bees of most species (*A. mellifera*, *A. cerana, A. florea, A. dorsat*a) seal cells containing ripened honey. However, in the *A. laboriosa* combs, we infrequently observed sealed honey cells. Observing the honey harvest of honey hunters on videos of two giant bee species, we have the following results. *A. laboriosa* bees rarely seal honey cells (38/39 colonies) while 303/409 *A. dorsata* colonies have sealed honey lids. Among colonies that have not yet seal their honey cells, there are some colonies that have only been in the nest for less than 10 days (no brood yet) and have had their honey cut off by honey hunters. Observing videos and photos of *A. laboriosa* honey hunting in Nepal and China, we also saw that the honeycomb were also unsealed.

Table 3. Number of colonies of *A. dorsata* and *A. laboriosa* with capped and uncapped of honey cells

Honey bee species		Number of combs with capped honey-cells	Number of combs with uncapped honey-cells	Total
Apis dorsata	Northern provinces	105	48	153
	Central provinces	83	31	114
	Southern provinces	125	17	142
	Total	303	96	409
Apis laboriosa		1*	38	39

** This colony has few (~ 2%) capped honey-cells*

Figure 1. Typical honeycomb of *A. dorsata* (left) with honey cells capped and uncapped and *A. laboriosa* honeycomb (right) with honey cells are uncapped.

3.4. Overlapping distributions between *Apis laboriosa* and *Apis dorsata*

In Vietnam, *A. dorsata* is very widely distributed, mostly in the northern and central mountainous areas as well as the flooded forests in the Southern Delta where rafter beekeeping is practiced (Phung and Vu, 1999). Sometimes they can be seen building nests on water towers in Ho Chi Minh City and Bien Hoa City, Dong Nai Province. They usually nest at low elevations, usually below 900 m.a.s.l, ranging from 0.8-1.2m in U Minh (Ca Mau) to 2000m in Hoang Lien Son National Park, Lao Cai. *A. laboriosa* bees have a narrower distribution, mainly in the northwestern mountainous provinces (Kitnya *et al.*, 2020), and recently documented in the northern and northeastern mountainous regions such as Ha Giang, Tuyen Quang, Cao Bang and Nghe An and at higher altitudes about 1,000m or more (Otis *et al*., 2024).

In Lao Cai, Yen Bai, Son La, Tuyen Quang and Cao Bang provinces, these two bee species are sometimes sympatric. *A. dorsata* usually nest in lower areas and are more numerous, while *A. laboriosa* nests in higher areas, but they often overlap with each other at altitudes of 800-1,200m. In Van Ban there are cases of nests only 300-400m apart, especially in Nam Lanh, Nghia Lo town, Yen Bai province. Mr. Thao A. Vang, a honey hunter, observed two nests only about 40m apart. While he was using smoke to harvest honey from the *A. dorsata* nest, he was stung by bees from the *A. laboriosa* colony. We have seen bees of these two species foraging for water from a moist stream bed in Van Ban without fighting. To find the nests of two giant bee species, experienced Vietnamese honey hunters often pour diluted salt water on the ground. Both species collect the salty water, then return to their nest. Honey hunters use binoculars to observe the direction of bees' flight to find their nests. Our observation of sympatric of these two species is similar to that of Kitnya *et al.* (2022) who discovered co-occurrence of *A. dorsata* and *A. laboriosa* at five sites in four districts of Arunachal Pradesh, India.

3.5. Harvesting honey sustainably

In *A. dorsata* that have nested on trees or rafters (made by humans), honey hunters only cut off the honey portion, leaving behind the part of the comb with brood to which the bees will return. With no loss in brood rearing, the population of the colony is maintained and the bees continue to collect and store honey. This can result in 2-3 honey harvests in one season. This constitutes sustainable honey harvesting and results in both high honey yield and quality. Because *A. laboriosa* often nests on cliffs at high elevations, when exploiting honey, honey hunters often use rope ladders to climb down from above https://www.youtube.com/watch?v=0YY6l-rqThA. They usually smoke the bees to get them to move off the comb, following which they cut off the part of the brood comb, then cut the part of the honeycomb. After cutting honey, the bees cluster for a few hours and then fly away. With this method of exploitation, the colony is greatly affected by the loss of brood and their investment in the comb and honey. Moreover, the honey hunter can only collect one honey harvest per season.

In the case of *A. laboriosa* nesting on trees, even if honey hunters apply the sustainable exploitation method of *A. dorsata*, which is to use smoke and then cut only the honeycomb, the bees will gather nearby for 1-2 days and then fly away. Even when honey hunters do not use smoke, only wear protective clothing, use their hands to move bees off the comb, and then cut off only the honeycomb and leave the brood untouched, the bees still abscond. This shows that *A. laboriosa* is somewhat less manageable than *A. dorsata.*

In some localities in Vietnam such as Na Hang (Tuyen Quang province) and Moc Chau (Son La province), experienced honey hunters have a way to harvest sustainably by stretching a plastic tarp ~10m^2 (3x3.5m) under the nests and then using poles to poke the nest to let the honey flow down to the plastic tarp. A funnel positioned directly below the honey comb guides the honey into a container. After completing the preparations, two honey hunters climb up a bamboo scaffold they have constructed and take turns poking the honeycomb with a stick. Using bamboo or wooden poles ~5-6m long, they poke uppermost honey portion of th comb. If a large nest has a lot of honey, they may poke 10-12 in it. A small nest need only be poked 2-3 times. If a nest is too small, they leave it. They recognize when a nest has a lot of honey by observing if the top part of the comb (close to the cliff) is bulged outward. After poking once and seeing a lot of honey come out, they poke again. If they pull out the pole and see no honey flowing, they stop.

Experienced honey hunters believe that if they use smoke, the bee colonies will not return the next year. The technique of poking the honey comb with a stick is to minimize the honeycomb falling to the ground. With this method of harvesting, the bee colony is conserved because the brood comb that remains after harvesting honey and the bees do not fly away. However, because the nests are located on high cliffs and the honeycombs are long, a few combs may fall during harvesting. When harvesting from a single nest, the honey hunter does not stretch out a large canvas. Instead he makes a basket about 1m in diameter to catch the honey or honeycomb and then uses a small stick to poke the honey to flow down.

In Quang Thanh commune, Nguyen Binh district, Cao Bang province, people of the Dao Tien ethnic group have protected two cliffs with over 50 colonies. They allow no one to collect honey or disturb the bees. When the bees seasonally migrate to other places, usually in July, they collect the empty combs to extract beeswax from them. In Tinh Pong commune, Tuan Giao district, Dien Bien province, Hmong people are conscious of preserving bees by not allowing anyone to take honey and wax from cliffs with over 30 colonies of *Apis laboriosa*.

3.6. Migratory behavior of *Apis laboriosa*

Like *A. dorsata, A. laboriosa* has seasonal migrations. They usually return around February, build nests, raise brood and store honey for about 5-7 months and then leave the empty comb to migrate to other places. Two cliffs with over 50 colonies of *A. laboriosa* in Nguyen Binh, Cao Bang, that are not disturbed by local people clearly show this. In July or August every year, they abscond and fly away. Residents then harvest the empty combs to extract wax. The same is true of bee colonies nesting on trees in Van Ban and Tram Tau. In July, when bee hunters harvest honey, the combs no longer have any brood. We do not know about the migration distance of *A. laboriosa* in Cao Bang, but according to honey hunters in Van Ban, *A. laboriosa* only migrate between 10-20km. Such close-distance migration is similar to the research results through polymorphism analysis of the COII gene sequence by Trung (2013), that suggested that *A. laboriosa* migrated from Tan Uyen district to Tam Duong district, Lai Chau province, about 30-40km away. *A. laboriosa* in Van Ban usually does not build comb in the winter, this is contrary to the observation of Woyke *et al.* (2001) that some *A. laboriosa* colonies on their nests and raise brood in winter at altitudes below 1500m in Nepal.

4. CONCLUSION

A. laboriosa build a single comb with sizes ranging from 0.8x0.6m to 1.6x1.5m. The width of worker cell is 5.9±0.02mm, the largest among honey bee species. The prevalence of *Tropilaelaps* sp. mites in capped brood cells was very low, from 0.66 to 1.33%. Unlike *A. dorsata* and other honey bee species that usually seal cells containing ripe honey, most honey cells in *A. laboriosa* colonies remain uncapped. In some regions, these two giant honeybee species are sympatric and worker bees have been observed foraging together at the same locations.

In addition to nesting on cliffs, *A. laboriosa* also nest on tree branches like *A. dorsata*, sometimes even more commonly on trees than on cliffs. *A. laboriosa* colonies migrate seasonally and our observations suggest that they disperse over relatively short distances of about 10-20km.

Honey is harvested by spreading a tarpaulin to collect honey under the nests and then using a pole to poke the honeycomb and allow the honey to drip out. With this harvesting technique *A. laboriosa* colonies not to fly away after harvest.

ACKNOWLEDGEMENTS

This research was partially funded by the Ministry of Agriculture and Rural Development's Agricultural genetic resource conservation program. The authors wish to thank the honey hunters: Mr. Nguyen Thanh Dinh, Mr. Tan Chan Dung, Mr. Thao A Vang and others for providing information, pictures, videos of Apis laboriosa to the authors. The authors would also like to thank Prof. Dr. Gard Otis for reading and editing the manuscript.

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EFFECT OF CONCENTRATE SUPPLEMENTATION ON HONEY YIELD AND QUALITY IN EUROPEAN HONEY BEES (*Apis mellifera*)

Lam Phuoc Thanh[1] and Tran Thi Thuy Hang[1]*

Submitted: 30/6/2025 - Revised: 27/7/2025

Accepted: 31/7/2025

ABSTRACT

The study aimed to determine the optimal level of concentrate supplementation for European honey bees (*Apis mellifera*) to achieve the best honey yield and quality. The experiment was conducted as a completely randomized design with 5 treatments and 3 replicates. Each replicate consisted of one Italian bee colony with 3 frames per colony. The feeding experiment lasted 8 weeks, with honey harvested and quality evaluated three times. Weekly, bees were fed a commercial concentrate at levels of 35, 50, 65, 80, and 95g per frame, corresponding to treatments T35, T50, T65, T80, and T95. The experimental results showed that different levels of concentrate supplementation significantly affected honey yield (P<0.05). Although the feed consumption of bees in T35 treatment was the lowest (P<0.001), the honey yield for this treatment was the highest (4.65 l/colony), compared to 2.20 l/colony in T80 treatment (P<0.05). There were no significant differences in honey quality parameters (Brix, moisture content, pH, and antioxidant activity) and honey color (L*, a*, b* indices) among the treatments. The profit from beekeeping in T35 treatment was the highest (657 thousand VND/colony), while that for T80 group decreased (P<0.05) to 253,000 đ/colony. In conclusion, supplementing concentrate at 35g per frame is optimal for European honey bees in terms of honey yield, quality, and profitability.

Keywords: *Concentrate, European honey bee, honey quality, honey yield.*

1. INTRODUCTION

The beekeeping industry requires low investment, minimal time for care, and low labor costs, yet it brings good economic returns for beekeepers (Nhung, 2010). For this reason, the honey bee population has been increasing in Vietnam in recent years. According to Decision No. 898 issued by the Ministry of Agriculture and Rural Development (2024), Vietnam aims to actively maintain between 1.3 and 1.5 million migratory bee colonies by 2030, based on the availability of floral and nectar sources. The target average honey yield is over 42 kg/colony/year for exotic bee species and over 18 kg/colony/year for native bee species. National honey production is expected to stabilize at 55,000-60,000 tons annually, with approximately 80% designated for export and the remaining 20% for domestic consumption.

Honey bees hold significant socio-economic importance as they play a crucial role in crop pollination and produce various products that are valuable to humans and multiple industries (Nhung, 2010). Bee products with high economic value include honey, beeswax, propolis, pollen, royal jelly, and venom (Kieliszek *et al.*, 2018; Aylanc *et al.*, 2021). Among these, honey is a natural product with numerous nutritional benefits and uses. Its diversity depends on several factors, including floral sources, environmental conditions, and beekeeping techniques (Hoan *et al.*, 2008).

The breed of bee is the primary factor affecting honey yield, but beekeeping techniques and nutrition also play a significant role in productivity. Previous study showed that supplementing feed to bees is essential and contributes to increased honey production. It also helps bee colonies survive during periods of food scarcity (Jaffé *et al.*, 2015). However, unbalanced

[1] Can Tho University

* Corresponding author: Assoc. Prof. Dr. Lam Phuoc Thanh, Can Tho University, Campus II, 3/2 street, Ninh Kieu ward, Can Tho city, Viet Nam, Phone: 0084 975763555, Email: phuocthanh@ctu.edu.vn.

supplement can negatively affect colony health and fail to deliver economic benefits. Up to date, studies on feed supplementation for honey bees in Vietnam remain very limited. Therefore, this study aims to determine the optimal level of concentrate inclusion for European honey bees (*Apis mellifera*) during the dry season to improve productivity, honey quality, and income over feed cost.

2. MATERIALS AND METHODS

2.1. Time and location

The experiment was conducted from February to April 2025 at the Faculty of Animal Science, College of Agriculture, Can Tho University (Campus II).

2.2. Animals

The experiment was conducted on European honey bees (*Apis mellifera*), using 15 colonies, each consisting of 3 frames. The bee colonies were kept in wooden hives (47×43×25cm-length×width×height) placed about 30cm above the ground, with a spacing of approximately 50 cm between hives. During the experiment, the bees were treated for parasitic mites using purple-yellow herbal mite medication.

2.3. Experimental design and diets

The feeding experiment was conducted over a period of two months using a completely randomized design with five treatments and three replicates, with each replicate consisting of one bee colony (hive). Treatments were developed by increasing levels of concentrate supplementation at 35, 50, 65, 80, and 95 g/frame/week, corresponding to treatments T35, T50, T65, T80, and T95. Concentrate was used as a commercial feed for European honey bees, which composed of 3.84% pollen, 38.1% roasted and ground soybeans, 57.2% cane sugar, 0.48% vitamin C, and 0.38% premix. Before feeding, feed was mixed with fresh water at a ratio of 5:1 and applied on the top bars of frames in the form of drawn strips.

2.4. Sample collection and calculation

Feed intake was calculated based on the average amount of feed consumed by each colony per week. Honey was harvested every three weeks in the 1st and 2nd harvests, and 2w in the 3rd harvest. After harvesting, the honey was weighed to determine the yield of each colony. Honey samples collected from each colony were stored at room temperature, then analyzed for Brix level, moisture content, pH, antioxidant capacity, and color.

2.5. Sample analyses

Brix and moisture content: The Brix and moisture content of honey were determined using a refractometer (Model: RBX4582/RH5890, Trans Instruments, Singapore).

pH value: 1ml of honey sample was dissolved in 7.5ml of distilled water, then the pH was measured using a digital pH meter (Model: HI5522, Hanna, USA) following the method of International Honey Commission (2009).

Antioxidant activity: The measurement was conducted using the DPPH method. 1.5 mL of honey sample was transferred into a test tube, followed by the addition of 1.5ml of 0.2mM DPPH solution in 95% ethanol. The mixture was shaken thoroughly and incubated in the dark for 30 minutes. After that, absorbance was measured using a spectrophotometer at a wavelength of 517nm, following the method described by Wu *et al.* (2003). Antioxidant activity was calculated using the formula: $DPPH(\%)=100\times(A_{blank}-A_{sample})/A_{blank}$, *where:* A_{blank} *is the absorbance of the control (0.2mM DPPH solution) and* A_{sample} *is the absorbance of the honey sample with DPPH.*

Honey color: According to the Vietnamese Standard TCVN 5262:1990 on sensory testing methods for bee products, issued by the Ministry of Science and Technology (1990), honey can range in color from nearly colorless to dark brown. This standard provides a visual assessment guideline for honey color as regulated by Vietnamese law. However, there are currently no specific regulations regarding the evaluation of honey color using a colorimeter. In this study, honey color was determined and evaluated based on the CIE L*a*b* color system. In this case L* defines the lightness of the sample, the higher value, the lighter the sample is. The a* coordinate determines the greenness/redness of the sample. In this case greenish color is in the negative range, while reddish color is in the positive range. The b* axis quantifies the blue or yellow color, where blueness is in the negative range and yellowness in the positive range (Bodor, 2021).

2.6. Statistical analysis

The experimental data were statistically analyzed with the GLM in Minitab 22.2 software (Minitab Inc., PA, USA). Statistical differences were considered significant when P<0.05, and experimental trends were declared when 0.05≤P<0.10. Differences between treatment means were tested using Tukey's multiple comparison method following a significant F-test.

3. RESULTS AND RESULTS

3.1. The growth performance of bees by weeks

Table 1. Changes in the number of bee frames

Number of frames	Treatment					SEM	P
	T35	T50	T65	T80	T95		
Week 1	3.00	3.00	3.00	3.00	3.00	-	-
Week 2	3.00	3.00	3.00	3.00	3.00	-	-
Week 3	4.00	4.00	3.33	3.33	3.00	0.33	0.205
Week 4	4.00	4.00	3.33	3.33	3.00	0.33	0.205
Week 5	4.33	4.67	4.33	3.33	3.33	0.49	0.251
Week 6	4.33	4.67	4.33	3.33	3.33	0.49	0.251
Week 7	5.00	5.00	4.33	4.00	3.33	0.76	0.517
Week 8	5.00	5.00	4.33	4.00	3.33	0.76	0.517
Mean (1-8)	4.08	4.17	3.75	3.42	3.17	0.38	0.354

Notes: T35, T50, T65, T80 and T95 were supplemented concentrate at 35, 50, 65, 80 and 95 g/frame/week.

The number of bee frames across all five treatments (T) showed no significant difference throughout the 8-week experiment (P>0.05, Table 1). However, the average number of frames from week 1 to 8 in treatment T50 was 1.32 times higher than that of T95. Notably, T95 exhibited the least variation in frame numbers among the five treatments over the 8 weeks.

3.2. Effect of concentrate on honey yield and quality

Supplementation with different levels of concentrate had a significant effect on honey yield after 3 weeks of the experiment (P<0.05, Table 2). Among the treatments, both T35 and T50 produced the highest honey yields, 1.44 and 1.08 l/colony, respectively. The lowest honey yield was recorded in treatment T80, with only 0.38 l/colony. For this reason, when an excessive amount of concentrate feed was provided in the hive, the bees reduced their nectar foraging activity, resulting in lower actual honey production. This reduced foraging activity may also have limited pollen collection, which in turn could suppress the queen's egg-laying behavior. This finding was supported by the data presented in table 1, where colonies receiving lower levels of concentrate feed exhibited a higher number of bee frames, which declined with increasing concentrate levels.

Table 2. Honey yield and quality at the 1st harvest

Item	Treatment					SEM	P
	T35	T50	T65	T80	T95		
Honey yd,l	1.44^{a}	1.08^{a}	0.77^{ab}	0.38^{b}	0.52^{b}	0.17	0.007
Brix, %	73.7	73.3	75.0	75.5	73.5	0.92	0.392
Moisture,%	24.5	24.8	23.7	22.7	24.9	0.87	0.399
pH	3.78	4.11	3.91	4.04	4.27	0.17	0.354
Color							
L*	29.9	30.0	30.2	28.6	30.9	0.59	0.168
a*	2.80	-0.18	-0.34	-0.09	-0.05	1.72	0.668
b*	7.20	7.69	7.14	7.42	8.52	0.47	0.304

Notes: Means within a row with different superscripts are significantly different (P<0.05).

The honey color values after 3 weeks of the experiment showed no significant differences among the treatments (P>0.05, Table 2). The lightness (L*) values ranged from 28.6 to 30.9, indicating that the honey color across all treatments fell within the light brown range. The redness level of honey (a*) values ranged from -0.34 to 2.8, suggesting that honey from the T35 treatment tended toward a reddish hue, while the other treatments leaned slightly toward green. All treatments produced yellowness level of honey (b*) values ranged from 7.14 to 8.52.

Supplementation with different levels of concentrate after six weeks of the experiment had a significant effect on honey yield ($P<0.05$, Table 3), with the highest yield was detected in T35 (1.56 l/colony) compared to T95 (0.52 l/colony). Quality parameters such as Brix, moisture content, pH, color, and antioxidant capacity were not significantly affected ($P>0.05$). Regarding honey color, the lightness level of honey (L* values) ranged from 30.0 to 31.9, indicating relatively uniform brightness across treatments. The redness level of honey (a* values) were negative and close to zero for all treatments, suggesting a subtle mixture of green and red tones. The yellowness level of honey (b* values) ranged from 6.1 to 6.9, reflecting a moderately pronounced yellow hue in the honey samples.

Table 3. Honey yield and quality at the 2nd harvest

Item	Treatment					SEM	P
	T35	T50	T65	T80	T95		
Honey yield, l	1.56^{a}	1.38^{ab}	0.95^{b}	0.70^{bc}	0.52^{c}	0.18	0.010
Brix, %	75.3	75.7	74.7	76.0	74.7	0.65	0.529
Moisture, %	22.8	22.6	23.5	22.3	23.6	0.61	0.542
pH	3.58	3.74	3.70	3.57	3.67	0.13	0.834
Color							
L*	31.4	30.8	31.9	31.6	30.8	0.78	0.814
a*	-1.05	-0.98	-0.87	-1.21	-1.20	0.12	0.304
b*	6.09	7.30	6.19	6.67	6.00	0.65	0.611
Antioxidant, %	93.0	90.5	89.5	91.2	91.4	1.02	0.237

Supplementing different levels of concentrate did not affect honey yield, quality, and color at the third harvest ($P>0.05$, Table 4). Specifically, honey yield ranged of 1.12-2.01 l/colony, Brix values varied 71.3-74.5%, and moisture content ranged of 23.7-26.7%. The pH values ranged of 3.29-3.47, indicating a relatively high acidity level in the honey.

Across the three harvests, the results showed that the level of concentrate had a significant impact on honey yield. In all three harvests, T35 consistently produced high yields, reaching 1.44, 1.56 and 1.66l. Although quality parameters such as Brix, moisture content, pH, color showed no significant differences among the treatments, honey from T80 was denser due to its higher Brix value, while T65 and T95 had higher moisture contents, which may affect honey's storability. According to TCVN 12605:2019, issued by the Ministry of Science and Technology (2019), the soluble solids content in honey must not be less than 60 g/100g, and the moisture must not exceed 23%. In this study, the Brix value of honey was consistently above 70%, regardless of the level of added concentrate, suggesting that concentrate feeding did not affect the overall honey quality.

Table 4. Honey yield and quality at the 3rd harvest

Item	Treatment					SEM	P
	T35	T50	T65	T80	T95		
Honey yield, l	1.66	2.01	1.92	1.12	1.42	0.38	0.492
Brix, %	73.3	72.7	71.3	74.5	72.7	0.71	0.098
Moisture, %	24.9	25.3	26.7	23.7	25.3	0.65	0.085
pH	3.29	3.37	3.38	3.36	3.47	0.06	0.363
Color							
L*	29.5	31.3	31.6	31.0	31.4	0.53	0.099
a*	-0.96^{a}	-1.09^{ab}	-1.10^{ab}	-1.03^{ab}	-1.21^{b}	0.04	0.016
b*	4.31	5.77	6.20	5.08	5.75	0.48	0.127

Notably, during the second honey harvest, there were no significant differences in antioxidant percentages among the treatments. However, treatment T35 exhibited a higher free radical scavenging activity, indicating that honey from this treatment has greater antioxidant activity toward the DPPH radicals. According to the study by Jaśkiewicz *et al.* (2025), the antioxidant activity of buckwheat honey was 82.7-94.7%, comparable to the antioxidant percentages observed in honey supplemented with concentrate in this experiment. Overall, feeding concentrate at 35 and 50 g/frame/week was considered optimal, as it enhanced honey production efficiency while maintaining honey quality standards.

3.3. Effect of concentrate on total feed intake, honey yield and income

In this study, bees from the T35 treatment consumed only 1.16kg of concentrate per colony over 8 weeks and produced 4.65l of honey per colony, resulting in a feed-to-honey conversion ratio of 0.25. In comparison, Vinh *et al.* (2023) reported a honey yield of 20 l/colony/8 weeks when supplementing European honey bees with concentrate as a pollen substitute, but this required 7.41kg of feed per colony. The feed-to-honey conversion ratio in the study of Vinh *et al.* (2023) was 0.37, much higher than that in the current study.

Feed costs increased with FI, ranging from 40,700VND (đ) in the T35 to 86,100đ in T95, this difference was not statistically significant (P=0.123). In contrast, honey yield differed significantly among treatments (P<0.05), with T35 producing the highest yield (4.65 l/colony) and T80 showing the lowest one (2.20 l/colony). Despite the greater amounts of supplemental feed used in T65, T80, and T95, honey production did not increase proportionally. On the contrary, T35 with the lowest level of supplementation achieved the highest yield, suggesting that moderate feed supplementation may allow bee colonies to produce honey more efficiently.

Table 5. Feed intake, honey yield and profit

Item	Treatment					SEM	P
	T35	T50	T65	T80	T95		
FI, g/frame/w	34.3^{d}	49.3^{cd}	65.4^{bc}	78.6^{ab}	96.5^{a}	5.01	<0.001
FI, kg/col/8w	1.16	1.76	2.00	2.21	2.46	0.32	0.123
Fcost, 1,000đ	40.7	61.5	70.0	77.2	86.1	11.3	0.123
∑honey yield, l	4.65^{a}	4.47^{ab}	3.64^{ab}	2.20^{b}	2.46^{ab}	0.58	0.042
Revenue, 1,000đ	698^{a}	670^{ab}	546^{ab}	330^{b}	370^{ab}	87.6	0.042
Profit, 1000đ	657^{a}	609^{ab}	476^{ab}	253^{b}	283^{ab}	82.5	0.018

The gradual decline in honey yield in the high-feed supplementation groups may be attributed to the bees' reduced ability to digest the excessive feed and a corresponding decrease in their nectar foraging activity, leading to lower-than-expected productivity. This indicates that supplementing feed for bee colonies does not always result in increased honey production and must be carefully considered in terms of feed type, quantity, environmental conditions, and seasonal timing to achieve optimal effectiveness.

Honey revenue significantly decreased (P<0.05) from 698,000đ/colony in T35 group to 330,000đ in T80 group. Consequently, profit was highest in T35 group (657,000đ), while it dropped sharply in T80 group to 253,000đ (P<0.05). Therefore, T35 achieved optimal economic efficiency with a moderate level of feed supplementation, whereas high supplementation treatments did not yield good economic returns due to high feed costs and disproportionally low honey production.

Some limitations of this study include natural variability, as honey yield and quality may be influenced by uncontrollable factors such as weather conditions and colony health. The experiment lasted only 8 weeks, which may not be sufficient to assess the long-term effects of different levels of concentrate supplementation on bee colonies. Finally, since the experiment was conducted under specific environmental conditions on the campus of Can Tho University, the results may not fully represent variations in yield and quality under different climates, crop cultivation methods, or colony management practices. These limitations should be addressed in future studies to enhance the applicability and accuracy of the findings.

4. CONCLUSION

Supplementation of concentrate for Italian honey bees at 35 g/frame/week during the dry season resulted in the highest honey yield and profit, but these parameters were reduced as increasing level of concentrate supplementation. Quality parameters of honey including Brix, moisture content, pH, antioxidant activity, and honey color were not affected by the level of supplement. Thus, feeding concentrate at 35 g/frame/week is considered optimal in terms of honey yield, quality, and economic return of Italian honey bees.

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INFLUENCE OF SOYBEAN RESIDUE AND RICE BRAN LEVELS IN DIETS ON GROWTH PERFORMANCE AND COOKING TIME ON POST-HARVEST FAT REDUCTION IN BLACK SOLDIER FLY LARVAE

Le Thuy Binh Phuong[1], *Nguyen Thanh Nhan*[2], *Nguyen Van Nghia*[3] *and Duong Nguyen Khang*[3]*

Submitted 28-Jun-2025 – Revised: 21-Jul-2025

Accepted: 31-Jul-2025

ABSTRACT

This study evaluated the effects of soybean residue (SBR) and rice bran (RB) levels in diets on the growth performance of black soldier fly larvae (BSFL) and investigated the impact of boiling time on post-harvest fat removal efficiency. Five different substrates were used in the trial including total SMR diet (100%), 75% SMR+25% RB, 50% SMR+50% RB and total RB diet (100%). Performance parameters that were assessed included size, weight of BSFL, feed conversion ratio (FCR) and economic efficiency. The results showed that the 30% SMR treatment gave the best growth performance, with large larvae and the lowest feed conversion ratio (FCR). Economically, the 30% SMR treatment was also the optimal solution due to its low cost and high biomass value. In addition, harvesting larvae on day 21 combined with boiling for 60 minutes produced a product rich in protein, low in fat and stable in mineral content. The analysis also showed that preservation methods such as drying and freezing can effectively maintain the nutritional value of larval meals. In conclusion, the use of rice bran mixed with 30% SMR combined with harvesting on the 21th day and fat removal is the optimal process in the production of BSFL powder. The research results contribute to a scientific basis for practical application in animal feed production, contributing to the development of sustainable alternative protein sources.

Key words: *Black soldier fly, rice bran, soybean meal, larval growth, fat removal.*

1. INTRODUCTION

Optimizing larval diets with agricultural by-products like soybean residue and rice bran enhances growth rates and reduces costs. Additionally, fat reduction via boiling post-harvest can improve nutritional value and storage. While previous studies have examined soybean residue and cooked rice effects on BSFL growth, comprehensive research combining diet formulation with post-harvest fat reduction is lacking. This study aims to address this gap.

The growing demand for high-quality, stable and sustainable protein sources in the livestock and aquaculture sectors has intensified research into alternative feed ingredients. Among these, black soldier fly larvae (BSFL, *Hermetia illucens*) have gained attention for their remarkable ability to convert organic waste into nutrient-rich biomass. While previous studies have established their efficiency in biomass conversion and their potential as an alternative protein source, critical gaps remain regarding the optimization of feed formulations-particularly the precise balance of protein and energy sources in their diet and the impact of post harvest processing on nutritional quality.

With a short life cycle-around 40-45 days from egg to adult, or 20-21 days from egg to harvestable larvae-they offer significant biological and economic advantages for large-scale production. BSFL can rapidly digest organic waste and convert it into protein- and fat-rich biomass while contributing to environmental waste reduction (Sheppard, 1983; Sheppard *et al.*, 1994; Yu *et al.*, 2009; Zheng *et al.*, 2012; Green *et al.*, 2012; Lalander *et al.*, 2013; Banks *et al.*,

[1] Nong Lam University of Ho Chi Minh City
[2] Department of Animal Husbandry, Veterinary and Aquatic Products, Long An Province
[3] Research and Technology Transfer Center, Nong Lam University of Ho Chi Minh City
* Corresponding author: Professor Dr. Duong Nguyen Khang, Nong Lam University of Ho Chi Minh City; Phone: 0884 989390179; Email: duongnguyenkhang@gmail.com.

2014; Lalander *et al*., 2015; Nguyen *et al*., 2015; Webster *et al*., 2016; Tomberlin *et al*., 2017; Hoa *et al.*, 2023; Giang *et al.*, 2023). This unique ability highlights the potential for utilizing agricultural by-products as larval feed, simultaneously addressing organic waste management and providing nutrient-rich feed ingredients. The black soldier fly larvae are already used to manage manure successfully (Sheppard *et al*., 2002), reducing odor and pest fly populations (Sheppard, 1983; Sheppard *et al*., 1994). However, to maximize this potential, further research is needed to define larvae nutritional requirements and optimize feed formulations.

Several studies have reported that BSFL contain 38 to 45% protein, depending on their diet (Newton *et al*., 2005; Fiala *et al*., 2020). They also supply essential fatty acids, vitamins, and minerals critical for animal growth. Yet, the nutritional value of agricultural by-products like rice bran for BSFL feeding remains under explored, especially concerning energy and protein contributions. Hence, identifying optimal dietary protein and energy levels is essential to enhance rearing efficiency and lower production costs.

In addition to feed formulation, post harvest processing methods are vital in determining the utility of BSFL. Boiling has been proposed as a practical method for fat reduction and preservation. Nevertheless, this process may alter protein levels and amino acid profiles, warranting careful analysis to ensure nutritional integrity post-processing (Liu *et al*., 2021).

The study aimed not only to optimize dietary formulations but also to evaluate post harvest preservation methods for maintaining BSFL nutritional quality. Methods such as drying, freezing, or using preservation solutions may extend shelf life. The findings are expected to contribute both scientific insights and practical applications for the sustainable integration of BSFL into animal feed value chains.

2. MATERIALS AND METHODS

2.1. Experimental site and materials

The experiments were conducted from February to April 2023 at the Cattle farm, Research and Technology Transfer Center, Nong Lam University, Ho Chi Minh City, Vietnam. Soybean residue (SBR) and rice bran (RB) were sourced locally and used in untreated form.

2.2. Experimental design

A completely randomized design with five dietary treatments was applied, corresponding to SBR inclusion rates of 0, 10, 20, 30 and 40%, balanced with RB. Each treatment was replicated three times, with 10g larvae per replicate housed in plastic trays (0.24m^2 per tray). On day 1, each tray received 10g of BSFL eggs and 200g of a starter feed mix. After 7 days, larvae were redistributed into experimental trays based on the intended feed treatment. Seven-day-old larvae were randomly selected, and the total weight of 100 larvae was measured in six replicates to determine the average weight and number of larvae used to initiate the experiment in each tray

2.3. Feeding management

Feeds were provided once daily at 7:30AM. To standardize larval access, the feed was distributed at a single location on the surface of each tray. The amount of feed was adjusted according to the larval growth stage and monitored to ensure minimal residue remained, thereby preventing spoilage and enabling accurate measurement of intake. The approximate feeding rate was 70mg per larva per day.

2.4. Growth performance measurements

Larval growth was assessed on days 7, 14, and 21 by measuring larval length, width, and weight. For each replicate, a random sample of 100 larvae was measured. Feed offer and frass was recorded daily to calculate feed conversion ratio (FCR). At the end of the feeding trial (day 21), total larval biomass was weighed both on a fresh and dry matter basis.

2.5. Post harvest boiling and fat removal study

Larvae harvested at day 21 from the optimal diet groups were subjected to boiling treatments at three different durations: 30, 45, and 60 minutes. After boiling, larvae were cooled, dried, and analyzed for proximate composition including crude protein, extract (lipid content), ash and dry matter.

2.6. Chemical analysis

Feed ingredients samples were analyzed for dry matter (DM), crude protein (CP), ether extract (EE), crud fiber, lipid, calcium, phospho and ash using AOAC (2005) standard methods.

2.7. Statistical analysis

All data were analyzed using analysis of variance (ANOVA) with the General Linear Model procedure in Minitab software (version 17.01). Tukey's Honest Significant Difference (HSD) test was used for comparisons when significant differences were detected (P<0.05). Regression analysis with linear and quadratic models was conducted using Microsoft Excel to evaluate response trends for key performance indicators such as feed intake, larval weight, and FCR.

3. RESULTS AND DISCUSSION

3.1 Chemical composition of feeds

The nutritional composition differed significantly between the individual feed ingredients (Table 1), with SBR containing more protein than RB. Although both ingredients provided similar energy levels, the lipid content in RB was nearly three times higher than in SBR. Despite being a by-product, SBR retained a notable amount of NFC. However, its relatively high lipid content may influence protein accumulation in the larval body, as the nutritional composition of BSFL is primarily protein and lipid (Li *et al.*, 2022). Therefore, mixing SBR and RB in BSFL diets may offer complementary nutritional benefits and support improved larval growth.

Table 1. Composition of diet ingredients (% DM)

Item	SBR	RB	Item	SBR	RB
DM (%)	15.71	89.98	Ash (%)	6.38	8.12
CP (%)	35.23	12.54	ME (kcal/kg)	3020	3140
CF (%)	8.04	8.08	Ca (%)	0.18	0.19
Lipid (%)	4.35	13.72	P (%)	0.62	1.89

Table 2. Proportion of ingredients and nutrients in diets

Item	SBR0	SBR10	SBR20	SBR30	SBR40
RB, %	100	90	80	70	60
SBR, %	0	10	20	30	40
Nutrients in diets					
DM, %	88.40	81.60	74.70	67.90	61.00
CP, %	14.00	15.00	16.00	16.00	17.00
CF, %	6.60	6.50	6.30	6.10	5.90
Lipid, %	11.80	10.10	9.80	8.80	7.90
Ash, %	8.20	7.30	6.50	5.70	4.90
ME, kcal/kg	2880	2920	2960	3000	3050
Ca, %	0.1	0.1	0.0	0.0	0.0
P, %	1.4	1.2	1.1	1.0	0.8
P:E	1:20.6	1:19.5	1:18.5	1:18.8	1:18

The nutritional composition of experimental diets is presented in table 2. The protein content was improved by incorporating soybean residue (SBR) at levels up to 40%, and the protein and energy ratio ranges from 1:18 to 1:20.

3.2. Diet composition effect on BSFL growth

The dietary inclusion of SBR at varying levels had a significant impact on the growth performance of BSFL.

3.2.1. Size of BSFL

In general, larval size was greatest in the SBR30 treatment at both 14 and 21 days of age. However, the length and width of larvae at 21 days varied among the different SBR supplementation levels. The longest larval length (19.65mm) was observed in the 30% SBR treatment, while the shortest (17.75mm) occurred in the 20% SBR treatment. In contrast, the greatest larval width was found in the 10% SBR treatment (5.47mm), and the smallest in the 0% SBR treatment (4.87mm) (Table 3).

Table 3. BSFL length and width (mm)

Item	SBR0	SBR10	SBR20	SBR30	SBR40	SEM	P
Length, mm							
7 days	2.59	2.52	2.54	2.53	2.52	0.012	0.326
14 days	9.73	9.31	9.72	9.85	9.61	0.097	0.308
21 days	18.93^{b}	19.11^{ab}	17.75^{c}	19.65^{a}	18.22^{c}	0.151	0.001
Width, mm							
7 days	0.78	0.74	0.72	0.75	0.74	0.005	0.424
14 days	2.64	2.70	2.78	2.87	2.82	0.030	0.346
21 days	4.87^{c}	5.47^{a}	5.23^{b}	5.29^{b}	5.15^{b}	0.045	0.01

Different letters (a, b, c) after each value indicate statistically significant differences at P<0.05

Larval size is influenced by various factors, including the ability to accumulate protein and lipid. However, an imbalance in the dietary protein-to-lipid ratio may adversely affect larval development. In this experiment, the 30% SBR+70% RB diet appeared to support better larval growth compared to the other mixing.

3.2.2. *Weight of BSFL*

The weight of BSFL presented in table 4 shows that, although there was no significant difference in the weight of 100 larvae at 14 days of age, a general trend was observed in which increasing SBR levels in the diet improved larval weight, reaching an optimum at 30% SBR before decreasing at 40% SBR. A similar trend was observed in the total larval weight, with a statistically significant difference at P<0.05.

Table 4. Weight of 100 BSFL and total weight of L/tray

Item	SMR0	SMR10	SMR20	SMR30	SMR40	SEM	P
Weight of 100 black soldier fly larvae, g							
7d	0.07	0.07	0.07	0.07	0.07	0.000	-
14d	2.58^{b}	2.94^{b}	3.24^{ab}	3.41^{a}	2.89^{b}	0.222	0.038
21d	17.46^{c}	18.76^{c}	21.17^{ab}	23.94^{a}	20.01^{b}	1.554	0.001
Total weight of larvae per tray, kg							
Fresh	1.73^{b}	1.85^{ab}	2.00^{ab}	2.41^{a}	1.90^{ab}	0.139	0.047
Dry	0.54^{b}	0.58^{ab}	0.63^{ab}	0.77^{a}	0.60^{ab}	0.044	0.031

3.2.3. *Feed conversion rate (FCR)*

Correlation between treatments and FCR are presented in figure 1. The relationship of FCR and fresh larvae weight in different treatments is shown in figure 2. FCR was inversely correlated with the dietary SBR level, with the most optimal FCR observed at 30% SBR inclusion. However, FCR increased when the SBR level was raised to 40%. This indicates that larvae in the treatment containing 30% SBR and 70% RB utilized the diet most efficiently for biomass conversion.

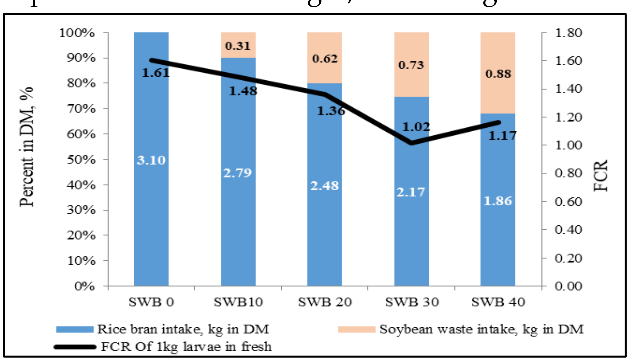

Figure 1. Correlation between treatments with FCR

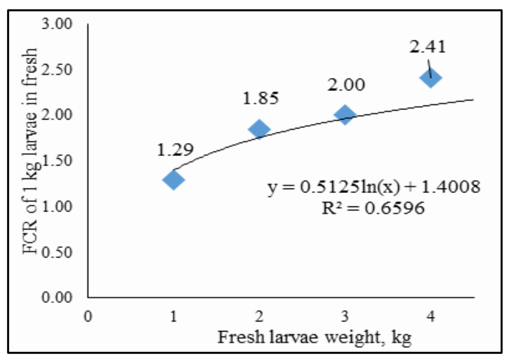

Figure 2. Effect of FCR on fresh larval weight

3.2.4. *Economic efficiency*

The profitability analysis demonstrated that the diet containing 30% SMR and 70% RB generated the highest economic return. With a total fresh larval (FL) weight of 2.41kg and production expenses of 20,813VND, this treatment yielded a net profit of 27,454VND. In comparison, the 0% SBR group generated a net profit of only 8,847VND. These findings underscore the advantage of using a 30% SMR inclusion level for maximizing growth performance and profitability in BSFL production. Overall, the results highlight that a balanced diet with 30% SMR and 70% RB optimizes both biological performance and economic return in BSFL production.

Table 5. Cost and profit according to treatments

Item	SMR0	SMR10	SMR20	SMR30	SMR40
Feed, VND	18,600	17,050	15,500	13,753	12,039
Labour cost/day	500	500	500	500	500
BSF eggs, 10gr	60	60	60	60	60
Total cost	25,660	24,110	22,560	20,813	19,099
Price 1kgFL,VND	20,000	20,000	20,000	20,000	20,000
Total price for WG	34,507	36,980	40,027	48,267	37,993
Benefit, VND	8,847	12,870	17,467	27,454	18,894

** The average price of RB and SBR are 6,000 and 1,000 VND/kg. The average price of fresh larvae is 20,000 VND/kg.*

3.3. Boiling time and fat reduction

Twenty-one-day-old larvae from each treatment were thoroughly mixed and analyzed for nutritional composition, including DM, ash, P), and EE, at different defatting times. The effect of boiling time on fat reduction in harvested BSFL was evaluated at 30, 45, and 60 minutes. Boiling significantly influenced the fat content of the larvae, with longer boiling times leading to greater fat reduction. At 30 minutes of boiling, a moderate reduction in crude fat content was observed, but a significant amount of lipid remained within the larvae. Increasing the boiling to 45 minutes resulted in a more substantial decrease in lipid content while retaining acceptable levels of protein and maintaining larval integrity. At 60 minutes provided the most effective fat removal, achieving the lowest residual lipid content among the tested durations. The extended boiling time facilitated thorough lipid extraction, without causing significant degradation of crude protein content or excessive moisture loss. However, excessive boiling beyond 60 minutes (as noted in a preliminary trial not included in the main dataset) risked texture deterioration and potential nutrient loss.

These results indicate that boiling for 60 minutes optimizes the balance between effective fat removal and preservation of nutritional quality. This finding supports the recommendation of a 60-minute boiling treatment as a practical post harvest processing method for BSFL aimed at producing a low-fat, high-protein feed ingredient.

Table 6. Effect of boiling time on nutritional composition of 21-day-old harvested larvae

Item	**Boiling time**, minutes				
(%)	0	30	45	60	P
DM	94,71^{b}±0,01	95,23^{a}±0,07	94,89^{b}±0,04	94,96^{b}±0,08	0,01
Ash	9,31±0,11	9,30±0,06	9,27±0,06	9,22±0,06	0,193
CP	47,47±0,17	47,66±0,01	47,54±0,12	47,24±0,33	0,100
EE	28,29^{a}±0,07	13,44^{b}±0,17	12,54^{b}±0,18	12,17^{b}±0,41	0,001

4. CONCLUSION

An optimal diet of 30% SMR and 70% RB enhances BSFL growth and feed efficiency. A 60-minute boiling treatment postharvest effectively reduces fat content. These findings provide a sustainable strategy for BSFL-based animal feed production.

ACKNOWLEDGEMENTS

The authors acknowledge support the fund for this research from the Department of Science and Technology of Ba Ria - Vung Tau province, Vietnam. They also acknowledge the Research and Technology Transfer Center, Nong Lam University of Ho Chi Minh City, Vietnam for providing infrastructure support.

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OPTIMIZING ENERGY LEVELS BASE ON BROKEN RICE FOR BLACK SOLDIER FLY LARVAE AND POST-HARVEST DEFATTING EFFICIENCY WITH PROTEASE

Le Thuy Binh Phuong[1], Bui Nguyen Phuong Thanh[2], Dinh Van Nam[1] and Duong Nguyen Khang[1]*

Submitted 28-Jun-2025 – Revised: 21-Jul-2025

Accepted: 31-Jul-2025

ABSTRACT

This study aimed to optimize dietary energy levels in soyabean meal-based diets for black soldier fly (*Hermetia illucens*) larvae and to evaluate the efficiency of post-harvest fat removal using protease. A completely randomized design was applied with four dietary energy levels: 2500, 2700, 2900, and 3100 kcal/kg. The larvae harvested from all treatments were pooled and subjected to biochemical analysis at 16, 18, and 21 days of age and fat extraction by mechanical pressing, either directly or following enzymatic hydrolysis with protease at 0.5, 1.0, and 1.5% concentrations. The results showed that higher dietary energy levels improved larval growth and feed conversion. The 2900 kcal/kg diet yielded the best biological performance, with larvae reaching an average length of 19.29mm, width of 4.81mm, and a 100-larvae weight of 13.83g. This group achieved the highest yields of 1.9 kg fresh weight and 0.57kg dry weight, with a feed conversion ratio (FCR) of 2.05, indicating efficient feed utilization. Biochemical analysis revealed that crude protein (CP) content increased with larval age, ranging from 42.41% at day 16 to 44.83% at day 21, while ether extract (EE) remained steady at 27.5-27.8% before fat removal. Protease-assisted hydrolysis prior to fat extraction significantly enhanced fat removal. At 1.5% protease, EE was reduced to 15.84-16.89%, corresponding to a 45% fat reduction compared to untreated larvae. The CP content of enzyme-treated larvae slightly decreased to 42.44-42.66% compared to mechanical pressing only, while ash content remained between 9.01-9.21%, indicating minimal nutrient loss. In conclusion, a dietary energy level of 2900 kcal/kg optimized the growth, yield, and feed efficiency of larvae fed broken rice-energy based diets. The application of 1.5% protease improved post-harvest fat extraction without significant protein loss, supporting its use to enhance the quality and stability of black soldier fly larvae meal in animal feed production.

Keywords: *Black soldier fly larvae, broken rice, energy level, growth, protease-assisted fat removal.*

1. INTRODUCTION

The search for sustainable, efficient, and environmentally friendly protein sources has gained global momentum due to the increasing pressure on conventional animal protein production and food security concerns. Insect farming, particularly using the black soldier fly (*Hermetia illucens)*, has emerged as a promising strategy to meet the growing demand for alternative protein sources while contributing to waste valorization and circular economy goals (Van Huis *et al.*, 2013; Makkar *et al.*, 2014).

The black soldier fly is a dipteran insect with a short lifecycle and high adaptability. Its larvae can convert a wide range of organic waste, including food residues and agro-industrial by-products, into valuable biomass rich in protein (30-40%) and lipids (20-30%) (Barragán-Fonseca *et al.,* 2017; Spranghers *et al*., 2017). These attributes make black soldier fly larvae (BSFL) an attractive ingredient for animal feeds, aquaculture, and even bioenergy production (Diener *et al.,* 2015; Lalander *et al*., 2019).

Among agro-industrial by-products, broken rice a by-product of rice milling widely available in Southeast Asia-is known for its favorable nutritional composition, including high-energy content, protein, and essential fatty acids (Tran *et al.,* 2022). broken rice has been used as a base substrate for BSFL with promising results; however, optimizing the dietary metabolizable energy (ME) levels

[1] Nong Lam University of Ho Chi Minh City
[2] Department of Animal Husbandry, Veterinary and Aquatic Products, Long An Province
[*] Corresponding author: Professor Dr. Duong Nguyen Khang, Nong Lam University of Ho Chi Minh City; Phone: 0084 989390179; Email: duongnguyenkhang@gmail.com.

is critical to enhance larval growth, feed conversion efficiency, and economic performance (Nguyen *et al*., 2020). Imbalanced energy levels may lead to sub-optimal growth rates, reduced biomass yield, and poor feed efficiency (Lalander *et al*., 2019).

Despite their nutritional value, BSFL have a relatively high lipid content-sometimes exceeding 30% of dry matter-posing challenges for long-term storage, feed stability, and formulation consistency (Spranghers *et al*., 2017; Wang and Shelomi, 2017). High-fat insect meals are prone to rancidity and may limit inclusion rates in certain animal diets. Therefore, post-harvest fat removal (defatting) is an essential step in BSFL processing to produce protein-rich, stable meal suitable for animal feed (Azzollini *et al.,* 2020; Gold *et al*., 2021).

Traditional mechanical pressing methods, while partially effective, often leave a significant amount of residual fat in the insect biomass. Recent studies have explored the combination of mechanical pressing with enzymatic hydrolysis, particularly using protease, to improve lipid extraction efficiency. Protease hydrolyze protein-lipid complexes, facilitating the release of bound lipids and increasing the yield of defatted protein meal (Ghosh *et al.,* 2020; Batish *et al.,* 2021). This approach not only enhances fat removal but also preserves the functional properties of proteins, making them more suitable for feed applications (Batish *et al*., 2021; Azzollini *et al*., 2020).

Furthermore, enzymatic pre-treatment offers a cost-effective and environmentally friendly alternative to chemical solvents, aligning with sustainable processing objectives. Ghosh *et al*. (2020) highlighted that protease-assisted extraction could increase lipid recovery by up to 40% compared to mechanical pressing alone, without significant degradation of protein quality.

In Vietnam, the interest in black soldier fly farming has expanded rapidly due to its potential for organic waste management and sustainable protein production (Zheng *et al*., 2012; Green *et al*., 2012; Lalander *et al*., 2013; Banks *et al*., 2014; Lalander *et al*., 2015; Nguyen *et al*., 2015; Webster *et al*., 2016; Tomberlin *et al*., 2017; Hoa *et al*., 2023; Giang *et al.,* 2023). However, studies that integrate nutritional optimization (e.g., BR energy levels) with advanced post-harvest processing (e.g., enzymatic fat extraction) remain scarce (Nguyen *et al.,* 2024).

The main objectives of this study are: (1) To determine the optimal dietary energy (DE) level in BR diets for BSFL, aiming to enhance biological performance indicators such as growth rate (GR), biomass yield, and FCR, (2) To evaluate the efficiency of post-harvest fat removal using protease-assisted hydrolysis combined with mechanical pressing, targeting improved lipid extraction and retention of protein quality in the larvae meal.

This study contributes to the growing body of knowledge on insect-based feed production, offering insights into practical applications of enzyme-assisted fat removal and energy optimization in BSFL farming. The findings aim to support circular agriculture models by promoting the effective utilization of agro-industrial by-products and enhancing the economic viability of insect meal production for feed industries.

2. MATERIALS AND METHODS

2.1. Experimental site and materials

The experiment was conducted from March to September 2024 at the Research and Technology Transfer Center, Nong Lam University, Ho Chi Minh City, Vietnam. Black soldier fly larvae (BSFL) used in the study were sourced from the research colony maintained by the center, broken rice served as the energy dietary substrate in soybean meal-based diet and fibrous ingredients to meet the target nutritional composition. The metabolizable energy (ME) levels of the diets were adjusted according to formulation. Commercially available protease enzyme powder was used for post-harvest fat removal treatments.

The eggs of black soldier fly were hatched on chicken feed until 07 days of age, then sieved through two layers of mesh with different pore sizes to remove individuals that were either too small or too large. Larvae of average size were collected and allocated to experimental trays. To estimate the number of larvae per tray, the weight of 100 seven-day-old larvae was measured and repeated six times. Each experimental tray was then assigned 100 g of 7-day-old larvae for the 14-day feeding trial.

2.2. Experimental design

A completely randomized design (CRD) was applied for both the feeding trial and the fat removal experiment.

***Dietary treatments*:** Four energy levels were tested of 2,500; 2,700; 2,900; 3,100 kcal/kg, corresponding to different BR diet formulations. Each treatment had three replicates, with identical rearing conditions for all groups.

***Harvesting ages*:** Larvae were harvested at 16, 18, and 21 days of age to assess GP and chemical composition.

***Fat removal treatments*:** After harvest, larvae were subjected to fat extraction by mechanical pressing, with or without prior incubation with protease at 0.5, 1.0, 1.5% (w/w) concentrations.

2.3. Feeding management

Larvae were reared in plastic containers (62,6×42,4×15cm) under controlled laboratory conditions, maintaining 28-30°C temperature and 65-75% relative humidity. Feed was offered *ad libitum***,** replenished daily according to larval consumption. The amount of feed offered and residuals were recorded to calculate feed intake. Containers were cleaned regularly to maintain hygienic conditions and prevent cross-contamination.

2.4. Growth performance measurements

At each harvest, larvae were randomly sampled from each replicate for growth performance evaluation (1) Body length (mm) and body width (mm) measured using digital calipers, (2) Weight of 100 larvae (g) determined using a precision scale, (3) Total biomass yield (kg) recorded for each container, (4) Feed Conversion Ratio (FCR) calculated as the total feed intake divided by the total larval biomass gain over the experimental period.

2.5. Fat removal study

After harvesting, larvae underwent fat removal in two phases: (1) Mechanical pressing only (Control group), (2) Enzyme-assisted fat removal (Treatment groups): Larvae were pretreated with protease solutions at 0.5, 1.0, and 1.5%**,** incubated for a standard period under controlled conditions, and then subjected to mechanical pressing. The extracted fat was collected, and the defatted larvae were dried and stored for further chemical analysis.

2.6. Chemical analysis

Samples of whole larvae and defatted larvae were analyzed for proximate composition using standard AOAC (2000) methods: (1) Dry matter (DM) by oven drying at 105°C, (2) CP by Kjeldahl nitrogen method (N×6.25), (3) EE by Soxhlet extraction method, (4) Ash content determined by incineration at 550°C in a muffle furnace. All analyses were performed in triplicate to ensure accuracy and repeatability.

2.7. Statistical analysis

Data was compiled using Microsoft Excel 2019 and analyzed with Minitab 17.1.0 software. Analysis of variance (ANOVA) was used to evaluate the effects of DE levels, harvesting time, and protease concentration on the measured parameters. Tukey's Honestly Significant Difference (HSD) test was applied for comparison at a significance level of $P<0.05$. Data are presented as Mean±SD.

3. RESULTS AND DISCUSSION

3.1. Chemical composition of feed ingredients and experimental diets

The chemical composition of the main feed ingredients of soybean meal (SBM) and BR is shown in table 1. Soybean meal contained notably higher CP (36.53%), CF (8.14%), and EE (4.52%) compared to BR, which had lower CP (7.84%), CF (0.63%), and EE (1.24%). Conversely, BR provided slightly higher ME (3,160 kcal/kg) than SBM (3,060 kcal/kg), reflecting its high starch content. The ash, calcium, and phosphorus contents were also higher in SBM.

The formulation of experimental diets aimed to maintain a CP level of 23% across treatments, while varying ME levels through adjustments in the proportions of BR and SBM (Table 2). As DE increased from 2,500 to 3,100 kcal/kg, the proportion of BR rose, while that of SBM and limestone powder decreased.

The nutrient composition of the diets across all treatments remained consistent for CP (23%), CF (2.3%), EE (1.2-1.3%), and ash (2.7-2.8%). However, ME increased as per the designed energy levels. Calcium and phosphorus were stabilized at 0.1 and 0.3%, respectively, to meet the nutritional requirements of BSFL.

This dietary formulation strategy ensured that energy level was the primary variable affecting larval GP, while other nutritional factors remained consistent across treatments.

Table 1. Composition of diet ingredients (% DM)

Item	SBM	BR
DM (%)	90.71	90.25
CP (%)	36.53	7.84
CF (%)	8.14	0.63
Lipid (%)	4.52	1.24
Ash (%)	6.48	0.72
ME (Kcal/kg)	3060	3160
Ca (%)	0.21	0.03
P (%)	0.65	0.19

Table 2. Proportion of ingredient and nutrient in diet

Item	ME2500	ME2700	ME2900	ME3100
Proportion of ingredients in diets (%)				
BR	28	39	41	48
SBM	44	42	43	41
Stone powder	28	19	16	11
Nutrients in diets				
DM (%)	89.0	88.6	88.6	88.3
CP (%)	23.0	23.0	23.0	23.0
CF (%)	2.3	2.3	2.3	2.3
Lipid (%)	1.2	1.2	1.2	1.3
Ash (%)	2.8	2.7	2.8	2.7
ME (kcal/kg)	2.500	2.800	2.900	3.100
Ca (%)	0.1	0.1	0.1	0.1
P (%)	0.3	0.3	0.3	0.3

3.2. Diet composition effect on BSFL growth

3.2.1. Effect of dietary energy on BSFL size

The ME level significantly influenced the body length and width of BSFL across different growth stages (Table 3).

Table 3. BSFL length and width (mm)

Item	ME2500	ME2700	ME2900	ME3100	SEM	P
Length, mm						
7 days	3,67	3,68	3,68	3,68	0,139	0,325
14 days	$10,42^{b}$	$10,93^{b}$	$12,50^{a}$	$11,05^{ab}$	0,560	0,019
21 days	$16,16^{b}$	$18,61^{ab}$	$19,29^{a}$	$17,67^{ab}$	0,651	0,015
Width, mm						
7 days	0,80	0,82	0,80	0,82	0,075	0,456
14 days	$2,77^{b}$	$3,03^{ab}$	$3,56^{a}$	$3,04^{ab}$	0,161	0,032
21 days	4,38	4,73	4,81	4,68	0,150	0,403

** Different letters (a, b, c) after each value indicate statistically significant differences at P<0.05*

By 14 days of age, differences in larval dimensions became apparent. Larvae fed the 2,900 kcal/kg diet showed the greatest increase in both length and width. Their average length reached 12.50mm, significantly higher than those in the 2,500 kcal/kg group (10.42mm, P<0.05). Similarly, the width of larvae in the 2,900 kcal/kg group was 3.56mm, markedly greater than the 2.77mm observed in the lowest energy group (P<0.05). At 21 days of age, the trend remained consistent. Larvae in the 2,900 kcal/kg treatment reached a maximum average length of 19.29mm, significantly longer than those in the 2,500 kcal/kg group (16.16mm, P=0.015). The 2,800 kcal/kg and

3,100 kcal/kg groups showed intermediate values. In terms of body width, although the highest measurement (4.81mm) was recorded in the 2,900 kcal/kg group, differences among treatments at this age were not statistically significant (P=0.403).

Overall, increasing dietary energy levels up to 2,900 kcal/kg improved larval growth in terms of size. However, further increasing ME to 3,100 kcal/kg did not result in additional growth benefits, suggesting that 2,900 kcal/kg may be the optimal energy level for maximizing larval size in RB diets.

3.2.2. Effect of dietary energy on larval weight

The dietary ME level exerted a significant effect on the weight of BSFL at various growth stages. At **7** days of age, the weight of 100 larvae showed no significant differences among treatments (P=0.132), with values ranging from 0.12g (2,500 kcal/kg) to 0.14g (2,900 kcal/kg). By 14 days of age, larvae fed the 2,900 kcal/kg diet exhibited a significantly higher weight (6.24g/100 larvae) compared to those in the 2,500 kcal/kg group (4.81g, P=0.003). The 2,900 kcal/kg group also showed significantly higher larval weight (5.84g) than the 2,500 kcal/kg group. At 21 days of age, larvae from the 2.900 kcal/kg treatment reached the highest recorded weight of 13.83g per 100 larvae, which was significantly superior (P=0.021) to the 10.51g observed in the 2,500 kcal/kg group. The weight difference between the 2,900 kcal/kg and 3,100 kcal/kg treatments was not significant, suggesting a plateau effect beyond the optimal energy level.

These results confirm that increasing dietary energy up to 2,900 kcal/kg effectively enhances larval weight gain. However, exceeding this level does not further improve larval weight, highlighting 2,900 kcal/kg as the optimal dietary energy concentration for maximizing BSFL biomass in rice bran-based feeding regimes. Energy and protein are two essential nutritional components that can interact during metabolism, potentially influencing larval development. Eggink *et al.* (2022) reported that larvae fed diets with a higher carbohydrate-to-protein ratio required a longer duration to reach their maximum body weight.

Table 4. Weight of 100 black soldier fly larvae (g)

Item	ME2500	ME2700	ME2900	ME3100	SEM	P
7d	0,16	0,16	0,16	0,16	0,001	0,962
14d	2,35^{b}	2,72^{b}	3,51^{a}	2,91^{b}	2,35	0,042
21d	9,45^{b}	12,73^{ab}	13,83^{a}	12,58^{ab}	0,917	0,001

3.2.3. Feed conversion rate

The dietary ME level significantly influenced feed conversion ratio (FCR) of BSFL (Figure 1, 2). The FCR improved (i.e., decreased) with increasing DE up to 2,900 kcal/kg. At this level, larvae exhibited the most efficient feed conversion with an FCR of 2.05, significantly better (P<0.05) than the 2.40 recorded in the 2,500 kcal/kg group. The 3,100 kcal/kg treatment did not result in further FCR improvement compared to 2,900 kcal/kg (FCR=2.08, P>0.05).

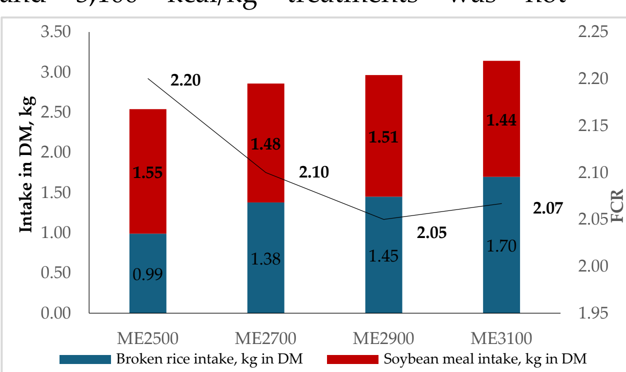

Figure 1. Correlation between treatments with FCR

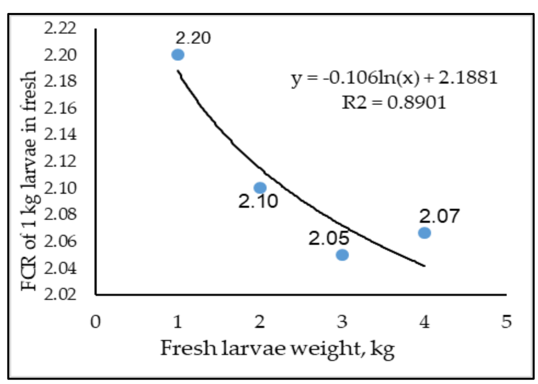

Figure 2. Effect of FCR on fresh larval weight

This finding indicates that 2,900 kcal/kg is the optimal DE concentration for maximizing feed efficiency in BSFL, enabling larvae to convert feed into biomass more effectively while minimizing excess intake.

3.3. Effect of protease concentration on fat removal in harvested BSFL

The application of protease at different concentrations significantly affected the lipid content of harvested BSFL. Across all tested harvest ages (16, 18, 21 days), increasing protease concentration prior to mechanical pressing effectively reduced the EE content of the larvae.

At 16 days of age, larvae treated with 1.5% protease showed the lowest EE content (16.89%), compared to the untreated control (30.82%), indicating a reduction of 45.2%. Similar trends were observed at 18 and 21 days of age, where EE content decreased from 29.35% and 27.46% (control) to 16.76 and 15.84% respectively with 1.5% protease treatment. The lipid reduction was statistically significant across all enzyme levels ($P<0.001$).

Regarding protein content, a slight decrease in crude protein (CP) was recorded with increasing protease concentration at all periods of BSFL harvesting. For instance, CP at 21 days declined from 44.83% (control) to 42.44% in the 1.5% protease group. However, these differences remained within acceptable limits for protein quality in animal feed applications. Dry matter (DM) content also decreased with higher enzyme concentrations, likely due to moisture retention during enzymatic hydrolysis, though ash content remained relatively stable (9.01–9.21%).

Overall, the use of 1.5% protease prior to mechanical pressing consistently achieved the highest fat removal efficiency, reducing EE content by 40-45% without severely affecting protein or ash content. These findings suggest that 1.5% protease is an effective level for enhancing lipid extraction in BSFL processing while maintaining desirable nutritional composition.

Table 6. Effect of protease concentration on nutritional composition of day-old harvested larvae

Item	Harvest (day)	Protease concentration (%)				
		0	0.5	1.0	1.5	P
DM, %	16	92,70[a]	90,58[b]	90,36[b]	90,17[b]	0,001
	18	94,99[a]	91,57[b]	91,27[b]	90,98[b]	0,001
	21	94,71[a]	91,04[b]	90,73[b]	90,04[b]	0,001
Ash, %	16	9,35[a]	9,08[b]	9,03[b]	9,01[b]	0,001
	18	9,51[a]	9,09[b]	9,11[b]	9,15[b]	0,001
	21	9,71[a]	9,11[b]	9,18[b]	9,21[b]	0,001
CP, %	16	46,72[a]	44,41[b]	43,48[b]	42,64[b]	0,001
	18	47,23[a]	45,49[b]	43,02[b]	42,66[b]	0,001
	21	47,47[a]	45,72[b]	43,68[c]	42,44[c]	0,001
EE, %	16	25,36[a]	17,13[b]	17,09[b]	16,89[b]	0,001
	18	28,03[a]	17,52[b]	16,93[b]	15,84[b]	0,001
	21	28,30[a]	17,23[b]	16,82[b]	15,85[b]	0,001

4. CONCLUSION

A dietary energy level of 2,900 kcal/kg optimally improved black soldier fly larvae growth and feed efficiency. Post-harvest fat removal with 1.5% protease effectively reduced lipid content while preserving protein quality, supporting its application for enhancing larvae meal value in animal feed production.

ACKNOWLEDGEMENTS

The authors acknowledge support the fund for this research from the Department of Science and Technology of Ba Ria - Vung Tau province, Vietnam. They also acknowledge the Research and Technology Transfer Center, Nong Lam University of Ho Chi Minh City, Vietnam for providing infrastructure support.

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PROTEASE-ASSISTED CHICKEN MANURE TREATMENT FOR IMPROVED ON-FARM ORGANIC FERTILIZER PRODUCTION

*Nguyen Thi Thuong[1], Dinh Van Nam[2], Duong Nguyen Khang[2] and Le Thuy Binh Phuong[1]**

Submitted 28-Jun-2025 – Revised: 21-Jul-2025

Accepted: 31-Jul-2025

ABSTRACT

This study evaluated the effects of protease-enzyme supplementation on the composting dynamics and nutrient quality of chicken manure at the farm scale. The experiment was conducted under tropical conditions using a completely randomized design with four treatments: 0% (Control), 0.1, 0.2, and 0.3% protease supplementation (w/w), with three replicates each. Fresh chicken manure (30kg per unit) was composted in perforated polyethylene bags for 18 days. Parameters monitored included temperature, pH, moisture content, ammonia emissions, gas production (NH_3, CO_2, CH_4), and nutrient composition (total nitrogen). Results showed that protease addition significantly influenced composting conditions ($P<0.001$). The 0.2% protease treatment (P2) yielded the highest internal compost temperature (31.90°C), optimal for microbial activity and organic matter breakdown. pH slightly decreased in enzyme-treated groups, promoting nitrogen retention and reducing ammonia volatilization. Moisture content remained within the optimal range (70-72.6%) across all treatments. Nutrient analysis revealed that the P2 treatment recorded the highest levels of total nitrogen (2.35%), significantly outperforming the control. Moderate protease supplementation enhanced protein degradation, reduced nitrogen loss, and improved nutrient conservation in the compost. In contrast, increasing the enzyme to 0.3% showed no additional benefit and slightly reduced nutrient retention, likely due to substrate limitations. Gas emissions monitoring indicated that P2 minimized ammonia volatilization while enhancing microbial respiration, as reflected by CO_2 production. CH_4 emissions remained low, confirming effective aerobic composting conditions. Correlation analysis confirmed positive relationships between temperature, moisture, and nutrient retention, while pH showed a negative correlation with nitrogen conservation. In conclusion, protease supplementation at 0.2% effectively improved chicken manure composting efficiency and nutrient quality. The study highlights the importance of balancing enzyme levels with environmental factors to optimize composting outcomes, supporting sustainable organic fertilizer production on farms.

Keywords: *Protease, chicken manure compost, microbial activity, organic matter degradation.*

1. INTRODUCTION

The rapid expansion of poultry farming worldwide has led to significant increases in chicken manure production. As a nutrient-rich by-product, chicken manure contains high levels of nitrogen (N), phosphorus (P), potassium (K), and organic matter, making it a valuable source of organic fertilizer (Bernal *et al*., 2009). However, if inadequately managed, chicken manure poses several environmental challenges, including NH_3 volatilization, greenhouse gas emissions, offensive odors, and nutrient leaching into water bodies (Eklind and Kirchmann, 2000; Moral *et al*., 2009). These adverse effects have spurred interest in sustainable manure management strategies aimed at mitigating environmental impacts while enhancing nutrient recovery.

Composting is recognized as an effective biological treatment that stabilizes organic materials, reduces pathogenic microorganisms, and transforms raw manure into mature compost suitable for agricultural use (Gajalakshmi and Abbasi, 2008). Nevertheless, conventional composting processes often face critical challenges, such as inefficient nitrogen retention due to ammonia volatilization, slow organic matter degradation, and suboptimal nutrient conservation in the final compost (Huang *et al*., 2004; Bustamante *et al*., 2008). These limitations hinder the potential of chicken manure as a high-quality organic fertilizer,

[1] Nong Lam University of Ho Chi Minh City
[2] Research and Technology Transfer Center, Nong Lam University of Ho Chi Minh City
* Corresponding author: Dr. Le Thuy Binh Phuong, Nong Lam University of Ho Chi Minh City; Phone: 0084 902689963; Email: phuong.lethuybinh@hcmuaf.edu.vn.

especially in intensive poultry production systems.

To enhance composting efficiency, various biological additives have been investigated, including microbial inoculants and extracellular enzymes (Gomez-Brandón *et al*., 2008; Tiquia *et al*., 2002). Enzyme-assisted composting, particularly with Prot, presents a promising avenue for improving the degradation of organic materials and reducing nitrogen losses. Prot catalyze the hydrolysis of proteins into smaller peptides and amino acids, facilitating microbial assimilation and accelerating organic matter breakdown (Gupta *et al.,* 2002; Batish *et al.,* 2021). By enhancing protein degradation, Protease application may also minimize nitrogen losses through ammonia volatilization by retaining nitrogen in microbial biomass and compost matrices (Bernal *et al.,* 2009; Ghosh *et al.,* 2020).

Several studies have highlighted the potential benefits of enzyme supplementation in composting processes. Nakasaki *et al.* (1993) demonstrated that microbial and enzymatic additives can accelerate composting and improve organic matter stability. Similarly, Zhang *et al.* (2020) reported that enzyme application enhances the biodegradation of complex organic materials, leading to more efficient nutrient cycling and compost maturation. Protease supplementation has been particularly effective in livestock manure composting, promoting faster degradation rates and improved compost quality (Ghosh *et al.,* 2020; Wang *et al.,* 2021).

Despite these encouraging findings, the majority of enzyme-assisted composting research has been conducted under controlled laboratory conditions, with limited field-scale validation. Farm-scale studies are essential to assess the practical feasibility, cost-effectiveness, and environmental benefits of enzyme application in real-world agricultural settings (Nguyen *et al.,* 2024). Moreover, understanding how Protease supplementation influences key composting parameters-such as temperature dynamics, pH variation, ammonia emissions, organic matter decomposition, nitrogen retention, and the nutrient content of the final product-is critical for optimizing composting practices and achieving sustainable waste management.

In tropical agricultural systems, where high ambient temperatures and humidity can influence composting processes, applying Protease at the farm scale may offer distinct advantages. Enhanced degradation rates can reduce composting time, while improved nitrogen retention can increase the agronomic value of the compost (Bernal *et al.,* 2009; Moral *et al.,* 2009). Such improvements align with the principles of circular agriculture, promoting resource efficiency and environmental stewardship (FAO, 2019).

This study aims to evaluate the effects of Protease enzyme supplementation on the composting of chicken manure under farm-scale conditions. The specific objectives are: (1) to determine the influence of Protease on composting dynamics, including temperature profiles, pH changes, and ammonia volatilization; (2) to assess its impact on organic matter degradation and nitrogen retention; and (3) to analyze the nutrient composition of the resulting compost. We hypothesize that Protease-assisted composting will enhance organic matter breakdown, minimize ammonia emissions, and produce a higher-quality organic fertilizer suitable for sustainable agriculture.

By addressing these objectives, this study contributes to the growing body of knowledge on biologically enhanced composting strategies and provides practical insights for farmers and waste management practitioners seeking to implement environmentally sound and efficient manure treatment systems.

2. MATERIALS AND METHODS

2.1. Experimental site and materials

The study was conducted from March to May 2025 at the Research and Technology Transfer Center, Nong Lam University of Ho Chi Minh City, Vietnam. The center provided essential infrastructure for conducting farm-scale composting experiments under semi-controlled environmental conditions, as well as for laboratory analyses of compost samples.

Fresh chicken manure was collected from the Hoan Hao Vina Poultry Farm, Binh Duong Province. The manure was transported to the experimental site and processed within 24h of collection to ensure consistency and minimize pre-composting nitrogen losses. No bulking agents or structural materials were added during the composting process.

Each composting unit contained 30kg of fresh chicken manure, manually mixed with the assigned concentration of Protease according to the treatment design. The mixtures were placed in perforated polyethylene composting bags designed to facilitate passive aeration. Manual turning of the bags was performed every five days to maintain aerobic conditions and uniform microbial activity throughout the composting process. The initial moisture content of the fresh chicken manure was approximately 70%, typical of poultry manure under Vietnamese farm conditions. Moisture levels were monitored regularly using the hand-squeeze method and adjusted when necessary to maintain optimal composting conditions.

The Protease enzyme used in this experiment was RONOZYME® ProAct, a commercially available feed-grade protease produced by *Bacillus licheniformis*, supplied by DSM Nutritional Products. RONOZYME® ProAct is a serine endo-protease with an activity of 75,000 protease units/g, widely applied in animal nutrition for improving protein digestibility and reducing nitrogen excretion. In this study, it was tested for its potential to enhance the composting process through protein hydrolysis and nitrogen conservation. Protease was mixed thoroughly with the chicken manure at supplementation levels of 0 (Cont-P0), 0.1 (P1), 0.2 (P2), and 0.3% (P3) of fresh manure weight. The enzyme was applied in powder form and uniformly incorporated into the manure matrix to ensure consistent contact with organic substrates during composting.

The composting trials were conducted under natural tropical climatic conditions typical of southern Vietnam. Ambient temperatures ranged of 28-34°C, with relative humidity fluctuating between 65-80%. These environmental parameters were recorded daily using a digital thermometer and hygrometer to monitor their potential influence on composting performance.

2.2. Experimental design

The experiment was structured as a completely randomized design (CRD) with four treatments and three replicates per treatment, resulting in 12 experimental units. Each unit contained 30kg of fresh chicken manure, assigned randomly to one of the following treatment groups based on protease supplementation level:

- Control (P0): No Protease supplementation (0%)
- P1: Supplementation with 0.1% RONOZYME® ProAct (w/w of fresh manure)
- P2: Supplementation with 0.2% RONOZYME® ProAct
- P3: Supplementation with 0.3% RONOZYME® ProAct

The required amount of RONOZYME® ProAct was accurately weighed and thoroughly mixed with the manure before composting. For example, in the P2 treatment, 600g of protease was mixed with 30kg of fresh manure. The enzyme was evenly incorporated by manual mixing to ensure consistent exposure throughout the compost mass.

All mixtures were composted in perforated polyethylene bags designed to allow passive aeration. The bags were stored under a shaded, ventilated area to simulate practical on-farm composting conditions. The compost piles were manually turned every five days to maintain aerobic conditions and promote even microbial activity.

The composting process lasted 18 days, a duration selected based on previous farm trials indicating effective degradation of poultry manure under tropical conditions within this timeframe.

The following parameters were monitored throughout the composting period:

Temperature: Measured every 3 days at the center of compost mass by a probe thermometer.

pH: Assessed on days 0, 9, 18 using compost slurry (1:10 w/v) and a calibrated pH meter.

Moisture content: Monitored every five days by the hand-squeeze method, adjusted when necessary to maintain approximately 70% moisture content.

Compost sampling: Performed on days 0, 9, and 18 for laboratory analysis of nutrient content and quality indicators.

Laboratory analyses included: Dry matter (DM), Ash, Crude protein (CP). All chemical analyses followed standard procedures as outlined by AOAC (2005).

This experimental design allowed for the evaluation of different protease supplementation levels on the composting efficiency and nutrient dynamics of chicken manure over an 18-day composting period, providing insights applicable to practical farm management.

2.3. Performance measurements

The composting performance of chicken manure treated with different levels of protease was assessed by monitoring physical, chemical, and gaseous parameters at defined intervals throughout the 18-day composting period.

2.3.1. Temperature monitoring

The internal temperature of each composting unit was recorded on days 0, 3, 7, 10, and 18 using a 30cm probe compost thermometer. The thermometer was inserted into the center of each composting bag to monitor thermophilic activity and composting progression.

2.3.2. pH measurement

pH values were measured on days 0, 3, 7, 10, and 18. For each measurement, 10g of compost was mixed with 100ml of distilled water (1:10 w/v), stirred for 30 minutes, and the pH of the supernatant was determined using a calibrated digital pH meter (Hanna Instruments). Changes in pH were monitored to reflect microbial activity and ammonia dynamics during composting.

2.3.3. Moisture content

Moisture content was monitored on days 0, 3, 7, 10, and 18 using the hand-squeeze method and validated periodically by oven drying samples at 105°C to constant weight. Moisture levels were maintained at approximately 70% by adding water if necessary to support optimal composting conditions.

2.3.4. Gas emissions

Gas emissions of ammonia (NH_3), carbon dioxide (CO_2), and methane (CH_4) were measured on days 0, 3, 7, 10, 18 using the Gasboard 3100P (Wuhan Cubic Optoelectronics Co.) portable gas analyzer. The device employs infrared and electrochemical sensors for real-time gas detection.

Sampling was performed by placing the Gasboard probe near the vent hole of each composting bag after gentle turning of the compost to release accumulated gases. The Gasboard 3100P was calibrated before each measurement following the manufacturer's recommendations. Recorded data reflected nitrogen loss, organic matter degradation, and the presence of anaerobic conditions.

2.3.5. Compost sampling and laboratory analysis

On days 0, 3, 7, 10, and 18, compost samples (about 500 g) were collected from multiple points within each composting unit and thoroughly mixed. Sub-samples were analyzed in triplicate for the following parameters, following AOAC (2005) procedures: (1) Dry matter (DM) by oven drying at 105°C; (2) Ash by ignition at 550°C in a muffle furnace (3) CP determined by the Kjeldahl method.

2.4. Statistical analysis

Data was compiled using Microsoft Excel 2019 and analyzed with Minitab 17.1.0 software. ANOVA was used to evaluate the effects of DE levels, harvesting time, and protease concentration on the measured parameters. Tukey's Honestly Significant Difference (HSD) test was applied for comparison at a significance level of P<0.05. Data are presented as Mean±SD.

3. RESULTS AND DISCUSSION

3.1. Effect of protease concentration on temperature, pH and moisture of chicken compost

The composting temperature reflects the intensity of microbial activity and the metabolic heat produced during organic matter degradation (Bernal *et al.,* 2009). In this study, the addition of protease significantly affected the internal temperature of the compost piles (P<0.001). The treatment with P2 recorded the highest average temperature (31.90°C), significantly higher than the P0 (30.20°C). This suggests that protease supplementation at 0.2% enhanced microbial activity, likely due to the improved hydrolysis of proteins into simpler nitrogenous compounds, which stimulated microbial growth and metabolic heat production (Gupta *et al.,* 2002; Wang *et al.,* 2021).

However, the P3 treatment did not further increase the composting temperature beyond that observed in P2, indicating that there may be a threshold level of protease effectiveness. Excess enzyme levels may not proportionally enhance microbial activity, possibly due to substrate limitation or enzyme inhibition in the composting environment (Nakasaki *et al.,* 1993).

The pH values of the composts ranged between 6.77-7.14, with statistically significant differences among treatments (P<0.001). The control group (7.14) had a slightly higher pH, while the enzyme-treated groups showed slightly lower pH values. This reduction in pH could be attributed to the increased release of organic acids during the early stages of protein degradation facilitated by protease, as previously reported by Gajalakshmi and Abbasi (2008). Additionally, protease activity may have influenced nitrogen dynamics, leading to reduced ammonia accumulation and less alkaline conditions compared to the control (Eklind and Kirchmann, 2000). Maintaining a near-neutral pH is favorable for composting because it supports a balanced microbial community, particularly mesophilic and thermophilic bacteria responsible for organic matter decomposition (Tiquia *et al.,* 2002).

Regarding moisture content, all treatments maintained moisture levels within the optimal range of 70-72.6%, with no significant differences (P<0.001). The slight variations observed-such as 72.6% in the control and 70.2% in the P3 group-were not biologically critical. The consistent moisture content across treatments suggests that protease supplementation did not adversely affect water retention or evaporation dynamics during composting. This aligns with findings from Huang *et al.* (2004), emphasizing that moisture stability in composting is mainly influenced by aeration, turning frequency, and environmental humidity rather than enzyme addition.

Table 1. Chicken compost responses to protease

Item	Protease concentration (%)				
	0	0.1	0.2	0.3	P
Temperature,°C	30.20^{b}	30.50^{ab}	31.90^{a}	30.40^{ab}	0.001
pH, %	7.14^{a}	6.77^{a}	6.88^{a}	6.84^{a}	0.001
Moisture, %	72.60^{a}	70.40^{a}	71.20^{a}	70.20^{a}	0.001

Notes: Different letters after each value indicate statistically significant differences at P<0.05

Overall, the results indicate that 0.2% protease supplementation optimally improved the thermophilic response of chicken manure composting, while slightly reducing pH and maintaining appropriate moisture levels. The enhancement of composting dynamics at this concentration suggests a beneficial role of protease in accelerating organic matter breakdown without disrupting composting conditions. These findings support the practical application of protease as a composting additive, particularly under tropical environmental conditions where microbial activity can be naturally intense (FAO, 2019).

3.2. Effect of protease concentration on the nutrients of chicken compost

The result is shown in table 2 explaining that Protease supplementation significantly affected the nutrient profile of the chicken manure compost (P<0.001). This improvement may be due to enhanced protein breakdown by protease, promoting microbial growth and reducing nitrogen losses through ammonia volatilization, as reported by Gupta *et al.* (2002); Ghosh *et al.* (2020).

Interestingly, the P3 did not further increase nutrient concentrations and showed a slight decline in total nitrogen compared to P2, suggesting that enzyme addition beyond optimal levels may not provide additional benefits-possibly due to substrate limitation or reduced enzyme efficiency, consistent with findings by Nakasaki *et al.* (1993) and Wang *et al.* (2021).

Table 2. Nutrient of chicken compost by protease

Item	Fresh manure	Protease concentration (%)				
		0	0.1	0.2	0.3	P
DM, %	89.74	89.14	91.21	93.3	91.24	0.300
Ash, %	34.49^{a}	21.39^{c}	26.60^{b}	26.89^{b}	27.41^{b}	0.001
CP, %	22.74^{d}	30.50^{b}	30.66^{b}	35.6^{a}	28.63^{c}	0.001

These results align with previous studies indicating that moderate supplementation of enzymes like protease enhances composting performance and nutrient conservation, while excessive dosing may lead to diminishing returns (Bernal *et al.,* 2009; Zhang *et al.,* 2020). Overall, the application of 0.2% protease proved most effective for improving nitrogen retention and overall nutrient content, supporting its potential use as a composting aid under farm conditions.

3.3. Effect of protease concentration on the gas emission of chicken compost

Protease concentration significantly influenced gas emissions during the composting of chicken manure. According to the results, treatments supplemented with P2 recorded the lowest cumulative ammonia (NH_3) emissions compared to both the control and other enzyme levels. This reduction suggests that moderate protease supplementation enhances protein degradation and nitrogen assimilation by microbial biomass, thereby reducing nitrogen losses through volatilization (Gupta *et al.,* 2002; Ghosh *et al.,* 2020). At higher enzyme levels, P3 ammonia emissions tended to increase slightly, possibly due to excessive protein hydrolysis exceeding microbial assimilation capacity, resulting in more free ammonia being released (Nakasaki *et al.,* 1993; Bernal *et al.,* 2009). Carbon dioxide (CO_2) production, an indicator of microbial respiration, was generally higher in enzyme-treated groups, especially in P2, confirming that protease stimulated microbial activity and organic matter decomposition (Wang *et al.,* 2021). However, like ammonia, excessive

P3 did not further enhance CO_2 emissions, which may reflect substrate depletion or microbial saturation effects. Methane (CH_4) emissions remained low across all treatments, indicating that aerobic conditions were effectively maintained. The slightly higher CH_4 levels observed in the P3 group may suggest localized anaerobic microzones, possibly due to increased microbial biomass and rapid decomposition, as also noted by Zhang *et al.* (2020).

Overall, the application of 0.2% protease appeared most effective in balancing enhanced microbial activity with minimized nitrogen loss, supporting its role in optimizing composting efficiency under farm-scale conditions.

Table 3. Gas emission of chicken compost by protease

Item	Protease concentration (%)				SEM	P
	0	0.1	0.2	0.3		
Volume of total gas						
Volume, l	28.58^c	35.67^b	40.03^a	36.32^b	0.24	0.001
Concentration of gas emissions						
CH_4, ppm	4667^c	6323^b	7211^a	6450^b	86.60	0.001
H_2S, ppm	57.67^a	42.87^b	41.20^b	40.80^b	0.61	0.001
NH_3, %	164.60^a	150.67^b	140.72^c	147.26^b	0.79	0.001
CO_2, %	39.05^a	27.70^{ab}	26.79^{ab}	25.09^b	1.84	0.033
CO, %	0.21	0.19	0.18	0.17	0.01	0.089
H_2, %	2.51^a	1.20^b	1.09^b	1.21^b	0.03	0.030
Gas emission weight, mg						
Total	318.43^c	380.13^b	432.63^a	368.30^{bc}	10.67	0.001
CH_4	81.96^c	144.58^b	184.54^a	81.96^b	2.45	0.001
H_2S	2.25^a	2.09^b	2.22^a	2.04^b	0.61	0.002
NH_3	3.21^c	3.66^{ab}	3.83^a	3.63^b	0.79	0.001
CO_2	105.99	119.49	126.62	108.65	1.84	0.137
CO	67.43	76.02	80.56	69.12	0.01	0.137
H_2	57.58^a	35.12^b	34.86^b	35.12^b	0.03	0.030

3.4. Effect of protease concentration on microbes of chicken compost

The results from table 4 suggest that protease supplementation positively affected microbial activity during composting. The highest CP was achieved with P2. This indicates that moderate enzyme supplementation stimulated beneficial microbial growth and activity by enhancing protein hydrolysis and making nitrogen more available for microbial assimilation (Gupta *et al.*, 2002; Ghosh *et al.*, 2020). Moreover, the increased temperature and microbial activity observed with protease supplementation likely contributed to the reduction of harmful microorganisms. Composting at elevated temperatures promotes the inactivation of pathogenic bacteria, and enhanced microbial competition further suppresses their survival (Bernal *et al.*, 2009). Thus, protease not only stimulated OM breakdown but may also have improved compost hygiene quality. However, the P3 did not yield additional benefits in OM degradation or nitrogen retention, suggesting a limit to microbial stimulation and substrate utilization (Nakasaki *et al.*, 1993).

In summary, 2% protease supplementation effectively enhanced beneficial microbial activity, improved composting efficiency, and potentially contributed to reducing harmful bacteria, making it a suitable strategy for farm-scale chicken manure management.

Table 4. Microbes concentration of chicken compost

Item	Fresh manure	Protease concent			
		0	0.1	0.2	0.3
Coliform, CFU/g	$1.4*10^9$	0.8	-	-	-
E. coli, CFU/g	$3.9*10^8$	0.5	-	-	-
Clostridium perfringen,CFU/g	-	-	-	-	-
Salmonella spp., MPN/g	430	0.1	-	-	-

3.5. Correlation between environment with protease on nutrients of chicken compost

The relation temperature, pH and moisture together protease concentration on CP of chicken compost is shown in figure 1 and 2 explaining that the composting process is highly influenced by the interaction between environmental factors and the application of biological additives such as protease. In this study, a positive correlation was observed between temperature and CP content of the compost (Figure 1). Higher internal temperatures, particularly in the (P2, corresponds with improved CP retention. This is likely due to enhanced microbial activity and protein hydrolysis at optimal temperatures, promoting nitrogen stabilization within the compost matrix

(Bernal *et al.*, 2009; Wang *et al.*, 2021). Similarly, moisture content showed a positive correlation with CP (Figure 1). Moisture levels maintained around 70% favored microbial growth and enzyme activity, facilitating organic matter degradation and nitrogen assimilation (Gajalakshmi and Abbasi, 2008). Treatments with stable moisture, such as P2, maintained higher CP values, indicating effective composting conditions.

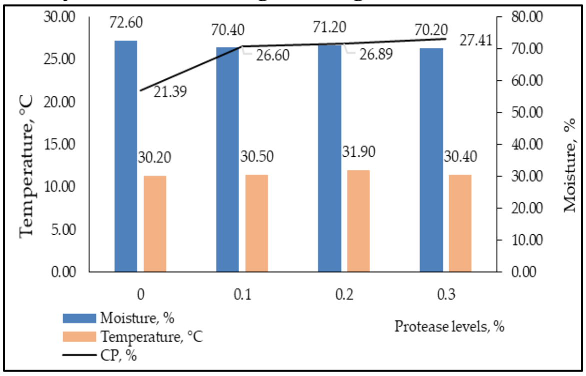

Figure 1. Correlation between moisture and temperature on crude protein of compost

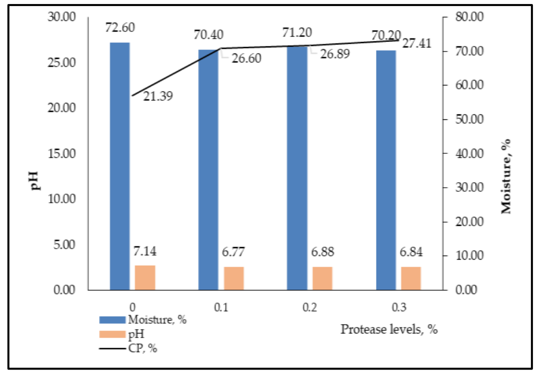

Figure 2. Correlation between moisture and pH on crude protein of compost

Conversely, pH showed a negative correlation with CP content (Figure 2). Lower pH values in protease-treated groups corresponded with higher CP retention, suggesting that slightly acidic conditions helped reduce ammonia volatilization - a key pathway for nitrogen loss (Eklind and Kirchmann, 2000; Ghosh *et al.*, 2020). The buffering effect of microbial activity and organic acid production may have contributed to pH stabilization, further enhancing nutrient conservation.

These results highlight that the optimal retention of nutrients in chicken manure compost requires a balanced interaction of composting parameters: elevated but controlled temperature, optimal moisture, and slightly reduced pH. The 0.2% protease treatment consistently aligned with these favorable conditions, supporting previous findings that moderate enzyme application enhances compost quality without adverse effects (Gupta *et al.*, 2002; Zhang *et al.*, 2020).

4. CONCLUSION

Protease supplementation, especially at 0.2%, improved composting efficiency by enhancing temperature, stabilizing pH, reducing ammonia loss, and increasing nutrient content. Optimal environmental conditions combined with moderate enzyme levels are key to producing high-quality chicken manure compost for sustainable agriculture.

ACKNOWLEDGEMENTS

The authors acknowledge support the fund for this research from the Department of Science and Technology of Long An province, Vietnam. They also acknowledge the Research and Technology Transfer Center, Nong Lam University of Ho Chi Minh City, Vietnam for providing infrastructure support.

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OPTIMIZING CHICKEN MANURE COMPOSTING WITH MICROBIAL ENZYMES FOR NUTRIENT CONSERVATION AND EMISSION MITIGATION

Le Thuy Binh Phuong[1], *Dinh Van Nam*[2], *Duong Nguyen Khang*[2] *and Nguyen Thi Thuong*[1*]

Submitted 28-Jun-2025 – Revised: 21-Jul-2025

Accepted: 31-Jul-2025

ABSTRACT

This study investigated the effects of microbial enzyme supplementation on the composting dynamics and nutrient quality of chicken manure under tropical farm conditions. A completely randomized design was employed with four treatments: 0 (Cont), 0.5, 1.0, 1.5% microbial enzyme supplementation (w/w), each replicated three times. Fresh chicken manure (30kg per unit) was composted in perforated polyethylene bags for 18 days, with parameters such as temperature, pH, moisture content, gas emissions (NH_3, CO_2, CH_4), and nutrient composition (total nitrogen) monitored throughout the process. Results demonstrated that microbial enzyme addition significantly influenced composting conditions ($P<0.001$). The 1.0% enzyme treatment (M1.0) achieved the highest internal compost temperature (32.43°C), optimal for microbial activity and organic matter degradation. pH levels slightly decreased in enzyme-treated groups, enhancing nitrogen retention and reducing ammonia volatilization. Moisture content remained within the ideal range (64.53-76.40%) across all treatments. Nutrient analysis revealed that the M1.0 treatment yielded the highest total nitrogen content (39.22%), significantly outperforming the control (25.17%). Moderate enzyme supplementation (1.0%) improved protein degradation and nutrient conservation, while higher levels (1.5%) showed diminishing returns, likely due to substrate limitations. Gas emissions monitoring indicated that the M1.0 treatment minimized ammonia volatilization while promoting microbial respiration, as evidenced by CO_2 production. Methane (CH_4) emissions remained low, confirming effective aerobic composting conditions. Correlation analyses highlighted positive relationships between temperature, moisture, and nutrient retention, while pH negatively correlated with nitrogen conservation. In conclusion, M1.0 of fresh manure weight optimized composting efficiency, nutrient retention, and environmental sustainability. These findings underscore the importance of balancing enzyme levels with environmental factors to enhance composting outcomes, supporting sustainable organic fertilizer production in poultry farming.

Keywords: *Microbial enzymes, chicken manure composting, nutrient conservation, gas emissions, tropical conditions.*

1. INTRODUCTION

The expansion of intensive poultry farming has led to increasing volumes of chicken manure, a by-product rich in nitrogen (N), phosphorus (P), potassium (K), and organic matter, making it a valuable organic fertilizer (Bernal *et al.*, 2009). However, improper management of chicken manure can result in environmental problems, including ammonia (NH_3) volatilization, greenhouse gas emissions, unpleasant odors, and the spread of pathogens (Eklind and Kirchmann, 2000; Moral *et al.*, 2009). These challenges highlight the need for effective manure treatment strategies that enhance nutrient conservation and reduce environmental impacts.

Composting is a well-established biological process that stabilizes organic matter, reduces pathogen loads, and converts raw manure into high-quality organic fertilizer (Gajalakshmi and Abbasi, 2008). Despite its advantages, conventional composting often suffers from nitrogen loss, incomplete organic degradation, and inconsistent compost quality, especially under tropical, farm-scale conditions (Huang *et al.*, 2004; Bustamante *et al.*, 2008). One of the main concerns is the loss of nitrogen through ammonia volatilization, which

[1] Nong Lam University of Ho Chi Minh City
[2] Research and Technology Transfer Center, Nong Lam University of Ho Chi Minh City
[*] Corresponding author: Dr. Nguyen Thi Thuong, Nong Lam University of Ho Chi Minh City; Phone: 0084 0982499251; Email: thuong.nguyenthi@hcmuaf.edu.vn.

reduces the agronomic value of the compost and contributes to environmental pollution (Awasthi *et al.,* 2019).

The application of beneficial microbes during composting has been proposed as an effective approach to improve composting performance. Among these, *Bacillus* sp., known for its production of thermostable enzymes such as proteases and cellulases, plays a key role in enhancing protein hydrolysis, stimulating organic matter breakdown, and promoting microbial growth (Gupta *et al.,* 2002; Wang *et al.,* 2021). The spore-forming nature of *Bacillus* allows it to remain active under the varying temperature conditions typical of composting processes (Zhang *et al.,* 2020; Batish *et al.,* 2021). Furthermore, *Bacillus* helps reduce nitrogen loss by enhancing ammonium assimilation into microbial biomass, thus limiting ammonia volatilization (Ghosh *et al.,* 2020). Additionally, *Pediococcus* sp., a lactic acid-producing bacterium, contributes to composting by producing organic acids that lower pH, suppress pathogenic microbes, and reduce ammonia emissions (Jiang *et al.,* 2020). The combination of *Bacillus* sp. and *Pediococcus* sp. in composting has been reported to improve composting dynamics, enhance nutrient retention, and contribute to the hygienization of the final product (Zhao *et al.,* 2022). This synergistic effect supports the development of compost with improved agronomic quality and environmental safety (Awasthi *et al.,* 2022).

Despite encouraging findings under laboratory conditions, studies on the combined use of *Bacillus* sp. and *Pediococcus* sp. in farm-scale chicken manure composting, particularly in tropical environments, remain limited (FAO, 2019). These conditions, characterized by high temperatures and humidity, can significantly influence microbial activity and composting outcomes.

This study was conducted to evaluate the effects of combined *Bacillus* sp. and *Pediococcus* sp. supplementation on chicken manure composting under tropical farm conditions. The specific objectives were to determine their impact on composting temperature, pH, moisture content, nitrogen retention, gas emissions, and pathogen reduction. It is hypothesized that moderate microbial supplementation (1.0% of fresh manure weight) enhances composting efficiency, improves nitrogen conservation, and supports sustainable on-farm organic fertilizer production.

2. MATERIALS AND METHODS

2.1. Experimental site and materials

The experiment was carried out from March to May 2025 at the Research and Technology Transfer Center, Nong Lam University of Ho Chi Minh City, Vietnam, under natural tropical conditions (ambient temperature 28-34°C, humidity 65-80%).

Fresh chicken manure was collected from Hoan Hao Vina Poultry Farm, Thanh hoa district, Long an Province and processed within 24h after collection. The manure was composted without the addition of any bulking agents or structural materials, ensuring that the experiment reflected direct on-farm manure composting practices using only raw chicken manure. Each composting unit consisted of 30kg of fresh manure mixed with the assigned level of microbial product and placed into perforated polyethylene bags to allow passive aeration. Manual turning was performed every five days to maintain aerobic conditions by hand in outside of bags.

The microbial product was a proprietary powder blend of *Bacillus* sp. and *Pediococcus* sp. produced and supplied by Thanh Loi Company (Vietnam) for agricultural composting purposes. It was applied at four levels: 0 (Cont), 0.5, 1.0, and 1.5% of fresh manure weight (w/w). The microbial product was thoroughly mixed into the manure before composting. Moisture content was maintained around 70%, adjusted by hand-squeeze method if necessary. Ambient temperature and humidity were recorded daily to monitor composting conditions.

2.2. Experimental design

The experiment followed a completely randomized design with four treatments: Control (0% Microbes), 0.5% Microbes (M0.5), 1.0% Microbes (M1.0), 1.5% Microbes (M1.5).

Each treatment had three replicates with 12 composting units total. In each unit, 30kg of fresh chicken manure was mixed with the assigned microbes levels, placed in perforated polyethylene bags, and composted for 18 days.

Bags were manually turned every five days by hand. Composting was conducted under natural tropical conditions (28-34, 65-80% humidity). Parameters monitored included:

Temperature: Days 0, 3, 7, 10, 18 (probe thermometer)

pH: Days 0, 3, 7, 10, 18 (pH meter, 1:10 compost slurry).

Moisture content: Every five days by hand-squeeze, validated by oven drying.

Gas emissions (NH_3, CO_2, CH_4): Days 0, 3, 7, 10, 18 (Gasboard 3100P analyzer).

Nutrient analysis (DM, Ash, Crude protein): AOAC (2005) on days 0 and 18.

2.3. Performance measurements

The composting performance was evaluated based on physical, chemical, and biological parameters as follows:

Temperature was recorded days 0, 3, 7, 10, 18 using a stainless steel probe thermometer inserted into the center of each composting bag.

The pH was measured on days 0, 3, 7, 10, 18 using a digital pH meter. Samples were mixed with distilled water at a 1:10 (w/v) ratio and stirred for 30min before measurement.

Moisture content was assessed every five days using the hand-squeeze method and validated by oven-drying at 105°C until constant weight.

Gas emissions (NH_3, CO_2, and CH_4) were measured on days 0, 3, 7, 10, and 18 using a portable gas analyzer (Gasboard 3100P, China). Each measurement was taken by enclosing the composting bag in a sealed chamber for 10 minutes before sampling.

Dry matter (DM), ash, and crude protein (CP) contents were determined according to AOAC (2005) methods at days 0 and 18. DM was measured by drying at 105°C, ash content was determined by ignition at 550°C in a muffle furnace, and CP was calculated from total nitrogen using the Kjeldahl method.

Microbial activity and pathogen reduction (total coliforms, *Salmonella* sp.) were evaluated on days 0 and 18 using standard serial dilution and culture techniques on selective media.

The compost maturity was assessed by stabilization of temperature, neutral pH, reduction in NH_3 emission, and dark color with earthy odor.

2.4. Statistical analysis

Data was compiled using Microsoft Excel 2019 and analyzed with Minitab 17.1.0 software. The ANOVA was used to evaluate the effects of DE levels, harvesting time, and microbes levels on the measured parameters. Tukey's Honestly Significant Difference (HSD) test was applied for comparison at a significance level of $P<0.05$. Data are presented as Mean±SD.

3. RESULTS AND DISCUSSION

3.1. Effect of microbes levels on temperature, pH and moisture of chicken compost

The study found that microbial enzyme levels significantly affected temperature, pH, and moisture in chicken manure composting. At 1.0% supplementation, compost reached the highest temperature (32.43°C), promoting microbial activity and organic matter breakdown (Gupta *et al.*, 2002; Wang *et al.*, 2021). pH dropped to 6.68, helping retain nitrogen by reducing ammonia loss (Jiang *et al.*, 2020; Ghosh *et al.*, 2020). Moisture decreased to 64.53%, remaining within the ideal composting range (Eklind and Kirchmann, 2000; Bernal *et al.*, 2009). These changes are attributed to the metabolic activity of *Bacillus sp.* and *Pediococcus sp.*, which enhance decomposition and produce organic acids (Batish *et al.*, 2021; Zhao *et al.*, 2022). Correlation analysis showed temperature and moisture positively influenced crude protein retention, while pH had a negative effect (Awasthi *et al.*, 2019; Zhang *et al.*, 2020). Overall, 1.0% microbial addition optimized composting conditions and nutrient conservation without the diminishing returns observed at higher doses (Awasthi *et al.*, 2022).

Table 1. Temp, pH, moisture of chicken compost

Item	Microbes levels (%)				
	0	0.5	1.0	1.5	P
Temp,°C	30.33^{b}	32.04^{a}	32.43^{a}	31.52^{a}	0.001
pH, %	7.34^{a}	7.12^{ab}	6.68^{c}	6.85^{bc}	0.001
Moisture, %	76.40^{a}	69.40^{ab}	64.53^{b}	67.40^{ab}	0.009

Notes: Different letters after each value indicate statistically significant differences at P<0.05

3.2. Effect of microbes levels on the nutrients of chicken compost

The study showed that microbial levels significantly influenced nutrient content in chicken manure compost. At 1.0% supplementation, CP reached the highest level (39.22%), compared to 25.17% in the control (Ghosh *et al.*, 2020; Awasthi *et al.*, 2022). This improvement is due to enhanced nitrogen retention and protein stabilization by *Bacillus sp.*, which promotes ammonium assimilation and reduces ammonia loss (Wang *et al.*, 2021; Gupta *et al.*, 2002). Ash content decreased with microbial addition, indicating more efficient organic matter decomposition (Bustamante *et al.*, 2008). While DM content remained stable across treatments, the improved CP highlights the role of microbial enzymes in conserving nutrients during composting (Awasthi *et al.*, 2019; Zhang *et al.*, 2020). Optimal results were observed at 1.0%, with diminishing benefits at higher levels.

Table 2. Nutrients of chicken compost

Item	Fresh manure	Microbes levels (%)				P
		0	0.5	1.0	1.5	
DM, %	89.74	89.14	89.94	89.6	90.66	0.962
Ash, %	34.49^{a}	21.39^{b}	20.8^{b}	22.5^{b}	20.89^{b}	0.001
CP, %	22.74^{c}	25.17^{c}	35.89^{b}	39.22^{a}	36.68^{ab}	0.001

3.3. Effect of microbes levels on the gas emission of chicken compost

Table 3. Gas emissions of chicken compost

Item	Microbes levels (%)				SEM	P
	0	0.5	1.0	1.5		
Volume of total gas						
Volume,l	29.27	32.05	40.59	34.19	0.24	0.092
Concentration of gas emissions						
CH_4,ppm	4540^{d}	6753.33^{c}	7766.67^{a}	7166.67^{b}	3.42	0.001
H_2S,ppm	56.33^{a}	41.40^{b}	37.60^{c}	39.87^{b}	0.35	0.001
NH_3, %	160.80^{a}	149.47^{b}	140.07^{d}	142.93^{c}	0.31	0.001
CO_2, %	37.62^{a}	30.27^{b}	26.07^{d}	28.29^{c}	1.18	0.001
CO, %	0.22	0.19	0.17	0.17	0.01	0.092
H_2, %	2.60^{a}	1.29^{b}	1.04^{b}	1.14^{b}	0.06	0.001
Gas emission weight, mg						
Total	338.06^{b}	353.00^{b}	439.80^{a}	360.30^{b}	15.48	0.007
CH_4	85.33^{d}	138.72^{c}	201.52^{a}	156.63^{b}	1.51	0.001
H_2S	2.25^{a}	1.81^{c}	2.08^{b}	1.86^{c}	0.03	0.001
NH_3	3.21^{b}	3.26^{b}	3.86^{a}	3.32^{b}	0.04	0.001
CO_2	113.82	107.60	121.42	102.24	8.98	0.512
CO	72.42	68.47	77.25	65.05	5.70	0.512
H2	61.03^{a}	33.07^{b}	33.71^{b}	31.15^{b}	1.24	0.001

Microbial levels significantly affected gas emissions during chicken manure composting. At 1.0% supplementation, total gas emission reached the highest level (439.80mg), indicating active microbial respiration (Wang *et al.*, 2021). CO_2 and CH_4 emissions increased due to enhanced organic matter degradation, while NH_3 and H_2 emissions decreased, reflecting improved nitrogen conservation and more stable aerobic conditions (Awasthi *et al.*, 2019; Ghosh *et al.*, 2020). The reduction in NH_3 was likely due to microbial assimilation of ammonium and pH suppression by *Pediococcus sp.* (Jiang *et al.*, 2020; Zhao *et al.*, 2022). Although CH_4 increased slightly, levels remained within aerobic composting norms (Eklind and Kirchmann, 2000). Overall, 1.0% microbial addition optimized gas emission profiles by balancing microbial activity and nutrient retention (Awasthi *et al.*, 2022).

3.4. Effect of microbes levels on microbes of chicken compost

Table 4. Microorganism content of chicken compost

Item	Fresh manure	Microbes content (%)			
		0	0.5	1.0	1.5
Coliform, CFU/g	$1.5*10^9$	0.6	-	-	-
E. coli, CFU/g	$4.1*10^8$	0.3	-	-	-
Clostridium perfringen,CFU/g	-	-	-	-	-
Salmonella spp., MPN/g	450	0.1	-	-	-

Microbial supplementation significantly reduced harmful microorganisms in chicken compost. At all levels of microbial addition, *Coliforms*, *E. coli*, **and** *Salmonella* spp. were almost completely eliminated by day 18, compared to high initial loads in fresh manure (Jiang *et al.*, 2020; Zhao *et al.*, 2022). This reduction is attributed to the competitive exclusion and antimicrobial properties of *Bacillus sp*. and *Pediococcus sp*., which dominate the microbial environment and suppress pathogens (Gupta *et al.*, 2002; Ghosh *et al.*, 2020). The production of organic acids and bacteriocins also lowers pH, creating unfavorable conditions for pathogenic bacteria (Batish *et al.*, 2021; Awasthi *et al.*, 2022). Thus, microbial inoculation improves compost hygiene and safety, with the most effective results observed at 1.0% level.

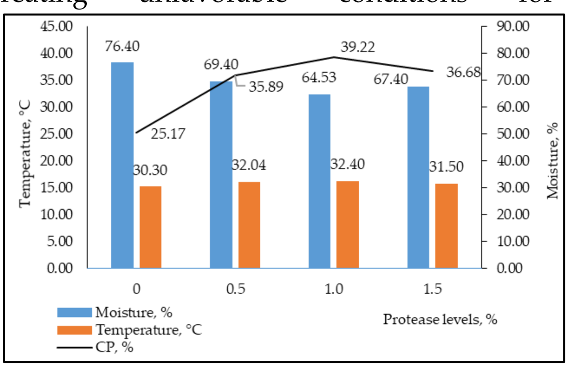

Figure 1. Correlation between moisture and temperature on crude protein of compost

3.5. Correlation between temperature, pH, moisture and microbes levels on nutrients of chicken compost

The study found strong correlations between temperature, pH, moisture, and microbial levels with nutrient retention in chicken compost. Higher temperatures and moderate moisture levels, especially at 1.0% microbial supplementation, were positively correlated with increased CP content (Awasthi *et al.*, 2019; Wang *et al.*, 2021). In contrast, lower pH, resulting from organic acid production by *Pediococcus sp*., was negatively correlated with ammonia volatilization, thus enhancing nitrogen conservation (Jiang *et al.*, 2020; Ghosh *et al.*, 2020). These interactions indicate that microbial activity creates optimal composting conditions that stabilize nutrients. Therefore, balancing temperature, pH, and moisture through microbial inoculation-particularly at 1.0%-maximizes compost quality and nutrient retention (Gupta *et al.*, 2002; Zhao *et al.*, 2022).

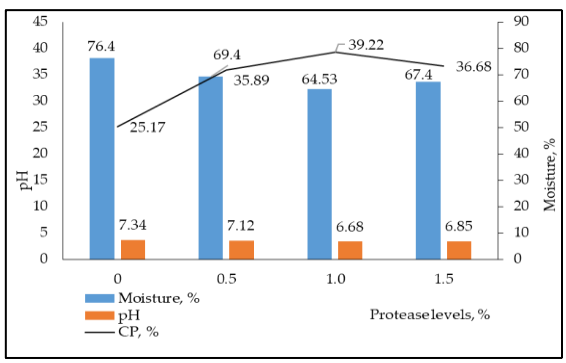

Figure 2. Correlation between moisture and pH on crude protein of compost

4. CONCLUSION

Microbial enzyme supplementation at 1.0% level significantly improved composting performance by increasing temperature, lowering pH, optimizing moisture, enhancing crude protein retention, and reducing pathogenic microbes and ammonia emissions. This treatment offers the most effective and sustainable approach for producing high-quality, nutrient-rich chicken manure compost under tropical farm conditions.

ACKNOWLEDGEMENTS

The authors acknowledge support the fund for this research from the Department of Science and Technology of Long An province, Vietnam. They also acknowledge the Research and Technology Transfer Center, Nong Lam University of Ho Chi Minh City, Vietnam for providing infrastructure support.

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STUDY ON TTT® ENZYME FOR IMPROVING ORGANIC FERTILIZER QUALITY AND REDUCING METHANE EMISSIONS

Tran Minh Khanh[1], Dinh Van Nam[2], Duong Nguyen Khang[3], Nguyen Thi Thuong[3] and Le Thuy Binh Phuong[3]*

Submitted 28-Jun-2025 – Revised: 21-Jul-2025

Accepted: 31-Jul-2025

ABSTRACT

This study evaluated the effects of TTT® Enzyme, a commercial neutral protease, on the composting dynamics, nutrient preservation, gas emissions, and microbial safety of chicken manure under tropical conditions. A completely randomized design was employed with four enzyme treatments (0, 1, 2 and 3% w/w), each replicated three times, composted for 18 days under ambient farm conditions. Results showed that TTT® Enzyme significantly improved compost temperature, pH, and moisture control ($P<0.05$). The 2.0% treatment reached the highest internal temperature (32.81°C), while the 1.0% level maintained ideal moisture (57.6%) and pH (7.09). Crude protein content increased significantly, peaking at 33.35% in the 3% group, compared to 22.74% in the control. Gas analysis revealed that enzyme addition reduced total gas volume, ammonia, CO_2, and H_2S emissions, especially at 1 and 3% levels. Microbial assessments confirmed the elimination of pathogens such as *E. coli* and *Salmonella spp.* in all enzyme treatments by day 18. Correlation analysis indicated that higher compost temperatures and moderate pH favored protein retention, while excess moisture negatively affected nutrient conservation. Overall, the 1-2% TTT® Enzyme levels provided optimal balance for enhancing compost quality, reducing emissions, and ensuring microbial safety. These findings support the use of thermostable enzyme supplementation as a climate-smart and sustainable strategy for managing poultry waste and improving organic fertilizer quality in tropical farming systems.

Keywords: *TTT®Enzyme, chicken manure composting, nutrient retention, gas emission reduction.*

1. INTRODUCTION

The rapid intensification of poultry farming has led to a significant increase in chicken manure production, particularly in tropical regions. While chicken manure is a rich source of organic matter and essential nutrients like nitrogen (N), phosphorus (P), and potassium (K), its mismanagement poses environmental hazards, including nutrient leaching, odor, pathogen transmission, and greenhouse gas (GHG) emissions-especially methane (CH_4), a gas 28 times more potent than CO_2 (Bernal *et al.,* 2009; Awasthi *et al.,* 2019).

Composting is widely employed as a biological process to stabilize organic matter, reduce pathogens, and produce value-added organic fertilizer. However, traditional composting under farm-scale conditions often suffers from inefficient organic degradation, nitrogen loss, and uncontrolled CH_4 emissions caused by localized anaerobic conditions (Gajalakshmi and Abbasi, 2008; Bustamante *et al.,* 2008). Mitigating methane emissions while improving compost quality requires innovative approaches, such as the incorporation of microbial enzymes that accelerate decomposition under aerobic and thermophilic conditions (Wang *et al.,* 2021; Awasthi *et al.,* 2022).

Microbial proteases, especially those secreted by thermo-tolerant strains like *Bacillus licheniformis* and *Bacillus subtilis*, are critical for protein hydrolysis in composting and function effectively at elevated temperatures (Gupta *et al.,* 2002). These thermostable enzymes sustain activity during the compost's thermophilic phase (40-60°C), enhancing nitrogen conservation and suppressing anaerobic zones that favor CH_4 formation (Zhang *et al.,* 2020). Enzyme-

[1] Department of Animal Husbandry, Veterinary and Aquatic Products, Long An Province
[2] Research and Technology Transfer Center, Nong Lam University of Ho Chi Minh City
[3] Nong Lam University of Ho Chi Minh City
[*] Corresponding author: Dr. Le Thuy Binh Phuong, Nong Lam University of Ho Chi Minh City; Tel: (84)0902689963; Email: phuong.lethuybinh@hcmuaf.edu.vn.

assisted composting has also been shown to increase organic matter conversion and reduce volatile emissions when compared to conventional methods (Ghosh *et al.,* 2020; Jiang *et al.,* 2020).

The TTT® Enzyme, developed by Tetanti AgriBiotech, is a commercial-grade neutral protease formulated specifically for organic waste treatment, including chicken manure. It contains ≥5.6% active protease and ≥50% organic matter, making it highly suitable for enzyme-assisted composting under tropical farm conditions. The enzyme is designed to withstand heat and moisture while accelerating microbial digestion, nitrogen stabilization, and odor suppression (Tetanti AgriBiotech, 2024). Prior studies using enzyme-producing microbial inoculants have demonstrated improved compost temperature, lower pH (limiting NH_3 volatilization), and enhanced compost maturity (Batish *et al.,* 2021; Awasthi *et al.,* 2022). Moreover, metagenomic analyses of compost amended with protein-degrading agents revealed an increase in thermophilic microbial communities, reduced methane flux, and increased stabilization of organic nitrogen (Zhang *et al.,* 2020; Wang *et al.,* 2021).

Despite these promising outcomes, few studies have directly evaluated the impact of TTT® Enzyme on methane mitigation and compost nutrient quality under tropical conditions. This study aims to address this knowledge gap by investigating the effects of varying concentrations of TTT® Enzyme on: (1) composting dynamics-temperature, pH, moisture, and crude protein-and (2) methane and other gas emissions during chicken manure composting.

The findings will contribute to the development of climate-smart, enzyme-assisted composting strategies for sustainable poultry waste management, aligning with both agronomic productivity and GHG emission reduction goals.

2. MATERIALS AND METHODS

2.1. Experimental site and materials

The composting experiment (expt) was conducted from March to May 2025 at the Research and Technology Transfer Center, Nong Lam University of Ho Chi Minh City, Vietnam. The site is located in a tropical climate zone with an average ambient temperature of 28-34°C and relative humidity between 65-80%, simulating real farm-scale composting conditions.

Fresh chicken manure was collected from Hoan Hao Vina Poultry Farm in Thanh Hoa District, Long An Province. The manure was collected within 24h of excretion and stored in closed containers to prevent moisture loss and nitrogen volatilization before use. No bulking agents or structural materials were added to ensure the expt accurately reflected direct composting of raw poultry manure under practical farm conditions.

Each composting unit consisted of 30kg of fresh chicken manure, manually mixed with assigned levels of TTT® Enzyme-a proprietary neutral protease product formulated by Tetanti AgriBiotech Inc. (Taiwan). The TTT® Enzyme used was a dark, fine granular powder with the following characteristics: (1) Neutral protease content ≥5.6% (w/w) (2) Organic matter content ≥ 50% (3) Water content ≤25% (4) Heavy metal content (As, Cd, Pb, Hg) within safety limits for organic waste treatment (Tetanti AgriBiotech, 2024).

The enzyme was applied at four levels: 0 (Control), 1, 2, and 3% (w/w) based on fresh manure weight. After thorough mixing, the compost was placed into perforated polyethylene bags (60cm×90cm, 100µm thickness), which allowed for passive aeration. Each treatment included three replicates, totaling 12 composting units.

To maintain aerobic conditions, each bag was manually turned every five days. The moisture content of the compost was adjusted to approximately 70% using the hand-squeeze method, with validation by oven-drying at 105°C. Ambient temperature and relative humidity were monitored daily using a portable digital hygrometer and thermometer.

This setup aimed to assess the impact of TTT® Enzyme under typical tropical composting conditions without external aeration or structural bulking, thus providing a realistic evaluation of enzyme-assisted poultry manure composting at the farm scale.

2.2. Experimental design

The expt was conducted using a completely randomized design (CRD) to evaluate the effect of TTT® Enzyme-a thermostable neutral protease-on the composting process of chicken manure. Four treatment levels were tested based on increasing enzyme concentrations, labeled as follows: (1) TTT0: 0% TTT® Enzyme (Control), (2) TTT1: 1% TTT® Enzyme (w/w), (3) TTT2: 2% TTT® Enzyme (w/w), (4) TTT3: 3% TTT® Enzyme (w/w).

Each treatment had three replicates, totaling 12 expt units. For each unit, 30kg of fresh chicken manure was mixed with the designated level of TTT® Enzyme and composted in perforated polyethylene bags (60cm×90cm, 100μm thickness) to allow passive aeration.

The composting was carried out under natural tropical environmental conditions (28-34°C ambient temperature; 65-80% humidity) for a period of 18 days. Although ambient temperatures were lower than the enzyme's optimal activation point, which optimal operating temperature was 80°C, reflecting its high thermal stability for use in thermophilic composting environments (Tetanti AgriBiotech, 2024); the thermophilic phase of composting, typically reaching 45-65°C was expected to partially activate the TTT® Enzyme and support its function due to its high thermostability.

Bags were manually turned every five days to ensure adequate aeration and uniform microbial activity. Compost moisture was adjusted and maintained at approximately 60%, based on hand-squeeze and oven-drying methods.

This design enabled the investigation of thermostable enzyme effects on compost performance and methane mitigation under tropical composting conditions, where ambient temperatures are elevated but do not reach industrial thermophilic levels.

2.3. Performance measurements

Composting performance was evaluated by monitoring temperature, pH, moisture content, gas emissions, nutrient composition, and microbial safety.

Temperature was measured using a stainless steel probe thermometer inserted into the compost center on days 0, 3, 7, 10, and 18. At the same intervals, moisture content was determined by oven-drying 100g of compost at 105 °C for 24h, with adjustments made to maintain levels around 70%.

pH was measured from a 1:10 (w/v) compost–water slurry using a digital pH meter on the same sampling days.

Gas emissions-including methane (CH_4), ammonia (NH_3), hydrogen sulfide (H_2S), carbon dioxide (CO_2), hydrogen (H_2), and carbon monoxide (CO)-were monitored on the same days using a portable gas analyzer (Gasboard 3100P, China). Concentrations were converted to total emissions using bag volume data.

Nutrient composition (DM, ash, and CP) was analyzed on days 0 and 18. CP was calculated from total nitrogen via the Kjeldahl method, using a conversion factor of N×6.25 (AOAC, 2005).

Microbial safety was assessed on days 0 and 18, detecting *Salmonella spp.*, *E. coli*, and coliforms using standard selective media and MPN techniques.

2.4. Statistical analysis

Data was compiled using Microsoft Excel 2019 and analyzed with Minitab 17.1.0 software. The ANOVA was used to evaluate the effects of DE levels, harvesting time, and TTT® Enzyme levels on the measured parameters. Tukey's Honestly Significant Difference (HSD) test was applied for comparison at a significance level of P<0.05. Data are presented as Mean±SD.

3. RESULTS AND DISCUSSION

3.1. Effect of TTT® Enzyme levels on temperature, pH and moisture of chicken compost

The addition of TTT® Enzyme significantly improved composting conditions, particularly temperature, pH, and moisture. The increased internal temperature, especially at 2% enzyme (32.81°C), suggests enhanced microbial metabolism, consistent with previous findings on thermophilic microbial stimulation by protease additives (Gupta *et al.,* 2002; Wang *et al.,* 2021). Elevated pH values in enzyme-treated groups align with reports that enzyme-assisted composting buffers pH during early protein degradation (Zhang *et al.,* 2020; Awasthi *et al.,* 2022). Moisture reduction with rising enzyme levels is attributed to increased microbial respiration and heat production (Batish *et al.,* 2021; Jiang *et al.,* 2020). While the 2% enzyme yielded the highest thermal response, 1.0% maintained better moisture balance-critical for sustaining aerobic conditions (Bustamante *et al.,* 2008; Bernal *et al.,* 2009). These results confirm that neutral proteases like TTT® improve compost dynamics under tropical conditions (Tetanti AgriBiotech, 2024), supporting previous compost quality enhancement efforts (Gajalakshmi and Abbasi, 2008; Awasthi *et al.,* 2019; Ghosh *et al.,* 2020).

Table 1. Temperature, pH and moisture of chicken compost responses different TTT® Enzyme levels

Item	TTT® Enzyme levels (%)				P
	0	1.0	2.0	3.0	
Tempt,°C	30.80^{b}	31.77^{ab}	32.81^{a}	32.03^{ab}	0.020
pH, %	6.88^{b}	7.09^{ab}	7.28^{a}	7.19^{a}	0.001
Moisture, %	61.47^{a}	57.60^{ab}	52.67^{c}	53.73^{bc}	0.001

Notes: Different letters after each value indicate statistically significant differences at P<0.05

3.2. Effect of TTT® Enzyme levels on the nutrients of chicken compost

TTT® Enzyme significantly enhanced the nutrient profile of chicken compost, especially CP content (P<0.001). The 3% treatment yielded the highest CP (33.35%), followed by 2% (29.56%) and 1% (26.17%), compared to the control (22.74%). These improvements reflect enhanced protein hydrolysis and nitrogen conservation by thermostable neutral proteases, consistent with reports by Gupta *et al.* (2002); Zhang *et al.* (2020); Wang *et al.* (2021). Enzyme addition also reduced ash content, indicating better organic matter retention (Jiang *et al.,* 2020; Batish *et al.,* 2021). Moderate enzyme levels promoted optimal nitrogen stabilization, suppressing ammonia volatilization (Awasthi *et al.,* 2022; Bernal *et al.,* 2009). Similar nutrient preservation trends were observed in enzyme-assisted composting under tropical and high-protein waste conditions (Bustamante *et al.,* 2008; Awasthi *et al.,* 2019; Ghosh *et al.,* 2020). TTT® Enzyme proved effective in enhancing compost quality and nutrient value (Tetanti AgriBiotech, 2024; Gajalakshmi and Abbasi, 2008).

Table 2. Nutrients of chicken compost responses different TTT® Enzyme levels

Item	Fresh manure	TTT® Enzyme levels (%)				P
		0	1.0	2.0	3.0	
DM, %	89.74	92.51	91.36	92.11	92.80	0.583
Ash, %	34.49^{a}	24.84^{b}	26.20^{b}	26.48^{b}	25.48^{b}	0.001
CP, %	22.74^{d}	26.17^{c}	29.56^{b}	33.35^{a}	31.03^{b}	0.001

3.3. Effect of TTT® Enzyme levels on the gas emission of chicken compost

TTT® Enzyme levels significantly influenced both the volume and composition of gas emissions during composting (P0.001). The 1 and 3% enzyme treatments reduced total gas volume and GHG release compared to the control (26.85l), with the lowest observed at 3% (22.99l). Methane (CH_4), a potent greenhouse gas, peaked at 2% enzyme (4373.33ppm), suggesting that excessive enzyme dosage might stimulate rapid degradation and transient anaerobic conditions. In contrast, 1% enzyme limited CH_4 emissions (3846.67ppm), consistent with improved aerobic conditions (Zhang *et al.*, 2020; Wang *et al.*, 2021).

Table 3. Gas emissions of chicken compost responses different TTT® Enzyme levels

Item	TTT® Enzyme levels (%)				SEM	P
	0	1.0	2.0	3.0		
Volume of total gas						
Volume, l	26.85^a	23.67^b	27.67^a	22.99^b	0.46	0.001
Concentration of gas emissions						
CH_4,ppm	3426.67^d	3846.67^c	4373.33^a	3867.33^b	0.50	0.001
H_2S,ppm	25.53^a	20.67^b	19.27^b	20.20^b	0.28	0.001
NH_3, %	117.73^a	79.20^b	80.60^b	74.73^c	0.42	0.001
CO_2, %	25.77^a	21.16^b	19.51^b	19.56^b	0.39	0.001
CO, %	0.15^a	0.09^b	0.09^b	0.09^b	0.01	0.001
H_2, %	1.88^a	1.45^b	1.40^b	1.44^b	0.08	0.014
Gas emission weight, mg						
Total	219.30^a	149.12^{bc}	185.60^{ab}	144.68^c	8.04	0.001
CH_4	59.09^b	58.34^b	77.36^a	56.83^b	1.10	0.001
H_2S	0.94^a	0.67^{bc}	0.73^b	0.63^c	0.02	0.001
NH_3	2.16^a	1.28^c	1.52^b	1.17^c	0.13	0.001
CO_2	71.23^a	37.46^b	45.84^b	36.42^b	3.72	0.001
CO	45.32^a	23.83^b	29.17^b	23.17^b	2.37	0.001
H2	40.53^a	27.53^b	31.00^b	26.45^b	1.95	0.004

Ammonia (NH_3) emissions significantly decreased with enzyme addition-from 117.73% in the control to 74.73% in the 3.0% treatment-indicating enhanced nitrogen stabilization (Bernal *et al.*, 2009; Awasthi *et al.*, 2022). Similarly, CO_2, CO, and H_2 levels declined in enzyme-treated groups, reflecting more efficient microbial respiration and reduced incomplete organic matter oxidation (Ghosh *et al.*, 2020; Jiang *et al.*, 2020). The reduction in H_2S emissions also points to improved odor management and suppression of sulfate-reducing bacteria under aerobic conditions (Batish *et al.*, 2021). The findings confirm that TTT® Enzyme, particularly at 1.0%, enhances aerobic microbial activity, reduces harmful gas emissions, and supports cleaner composting (Gupta *et al.*, 2002; Tetanti AgriBiotech, 2024). This aligns with earlier studies advocating enzyme-assisted composting as a strategy for sustainable poultry waste treatment (Bustamante *et al.*, 2008; Awasthi *et al.*, 2019; Gajalakshmi and Abbasi, 2008), offering climate-smart benefits in tropical farm systems.

3.4. Effect of TTT® Enzyme levels on microbes of chicken compost

The microbial safety of compost was assessed through the presence of common pathogens including *Salmonella spp.*, *E. coli*, coliforms, and *Clostridium perfringens*. In fresh chicken manure, high microbial loads were recorded—*Salmonella spp.* at 430 MPN/g, *E. coli* at 3.9×10^8CFU/g, and coliforms at 1.4×10^9CFU/g. After 18 days of composting with TTT® Enzyme, these pathogens were no longer detected in any treatment, indicating complete microbial inactivation.

This outcome demonstrates the efficiency of TTT® Enzyme-assisted composting in pathogen reduction, attributable to increased thermophilic microbial activity and elevated compost temperatures (Gupta *et al.*, 2002; Bernal *et al.*, 2009). Enzymatic degradation of organic matter enhanced aerobic conditions, suppressing survival of anaerobic and mesophilic pathogens (Zhang *et al.*, 2020; Batish *et al.*, 2021). These findings align with prior studies confirming that enzyme-assisted composting supports hygienic, safe organic fertilizer production (Gajalakshmi and Abbasi, 2008; Awasthi *et al.*, 2022; Tetanti AgriBiotech, 2024).

Table 4. Microorganism content of chicken compost responses different TTT® Enzyme levels

Item	Fresh manure	TTT® Enzyme levels (%)			
		0	1	2	3
Coliform, CFU/g	$1.4*10^9$	-	-	-	-
E.coli, CFU/g	$3.9*10^8$	-	-	-	-
Clostridium perfringens, CFU/g	-	-	-	-	-
Salmonella spp., MPN/g	430	-	-	-	-

3.5. Correlation between temperature, pH and moisture together TTT® Enzyme levels on nutrients of chicken compost

The relation temperature, pH and moisture together TTT® Enzyme levels on crude protein of chicken compost is shown in Figure 1 and 2.

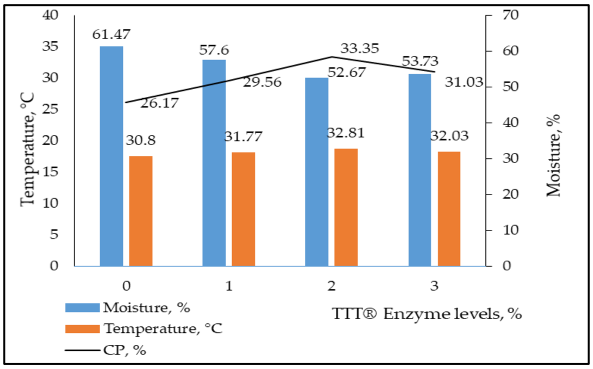

Figure 1. Correlation between moisture and temperature on crude protein of compost

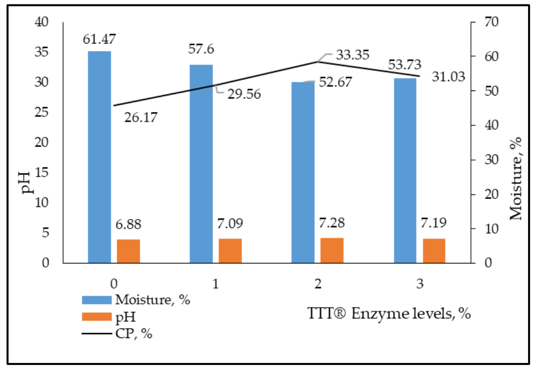

Figure 2. Correlation between moisture and pH on crude protein of compost

Correlation analysis revealed important relationships between composting parameters and nutrient preservation. As shown in Figures 1 and 2, temperature and pH were positively correlated with crude protein content, while moisture content exhibited a negative correlation. Specifically, higher compost temperatures stimulated microbial activity and enzymatic breakdown of proteins, increasing nitrogen retention in the form of crude protein (Gupta *et al.*, 2002; Wang *et al.*, 2021).

Moderate increases in pH (from 6.88 to 7.28) also supported protein stability by reducing ammonia volatilization, a key factor in nitrogen conservation (Awasthi *et al.*, 2019; Zhang *et al.*, 2020). Conversely, excessive moisture levels diluted microbial activity and limited oxygen availability, hindering efficient decomposition and nutrient stabilization (Bustamante *et al.*, 2008).

The 1 and 2% TTT® Enzyme treatments achieved an optimal balance of temperature, pH, and moisture, resulting in the highest protein recovery. These findings reinforce that TTT® Enzyme enhances compost nutrient quality by optimizing biophysical conditions (Bernal et al., 2009; Tetanti AgriBiotech, 2024).

4. CONCLUSION

TTT® Enzyme supplementation, particularly at 1-2%, enhanced composting efficiency by improving temperature, pH balance, and protein retention while reducing gas emissions and pathogens. This enzyme-assisted approach offers a sustainable, effective solution for nutrient conservation and methane mitigation in chicken manure composting under tropical conditions.

ACKNOWLEDGEMENTS

The authors acknowledge support the fund for this research from the Department of Science and Technology of Long An province, Vietnam. They also acknowledge the Research and Technology Transfer Center, Nong Lam University of Ho Chi Minh City, Vietnam for providing infrastructure support.

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EFFECTS OF BANANA PSEUDO-STEM AND LEAF ON NUTRIENT DIGESTIBILITY AND WEIGHT GAIN OF GROWING GOATS

Nguyen Thanh Dat[1], Tran Thi Thuy Hang[1] and Nguyen Thiet[1]*

Submitted: 30/6/2025 - Revised: 25/7/2025

Accepted: 31/7/2025

ABSTRACT

This experiment aimed to evaluate the effects of banana pseudo-stem and leaf on nutrient digestibility and weight gain in growing goats. The first experiment was arranged in a 4×4 Latin square design, comprising four periods and four treatments applied to four Boer crossbred goats. The treatments were as follows BLS0: 70% natural grass+30% concentrate; BLS20: 14%BLS+56% natural grass+30% concentrate; BLS40: 28%BLS+42% natural grass+30% concentrate; and BLS60: 42%BLS+28% natural grass+30% concentrate. Each experimental period lasted 14 days, including 7 days for adaptation and 7 days for data collection. The second experiment used a completely randomized design with three treatments and four replicates. The treatments included control (C): 70% natural grass+30% concentrate; BLF: 28% banana pseudo-stem leaf (fresh form)+42% natural grass+30% concentrate; and BLS: 28% banana pseudo-stem leaf silage+42% natural grass+30% concentrate. Results from the first experiment showed that nutrient intake significantly decreased as BLS levels increased (P<0.05), while nutrient digestibility and nitrogen retention remained unaffected across treatments. In the second experiment, feeding goats with 40% banana pseudo-stem leaf, whether fresh or ensiled, did not significantly affect dry matter intake, body weight gain, or feed conversion ratio (FCR). These findings suggest that banana pseudo-stem and leaf can effectively replace up to 40% of natural grass in the diet of growing goats without compromising growth performance or nutrient digestibility.

Keywords: *Agricultural by-product, digestibility, FCR, goats, silage.*

1. INTRODUCTION

Effective use of agro-industrial by-products as animal feed is a good strategy for decreasing the feed cost and avoid to compete between animals and human foods. As a result, integration of crop residues, agro-industrial by-products in ruminant production system are important role in Mekong delta provinces of Vietnam. Banana tree is one of the most important fruit tree in Vietnam. Accordingly, Amarnath and Balakrishnan (2007) reported that each hectare banana crop produced 13 to 20 tons/ha/year of banana pseudo-stem and leaf. In Mekong delta, banana pseudo-stem and leaf normally use for animal without any processing, particularly in fattening goat farm when farmer provide natural grass plus banana pseudo-stem and leaf together with concentrate from technician' recommendation. Importantly, there is a lot of banana pseudo-stem and leaf at the harvesting time. Therefore, developing suitable preservation methods is essential to make full use of this feed resource. The ensiling technique is a suitable approach for the preservation of animal feed resources, and the method is extensively used worldwide. Previous study confirmed that banana pseudo-stems could be improved by making silage with 5% sugar cane molasses and could substitute 10% rice bran in the diet of growing wild crossbred boars without negative impacts on performance (Nguyen Thiet *et al.*, 2022). Until now, there is little information on the use of banana pseudo-stem and leaf as silage or fresh status in the diet of crossbred Boar goats. Thus, the aims of present study were to determine the effect of banana pseudo-stem and leaf silage substituted natural grass on nutrient digestibility and to compare between fresh or

[1]Can Tho University

*Corresponding author: Assoc. Prof. Dr. Nguyen Thiet, Can Tho University, Campus II, 3/2 street, Ninh Kieu district, Can Tho city, Viet Nam, Phone: 0084 932147900; Email: nthiet@ctu.edu.vn.

silage status of banana pseudo-stem and leaf on weight gain of growing goats.

2. MATERIALS AND METHODS

2.1. Collecting banana pseudo-stem and leaf, making silage

After harvesting, banana tree was collected from the farmer and chopped into 3-5cm, then directly offer for the goats as fresh status. Additionally, our previous study showed that banana pseudo-stem and leaf (BL) mixed with 5% sugar cane molasses (BLS) is a good choice for making the silage (Nguyen Thiet *et al.,* 2022). Therefore, this experiment (Expt) used this formula (BLS) to substitute the natural grass in the diets of Boer crossbred goats. BLS from this study was ensiled with 5% sugar cane molasses within 21 days before feeding for the goats.

2.2. Effects of levels of natual grass replacement by banana pseudo-stem leaf silage goats

Experimental design: The Expt was arranged in 4x4 Latin square design, including 4 periods and 4 treatments (Tr) corresponding to 4 crossbred Boer goats, 4 Tr as:

BLS0: 70% natural grass+30% concentrate

BLS20: 14% BLS+56% natural grass+30% concentrate (20% replacement)

BLS40: 28% BLS+42% natural grass+30% concentrate (40% replacement)

BLS60: 42% BLS+28% natural grass+30% concentrate (60% replacement)

Table 1. **Chemical composit of ration, concent**

Items	BLS0	BLS20	BLS40	BLS60	Concent
DM,%	17.40	16.80	16.00	14.90	89.90
CP,%	10.10	10.00	9.30	8.60	16.90
NDF,%	59.70	56.30	56.6	59.30	37.80
ADF,%	34.10	35.00	36.22	36.20	24.70
Ash,%	10.00	11.30	10.60	11.40	7.20

Each Expt period consisted of 14 days, including 7 days for adaptation and 7 days for data collection. All expt goats were fed a complete mixed ration (TMR) with different proportions of silage from banana stems and leaves depending on the Tr, including 70% roughage and 30% concentrate. The goats were fed twice daily at 07:00 and 15:00 and had free access to water.

Management and measurements: All feed offer, water and feed residues were recorded daily, and feed residues were collected daily throughout the expt. At the end of the expt, feed offer and residues were mixed and analyzed for DM, OM and CP according to the method of AOAC (1990) and NDF, ADF according to the method of Van Soet *et al.* (1991). At the end of each period, all feed samples were thawed and mixed thoroughly, and subsamples were dried at 65°C for approximately 36h for CP and ash analysis according to AOAC (1990). Analysis of ADF and NDF followed the method of Van Soest *et al.* (1991). Goats were weighed at the beginning and end of period of the expt, in the morning before feeding.

Nutrition digestibility: All feces and urine were collected continuously during the last 5 days of each period (day 10 to day 14 of each period). After daily recording, 10% of the daily feces mas or urine were collected and stored at -20^{0}C for later analysis. Feed offer, refusals and feces samples were analyzed for DM, CP, ADF and NDF, whereas urine samples were analyzed for CP.

2.3. Comparison of using banana pseudo-stem and leaf as fresh or silage status

After the end of first expt, a suitable silage formula were selected to raise crossbred Boer goats and evaluated in 2nd expt. The results of BLS40 was a good choice for growing goats.

Experimental design: The study was arranged into a completely randomized design, consisting of 3 Trs and 4 replicates. The Trts were control diet (C): 70% natural grass + 30% concentrate; Trt1 (BLF): 28% banana pseudo-stem leaf without silage (fresh status) and Trt2 (BLS): 28% banana pseudo-stem leaf silage. The animals were offer the feed twice daily at 07:00 and 15:00 and had free access to water. For body weight measurement, all animals were weighed before morning feeding at the beginning and the end of the expt. The expt was lasted in 35 days.

Management and measurements: Feed offered and refusals (10%) were recorded daily in the morning starting from day 1st to 35th of the expt. Feed and refusal samples were collected daily from day 1st to 35th and were divided into 2 parts: one half was immediately dried in the oven at 105°C until its weight remained constant to determine DM, and the remaining samples were kept frozen at -20°C until chemical analysis. At the end of the expt, all feed samples were thawed and mixed thoroughly, and subsamples were dried at 65°C for approximately 36h for CP and ash analysis according to AOAC (1990). Analysis of ADF and NDF followed the method of Van Soest *et al.* (1991).

2.4. Statistical analysis

The data are presented as the mean ± SEM. Statistical analysis was performed using the General Linear Model procedure. The significance of pairwise comparisons was determined by Tukey posttest. Significance was declared at P<0.05.

3. RESULTS AND DISCUSSION

3.1. Effects of levels of natual grass replacement by banana pseudo-stem leaf silage goats

There was an effect of BLS silage replaced natural grass on nutrient intakes of growing goats (Table 2; P<0.01). Nutrient intakes decreased as the BLS levels increased, particularly between BLS0 and BLS60 (Table 2). Similar finding was found by Foulkes and Preston (1999), who reported that there was a linear decrease in the voluntary intake of pseudo-stem - leaves mixture diets as the proportion of pseudo-stem increased. Additionally, there were curvilinear trends showing a decrease in daily DMI (Figure 1; R^2=0.9732) as the BLS levels increased from current study. Some studies found that increasing the fiber content in diet decreased the feed intake and nutrient digestibility (Ngoc *et al.,* 2013; Lindberg, 2014) or low nutrient digestibility. This study found that levels of fiber in diets or nutrient digestibility were similar among treatments (Table 1 and 3; P>0.05). The reduction in DMI may be due to the high water content of silage (Table 1) or its low of palatability compared to fresh grass. In addition, figure 2 showed that there was a decrease in NDF digestibility as the BLS levels increased (R^2=0.892) and its result may explain for lower DMI from higher levels of BLS.

Table 2. Effect of BLS silage replaced natural grass

Items,g/h/c	BLS0	BLS20	BLS40	BLS60	SE	P
DMI	425.68^{a}	404.88^{ab}	400.11^{b}	393.90^{b}	4.73	0.01
CPI	55.01^{a}	52.81^{b}	50.74^{bc}	48.65^{c}	0.45	0.001
NDFI	215.22^{a}	195.00^{b}	193.01^{b}	173.68^{c}	2.58	0.001
ADFI	128.43^{a}	123.37^{a}	113.77^{b}	113.91^{b}	1.54	0.001
OMI	388.04^{a}	366.33^{b}	363.65^{b}	356.47^{b}	4.14	0.01

Notes: Mean values with different superscripts within the same row are different at P<0.05.

DMI, CPI, NDFI, ADFI and OMI: dry matter, crude protein, neutral detergent fiber, acid detergent fiber and organic matter intake.

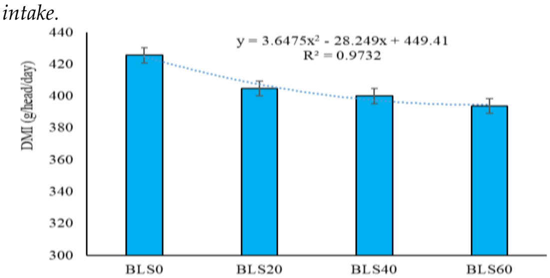

Figure 1. Negative relationship between DMI-BLS

There was no effect of BLS silage replaced natural grass on nutrient digestibility in growing goats (Table 3; P>0.05). But CP, NDF and ADF digestibility decreased as BSL levels increased as showing in Figure 2 (R2=0.8944), Figure 3 (R^2=0.892) and Figure 4 (R^2=0.9909). Lower CP digestibility from high BLS inclusion may be a high concentration of tannin from BLS can combine with dietary protein to form insoluble complexes in the rumen, which decreased CP digestibility. Additionally, lower fiber digestibility may be the present of lignin in the leaf of banana. Because the presence of lignin may interfere with the use of fibrous carbohydrates (Moore and Jung, 2001). Previous studies found that higher inclusion of BLS levels which decreased nutrient digestibility (Ngoc *et al.*, 2013 and Lindberg, 2014). Besides, adding 0.6% formic acid and 10% corn flour supplementation to banana pseudostem silages improved apparent nutrient digestibility of Nubian black castrated goats (Zhang *et al.*, 2021).

Table 3. Effect of BLS silage replaced natural grass

Nutrient digestibility,%	BLS0	BLS20	BLS40	BLS60	SE	P
DM	74.09	70.37	70.63	70.51	2.06	0.56
CP	78.57	75.22	75.31	73.56	1.46	0.21
NDF	74.17	68.97	69.13	66.21	1.96	0.13
ADF	66.68	62.53	59.81	60.77	2.41	0.29
OM	80.81	80.80	80.45	80.12	0.54	0.78

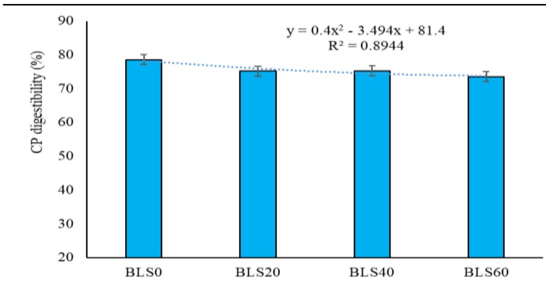

Figure 2. Negative relationship between DCP-BLS

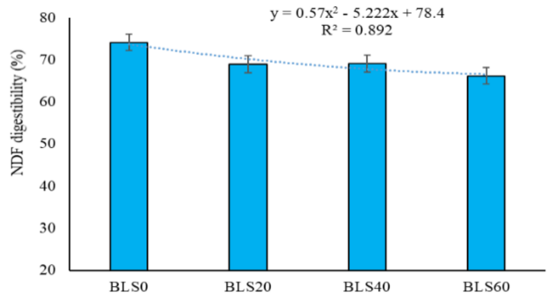

Figure 3. Negative relationship between DNDF-BLS

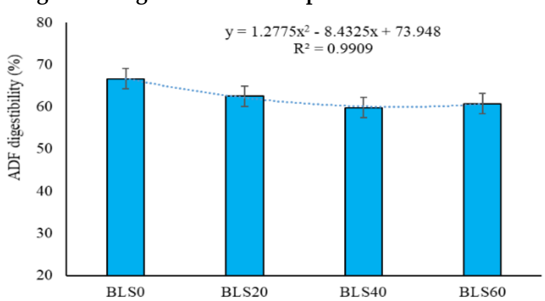

Figure 4. Negative relationship between DADF-BLS

Nitrogen intake differed significantly among treatments and was highest in the control group (BLS0) (Table 4; P<0.05). However, BLS silage levels had no significant effect on nitrogen retention in growing goats. A negative relationship between nitrogen retention (g/head/day) and the level of BLS silage in the diet was observed (Figure 5; R^2=0.9115). This trend may be attributed to the higher tannin content associated with increasing levels of BLS silage, which potentially reduced crude protein digestibility. A similar negative relationship between CP digestibility and BLS inclusion levels was observed in this study (Figure 2; R^2=0.8944).

Table 4. Effects of BLS silage replaced natural grass on nitrogen retention

Items,g/h/d	BLS0	BLS20	BLS40	BLS60	SE	P
N intake	8.80^{a}	8.45^{b}	8.12^{bc}	7.78^{c}	0.07	0.001
N feces	1.88	2.07	2.00	2.06	0.13	0.74
N urine	4.80	4.33	4.88	4.61	0.59	0.92
N retention	2.12	2.03	1.23	1.01	0.62	0.57

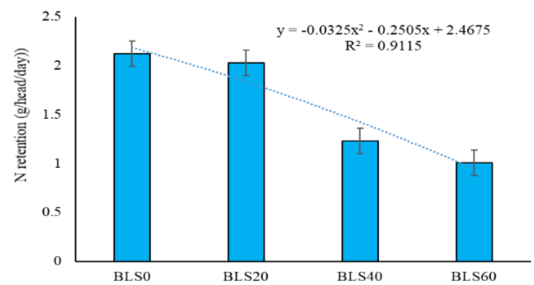

Figure 5. Negative relationship between N retention-BLS

3.2. Comparison of using banana pseudo-stem and leaf as fresh or silage status

There was no significant effect of banana pseudo-stem and leaf, whether fed fresh or as silage, on DMI, body weight, weight gain, or feed conversion ratio (FCR) among treatments (Table 5; P>0.05). These findings are consistent with those reported by Barbera *et al.* (2018), who found similar results in sheep fed banana by-products. Viswanathan *et al.* (1989) revealed that inclusion of banana pseudo-stem replacing *Brachiaria mutica* hay up to 40% level did not have any negative effect on growth performance of sheep. However, Zhang *et al.* (2021) reported that supplementing banana pseudo-stem silage with 0.6% formic acid and 10% corn flour improved the growth performance of Nubian black castrated goats. The observed improvement was likely due to enhanced fiber digestibility as a result of the additives.

Table 5. Comparison of using banana pseudo-stem and leaf as fresh or silage status on DMI and WG

Items	C	BLF	BLS	SE	P
DMI, g/h/d	470.34	499.49	483.41	10.38	0.194
Initial BW,kg/h	13.40	13.60	13.67	0.85	0.974
Final BW, kg/h	16.33	16.67	16.40	0.85	0.959
WG, g/h/d	83.81	87.62	78.10	5.38	0.482
FCR	5.73	5.79	6.30	0.53	0.71

4. CONCLUSIONS

The results indicated that banana pseudo-stem and leaf can effectively replace up to 40% of natural grass in the diet of growing goats without negatively affecting growth performance or nutrient digestibility. For long-term preservation, ensiling banana pseudo-stem and leaf with 5% sugarcane molasses is recommended, as it improves feed quality and ensures better utilization of this abundant by-product.

ACKNOWLEDGMENTS

This study was funded by Can Tho university (TĐH2023-03). The author would like to thank the manager of the Experimental farm at College of Rural Development, Can Tho university for supplying all the experiment materials and sincere gratitude thanks to Mr. Binh, Phi for taking care the goats.

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METHOD OF MAKING STUFFED MODELS OF SOME OF VERTEBRATES IN THE MEKONG DELTA

Le Thi Thanh[1]*

Submitted 17-Jan-2025 – Revised: 17-Feb-2025

Accepted: 03-Mar-2025

ABSTRACT

Building a collection of stuffed specimens is considered evidence of the existence of locally distributed animals. The successful construction and display of the taxidermy collection will support in widely disseminating the value of local biodiversity, environmental protection, nature protection and raising awareness of natural resource conservation to limit over-exploitation and trade of wild animals. This study has established a general process for making stuffed specimens of vertebrates. At the same time, a separate process established to guide the making of stuffed specimens for 4 species representing 4 orders in 3 classes of vertebrates in the Mekong Delta. Successfully built a collection of stuffed specimens. At the same time, instructions on display, preservation and use of the stuffed specimen collection after complete reconstruction so that the specimens can be preserved and used for a long time in study and research at the Faculty of Natural Science Education, Dong Thap University. The study also listed common errors in the process of making stuffed specimens. Analyzed the causes of errors in the process of making stuffed specimens and proposed solutions to overcome common errors in the process of making stuffed specimens.

Keywords: *Stuffed animals, vertebrates, biodiversity, Mekong Delta.*

1. INTRODUCTION

Experimental zoology comparative anatomy and diversity of living things, theoretical learning is always linked with observation and practice as methodology. Therefore, to keep theoretical knowledge firmly in learners, learning activities must be closely linked with observation and practice on specimens. Making stuffed specimens also helps learners practice the skills of using animal dissection kits and practice tools. Practice observation skills, know how to arrange and record, synthesize information, synthesize materials from experiments. This helps to consolidate theory, increase interest and deepen knowledge for learners. While making stuffed animal specimens, it also promotes creativity and proactive acquisition of knowledge, stimulates interest in observation. Know how to love specimens and have awareness of protecting and developing biodiversity resources of vertebrates, diversity of the living world and biodiversity conservation, stimulates learning about animal body biology in the new general education program. Building a collection of stuffed specimens is considered evidence of the existence of locally distributed animals. The successful construction and display of the taxidermy collection will support in widely disseminating the value of local biodiversity, environmental protection of wild animals. This paper is the result of the topic "Building a collection of stuffed specimens of some vertebrates in the Mekong Delta region".

2. MATERIALS AND METHODS

2.1. Material research

Species of Reptilia, Aves, Mammalia in the Mekong Delta (Dong Thap, Long An, An Giang). The selected species are usually convenient for specimen collection, common species. Vertebrates selected for stuffed specimens have characteristics typical of each animal class, are common or typical of the research area, are easy to collect samples, and are not rare species. Species were collected directly or indirectly from the wild or from weakened or old specimens culled from livestock in the provinces of the Mekong Delta. We also used newly dead specimens for stuffing.

[1] Dong Thap University

* Corresponding author: Dr. Le Thi Thanh, Faculty of Natural Science Education, Dong Thap University. Phone: 0084.906798589; Email: lethithanh@dthu.edu.vn.

2.2. Methods

Research specimens are collected directly, or by local people. In addition, research specimens are also purchased at local markets, or can be ordered through animal trapper. Specimens for taxidermy must meet a number of criteria: The specimen is not deformed, the specimen is not damaged, and the species is alive and healthy. To have specimens with the best external morphology and easy observation, research specimens are often selected from mature individuals. Specimens are neither old nor young because young individuals have an incomplete external morphology, old individuals often have defects, injuries, and scratches.

The stuffed specimens were observed, photographed, recorded, identified by scientific name and described in morphology based on the morphological characteristics of the specimens combined with references to related documents: Vietnamese zoology (IUCN, 2024; Tran Kien và Tran Hong Viet, 2005); Animals around the planet: Birds (Elicom, 2008); Amphibian - reptile fauna in An Giang and Dong Thap regions (Tran Hong Viet *et al*., 2004); Vertebrate zoology (Miller, 2001; Ministry of Science and Technology, 2007 and 2008; Nguyen Van Sang *et al*., 2009).

Based on the general guidelines for making stuffed specimens and experiments, we have completed the general procedure and proposed instructions for making stuffed specimens for each representative of the vertebrate classes. At the same time, instructions on how to preserve and display the collection of stuffed specimens of some representative vertebrate species in the Mekong Delta region.

3. RESULTS AND DISCUSSION

3.1. General procedure for making stuffed

By experimenting with making stuffed specimens on 20 individuals of vertebrates belonging to 4 species (Trun snake, pigeon, rabbit, field mouse) of 4 orders, in 3 classes of vertebrates. We have compiled and proposed a general 8 step process for making vertebrate taxidermy specimens as follows:

Step 1: Observe and photograph the specimen

Observe the overall parts of the specimen. Record the morphological characteristics of the specimen. Place the specimen in a natural position, easily visible for the characteristic features of the species, then photograph the dorsal, lateral, and ventral surfaces.

Step 2: Kill and prepare the specimen

The specimen should be wiped/washed gently to remove any dirt on the skin/fur. Only wipe/wash the dirt and dry immediately, do not wash the fur. Then, kill the specimen by applying cotton soaked in diethyl ether to the specimen's nose or using concentrated alcohol to anesthetize specimen. For some reptiles, the specimen can be placed in a plastic glue containing a concentrated alcohol solution, sealed in a box to suffocate and kill the specimen, however, this method will take longer than the above method.

Step 3: Separate the skin from the muscle

After anesthetizing the specimen, start dissecting the specimen (Figure 1, appendix). Next, peel the skin and wash away the blood. For birds, the blood must be allowed to clot to prevent blood from spreading and staining the bird's feathers. Instructions for peeling the belly skin: Place the specimen on its back on a dissecting tray, use a knife or scissors to cut the skin in a straight line from the belly to the vent. Depending on the species and characteristics of the specimen, the length of the cut will also be different. Use the knife handle to slide between the skin and muscle, gently separate the entire skin from the underlying muscle layer, avoiding tearing the skin. Separate the skin around the back and continue to separate down the belly to the cut line. Use the knife handle to peel the skin to avoid tearing the skin. Skinning the body and forelimbs: Peel the skin back towards the chest, use the knife handle to separate the skin from the muscles to the armpit level, then start to skin the two forelimbs. When skinning around the two forelimbs, be careful not to let the wrist bones separate from the skin. Cut away the joints that connect to the body. Skinning the neck and head area: Peel the skin back towards the neck, to skin the head. Use scissors to cut off the cervical vertebrae, which is where it connects to the skull, then completely separate the skin from the body.

Step 4: Head treatment

Remove all the flesh around the occipital area and use a steel wire to bend at the head and wrap a piece of cotton tightly around the end of the wire and insert the cotton ball into the skull through the occipital foramen to remove the brain, changing the cotton several times until the brain is completely cleaned. Remove the tongue, esophagus, trachea, remove all the flesh in the oral cavity, do not separate the lower jaw from the skull. Next, remove the fat and meat that is still attached to the skin. The more effective the process of removing the brain, fat, and meat, the longer the stuffed sample can be preserved and stored. However, the skull bone still retains its original shape. The skull plays a very important role in shaping the head, so it should be retained. The head area needs to be treated carefully to avoid rotting during preservation and storage.

Step 5: Clean the skin, remove grease, and treat the specimen with chemicals

During skin peeling, the skin or fur of the specimen may be dirty or stained with blood, so it must be wiped clean or gently washed to clean it. Usually wash with water mixed with salt to sterilize the skin. Mix 20g of salt with one liter of water. If the skin has a lot of fat, it is necessary to remove the fat by soaking the skin in hot water mixed with NaOH at about 30-40^{0}C, for every 1 liter of water, add 15g of NaOH and soak for 2-3 hours. Or use a spoon to scrape off the fat on the skin before soaking. For specimens with thin skin and little fat, soak for only about 1-1,5 hours. The peeled skin needs to be washed under running water or use a dry towel to wipe off blood and body fluids. After washing, the skin needs to be wiped dry and drained by mechanical methods such as wiping gently, using a dryer or fan. Before stuffing and mounting the specimen, the specimen must be disinfected with an antiseptic into the eye sockets, skull, limb bones, and the entire inner surface of the skin. The antiseptic includes 99^{0}C alcohol for preliminary disinfection, and a mixture of crushed salt and copper sulfate CuSO4 in a ratio of 1:2 to disinfect the skin. Add a few drops of 99^{0}C alcohol to the disinfecting salt mixture to create a smooth, thick chemical mixture. Use a small cotton ball or a small soft cloth to the antiseptic chemical mixture to rub it many times on the inner skin surface.

Step 6: Create a characteristic shape and stuff to recreate the natural shape of the specimen

Accurately measure the length of the neck, body, limbs, and tail. Use a steel wire of the same length as measured to make a mold to create the characteristic shape of the specimen. The cross-section of the steel wire used depends on the size of the specimen, large and long specimens have a large cross-section of steel wire and vice versa. This step requires determining the exact length of steel wire needed to build the neck, body, tail, legs, and wings. Depending on the size of the specimen and the cross-section of the steel wire, a frame will be created consisting of more or less steel sections connecting the body parts together. It is recommended to measure the excess steel wire from the skull and tail by about 2-4cm so that the tip of the excess steel can be bent back to help the head and tail be firm and in the natural position of the species.

Continue to bend the steel wire to make a frame for the body, tail and limbs. When constructing the neck, wrap a small cross-section steel wire (lead wire) or a tough thread to fix the neck specimen frame to the body and stabilize the limbs. The size of the body stuffing block depends on the size of the specimen. If it is a large stuffing block, a hole can be made in the cardboard to measure so that the stuffing block is equal to the size of the body that needs to be stuffed after skinning. Finally, in this step, place the stuffed specimen on a tray or wooden shelf or on a piece of cardboard. Adjust the body parts so that the cotton is more even and the specimen has a natural posture of the species as when the animal is performing life activities. It is necessary to create a natural posture for the specimen before drying, because when the specimen is dry, the body shape is stable, so it will be difficult to change the posture of the specimen (Figure 6, appendix). The completed stuffed specimen is dried or sun-dried.

Step 7: Inject the chemical solution to preserve and dry the stuffed sample

Before drying, the sample needs to be injected with 10-30% formalin depending on the species in the neck, tail, limbs, some points in the abdomen, and body. The concentration of formalin used to inject into the sample depends on the size of the sample and the developmental stage of the species.

After injection, the sample will be dried by drying in the sun to let the sample dry naturally, or placing the sample in a drying cabinet with a large enough cross-section to dry at a temperature of 35-45 degrees. The drying temperature of the sample varies depending on the size of the sample and the developmental stage of the sample. During the drying process, formalin will be injected into the parts that have not been completely removed, such as the head area, every 2-3 days, and then the sample will be dried. Inject formalin several times during the drying process. Continue drying until the sample is completely dry and the smell of formalin on the sample has disappeared.

Step 8: Preservation, display and use of stuffed specimens

The completed stuffed specimen should be placed on a wooden shelf, and the final posture of the specimen should be adjusted to be as natural as the posture of the species when alive before long-term preservation in a glass box or glass cabinet. The dried stuffed specimen should be stored in a cool, dry place. Avoid dust, avoid contaminated environments and avoid mechanical impacts on the specimen. The stuffed specimen should be placed in a glass cabinet with lighting to keep the specimen dry. In the specimen storage area, anti-humidity drugs and anti-bacterial drugs such as mothballs should be placed. Regularly check specimen and clean the storage cabinet.

Make a label containing the specimen information and stick it on the wall of the storage device. The label must have information about the scientific name of the species and the common name of the species. The area displaying stuffed animal specimens should avoid locations that are easily bumped into and near water tanks. The stuffed specimen display cabinet should be placed in a convenient location for observing and researching the specimen.

3.2. Complete collection of stuffed in the Mekong Delta River

The collection of stuffed specimens of vertebrates is displayed and preserved at the animal practice room, Faculty of Natural Science Education, School of Education, Dong Thap University. The specimens are built based on the natural shape of the species. The complete specimens will be placed on a wooden pedestal and stored in a glass cabinet to avoid termites and dirt. The stuffed specimens are placed in an easy-to-observe location, to avoid walkways, collisions or near chemicals, sinks because they can cause the specimens to fall or be damaged.

Synthesizing the data during the process of making the stuffed specimen collection, it is found that the stuffed specimens of the Trun Snake that meet the requirements (complete stuffed specimens) account for 60%, pigeons reach 60%, domestic rabbits 100%, and small field mice reach 33.3%. In general, the stuffed specimens made have a success rate of 60%.

It can be seen that the success rate of some stuffed specimens of small field mice, trun snakes, and pigeons is low. This may be due to the small size of the species, or because during the process of making stuffed specimens, very weak or dead specimens were used, or because the storage conditions for stuffed specimens were not good, or there was no storage facility. The stuffed specimen of domestic rabbits had the highest success rate (100%), possibly due to the large size of rabbits and thick skin, making it easy to follow the steps in the process.

After the specimen is modeled, the specimen needs to be dried in the sun or dried in a drying cabinet at a suitable temperature. Only use a drying cabinet for large specimens or on days when the specimen is exposed to sunlight. Every 2-3 days, inject formalin into the parts where the meat has not been removed, especially the head, limbs and abdomen. The specimen continues to be dried continuously for 1-3 weeks depending on the size of the specimen. Stuffed specimens are checked and labeled, arranged according to the principles of animal classification and placed in the location of the specimens.

Specimens need to be kept in a cool, dry place, away from sunlight and dust. Therefore, stuffed specimens are stored in glass cabinets with desiccants (silica gel), mold and bacteria inhibitors such as mothballs. During storage, specimens must be regularly checked and storage equipment and storage areas cleaned. The place where the specimen is displayed should avoid places that are often prone to collisions, walkways, damp places, polluted places or near water tanks. The specimen should be positioned naturally to facilitate observation and research on the specimen. When using stuffed specimens, be gentle, avoid scratches and deformation. Only observe the specimen, limit direct contact or impact on the stuffed specimen.

4. CONCLUSIONS

This study has established a general process for making stuffed specimens of vertebrates in the following order: Observing and photographing specimens; Killing and preparing specimens; Separating the skin from the muscles; Processing the head area; Cleaning the skin, removing grease, and chemically treating specimens; Creating a characteristic shape and stuffing to recreate the natural shape of the specimen; Injecting preservative chemicals and drying the stuffed specimen; Preserving and displaying stuffed specimens. At the same time, a separate process established to guide the making of stuffed specimens for 4 species representing 4 orders in 3 classes of vertebrates in the Mekong Delta. Successfully built a collection of stuffed specimens of some representative vertebrates in the Mekong Delta. At the same time, instructions on display, preservation and use of the stuffed specimen collection after complete reconstruction so that the specimens can be preserved and used for a long time in study and research at the Faculty of Natural Science Education, Dong Thap University.

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AAAP21 IN HANOI-VIETNAM 2026

Assoc. Prof. Dr. Nguyen Van Duc
Head of AAAP21 Scientific Board

The 21st Congress - Vietnam - 2026
ASIAN - AUSTRALIA ASSOCIATION OF ANIMAL PRODUCTION SOCIETIES
Hội nghị Khoa học Chăn nuôi Á - Úc lần thứ 21 tại Hà Nội, Việt Nam, năm 2026

HỘI CHĂN NUÔI VIỆT NAM
AHAV

BRIEF INTRODUCTION OF AAAP21 SCIENCE CONFERENCE IN VIETNAM

1. Time: 28-31/10/2026

2. Venue: National Convention Center: 57 Pham Hung Street, Tu Liem Ward, Hanoi City

3. Scheduled agenda

Oct. 28: Opening ceremony; plenary session; Concurrent sessions

Oct. 29: Concurrent sessions

Oct. 30: Concurrent sessions; Gala dinner and Closure ceremony

Oct. 31: Pre-registered sightseeing tours with options such as: Ha Long Bay World Natural Heritage; Hoa Lu Ancient Capital, Trang An World Heritage Complex; Thang Long Imperial Citadel; Family dairy farming model, Hill chicken farming households.

4. Expected attendees: 1,200-1,400 of which:

Foreigners: 400-500 participants

Domestic: 800-900 participants

5. The Theme of the Conference: Sustainable Livestock Development, with 11 Concurrent sections including:

1. Animal breeding and genetics
2. Animal production systems
3. Animal nutrition
4. Sustainable livestock systems
5. Digital management & smart farming
6. Animal welfare
7. Carbon emission management
8. Food safety & security
9. Companion animals
10. Smallholder livestock farming
11. Agricultural biomass utilization

6. Registration fee:

USD 400/participant

USD 200/Accompanying Person

7. Contact:

vanphong@hoichannuoi.vn
ddluc@vnua.edu.vn
thangnt49@gmail.com
nvanduc48@gmail.com