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ANIMAL GENETICS AND BREEDING

- Do Thi Thu Huong, Nguyen Thai Anh, Tran Thi Binh Nguyen, Nguyen Thi Vinh, Nguyen Thi Chau Giang, Do Duc Luc, Hoang Anh Tuan, Pham Kim Dang, Bui Huu Doan and Nguyen Hoang Thinh. Growth performance of Lien Minh chicken with AA genotype of G1705a polymorphism in GH gene 2
- Nguyen Khanh Van, Vu Thi Thu Huong, Hoang Thi Au, Pham Thi Kim Yen, Le Van Dat, Nguyen Thi Lan Anh and Pham Doan Lan. Influence of oocytes recovery techniques on recovery and in vitro maturation of Cỏ goat oocytes 9
- Nguyen Thi Kim Khang, Tran Quoc Khanh and Le Thanh Phuong. Morphology and fighting behaviors of gamecock chickens 13
- Ho Thieu Khoi, Vo Cong Thi, Lam Trung Nghia, Nguyen Thi Kim Khang and Nguyen Thi Thuy. Effect of difference extender on extended boar semen quality in the long-term storage 19
- Ho Thieu Khoi, Lam Trung Nghia, Vo Cong Thi, Nguyen Thi Kim Khang and Nguyen Thi Thuy. The effect of long-term storage at storage temperatures on quality of extended boar sperm 24

ANIMAL FEEDS AND NUTRITION

- Mai Truong Hong Hanh, Lam Phuoc Thanh and Ho Thanh Tham. Effects of fermented total mixed rations (FTMR) on in vitro nutrient digestibility and ruminal fermentation patterns 29
- Jay-r J. Dapar, Abeljino R.Ermac, Richelle A. Niepes, Mechie Ann C. Florida, and Jerico M. Consolacion. Voluntary feed intake and growth performance of growing goats and nutrient digestibility of IPIL-IPIL and napier grass in different feeding ratios 35
- Truong Thanh Trung and Nguyen Binh Truong. Effects of feeding frequency on feeding behavior, feed efficiency, weight gain and economic returns of growing crossbred rabbits 42
- Nguyen Huu Tinh, Le Van Tho, Phan Thi Ngoc Thu, Ha Hai Van, Nguyen Phan Quynh Nhu and Truong Thanh Trung. Effects of vitamin e supplementary levels in diets on the reproductive performance of crossbred (Newzealand white x local) rabbits in Vietnam 49

ANIMAL PRODUCTION

- Nguyen Thi Kim Khang, Thach Vila and Tang Thi To Nguyen, Nguyen Thi Kim Khang, Thach Vila and Tang Thi To Nguyen. Effects of different levels of garlic powder (Allium Sativum) on growth, carcass traits and drip loss of ross 308 broiler chickens 57
- Nguyen Thi Thuy, Nguyen Thi Ngoc Linh and Ho Thieu Khoi. Effects of premix-vitamin supplementation in drinking water on egg performance and quality of isa brown laying hens from 44-52 weeks of age 66
- Lam Thi Hon, Le Thanh Phuong, Tran Vuong Khang, Sutisa Khempaka and Nguyen Thi Kim Khang. Effects of different diets on growth performance of Thai crickets 72
- Nguyen Thi Kim Khang, Nguyen Thi Minh Thu and Ngo Thi Minh Suong. Effects of turmeric and cinnamon powder supplementation on growth performance in Japanese quail 78
- Nguyen Duc Dien, Nguyen Van Thai and Le Van Khoa. Effects of antimicrobial peptides supplementation on commercial pig production efficiency 85
- Lam Phuoc Thanh, Tran Thi Thanh Khuong and Tran Thi Thuy Hang. Effect of increasing concentrate level in the diet on intake, digestibility and ruminal fermentation in non-lactating goats 90
- Ho Thanh Tham, Nguyen Minh Thu and Nguyen Thi Tuyet Nhung. Effect of supplementation rates of fermented total mixed ration (FTMR) from jackfruit by-products on the growth of crossbred boer goats 95

SCIENTIFIC NEWS

College of agriculture - 55 years of construction and continuous development 102

GROWTH PERFORMANCE OF LIEN MINH CHICKEN WITH AA GENOTYPE OF G1705A POLYMORPHISM IN GH GENE

Do Thi Thu Huong¹, Nguyen Thai Anh², Tran Thi Binh Nguyen³, Nguyen Thi Vinh²,
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 Nguyen Hoang Thinh^{2*}

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ABSTRACT

The study was conducted to evaluate the growth performance of Lien Minh chicken with AA genotype of G1705A/GH polymorphism after selection for improving growth. The experiment was designed according to a completely randomized one-factor model, including of 2 groups: (1) AA genotype group - Lien Minh chicken with AA genotype of G1705A/GH polymorphism, and (2) Control group-Lien Minh chicken from general population. A total of 120 chickens, including 60 (30 males and 30 females) for AA genotype and 60 (30 males and 30 females) for control groups were used with 3 replications. Animals were raised from 1 day to 20 weeks of age. The results showed that body weights of males (2430.47g) and females (1784.77g) in AA genotype group at 20 weeks of age were higher than those of males (2162.87g) and females (1694.93g) without genotyping selection ($P<0.05$). The absolute growth of chickens in AA genotype group was higher than that in the control group at 4-5, 10-11 and 13-15 weeks of age for males, 7-8 weeks of age for females ($P<0.05$). Feed conversion ratios of males (3.75kg) and females (4.33kg) in AA genotype group after 20 weeks of age were lower than those of males (3.98kg) and females (4.53kg) in control group ($P<0.05$).

Keywords: Lien Minh chicken, growth, G1705A/GH.

TÓM TẮT

Khả năng sinh trưởng gà Liên Minh mang kiểu gen AA thuộc đa hình G1705A gen GH

Nghiên cứu được thực hiện nhằm đánh giá khả năng sinh trưởng của gà Liên Minh thương phẩm (mang kiểu gen AA của đa hình G1705A/GH sau chọn lọc kiểu gen để nâng cao khả năng sinh trưởng). Thí nghiệm được thiết kế theo mô hình một nhân tố hoàn toàn ngẫu nhiên, gồm 2 lô: Lô thí nghiệm (TN) là gà Liên Minh mang kiểu gen sinh trưởng nhanh AA thuộc đa hình G1705A của gen GH và lô đối chứng (ĐC) là gà Liên Minh được chọn ngẫu nhiên từ quần thể. Tổng số 120 gà, bao gồm lô TN 60 con (30 trống và 30 mái) và lô ĐC 60 con (30 trống và 30 mái) với 3 lần lặp lại. Gà được theo dõi từ 1 ngày tuổi đến 20 tuần tuổi. Kết quả nghiên cứu cho thấy ở 20 tuần tuổi; khối lượng cơ thể của gà trống (2430,47g) và mái (1784,77g) mang kiểu gen AA thuộc đa hình G1705A cao hơn so với khối lượng gà trống (2162,87g) và mái (1694,93g) không được chọn lọc kiểu gen ($P<0,05$). Sinh trưởng tuyệt đối của gà trống mang kiểu gen AA cao hơn so với gà trống đàn quần thể ở các giai đoạn 4-5, 10-11 và 13-15 tuần tuổi; đối với con mái tương ứng là giai đoạn 7-8 tuần tuổi ($P<0,05$); tiêu tốn thức ăn/kg tăng khối lượng của gà trống (3,75kg) và mái (4,33kg) mang kiểu gen AA thuộc đa hình G1705A thấp hơn so gà trống (3,98kg) và mái (4,53kg) của đàn quần thể ($P<0,05$).

Từ khóa: Gà Liên Minh, sinh trưởng, G1705A/GH.

1. INTRODUCTION

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Lien Minh chicken originated in Lien Minh village, Tran Chau commune, Cat Hai district, Hai Phong city. The consumers prefer this breed due to their morphology characteristics, as well as meat quality. The Lien Minh chicken breed is only raised on Cat Ba Island with limited individuals. To conservation, exploitation and development of this chicken breed, also to meet demand of

local market, multiplication and selection for improve productive performance of this breed is especially important.

Previous studies have shown that there was a relationship between polymorphisms of several genes such as GH, GHR, IGFBP2, INSULIN and the growth performance of chickens (Nie *et al.* (2005); Ouyang *et al.* (2008); Lei *et al.* (2005); Qiu *et al.* (2006); Do Vo Anh Khoa *et al.* (2013). Based on this research direction, the study found that the G1705A polymorphism of GH gene related to increase growth rate of Lien Minh chicken. Body weight of birds carrying AA genotype was heavier than that carrying two other genotypes. From this finding, a new line of Lien Minh chickens with high growth rate was created. Commercial Lien Minh chicken were born from this line. This study was conducted to evaluate productive performance of commercial Lien Minh chickens carrying AA genotype.

2. MATERIALS AND METHODS

2.1. Experimental design

Experiment was setup according to a completely randomized one-factor design with 2 groups: (1) AA genotype group with 60 Lien Minh chickens at one-day of age carrying AA genotype of G1705A polymorphism in GH gene, and (2) control group of 60 Lien Minh chickens at one-day of age selected from the general population without genotyping. There were 3 replications for each treatment group, including 20 birds (10 males and 10 females) for the first 4 weeks of age and 10 chickens, for each sex in the period from the fifth weeks of age to the end of experiment. All birds were individually numbered on legs and raised to 20 weeks of age for collecting data. From the first day to 4 weeks of age, males and females were raised together. After this period, males and females were raised separately under semi-intensive conditions. All chickens were raised according to the commercial-colored broilers procedure of National Institute of Animal Science. The raised conditions such as

density, nutrition values, vaccination program were similar between two treatment groups. The experiment was conducted at the chicken farm belong to Thien Thuan Tuong Mining Joint Stock Company, Quang Ninh province. The nutritional values of feeds are presented in Table 1.

Table 1: Feed nutritional values according to periods

Nutritional value	0-4 weeks	5-8 weeks	9-20 weeks
ME (Kcal/kg)	2900	3000	3050
Protein (%)	21.0	20.0	17.0
Fiber (%)	4.0	4.2	5.0
Calcium (%)	1.0	0.95	0.9
Phosphorus (%)	0.5	0.45	0.45
Methionine (%)	0.54	0.45	0.35
Lysine (%)	1.1	1.0	0.75

2.2. Methods and studied traits

Each individual was weighed on a fixed day of a week in the morning. For the first 4 weeks of age, the body weights (BW) were recorded by using an electronic balance of $3\text{kg}\pm 0.5\text{g}$ (FEH, Taiwan). After this period, BW was recorded by mechanical balance of $5\text{kg}\pm 10\text{g}$ (Nhon Hoa, Vietnam). Based on body weight, absolute growth and relative growth were calculated. For feed conversion ratio (FCR), amounts of supplied feed and of remain feed were recorded every day by electronic balance of $3\text{kg}\pm 0.5\text{g}$ (FEH, Taiwan) and the feed intake was accumulated from supplied feed minus remain feed during period. FCR was calculated from BW gain and feed intake according to week or periods based in the values from replications. BW and FCR were determined according to the method of Bui Huu Doan *et al.* (2011).

2.3. Data analysis

Data was performed using SAS software version 9.0 (2002) with one-way ANOVA. The statistical parameters were number of observation (n), arithmetic mean (Mean), standard deviation (SD). Pairwise comparison was made using Duncan test with significant level of $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Growth performance

The cumulative growths of experimental chickens are presented in Table 2 and Figure 1. Observed results show that body weight gain of Lien Minh chickens in this study followed the trend of growth, BW increased gradually through the weeks of age. BW of chickens in AA genotype group was significantly higher than that in control group between 7-20 weeks for males and 8-20 weeks of age for females ($P<0.05$). At the 20 weeks of age, BWs of AA genotype group were 2430.47g and 1784.77g for cocks and hens respectively; while these values in control group reached 2162.87g and 1694.93g respectively. BW in AA genotype group was higher than that in control group ($P<0.05$). The difference in BW was 267.6g

(12.37%) and 89.84g (5.30%) for males and females, respectively.

According to Trinh Phu Cu *et al.* (2012), BW of Lien Minh chickens at 20 weeks of age were 1886.53g and 1565.42g for males and females, respectively. The results from Vu Cong Quy *et al.* (2016) on Lien Minh chickens shown that BW of males and females at the third generation were 2001,68g and 1727.31g, respectively. In our study, BWs of both males and females in AA genotype group were higher than those in study of Vu Cong Quy *et al.* (2016). However, BW of males in Control group was higher but not difference for female in comparison with results of Vu Cong Quy *et al.* (2016). Lien Minh chickens in AA genotype group selected in combination with the morphology characteristics over 3 generations had BW higher than Lien Minh chickens in the previous studies.

Table 2. Accumulative growth of Lien Minh chickens (Mean \pm SD, g)

Age (week)	AA genotype		Control	
	Male and Female (n=60)		Male and Female (n=60)	
1 day	32.81 \pm 2.92		32.44 \pm 2.88	
1	62.25 \pm 6.56		57.92 \pm 6.41	
2	111.67 \pm 16.23		105.20 \pm 13.19	
3	198.12 \pm 32.43		191.33 \pm 26.91	
4	311.52 \pm 39.09		284.37 \pm 42.74	
	Male (n=30)	Female (n=30)	Male (n=30)	Female (n=30)
5	448.77 \pm 57.74	410.50 \pm 46.44	400.97 \pm 57.18	363.60 \pm 47.72
6	593.00 \pm 74.68	509.10 \pm 55.33	526.90 \pm 71.92	445.37 \pm 44.9
7	744.73 ^a \pm 101.52	607.83 \pm 72.01	656.20 ^b \pm 64.92	544.53 \pm 51.63
8	910.47 ^a \pm 131.15	742.93 ^c \pm 88.15	830.63 ^b \pm 72.09	655.00 ^d \pm 51.48
9	1090.97 ^a \pm 151.44	870.30 ^c \pm 100.39	1015.40 ^b \pm 90.76	796.77 ^d \pm 121.98
10	1242.90 ^a \pm 161.19	984.00 ^c \pm 117.33	1129.07 ^b \pm 110.47	906.80 ^d \pm 126.81
11	1415.97 ^a \pm 164.9	1094.33 ^c \pm 127.1	1250.47 ^b \pm 116.59	1008.07 ^d \pm 129.95
12	1580.60 ^a \pm 167.45	1187.97 ^c \pm 130.02	1412.77 ^b \pm 147.64	1082.93 ^d \pm 132.2
13	1731.47 ^a \pm 193.38	1278.50 ^c \pm 129.47	1553.77 ^b \pm 156.07	1168.20 ^d \pm 138.86
14	1858.17 ^a \pm 205.16	1381.67 ^c \pm 140.53	1650.03 ^b \pm 153.98	1249.10 ^d \pm 171.49
15	1993.03 ^a \pm 224.24	1481.13 ^c \pm 147.19	1754.27 ^b \pm 152.38	1327.03 ^d \pm 171.73
16	2112.43 ^a \pm 240.32	1576.93 ^c \pm 147.54	1857.30 ^b \pm 157.7	1421.20 ^d \pm 173.23
17	2186.83 ^a \pm 253.86	1648.20 ^c \pm 148.21	1932.73 ^b \pm 155.74	1509.97 ^d \pm 174.91
18	2252.10 ^a \pm 246.64	1701.63 ^c \pm 149.57	1988.60 ^b \pm 155.06	1573.67 ^d \pm 175.57
19	2345.80 ^a \pm 250.67	1744.20 ^c \pm 150.35	2075.07 ^b \pm 154.43	1630.53 ^d \pm 175.7
20	2430.47 ^a \pm 263.79	1784.77 ^c \pm 154.17	2162.87 ^b \pm 154.25	1694.93 ^d \pm 174.86

Means in a row with the same gender followed by different letters are significantly different ($P<0.05$)

In comparing with BW of Ho chicken at 20 weeks of age (2530g for cocks and 1880g hens) in Nguyen Hoang Viet (2013), Dong Tao chickens at 20 weeks of age (2506.33-2588.55g for cocks and 1920.67-1950.33g for hens) in Le Thi Thu Hien *et al.* (2015), BW of Lien Minh chickens in AA genotype group and control groups of our study were lower. Inversely, BWs in our study were higher than those in other local chicken breeds such as Lac Thuy (1389.33g for males, 1273.33g for females) in Vu Ngoc Son *et al.* (2015), Te (1458.04g and 1118.40g for males and females respectively) in Dang Vu Hoa *et al.* (2015). Hoang Anh Tuan (2022) reported that for commercial Mia chickens carrying GG genotype of G662A polymorphism in GH gene after 20 weeks of age, BWs of males (2419.13kg)

and females (1742.26kg) were higher than individuals without genotyping selection ($P<0.05$). Observed results on Lien Minh chickens carrying AA genotype of G1705A polymorphism in GH gene in this study also showed similar trends.

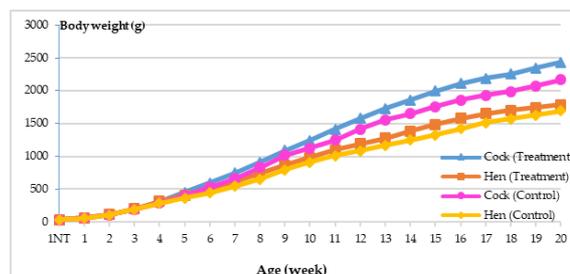


Figure 1. Body weight of Lien Minh chickens

3.2. Absolute growth

Table 3. Absolute and relative growth of Lien Minh chickens (Mean±SD, g/head/day)

Period (week)	Absolute growth				Relative growth			
	AA genotype		Control		AA genotype		Control	
	Male and Female (n=60)	Male and Female (n=60)	Male and Female (n=60)	Male and Female (n=60)	Male and Female (n=60)			
1d-1	4.21±0.86		3.64±0.93		61.65±8.59		57.55±8.80	
1-2	7.06±1.74		6.75±1.79		56.19±8.95		57.51±9.70	
2-3	12.35±3.06		12.30±3.96		55.24±9.34		55.99±9.79	
3-4	16.20±3.63		13.29±3.03		45.00±8.09		38.69±7.34	
	Male (n=30)	Female (n=30)	Male (n=30)	Female (n=30)	Male (n=30)	Female (n=30)	Male (n=30)	Female (n=30)
4-5	18.58 ^a ±4.91	15.17±3.74	14.72 ^b ±5.61	13.26±6.78	33.98±6.17	29.60±4.46	29.68±6.24	29.11±6.88
5-6	20.60±4.59	14.09±3.61	17.99±8.76	11.68±5.11	27.72±5.63	21.47±3.55	27.04±6.74	20.50±5.23
6-7	21.68±5.88	14.11±3.74	18.47±6.24	14.17±5.25	22.53±4.55	17.53±3.02	22.19±5.82	19.98±4.58
7-8	23.68±7.16	19.30 ^c ±4.41	24.92±3.51	15.78 ^d ±6.97	19.91±4.48	19.99±2.68	23.51±5.88	18.49±4.61
8-9	25.79±7.31	18.19±3.12	26.40±6.12	20.25±5.18	18.11±4.59	15.82±2.30	19.96±4.29	18.64±4.59
9-10	21.71±6.95	16.24±4.33	16.24±6.55	15.72±6.44	13.12±4.28	12.23±2.5	10.50±3.80	13.03±3.57
10-11	24.72 ^a ±6.78	15.76±3.75	17.34 ^b ±6.75	14.47±6.16	13.14±5.35	10.66±2.51	10.26±3.26	10.70±2.88
11-12	23.52±5.05	13.38±4.14	23.19±7.14	10.69±6.57	11.11±2.59	8.30±2.72	12.09±3.59	7.25±1.87
12-13	21.55±6.03	12.93±3.40	20.14±6.82	12.18±5.62	9.04±3.02	7.43±2.13	9.54±2.02	7.62±1.92
13-14	18.10 ^a ±4.56	14.74±3.94	13.75 ^b ±6.49	11.56±3.46	7.07±1.66	7.75±1.99	6.07±1.92	6.50±1.90
14-15	19.27 ^a ±5.99	14.21±3.53	14.89 ^b ±3.48	11.13±2.44	6.98±1.86	6.97±1.76	6.18±1.84	6.14±1.57
15-16	17.06±4.01	13.69±3.72	14.72±2.42	13.45±2.67	5.81±1.12	6.32±1.80	5.72±1.98	6.94±1.59
16-17	10.63±5.84	10.18±3.71	10.78±2.31	12.68±2.50	3.44±1.80	4.45±1.66	4.01±0.63	6.12±1.91
17-18	9.32±3.55	7.63±2.06	7.98±1.87	9.10±1.28	3.01±1.26	3.21±0.91	2.87±0.41	4.17±1.70
18-19	13.39±4.71	6.08±3.07	12.35±2.10	8.12±1.91	4.11±1.47	2.48±1.25	4.28±0.52	3.59±0.90
19-20	12.09±5.67	5.8±2.06	12.54±1.16	9.20±2.40	3.53±1.51	2.30±0.77	4.17±0.49	3.91±1.08
Average	17.08±4.91	12.57±3.28	15.12±4.35	11.97±4.02	21.03±4.32	19.73±3.55	20.39±4.25	19.62±4.12

The results in Table 3 shows that the absolute growth of chickens in the experiment

increased gradually from the first week, the highest was 25.79g for males in AA genotype

group at 8-9 weeks of age and 26.40g for females in control group at the same period of 8-9 weeks of age. For females, these values were 19.30g at 7-8 weeks and 20.25g at 8-9 weeks of age for AA genotype and control groups, respectively. After these periods, absolute growth tended to decrease. Comparing between two groups, absolute growth of males in AA genotype group was higher than that in control group at the periods of 4-5, 10-11 and 13-15 weeks of age ($P < 0.05$). Similarly, absolute growth of females in AA genotype was higher than that in control groups at 7-8 weeks of age ($P < 0.05$). The absolute growths for whole period of males (17.08g) and females (12.57g) in AA genotype group were higher than those of males (15.12g) and females (11.97g) in control group.

In comparison with Ri Lac Son chickens in the study of Nguyen Hoang Thinh *et al.* (2020) with the highest absolute growth at 8-9 weeks of age in males (37.41g) and at 6-7 weeks of age in females (18.87g), Lien Minh chickens in both groups in this study had a lower absolute growth in males and similar in females. According to Nguyen Thi Phuong *et al.* (2017), the absolute growth of H'Mong chickens at 12 weeks of age was 14.9 and 13.2g for males and females, respectively. Lien Minh chickens in two groups of this study had higher absolute growth in males and similar in females in comparison with Nguyen Thi Phuong *et al.* (2017).

3.3. Relative growth

The relative growths of Lien Minh chicken are shown in Table 3. Birds in both groups had a relative growth following the general rule. Relative growth was the highest in the period from one-day to one week of age. These values in AA genotype and control groups were 61.65% and 57.55% respectively; then gradually decreased in the following weeks, and tended to decrease from the 10th week of age. There was a negative relationship between raised duration and relative growth. For whole period, the relative growth in AA

genotype group were 21.03% for males and 19.73% for females; in control group 20.39 and 19.62% for males and females, respectively. The relative growth was not significantly different between two groups for both males and females ($P > 0.05$).

The relative growth of Lien Minh chickens in the two groups were similar to the results of Nguyen Thi Phuong *et al.* (2017); Ngo Thi Kim Cuc and Tran Trung Thong (2018). In the study of Nguyen Thi Phuong *et al.* (2017) on H'Mong chicken, at 12 weeks of age, the relative growth of males was 9.3%, and 9.5% for females. In the study of Ngo Thi Kim Cuc and Tran Trung Thong (2018), the relative growth of Mia chicken at 8 weeks of age was 17.28-18.10%, at 15 weeks of age was 6.07-7.20%. However, the results in our study were lower than that of Noi chicken in the study of Nguyen Huu Van *et al.* (2021) whose relative growth at 9-12 weeks of age was 18.1%.

3.4. Feed conversion ratio

Feed conversion ratio of Lien Minh chickens according to groups and genders are presented in Table 4. The results showed that FCR increased gradually over raised time. FCR of males and females in AA genotype group was lower than that of control group at different periods. In the period from one day to 4 weeks of age, FCR in AA genotype group (1.98kg) was lower than that in control group (2.05kg) with the difference between 2 groups of 0.07kg (3.54%). At the end of the experiment at 20 weeks of age, FCR of males in AA genotype group (3.75kg) was lower than that in control group (3.98kg) ($P < 0.05$). This trend was observed for the females ($P < 0.05$).

FCR was related to growth rate. Chickens with fast growth rate, high body weight had high feed intake and feed efficiency. Consequently, FCR was reduced. FCR in our study was completely consistent with the above rule.

Our results were correspondent to the results of Hoang Anh Tuan (2022) on Mia commercial chicken at 20 weeks of age (3.79-

3.92kg for male and 4.29-4.54kg for female), but lower than the results in the study of Dang Vu Hoa *et al.* (2015) on Te chicken at 20 weeks of age (4.72kg).

Table 4. Feed conversion ratio (kg)

Period (week)	AA genotype		Control	
	Male and Female (n=3)		Male and Female (n=3)	
1d-1w	1.75±0.05		1.77±0.03	
1-2	1.84±0.04		1.92±0.07	
2-3	1.92±0.04		1.98±0.05	
3-4	2.17±0.02		2.25±0.08	
1d-4w	1.98±0.03		2.05±0.05	
	Male (n=3)	Female (n=3)	Male (n=3)	Female (n=3)
4-5	2.09±0.05	2.44±0.07	2.27±0.08	2.48±0.09
5-6	2.24±0.06	2.72±0.03	2.35±0.07	2.76±0.05
6-7	2.38±0.08	2.91±0.05	2.51±0.12	3.01±0.09
7-8	2.55±0.12	3.02±0.08	2.70±0.13	3.11±0.11
8-9	2.74±0.08	3.33±0.09	3.08±0.10	3.4±0.12
9-10	3.26±0.05	3.80±0.04	3.31±0.15	3.86±0.08
10-11	3.33±0.07	3.90±0.05	3.69±0.08	3.96±0.07
11-12	3.56±0.11	4.62±0.08	3.76±0.11	4.65±0.08
12-13	4.08±0.09	5.06±0.04	4.30±0.09	5.11±0.11
13-14	4.55±0.10	5.89±0.03	4.71±0.12	5.92±0.08
14-15	4.84±0.05	6.46±0.14	4.93±0.06	6.54±0.12
15-16	5.37±0.10	6.78±0.07	5.48±0.09	6.83±0.06
16-17	6.80±0.04	7.86±0.11	7.08±0.07	7.89±0.05
17-18	7.60±0.07	8.50±0.10	7.80±0.06	8.55±0.09
18-19	7.65±0.08	8.56±0.03	7.88±0.05	8.61±0.11
19-20	8.66±0.05	8.85±0.09	8.79±0.06	8.89±0.08
Aver.	3.75 ^a ±0.04	4.33 ^b ±0.03	3.98 ^b ±0.08	4.53 ^d ±0.05

4. CONCLUSION

Body weights of males (2430.47g) and females (1784.77g) with AA genotype of G1705A polymorphism in GH gene at 20 weeks of age were higher than those of males (2162.87g) and females (1694.93g) without genotyping selection. The absolute growth of chickens with AA genotype was higher than that in the general population at 4-5, 10-11 and 13-15 weeks of age for males, 7-8 weeks of age for females. Feed conversion ratios of males (3.75kg) and females (4.33kg) with AA genotype after 20 weeks of age were lower than those of males (3.98kg) and females (4.53kg) in general population.

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INFLUENCE OF OOCYTES RECOVERY TECHNIQUES ON RECOVERY AND *IN VITRO* MATURATION OF CỎ GOAT OOCYTES

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ABSTRACT

The goat oocytes matured is a valuable material to generate goat clones and goat embryos *in vitro*. However, the efficiency of the goat oocytes recovery techniques is low and the goat oocytes quality and quantity represent important obstacles on the success of production goat embryos. Therefore, the study was aimed to evaluate the influence of oocytes recovery techniques on *in vitro* maturation of Cỏ goat oocytes. In this study, the Cỏ goat oocytes recovered by two techniques ovary slicing and follicular aspiration of Cỏ goat ovaries obtained from slaughter house. It was observed that follicular aspiration showed the average of Cỏ goat oocytes collected per ovary was 3.4 while ovary slicing showed the average of Cỏ goat oocytes collected per ovary was 6.78. There was a significant difference between the two methods ($P < 0.05$). The average of grade A, B Cỏ goat oocytes recovered per ovary of slicing group was higher than follicular aspiration group (respectively 2.68 vs 5.14, $P < 0.05$). However, no difference on the oocytes matured rate was observed between the two methods ($P > 0.05$). In conclusion, we propose that ovary slicing is the method of choice for Cỏ goat oocytes *in vitro* maturation.

Keywords: Cỏ goat, oocyte recovery, goat oocytes maturation, ovary slicing, follicular aspiration.

TÓM TẮT

Ảnh hưởng của kỹ thuật thu tế bào trứng đến sự thành thực *in vitro* tế bào trứng dê Cỏ

Tế bào trứng dê thành thực là nguồn vật liệu quý để tạo dê nhân bản và phôi dê *in vitro*. Tuy nhiên, hiệu quả của các kỹ thuật thu tế bào trứng dê còn thấp và chất lượng cũng như số lượng tế bào trứng dê là những trở ngại lớn đối với sự thành công của việc tạo phôi dê. Do vậy, mục đích của nghiên cứu này là đánh giá ảnh hưởng của kỹ thuật thu tế bào trứng dê đến sự thành thực của tế bào trứng dê Cỏ. Trong nghiên cứu này tế bào trứng dê Cỏ được thu bằng hai kỹ thuật cắt lát buồng trứng và chọc hút từ buồng trứng dê Cỏ thu ở lò mổ. Kết quả thu được cho thấy trung bình số tế bào trứng/buồng trứng thu bằng kỹ thuật chọc hút đạt 3,4 trong khi đó ở kỹ thuật cắt lát buồng trứng trung bình số tế bào trứng/buồng trứng đạt 6,78. Sự khác nhau giữa hai phương pháp này là có ý nghĩa ($P < 0,05$). Trung bình số tế bào trứng dê Cỏ loại A thu được/buồng trứng của nhóm cắt lát là cao hơn có ý nghĩa so với nhóm chọc hút (tương ứng 2,68 so với 5,14, $P < 0,05$). Tuy nhiên không nhận thấy sự khác nhau về tỷ lệ thành thực giữa hai phương pháp này ($P > 0,05$). Trong phần kết luận, chúng tôi đề xuất cắt lát buồng trứng là một phương pháp được lựa chọn cho quá trình nuôi thành thực *in vitro* tế bào trứng dê Cỏ.

Từ khóa: Dê Cỏ, thu tế bào trứng, nuôi thành thực tế bào trứng dê, cắt lát buồng trứng, chọc hút nang trứng.

1. INTRODUCTION

In the oocytes maturation, the oocytes must to undergo nuclear and cytoplasmic

maturation that are responsible for early embryonic development, that why the oocytes maturation is considered one of the most important steps of *in vitro* production of embryos (Marques *et al.*, 2015). Slaughter house ovaries are alternative source of oocytes for *in vitro* studies, such as *in vitro* maturation, *in vitro* fertilization and somatic cell nuclear transfer (Marques *et al.*, 2015). The obvious advantage of oocytes recovery technique is

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speed of operation, quality of oocytes and quantity of oocytes (Martino *et al.*, 1995).

There are several techniques used for collecting the oocytes from slaughter house ovaries. These techniques include follicular aspiration, ovary slicing and puncture (Martino *et al.*, 1995; Wani *et al.*, 2000; Alm *et al.*, 2008; Rahman *et al.*, 2009 and Hoque *et al.*, 2011); being the follicular aspiration or ovary slicing the most used (Marques *et al.*, 2015).

In Vietnam, Cỏ goat ovaries from slaughter house are less and most of slaughtered females are pre-pubertal, which makes the recovery of goat oocytes by follicular aspiration with low recovery rates. Therefore, ovary slicing would be an alternative for faster recovery. The comparison between follicular aspiration and ovary slicing techniques has not been performed on goat in Vietnam. The objective of our study was to evaluate two techniques of oocytes collection from slaughter house Cỏ goat ovaries and the effect on oocytes recovery efficiency, *in vitro* maturation rate.

2. MATERIAL AND METHODS

2.1. Materials

Unless otherwise stated, all chemicals in this study were purchased from Sigma (Sigma-Aldrich Corp., St Louis, MO, USA).

Cỏ goat ovaries were collected from a local slaughter house in Ha Noi and transported to the Key Laboratory of Animal Cell Biotechnology within one to two hours slaughter in DPBS supplemented with antibiotic at 35°C. The reproduction status of animals was not known.

2.2. Methods

2.2.1. Cỏ goat ovaries recovery

The Cỏ goat ovaries were cut by sterile seizer and freed from surrounding tissues and overlying bursa. Each ovary was treated to four washings in normal saline and three washing in DPBS with antibiotic.

2.2.2. Oocytes recovery

Each Cỏ goat ovary was treated to three washing in collecting media (TALP-Hepes

supplemented with Calf serum and antibiotic). Then ovaries were subjected to ovary slicing or follicular aspiration techniques.

For the ovary slicing method, 5 ovaries were held with the forceps inside a 20ml TALP-Hepes and follicles (2-8mm) on the ovarian surface were incised with a scalpel blade. After every 5 sliced ovaries, the TALP-Hepes containing follicular fluid was transferred to 50ml centrifuge tubes. For the aspiration method, a 5ml syringe containing 2ml of collection medium with a 18G needle was used to aspirate oocytes and follicular fluid from 2-8mm follicles on the surface of the Cỏ goat ovary. Follicular fluid of each ovary was transferred to 50ml centrifuge tube containing 10ml collection medium.

Tubes from both recovery techniques were placed in water bath at 35°C for 15min for settling of cumulus oocytes complexes (COCs). The supernatant was removed, then the sediment containing the oocytes was dissolved in the collection medium (TALP-Hepes) and COCs selected under a stereomicroscope (40xof magnification) and then the total number of collected oocytes were counted.

2.2.3. Oocyte classification method

The collected oocytes were graded according to Wani *et al.* (2000) as good (grade A), fair (grade B), and poor (grade C) on the basis of cumulus cells and cytoplasm.

Grade A: Oocytes with uniform cytoplasm surrounded by many complete layers of cumulus cells.

Grade B: Oocytes with uniform cytoplasm surrounded by thin or incomplete layers of cumulus cells.

Grade C: Oocytes with uneven cytoplasm surrounded by few or no cumulus cells.

2.2.4. *In vitro* maturation

Only grade A and B oocytes were selected for *in vitro* maturation in our study. The Cỏ goat oocytes were washed three times in maturation medium either TCM 199

supplemented with 10% FBS, 50ng/ml FSH, 10ng/ml EGF and 100 μ M Cysteamine and then incubated in the maturation medium in 38.5°C, 5% CO₂ and high humidity for 24hrs. Presence of first polar body was a good criterion for *in vitro* maturation (IVM).

2.3. Statistical analysis

The data was analyzed using Microsoft Excel 2010 with ANOVA tested differences between groups with less than 5% probability were considered significant.

Table 1. Influence of collection techniques on oocytes recovery in Cỏ goat

Method of collection	No. of ovaries	No. of oocytes	No. of oocytes collected per ovary	No. of Grade A, B oocytes collected per ovary	No. of Grade C oocytes collected per ovary
Aspiration	42	142	142/42 3.4 ^a ±1.02	112/42 2.68 ^a ±0.98	30/42 0.79±0.72
Slicing	44	298	268/44 6.78 ^b ±1.46	226/44 5.14 ^b ±1.71	72/44 1.64±1.12

Five replicates were performed. Different superscripts (a, b) denote a significant difference in the same column (P<0.05).

The results in Table 1 showed the average of oocytes collected per ovary of ovary slicing method higher than follicular aspiration method (respectively 6.78 vs 3.4, P<0.05). Our result similar observation has been made by Wang *et al.* (2007), Sogorescu *et al.* (2010), Majeed *et al.* (2011), indicating that ovary slicing method yielded significantly more oocytes per ovary than follicular aspiration method.

It has been shown that follicular aspiration method is also less efficient when compared to whole ovary slicing method. Wang *et al.* (2007) observed that puncturing the visible follicles with a scalpel blade yielded higher number oocytes per ovary as well as good oocytes compared to follicular aspiration technique. High recovery rate with ovary slicing method might due to when ovary slicing method is used, oocytes collection could be performed with small diameter follicles.

Visual assessment of morphological features remains the most important vehicle for selection of oocytes before maturation during oocytes recovery. There was a

3. RESULTS AND DISCUSSION

3.1. Influence of collection techniques on oocytes recovery in Cỏ goat

The results in Table 1 showed the influence of collection techniques on recovery rates and grade of oocytes recovered. The average of oocytes and grade A, B oocytes recovery of each technique was determined by the ratio between number of selected oocytes and used ovaries. Our results showed a significant difference between ovary slicing and follicular aspiration methods (P<0.05).

significant difference in recovery rate of different oocytes collection methods. Our results showed that higher recovery rate were obtained of grade A, B oocytes of ovary slicing method when compared to follicular aspiration method. The average of grade A, B oocytes collected per ovary of follicular aspiration method was 2.68, while ovary slicing method showed 5.14 (P<0.05). Similar observations have been made by Wani *et al.* (1999) and Rahman *et al.* (2009).

3.2. Influence of collection techniques on *in vitro* maturation in Cỏ goat

The maturation rates of oocytes recovered by the different techniques were checked with the expression of the first polar body at 24hrs after *in vitro* maturation. In this study, no differences between oocyte recovery methods were observed on the expression of the first polar body. The results showed in Table 2 that no significant difference in maturation rates of oocytes between ovary slicing method and follicular aspiration method (respectively 72.41 vs 72.18, P>0.05).

Table 2. Influence of collection techniques on *in vitro* maturation in Cò goat

Method of collection	Total No. of oocytes	No. of matured oocytes	Matured (%)
Aspiration	112	81	72.41±2.34
Slicing	226	163	72.18±2.45

Five replicates were performed. Percentages are presented as Mean±SE

Our results similar observations have been made by Marques *et al.* (2015), but no similar to those of Majeed *et al.* (2011). According to Marques *et al.* (2015) no differences between pig oocytes recovery by ovary slicing method and follicular aspiration method were observed on metaphase II at 44hrs after *in vitro* maturation. Meanwhile, according to Majeed *et al.* (2011), there was a significant difference in maturation rate between the follicular aspiration and ovary slicing methods at 24-26hrs after *in vitro* maturation. The difference between the study results may be due to the difference in oocytes quality before *in vitro* maturation.

4. CONCLUSION

The recovery of Cò goat oocytes using the ovary slicing technique yielded more oocytes per ovary than follicular aspiration technique, and no significant difference in matured Cò goat oocytes rate between different technique of recovery oocytes. In conclusion, we propose that ovary slicing is the method of choice for Cò goat oocytes *in vitro* maturation.

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MORPHOLOGY AND FIGHTING BEHAVIORS OF GAMECOCK CHICKENS

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ABSTRACT

The study was conducted to compare the appearance, morphology, and fighting behaviors of the Asil and American crossbred roosters. A total of 30 roosters 8-10 months of age were arranged in a completely randomized design with 2 treatments corresponding to 2 breeds: Asil and American crossbred. Each treatment was reproduced 15 times with 1 rooster per time. Each rooster was isolated in an individual cage at the household in Chau Hoa commune, Giong Trom district, Ben Tre province. The appearance characteristics were observed directly and the chicken's body indexes were measured directly. The fighting behavior was assessed by the recorded videos. The results showed that the Asil crossbred roosters had red and white neck feathers (26.67%), red-orange body feathers (33.33%), blue-black tail feathers (60%), strawberry comb (66.67%), yellow in legs (33.33%) and beak (40%), and orange eyes (46.67%). American crossbred roosters had red feathers in the neck (40%) and body (40%), blue-black tail feathers (73.33%), strawberry comb (66.67%), yellow in legs (53.33%) and eyes (73.33%), and yellow-black beak (46.67%). There was a statistically significant difference in some morphological characteristics between American and Asil crossbreds ($P < 0.05$). Specifically, body weight, head height, breast length, breast circumference, leg length, and height in American crossbred roosters were larger than in Asil crossbred roosters, in contrast, Asil crossbred roosters had larger leg circumference. The differences in fighting behavior between Asil and American crossbred roosters were not statistically significant ($P > 0.05$).

Keywords: Appearance characteristics, morphology, fighting behavior, rooster.

TÓM TẮT

Hình thái và tập tính chiến đấu ở gà trống Nòi lai

Thí nghiệm được thực hiện so sánh đặc điểm ngoại hình, hình thái và một số tập tính chiến đấu ở gà trống Nòi lai Asil và lai Mỹ. Tổng số 30 gà trống từ 8 đến 10 tháng tuổi được bố trí hoàn toàn ngẫu nhiên với 2 nghiệm thức tương ứng với 2 giống gà lai: Asil và Mỹ. Mỗi nghiệm thức được lập lại 15 lần và mỗi lần lập lại là 1 gà trống. Mỗi gà trống được nuôi ở một chuồng riêng biệt ở điều kiện nông hộ tại xã Châu Hòa, huyện Giồng Trôm, tỉnh Bến Tre. Bằng cách quan sát ngoại hình và đo trực tiếp các chỉ số hình thái trên cơ thể gà thí nghiệm, các tập tính chiến đấu của chúng cũng được ghi nhận qua các video clip. Kết quả thí nghiệm cho thấy gà trống Nòi lai Asil có màu lông cổ trắng đỏ (26,67%), lông thân màu đỏ cam (33,33%), lông đuôi xanh đen (60%), kiểu mào dậu (66,67%), màu vàng ở chân (33,33%) và mỏ (40%) và mắt màu cam (46,67%). Gà trống Nòi lai Mỹ có màu lông cổ đỏ (40%), lông thân màu đỏ (40%), lông đuôi xanh đen (73,33%), kiểu mào dậu (66,67%), chân màu vàng (53,33%), màu mắt vàng (73,33%) và màu mỏ vàng đen (46,67%). Có sự khác biệt có ý nghĩa thống kê về một số đặc điểm hình thái giữa gà trống Nòi lai Mỹ và lai Asil ($P < 0,05$), trong đó gà trống Nòi lai Mỹ có khối lượng, cao đầu, dài lườn, vòng ngực, dài đuôi và cao chân lớn hơn gà Nòi lai Asil, ngược lại gà Nòi lai Asil có vòng chân lớn hơn gà Nòi lai Mỹ. Sự khác biệt về tập tính chiến đấu giữa giống gà lai Asil và lai Mỹ không có ý nghĩa thống kê ($P > 0,05$).

Từ khóa: Đặc điểm ngoại hình, hình thái, tập tính chiến đấu, gà trống.

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1. INTRODUCTION

Noi chicken is a local chicken breed raised in the Mekong Delta, popular in some provinces such as Ben Tre, Tien Giang, Dong Thap, etc. This is one of the chicken breeds that is well adapted to the harsh environmental conditions of Vietnam, and resistance to disease (Khang *et al.*, 2010). This chicken breed is popular due to its delicious meat quality, firmness, low fat, and large thighs, suitable for consumers' tastes. In addition to nutritional value such as meat and eggs, Noi chickens are also bred with other imported chicken breeds such as Asil chicken, and American chicken to improve productivity and also to entertain people.

Cockfighting is one of the popular forms of entertainment but is considered illegal in Vietnam and some other countries. This may be why scientific reports on the social and cultural aspects of cockfighting are scarce, and empirical studies on cockfighting behavior during fighting are almost nonexistent. Some observations on fighting behavior during fighting show that the Asil chicken originates from India with advantages such as bounce, good endurance and extremely fast fighting style, extremely skilled strikes, and steps to dodge attacks (Singh, 2001). However, this breed of chickens has the disadvantage that the body is quite slender and a bit short. Compared to the American breed of chickens originating from the US with the advantage of being aggressive, the ability to go to battle quickly makes the opponent unable to react, but their disadvantage is the ability to dodge attacks weaker than other fighting chicken breeds, and poorly adapted to the climatic conditions of Vietnam.

Recording the morphological characteristics of the Noi crossbred chickens published by Khang *et al.* (2022). Continuous sampling is one of the methods used in behavioral recording in animals. This method is considered the gold standard used across all species, as it provides the most complete assessment of animal behavior by providing

a complete profile of all behavior, and is observed over a period of time (Lehner, 1992). Continuous sampling, however, requires the scientist to invest considerable time, effort, and resources. This is especially evident when assessing large animal populations or long observation periods.

The objective of this study was to compare the appearance, morphology, and some fighting behaviors of the Asil and American crossbred roosters.

2. MATERIALS AND METHODS

2.1. Animals and management

This study was conducted at the household in Chau Hoa commune, Giong Trom district, Ben Tre province from February to April 2021. A total of 30 roosters from 8 to 10 months of age were used in the study. Birds were raised in individual barns (1.0×2.0×2.0m) with a cement wall 1m high, covered with a B40 net, the distance between 2 cages was 40cm; the flying cage has the same area as the jogging cage, with 1 perch 1.5m high from the ground, the distance between the 2 flying cages was 45cm, covered with a silver plate in the middle. In addition, there were 45 conical iron cages with a diameter of 1m and a height of 1m, including 30 cages for confinement and 15 cages for sunbathing.

The feed for roosters during the training period was used based on the local formula for fighting cocks consisting of rice, green beans, and mixed feed at a ratio of 10:1:1. Feeding twice a day, in addition, the supplementary feed included green vegetables, bean sprouts, fresh beef, eel, and black soldier fly pupae (Figure 2). The nutritional composition of the mixed chicken feed was 24.69% protein, respectively; 6.1% minerals; 5.19% fat; 6.67% fiber; 1.66% Ca; and 1.04% P (Analysis results at the Animal Nutrition and Feed Technology Laboratory, CTU).

All experimental roosters were trained daily according to the activities described in Table 1.

Table 1. Care and training of the experimental roosters

Activities	Time (h)	Note
Sunbathing for rooster	7:00-7:15	Daily
Chicken bath	7:15-9:30	Daily
Give chickens honey to drink	7:15-9:30	Every 2 days
Feeding 1	9:30-10:00	Daily
Supplement feed	13:00-13:20	Cont. 4 days/ week
Running training	14:00-14:20	Every 3 days*
Flight training	14:00-14:30	Every 3 days*
Live fighting cock training	15:30-16:00	end of every week
Turmeric bath for chickens	16:00-16:30	end of every week
Feeding 2	16:30-17:00	Daily
Put perch for chicken to sleep	17:00	Daily

2.2. Experimental design and data collection

A total of 30 roosters from 8 to 10 months of age were arranged in a completely randomized design with 2 treatments corresponding to 2 chicken breeds: Asil and American (Ame) crossbred roosters. Each treatment was repeated 15 times, each repetition was one rooster.

The experimental cockerels were weighed at the beginning (8 months of age) and at the end (10 months of age) of the experiment. Roosters were fed a restricted diet of 50 g/bird/day and 10g of supplemental feed. Visual observations of the most important qualitative parameters of the birds (comb type, colors of beak, eyes, shank and foot, feathers in neck, body and tail) were recorded according to FAO guidelines (FAO, 2012). Quantitative traits were body weight (BW, gram) and skull length (SL, cm), skull width (SW, cm), neck length (NL, cm), neck circumference (NC, cm), back length (BL, cm), keel length (KL, cm), breast circumference (BC, cm), breast angle (BA, cm), breast depth (BD, cm), wing length (WL, cm), thigh length (TL, cm), shank length (ShL, cm) and shank circumference (SD, cm) according to the standard descriptors (FAO, 2012).

The behavior and aggression of roosters during the fighting were collected using a continuous sampling methodology by

observation and video recording over a period of nearly 1 minute. To ensure observer reliability between 2 observers, a subset of the video was randomly selected, observed, and compared until 90% accuracy was achieved. The fighting behaviors of cockerels included Standing neck feathers (seconds) are a reaction when facing an opponent, the neck feathers will stand up; Approaching (seconds) is the chicken walking toward the opponent. If the opponent also approaches, the head and tail are usually lowered so that they are parallel to the ground and the hackles raised; Spurring (seconds) is the action of a rooster leaping into the air from a standing position and kicking the opponent so that the spurs on the legs injure the adversary; Wing flashing: a brief raising of the wings, sometimes accompanied by a simultaneous lifting of the tail; Mouth kick (seconds) is the act of directly pecking the opponent with the mouth when fighting.

2.3. Statistical analysis

The collected raw data were recorded and processed by Microsoft Excel software, then statistically processed by Minitab Version 16 software, in which the indicators of appearance were processed by Chi-square test, the difference between values was considered reliable when $P < 0.05$, dimensional indicators were expressed as the numerical mean. Indicators with a low rate (<5%), were not presented in the results. The morphological parameters and fighting behavior of the roosters were treated with T-Test.

3. RESULTS AND DISCUSSION

3.1. Appearance characteristics of the Asil and American crossbred roosters

The results of appearance characteristics of 30 crossbred roosters were shown in Table 2 showed that the neck feather colour of Asil crossbred roosters was mostly white-red (26.67%), while the Ame crossbred roosters were mostly red (40%) and the remaining colours were red black, red orange and yellow. Similarly, Asil crossbred chickens had mainly

red-orange body feather colour (33.33%) and Ame crossbred chickens were mainly red (40%). The Asil and Ame crossbreds were mostly blue-black tail feather colour (60% Noi×Asil, 73.33% Noi×Ame) and strawberry comb type (66.67%). Red and orange eye colour predominated in Asil (46.67%) and Ame (73.33%) crossbred chickens and white eye colour accounted for 20% in both Noi×Asil and Noi×Ame chickens. Besides, Noi×Asil roosters mostly had yellow beak colour, accounting for 40%, in Noi×Ame roosters had yellow-black colour accounting for 46.67% compared to other beak colors such as black and white. Shank and foot colour in Noi crossbred roosters was very diverse, the results showed that most of the Noi×Asil and Noi×Ame cocks had yellow legs (Noi×Asil 33.33%; Noi×Ame 53.33%), besides the colors others like white-yellow, yellow-green, white, silver.

Table 2. Qualitative trait of Noi crossbred roosters

Qualitative trait	Noi×Asil (n=15)		Noi×Ame (n=15)	
	n	%	n	%
Comb type				
Strawberry	10	66.67	10	66.67
Others	5	33.33	5	33.33
Beak colour				
Black	3	20	4	26.67
Yellow	6	40	3	20
Yellow + black	5	33.33	7	46.67
Eye colour				
Orange	5	33.33	11	73.33
Red	7	46.67	1	6.67
White	3	20	3	20
Shank & foot colour				
Yellow	5	33.33	8	53.33
Green + yellow	4	26.67	4	26.67
Others	6	40	3	20
Neck feather colour				
Red	2	13.33	6	40
White + red	4	26.67	1	6.66
Red + black	3	20	4	26.67
Red + orange	3	20	4	26.67
Others	3	20	-	-
Body feather colour				
Red	3	20	6	40
Red + orange	5	33.33	4	26.67
Red + black	3	20	4	26.67
White + red	4	26.67	1	6.66
Tail feather colour				
Green + black	9	60	11	73.33
Others	6	40	4	26.67

In general, the Asil and Ame crossbred roosters had similar characteristics in tail

feather colour, shank and foot colour, and comb type. There were also differences in neck feathers, body feathers, beak and eye colours between the two chicken breeds. These differences may be due to specific characteristics in each chicken breed or due to selection factors in each household, each location. Similar to the present study, yellow beak colour was predominantly found in both Asil from Bangladesh (Sarker *et al.*, 2012), India (Rajkumar *et al.*, 2017), and Vietnam (Khang *et al.*, 2022). Moreover, similar observations for strawberry combs (24.05%) in Asil from Bangladesh were reported by Sarker *et al.* (2012), and Noi and Asil from Vietnam reported by Khang *et al.* (2022). Khang *et al.* (2022) revealed that most Asil birds in Vietnam were orange (32.2%) and yellow (25.1%) eye colours while Noi cocks with yellow + orange (35.7%) and yellow (32.8%) eye colours. It was not in agreement with the observations of Rajkumar *et al.* (2017), most Asil birds in India were black (99%) and white (1%). In the present study, the colours of the neck, body, and tail in both chicken breeds were in line with the report of Khang *et al.* (2022). The various plumage colors observed in the indigenous chicken populations of Mekong in general and particularly in this study might be due to the fact that, the preference of people for fighting towards red, black and green plumages which accounted for the largest occurrence of these plumage colors across this area.

3.2. Morphological characteristics of Noi crossbred roosters

The morphological characteristics of the Asil and Ame crossbred roosters in Table 3 showed that there was a statistically significant difference in some morphological characteristics between Noi×Ame and Noi×Asil chickens ($P < 0.05$), in which BW, SL, KL, TL and ShL in the Noi×Ame chickens were higher than that of the Noi×Asil chickens, on the contrary, the Asil crossbred chickens had a larger SD than the Ame crossbred chickens.

Table 3. Morphological characteristic Noi crossbred

Parameters	Treatments (Mean±SD)		P
	Noi×Asil	Noi×Ame	
InBW (g)	2822.7±199.7	2972±170.7	0.036
FiBW (g)	2859.3±174.1	3014.7±166.2	0.019
Age (month)	8.67 ± 0.82	9.0 ± 0.93	0.305
SL (mm)	27.8 ± 1.70	29.1 ± 1.67	0.049
SW (mm)	32.5 ± 3.09	31.3 ± 1.83	0.183
NL (cm)	15.3 ± 1.23	15.4 ± 1.24	0.884
NC (cm)	13.5 ± 1.41	12.5 ± 1.46	0.085
BL (cm)	20.1 ± 1.30	20.9 ± 0.96	0.066
KL (cm)	12.3 ± 0.96	13.0 ± 0.01	0.006
BC (cm)	31.9 ± 2.88	34.2 ± 1.82	0.013
BA (°)	75.5 ± 5.11	78.0 ± 5.89	0.231
BD (cm)	13.0 ± 1.16	13.7 ± 0.98	0.137
WL (cm)	28.4 ± 1.88	28.6 ± 0.91	0.714
TL (cm)	15.8 ± 1.57	17.3 ± 1.54	0.012
ShL (cm)	8.4 ± 1.40	9.7 ± 1.44	0.016
SD (cm)	5.0 ± 0.01	4.53 ± 0.52	0.002

The overall average body weights of Noi×Asil and Noi×Ame were higher than those reported by Khang *et al.* (2022) for Noi and Noi×Asil cocks from the Mekong area. In addition, the TL, KL, and SD in this present study are consistent with the study results of Khang *et al.* (2022), while the SL and BA in Noi and Noi×Asil were longer than those of the current study.

3.3. Some fighting behaviors of the Asil and American crossbred roosters

The rooster’s actions when fighting, through observation and video recording, such as raising neck feathers (Fig 1a), approaching (Fig 1b), spurring (Fig 1c), wing flashing (Fig 1d), and kicking the mouth (Fig 1e). The results in Table 4 showed that the performance indicators of fighting behavior between Noi×Asil and Noi×Ame cocks were not statistically significant (P>0.05).



(a) Raising neck feathers



(b) Approaching



(c) Spurring



(d) Wing flashing



(e) Kicking the mouth

Fig 1. Fighting behaviors of the experimental roosters

However, the response to fighting behaviors in Noi×Ame chickens was faster than that of Noi×Asil chickens. There are very few detailed descriptions of the fighting chicken behavior. Some authors suggested that the

importance of various factors in determining the winners was the absence of molt, comb size, social rank, and weight, however, these four factors accounted for only 56% of the variance in fight outcome (Herzog, 1979). The rate of

change in behavior during fights varied, and in more prolonged fights seems to slow down as the animal was tired, the rate of change in the fighting behaviors varied between 41.4 and 90.2 per min (Herzog, 1979).

Table 4. Fighting behaviors of Noi crossbred rooster

Parameters, times	Noi×Asil	Noi×Ame	SEM	P
Raising neck feathers	0.93	0.93	0.25	1.000
Approach	2.00	2.00	0.46	1.000
Wingflash	3.53	2.93	0.60	0.479
Spur	4.73	3.2	0.79	0.183
Mouth kick	15.4	12.7	3.75	0.619

4. CONCLUSION

There was a similarity in tail feather colour, shank and foot colour, and comb type between Noi×Asil and Noi×Ame roosters. However, BW, SL, TL and ShL were higher in Noi×Ame than in Noi×Asil, in contrast, Noi×Asil had a larger SD than Noi×Ame. There was no significant difference in fighting behaviors between the two chicken breeds.

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EFFECT OF DIFFERENCE EXTENDER ON EXTENDED BOAR SEMEN QUALITY IN THE LONG-TERM STORAGE

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ABSTRACT

The purpose of this study was to assess the effects of three different extenders as Modena, Beltsville Thawing Solution (BTS), and Kiev on the quality of boar semen preserved in some boar breeds in Vietnam. Semen samples of 6 Yorkshire boars were collected then diluted in three extenders and stored at 17°C for up to 144h. The semen parameter as viability, motility, and extender quality as pH, and bacteria status were assessed and compared after 72, 120, and 144h of preservation. Results showed that Modena extended boar semen had better motility, and survival rate than BTS and Kiev extended boar semen after 144h preservation. The value of pH was stable and acceptable during the experiment period, Modena was a higher value of pH than BTS and Kiev. Moreover, bacteria status was higher in semen preservation in Kiev than in Modena and BTS. Thus, the Modena had a more beneficial effect on stored boar semen during 120h preservation, and BTS and Kiev had the ability to preserve boar semen within 72h in the current study.

Keywords: Semen, extender, pig, bacteria status, storage.

TÓM TẮT

Ảnh hưởng của các dung dịch bảo quản khác nhau lên chất lượng tinh trùng pha loãng của lợn khi được bảo quản trong thời gian dài

Mục tiêu của nghiên cứu nhằm đánh giá tác động của ba loại dung dịch bảo quản như Modena, Beltsville Thawing Solution (BTS) và Kiev lên chất lượng của tinh trùng pha loãng của một số giống lợn thương mại được nuôi tại Việt Nam. Tinh nguyên của 6 lợn giống Yorkshire được khai thác và pha loãng với ba dung dịch bảo quản và bảo quản ở 17°C đến 144h. Các chỉ tiêu về chất lượng tinh trùng như tỷ lệ sống và hoạt lực cũng như chất lượng của dung dịch bảo quản như pH, mật độ vi khuẩn được phân tích và so sánh sau 72h, 120h và 144h bảo quản. Kết quả cho thấy tinh trùng được pha loãng với Modena có tỷ lệ sống và hoạt lực tốt hơn BTS và Kiev sau 144h bảo quản. Chỉ số pH tương đối ổn định trong quá trình bảo quản và nằm trong mức cho phép, Modena có chỉ số pH cao hơn BTS và Kiev sau 144h bảo quản. Thêm vào đó, mật độ vi khuẩn của tinh trùng cao hơn ở Kiev khi so sánh với Modena và BTS. Do đó, trong thí nghiệm này, khả năng bảo quản tinh phù hợp nhất là 120h đối với Modena, 72h đối với BTS và Kiev.

Từ khóa: Tinh trùng, dung dịch bảo quản, lợn, mật độ vi khuẩn, bảo quản.

1. INTRODUCTION

Artificial insemination (AI) is used widely in swine production in Vietnam. AI increased the production rates and carcass homogeneity as well as the application of new management systems. Currently, AI centers and pig farms use fresh diluted semen for the insemination of sows. Therefore, short-term extenders are

applied popular, also in a longer preservation period of semen.

The quality of boar semen is evaluated by several parameters. Sperm morphology represents sperm viability and fertility and can be routinely examined (Britt *et al.*, 1999). Sperm motility shows an active metabolism and membrane integrity (Johnson *et al.*, 2000) and is important for fertilizing. Semen with motility scores <60% has fewer fertilized eggs and lower farrowing rates (Britt *et al.*, 1999). Long-term extenders must ensure not only sperm cell viability but also sperm motility during preservation time.

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pH is one of the important requirements for extenders, due to the ability to neutralize metabolic wastes and osmolality (Britt *et al.*, 1999). In addition, limiting bacterial development in extended semen is another requirement for semen extenders, because bacteria are not only damaging the semen quality and the survival time of semen and manipulate the pH level (Hofmo *et al.*, 1999). Therefore, short-long-term extenders should maintain pH levels and limit bacteria which leads to an increment in the quality of boar semen.

This study aims to investigate the differences between three semen extenders to evaluate boar sperm survival and ability. Therefore, boar sperm extension in one long-term extender and two short-term extenders were examined for sperm survival rate, morphology, motility, pH, and bacterial status during a period of 144h.

2. MATERIALS AND METHODS

2.1. Experimental design

Semen was extended in Beltsville Thawing Solution (BTS), Kiev, and Modena Split samples were stored at 17°C, and analyzed after 72, 120, and 144h of storage. The motility, survival rate, pH, and bacteria status of the extended semen were assessed after a 30min incubation period of the samples at 38°C.

The composition of extenders used in the experiment is shown in Table 1 (GadeaJ, 2003).

Table 1. Ingredients of Extenders

Contents (g/l)	Kiev	BTS	Modena
Glucose	60	37	25
Sodium citrate	3.7	6.0	6.90
EDTA	3.7	1.25	2.25
Sodium bicarbonate	1.2	1.25	1.00
Potassium chloride		0.75	
BSA			3.00
Tris			5.65
Citrate			2.00
Cysteine			0.05

2.2. Semen processing

Semen was collected from six healthy, mature boars housed at commercial farms.

From each boar, one ejaculate was collected by the gloved hand method into disposable collection bags with an integrated filter to remove the gel fraction. Collection bags were mounted in pre-warmed thermos cups (38°C) to prevent any cold-shock cold shock for the semen. After collection, the semen was immediately transferred to the laboratory of Non-ruminant Animal Production Techniques, Faculty of Animal Sciences, Can Tho University in Styrofoam boxes.

The raw semen concentration was assessed by Porcine Sperm Photometer III (Fujihika industry, Tokyo, Japan). Only ejaculates with $\geq 70\%$ motile and $\leq 25\%$ morphologically abnormal sperm were used in this study. After that, semen was diluted to 20×10^6 sperm/mL with three extenders. The samples were cooled stepwise to the desired storage temperature. Samples were kept for 90min at room temperature and then placed in a 17°C storage unit (BioPlus ER500, Grambioline, Vojens, Denmark) (Henning *et al.*, 2022). All samples were stored in the dark.

2.3. Parameters of comparison

Four different parameters were used to investigate the extended semen samples, namely percentage of live semen, motility, pH, and bacterial contamination. Semen viability was evaluated by the eosin-nigrosin staining method (Kondracki *et al.*, 2017). Every sample was investigated three times on eosin-nigrosin staining and an average percentage of the parameter values was calculated. After gentle mixing, a portion of each dilution was thawed at 37°C for 30min and motility was assessed visually using a microscope (BB 4260, Euromex, Arnhem, Netherlands) at 40x magnification. Assessments were made by the same person to minimize differences in interpretation.

The pH of all the samples was measured by means of an Orion Star™ A214 (Thermo Fisher Scientific, Massachusetts, United States), calibrated with pH 4,00; 7,00 and 10,00 standard solutions. The bacteriological status of the extended sperm was evaluated by the number of Colony form units (CFU) on a pour

plate prepared with plate count agar (HiMedia, Mumbai, India) (Vyt *et al.*, 2004). The number of CFU was counted by Haloes Caliper (IUL Instruments SA, Barcelona, Spain).

2.4. Statistical analysis

All data are shown as the Mean \pm standard error of the mean (SEM). Analysis of variance (ANOVA) and Tukey's test were performed using R (version 3.5.3; <https://www.r-project.org/>). Statistical significance was set at $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Effect of extenders on the viability of boar semen during long-term storage

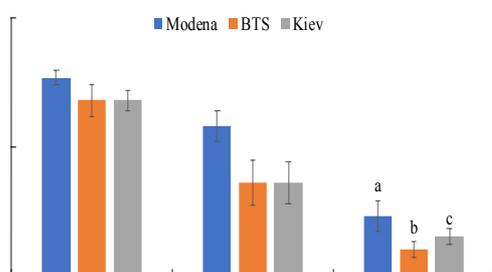


Figure 1. The average percentage of live semen

Bar marked by different letters are significantly different ($P < 0.05$)

The percentage of live semen (Figure 1) shows a clear decrease pattern during the experiment period. The highest viability was observed in Modena during the experiment, i.e. 77.32 \pm 3.47% at 72h, 57.81 \pm 5.93% at 120h, and 23.05 \pm 5.67% at 144h. The ratio of live semen of both short-term extenders as BTS and Kiev was similar during 72h (67.73 \pm 5.81% for BTS; 67.57 \pm 4.15% for Kiev) and 120h (36.06 \pm 9.40% for BTS; 36.38 \pm 7.88%). At 144h storage, the viability of semen dilution with BTS and Kiev was 9.58 \pm 2.56 and 14.94 \pm 2.79%, respectively. The viability of extended boar semen was not a significant difference when comparing short- and long-term extenders at 72 and 120h storage ($P > 0.05$). However, Modena had a significantly higher number of live semen than BTS and Kiev at 144hrs ($P < 0.05$). Similar to our result, long-term extender as Androhep and Zorlesco also had higher viability than BTS

and Kiev when storing for up to 13 days (Huo *et al.*, 2002). In addition, Modena was the only Tris-based extender among three extenders in this experiment. A previous study found that Tris had an effect on the viability of rabbit semen when stored at 15°C (Roca *et al.*, 2000). In conclusion, long-term extenders had better viability than short-term extenders, these differences may be explained by Tris content on the Modena formulator.

3.2. Effect of extenders extender on the motility of boar semen during long-term storage

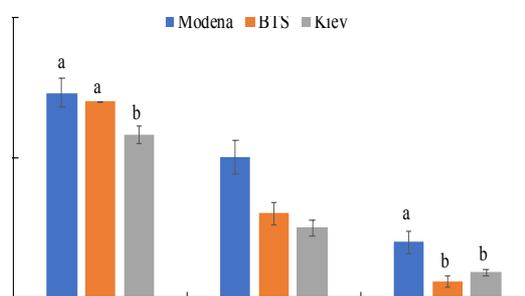


Figure 2. The average percentage of motility

Motility has a similar pattern with the viability of extended boar semen when motility of all treatment groups was linearly reduced from 72 to 144hrs of the experiment period (Figure 2). The average motility of all extenders was higher than 50% for semen stored until the third day, and it was lower than 20% at the end of the experiment (Figure 2). After 72hrs, short-term extenders such as Kiev significant decrease the motility of boar semen to 57.50 \pm 2.50%, but the motility of Modena and BTS extended semen still maintain at 72.50 \pm 4.79 and 70.00%, respectively ($P < 0.05$). After 144hrs, motility was significantly higher for semen dilution with Modena (20 \pm 4.08%) than for those stored with BTS (6.25 \pm 2.39%) and Kiev (8.75 \pm 1.25%) ($P < 0.05$). In boar, semen motility is one of the main parameter quality (Britt *et al.*, 1999; Johnson *et al.*, 2000). The finding of Huo *et al.* (2002) that on long-time storage, long-term extender had better semen motility than BTS and Kiev. BSA composition in Modena extender can decline the metabolic by-products from semen and microorganisms and may inhibit peroxidative activity (Alvarez

et al., 1983; Bamba and Sone, 1981). Therefore, BSA content in Modena may be the main reason for maintaining better motility than BTS and Kiev.

3.3. Effect of extenders on pH of boar semen during long-term storage

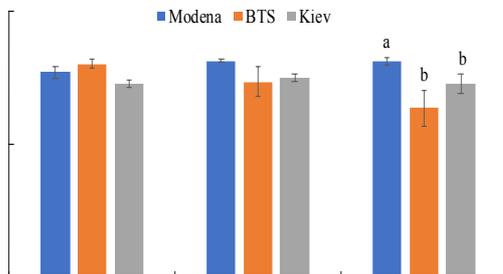


Figure 3. The average of pH

During the experiment period, average pH levels of short- and long-term extenders varied from 6.80 to 7.87 (Figure 3). pH of Modena extender was slightly increased during the experiment, it was 7.62 ± 0.14 at 72h, 7.87 ± 0.04 at 120h, and 7.87 ± 0.08 at 144h. However, BTS extender showed a clear reduction pattern of pH level, it was 7.81 ± 0.10 at 72h, 7.40 ± 0.33 at 120h, and 6.80 ± 0.41 at 144h. During storage time, the pH value of Kiev induced a stable trend from 72h (7.36 ± 0.08) to 144h (7.36 ± 0.23), and it was highest at 120h (7.50 ± 0.09). Extender characteristics did not affect pH value after 72 and 120h storage ($P > 0.05$). After 144h, both of BTS and Kiev extender had a lower pH value than Modena ($P < 0.05$). Previous study about boar semen storage, the pH value of five different short- and long-term extenders was 7.07 to 7.82, which is similar to the current study (Vyt *et al.*, 2004). Lactic acid is the main product of glycolytic metabolism and the increase of lactic acid lead to a decrease in pH to acidic levels (5.7-6.4) (Rigau *et al.*, 1996). Therefore, the reduction of pH level during storage can explain by glycolytic metabolism, which suppresses the motility of boar semen (GadeaJ, 2003). Moreover, Modena contains Tris which is a pH regulator over a wider range (GadeaJ, 2003). Modena had better control pH

ability than BTS and Kiev after 144h storage due to the Tris-based formulator, and the pH level of the two short-term extenders was not acidic. Thus, pH level may have a low influence on semen motility and viability in the current study.

3.4. Effect of extenders on bacteria status of boar semen during long-term storage

The number of CFU was linearly increased during the experiment period for Modena, BTS, and Kiev extenders (Figure 4). After 72h storage at 17°C in the absence of antibiotics, bacterial counts after culture on PCA was $0.20 \pm 0.06 \times 10^3$ CFU/ml in Modena, $0.17 \pm 0.08 \times 10^3$ CFU/ml in BTS and $0.18 \pm 0.06 \times 10^3$ CFU/ml in Kiev. Short- and long-term extenders did not affect on bacteria level of boar semen after 72h storage ($P > 0.05$). After 120h, the number of bacteria was higher in Kiev ($0.57 \pm 0.06 \times 10^3$ CFU/ml) than in Modena ($0.31 \pm 0.09 \times 10^3$ CFU/ml) and BTS ($0.39 \pm 0.06 \times 10^3$ CFU/ml), but there was not a significant difference ($P > 0.05$). At the end of the storage period, CFU of semen dilution with Modena ($0.49 \pm 0.14 \times 10^3$ CFU/ml) and BTS ($0.43 \pm 0.12 \times 10^3$ CFU/ml) was significantly lower than semen dilution with Kiev ($0.69 \pm 0.07 \times 10^3$ CFU/ml) ($P < 0.05$). The high glucose level in the component of Kiev can explain the increase of the bacteria population in this extender (GadeaJ, 2003). Moreover, bacterial contamination was the main reason for a decrease in semen motility and an increased proportion of pH lowering to acidic levels (5.7-6.4) (Althouse *et al.*, 2000). The concentration of potassium in BTS may play an important role in maintaining physiological levels of potassium ions during storage (Johnson *et al.*, 2000). Therefore, the high CFU of Kiev is due to the highest glucose composition. On the contrary, low CFU of Modena and BTS may come from advanced components in the formulator as BSA or potassium. These results suggest an influence of bacterial growth on semen viability and motility in the current trial.

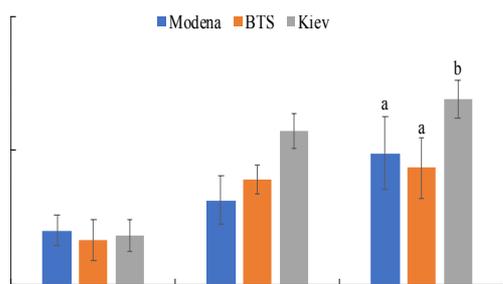


Figure 4. The average CFU of the three extenders

4. CONCLUSION

Based on this *in vitro* trial, Modena is the most suitable extender for using fresh boar semen for more than 120h. to avoid disappointing fertility results, semen BTS and Kiev should be used within 72h. Our data indicate that the pH and bacteria status may influence semen viability and motility. Therefore, the formulator of the boar semen extender should be enhanced by maintaining metabolism, regulating pH level, and inhibiting microbial growth.

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THE EFFECT OF LONG-TERM STORAGE AT STORAGE TEMPERATURES ON QUALITY OF EXTENDED BOAR SPERM

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ABSTRACT

In this study the effect of long-term storage of boar sperm at various storage temperatures on sperm motility, survival rate, pH, and bacteria status. Modena-diluted sperm from 6 boars (Duroc) were stored at 4, 17, and 25°C, respectively. After 24, 72, 120, and 144h of storage, all of the parameters were determined. The motility, survival rate, and bacteria status of boar sperm were lowest at 4°C and highest at 25°C. However, the pH of extended sperm was lowest at 25°C and highest at 4°C. In conclusion, the results from this study show that 17°C is the least harmful of the three temperatures tested for the long-term liquid storage of boar sperm. The 4°C provided an advantage in inhibiting the bacteria activity, but it induced cold-shocked injury for boar sperm. Therefore, we need a further optimum formulator extender for the preservation of boar sperm below 17°C.

Keywords: *Sperm, temperature, pig, bacteria status, storage.*

TÓM TẮT

Ảnh hưởng của nhiệt độ bảo quản lên chất lượng tinh trùng pha loãng của lợn khi được bảo quản trong thời gian dài

Mục tiêu của nghiên cứu nhằm xác định ảnh hưởng của bảo quản tinh trùng lợn ở nhiều nhiệt độ khác nhau trong thời gian dài lên hoạt lực, tỷ lệ sống, pH và mật độ vi khuẩn. Tinh trùng được pha loãng từ 6 lợn đực (Duroc) được bảo quản ở 4, 17 and 25°C. Các chỉ tiêu được đánh giá tại thời điểm 24, 72, 120 và 144h. Tỷ lệ sống, hoạt lực, và mật độ vi khuẩn thấp nhất ở 4°C và cao nhất ở 25°C. Tuy nhiên, pH của tinh trùng pha loãng lại thấp nhất khi bảo quản ở nhiệt độ 25°C và cao nhất ở 4°C. Có thể kết luận rằng 17°C là nhiệt độ bảo quản ít có tác động tiêu cực đến tinh trùng trong thời gian dài. 4°C có nhiều thuận lợi để áp dụng bảo quản tinh trùng với khả năng làm giảm mật độ vi khuẩn, nhưng lại tăng thương tổn cho tinh trùng. Do đó, cần phải có nhiều nghiên cứu hơn về tối ưu hoá dung dịch bảo quản để bảo quản tinh trùng ở nhiệt độ dưới 17°C.

Từ khóa: *Tinh trùng, nhiệt độ, lợn, mật độ vi khuẩn, bảo quản.*

1. INTRODUCTION

Liquid-preserved sperm is the most efficient and widely used assisted artificial insemination in pig breeding (Waberski *et al.*, 2019). In commercial farms, 17°C (15-20°C) is recommended storage temperature for sperm in extenders (Waberski *et al.*, 2019, Johnson *et al.*, 2000). 10 and 12°C have been suggested to be the lower temperature limits for boar

sperm storage (Althouse *et al.*, 1998, Schmid *et al.*, 2013). However, many studies found that sperm can store at 5°C without effect on sperm quality or fertility (Waberski *et al.*, 2019, Menezes *et al.*, 2020). Lower temperature storage has limited when warming in the temperature range between 20-38°C, due to sublethal chilling-related injury (Bamba and Cran, 1988). In opposite to the temperature range below 15°C, storage temperatures above 20°C can not fully limit the metabolism of sperm, thus increasing the depletion of sperm energy reserves and an accumulation of metabolic (by-)products (Althouse *et al.*, 1998). On the other hand, viability, motility,

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or acrosome integrity of sperm stored at 25°C than at 17 or 15°C (Paulenz *et al.*, 1999, Gaczarzewicz *et al.*, 2015). Therefore, storage temperatures above 20°C and lower 17°C have advantages and disadvantages, especially storage temperatures above 20°C improved sperm quality.

This study investigates the effect of different storage temperatures (25, 17, and 4°C) on quality boar sperm during long-term storage.

2. MATERIALS AND METHODS

2.1. Experimental design

Sperm was diluted in Modena to a concentration of 20×10^6 sperm/ml. Split samples were stored at 25, 17, or 4°C and analyzed after 24, 72, 120, and 144h of storage. All parameters were assessed after a 30min incubation period of the samples at 38°C.

The composition of the extender used in the experiment is shown in Table 1 (Gadeaj, 2003).

Table 1. Ingredients of Extenders

Contents (g/l)	Modena
Glucose	25
Sodium citrate	6,90
EDTA	2,25
Sodium bicarbonate	1,00
Bovine serum Albumin (BSA)	3,00
Tris	5,65
Citrate	2,00
Cysteine	0,05

2.2. Sperm processing

Six healthy, mature boars at a commercial farm were used for sperm collection. From each boar, one ejaculate was collected by the gloved hand method into disposable collection bags with an integrated filter to remove the gel fraction. Collection bags were mounted in pre-warmed thermos cups (38°C) to prevent any cold-shock for the spermatozoa. After collection, the sperm was immediately transferred to the laboratory of Non-ruminant Animal Production Techniques, Faculty of

Animal Sciences, Can Tho University in Styrofoam boxes.

The raw sperm concentration was assessed by Porcine Sperm Photometer III (Fujihika industry, Tokyo, Japan). Only ejaculates with $\geq 70\%$ motile and $\leq 25\%$ morphologically abnormal sperm were used in this study. The samples are cooled stepwise to the desired storage temperature (Henning *et al.*, 2022). Samples designed for storage at 25°C are kept for 60min at room temperature (RT, 20-22°C) and then transferred to an incubator (MIR-154-PJ, Fujihira, Tokyo, Japan) set at 25°C. Samples designed for storage at 17°C are kept for 90min at RT and then placed in a 17°C storage unit (BioPlus ER500, Gram-bioline, Vojens, Denmark). Samples designed for 4°C storage are kept for 90min at RT, then for 60min at 17°C, followed by 60min at 10°C, and then transferred to a 4°C storage unit (BioPlus ER500, Gram-bioline, Vojens, Denmark). All samples are stored in the dark.

2.3. Measurements

Sperm samples were analyzed for viability, motility, pH, and bacteria status. The eosin-nigrosin staining method was used for the evaluation of sperm viability, this evaluation was replicated three times to calculate the average percentage (Kondracki *et al.*, 2017). The percentage of motility was analyzed by a microscope (BB 4260, Euromex, Arnhem, Netherlands) at 40x magnification, and it was assessed by the same person to reduce differences in interpretation. Orion Star™A214 (Thermo Fisher Scientific, Massachusetts, United States) calibration with pH 4.00, 7.00, and 10.00 standard solutions were used for measurement of pH value. Sperm samples were prepared with plate count agar (HiMedia, Mumbai, India) and counted number of colony form units (CFU) after incubation 3 days at 37°C (Vyt *et al.*, 2004). The number of CFU was counted by Haloes Caliper (IUL Instruments SA, Barcelona, Spain). CFU was assessed by Haloes Caliper (IUL Instruments SA, Barcelona, Spain).

2.4. Statistical analysis

Results were illustrated as the Mean±standard error of the mean (SEM). Analysis of variance (ANOVA) and Tukey's test were analyzed using R (version 3.5.3; <https://www.r-project.org/>) with a significant difference at $P<0.05$.

3. RESULTS AND DISCUSSION

3.1. Effect of storage temperatures on the viability of sperm during long-term storage

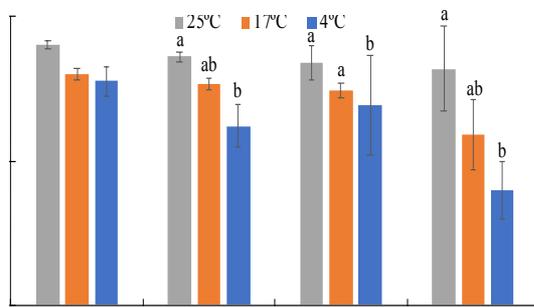


Figure 1. The average percentage of live sperm

Bar marked by different letters are significantly different ($P<0.05$).

Preservation at 17°C has been considered as best for liquid storage of boar sperm, so this condition may consider a control treatment (Waberski *et al.*, 2019). The viability of sperm was higher than 65% at all storage temperatures after 120h (Figure 1). The viability of extended boar sperm did not differ between sperm preserved at 25°C (90.25±1.38%), 17°C (79.95±1.78%), and 4°C (77.53±5.11%) after 24h storage ($P>0.05$). Viable of semen has fewer in 4°C (68.98±17.25%) than 17°C (74.21±2.46%) and 25°C (84.17±5.95%) at 120h ($P<0.05$). At 144h, the ratio of survival sperm at 25°C was 81.75±14.71%, and it was significantly higher than others at 4°C with only 40.00±9.91% sperm alive. However, sperm survival at 17°C was 59.05±12.28%, which did not differ at 25 and 4°C ($P>0.05$). Consistent with our result, a previous study found that preservation was higher at 17°C without influencing the percentage of live sperm (Johnson *et al.*, 2000, Aalbers *et al.*, 1961). On the opposite, the decrease in the number of sperm under low

storage temperatures may explain by the cold-shocked injury of sperm (Mann and Lutwak-Mann, 1955). In conclusion, boar semen is safe when preserved at between 17 and 25°C, and it needs special methods to prevent the cold-shocked at 4°C.

3.2. Effect of storage temperatures on the motility of sperm during long-term storage

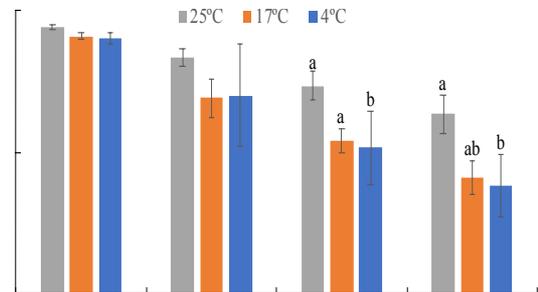


Figure 2. The average percentage of motility

The average motility was higher than 65% for sperm stored up to 72h at 25, 17, and 4°C (Figure 2). From 24h to 72h, motility of sperm preservation at 25, 17, and 4°C was reduced from 94±1 to 83±3%, from 91±1 to 69±7%, from 90±2 to 70±18%, respectively ($P>0.05$). After 120h, movement ability was consistently slower for sperms stored at 4°C (51.67±13.00%) than for those stored at 17°C (54.00±4.00%) or 25°C (73.33±5.00%) (both $P<0.05$). At the end of storage time, the motility of sperm at 4°C was 38.33±11.00%, it was lower than others at 25 and 17°C ($P<0.05$). However, the motility ratio at 25°C and 17°C were 63.33±7.00% and 41.00±6.00%, respectively, and it did not differ between the two groups after 144h ($P>0.05$). As expected, the simple extender reduced sperm motility and chilling storage temperature. It is known that cold shock induced extremely damage to the sperm of the boar (White, 1993). In addition, long-term storage sperm must spend more energy to reactivate their motility (Henning *et al.*, 2022). Therefore, the lack of energy for reactivation from cold storage temperature and injury from cold shock may influence sperm motility after 4°C storage. This could conclude that the reduction of sperm motility by low-temperature storage is due to cold-shocked damage and an imbalance of energy.

3.3. Effect of difference storage temperatures on pH of sperm during long-term storage

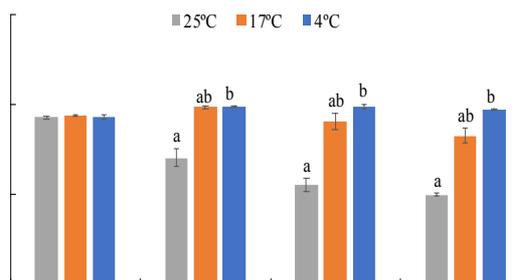


Figure 3. The average pH

After 24h, pH values of sperm at 25, 17 and 4°C were 8.56 ± 0.05 , 8.61 ± 0.02 , and 8.57 ± 0.06 , temperature did not affect pH value (Figure 3). The initial pH reduced significantly ($P<0.05$) on 72, 120, and 144h of semen storage at 25°C, reaching 5.97 ± 0.05 in the last hour of the experiment. pH value of sperm storage at 4°C was stable from 72h (8.92 ± 0.03), 120h (8.92 ± 0.07), and 144h (8.83 ± 0.02). During the experiment, the 17°C group had a reduction trend in pH value, it was 8.90 ± 0.05 at 72h, 8.42 ± 0.26 at 120h, and 7.95 ± 0.25 at 144h. The pH level of sperm stored at 17°C and 4°C in this study did not show a significant difference ($P>0.05$). The 25°C groups had the lowest pH level after 24h storage, it was 7.20 ± 0.29 at 72h, 6.30 ± 0.20 at 120h, and 5.97 ± 0.05 at 144h. Sperm preservation at 25°C had a significantly lower pH level than 4°C ($P<0.05$), but it did not significant difference with 17°C ($P>0.05$).

Similar to the current study, Paulenz *et al.* (2000) found a significant decrease in the pH of semen stored at 25 and 20°C, a nonsignificant increase when stored at 15°C. Previous studies found that lower pH extenders are better quality at preserving sperm (Vyt *et al.*, 2004; Fantinati *et al.*, 2009). Depression of motility by high-level pH of extenders may explain by pH sensitivity enzyme systems responsible for premature activation of motility (Vyt *et al.*, 2004; Fantinati *et al.*, 2009). In conclusion, pH level influenced sperm motility under cool and warm storage temperatures.

3.3. Effect of storage temperatures on bacteria status of boar sperm during long-term storage

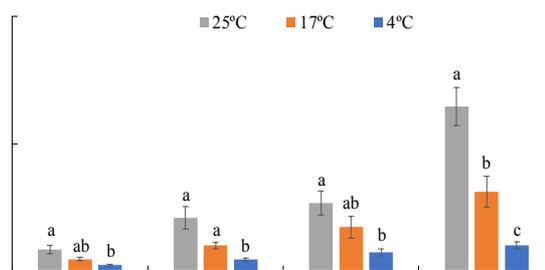


Figure 4. CFU of difference storage temperatures

The bacterial load was greater in sperm preservation at 25°C than those at 4°C at all-time points of this study ($P<0.05$). The quantification of bacteria was similar at 25 and 17°C until 120h storage ($P>0.05$). However, until 144h preservation, doses stored at 25°C had a greater bacterial load than those stored at 17°C ($P<0.05$). The lower number of bacteria preservation at 4°C may explain by the decrease in bacterial generation time under hypothermic storage (Althouse *et al.*, 2008). During sperm storage, bacteria may induce toxicity for sperm cells and the growth of bacteria leading to low pH levels mentioned by several studies (Althouse *et al.*, 2000; Althouse *et al.*, 2005; So *et al.*, 2011; Prieto-Martínez *et al.*, 2014). In addition, the number of bacteria in all of the storage temperatures was below the critical value of 10^3 CFU/ml at all tested time points (Maroto *et al.*, 2010; Waberski *et al.*, 2019). The present result showed a negative relationship between bacteria status and pH level under different storage temperatures in long-term storage, but bacteria growth during the preservation period did not influence the quality of sperm.

4. CONCLUSION

Our findings indicate that storage temperature is nearly related to the quality of extended boar sperm in the long term. During long-term storage, the change in pH level and bacteria status may correlate and are likely the main contributor to viability and motility. Preservation at 17°C is the most suitable temperature for using fresh boar sperm for more than 120h. To avoid contamination with

bacteria, sperm preservation at 25°C should be used within 120h. Cooling semen to 4°C has the advantage on prevent bacteria growth but it also induced cold-shocked damage and negative energy balance in long-term storage. Therefore, implementing a low-temperature method for boar sperm storage should focus on the optimum extender formulator to inhibit cold shock and energy metabolism.

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EFFECTS OF FERMENTED TOTAL MIXED RATIONS (FTMR) ON *IN VITRO* NUTRIENT DIGESTIBILITY AND RUMINAL FERMENTATION PATTERNS

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ABSTRACT

The study aimed to assess the effect of fermented total mixed ration (FTMR) on the *in vitro* nutrient digestibility and ruminal fermentation patterns. The experiment was arranged in a completely randomized design with 3 treatments corresponding to three FTMR formulas: FTMR1, FTMR2, and FTMR3. It was found that the digestibility of dry matter, organic matter, and neutral detergent fiber showed no significant differences ($P>0.05$) among the treatments at 24, 48, and 72h post-incubation. However, the digestibility of crude protein showed significance ($P<0.05$) at 24 and 48h across the treatments. At 24h incubation, FTMR3 demonstrated the highest value and FTMR1 showed the lowest one; however, at 48h incubation, FTMR1 showed the highest value and FTMR3 the lowest value. Ruminal pH value generally decreased from 0 to 24h during incubation, while NH_3 and VFAs values increased over the times of incubation, though these changes were not statistically significant across the three treatments ($P>0.05$). Regarding individual VFA proportions, no changes were seen in acetic, butyric, and propionic from 0 to 3 hours of incubation, but variations appeared at 24h. In summary, varying FTMR formulas did not affect nutrient digestibility and ruminal fermentation patterns.

Keywords: Digestibility, FTMR, ruminal fermentation patterns, sweet potato by-products.

TÓM TẮT

Ảnh hưởng của khẩu phần hỗn hợp hoàn chỉnh được lên men (FTMR) đến tiêu hóa dưỡng chất và các thông số lên men dạ cỏ *in vitro*

Nghiên cứu nhằm đánh giá ảnh hưởng của khẩu phần hỗn hợp hoàn chỉnh được lên men (FTMR) đến tỷ lệ tiêu hóa chất dinh dưỡng và các thông số lên men dạ cỏ *in vitro*. Thí nghiệm được bố trí theo thể thức hoàn toàn ngẫu nhiên với 3 nghiệm thức tương ứng với 3 công thức FTMR: FTMR1, FTMR2 và FTMR3. Kết quả cho thấy tỷ lệ tiêu hóa vật chất khô, vật chất hữu cơ và xơ trung tính khác biệt không có ý nghĩa thống kê ($P>0,05$) giữa các nghiệm thức ở thời điểm 24, 48 và 72 giờ sau khi ủ. Tuy nhiên, tỷ lệ tiêu hóa của protein thô khác biệt có ý nghĩa thống kê ($P<0,05$) ở thời điểm 24 và 48 giờ qua các nghiệm thức. Sau khi ủ 24 giờ, FTMR3 thể hiện giá trị cao nhất và FTMR1 cho thấy giá trị thấp nhất; tuy nhiên, sau 48 giờ ủ, FTMR1 cho thấy giá trị cao nhất và FTMR3 cho giá trị thấp nhất. Giá trị pH dạ cỏ nhìn chung giảm từ 0 đến 24h trong quá trình ủ, trong khi giá trị NH_3 và VFAs tăng theo thời gian ủ mặc dù những thay đổi này khác biệt không có ý nghĩa thống kê qua 3 nghiệm thức ($P>0,05$). Về tỷ lệ VFA riêng lẻ, không thấy sự thay đổi về axit acetic, butyric và propionic từ 0 đến 3 giờ ủ, nhưng sự biến động xuất hiện sau 24 giờ. Tóm lại, các công thức FTMR khác nhau không ảnh hưởng đến khả năng tiêu hóa dưỡng chất và các thông số lên men dạ cỏ.

Từ khóa: Tỷ lệ tiêu hóa, khẩu phần hỗn hợp hoàn chỉnh được lên men (FTMR), thông số dạ cỏ, phụ phẩm khoai lang.

1. INTRODUCTION

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The production of beef cattle is steadily growing in several Mekong Delta provinces including Ben Tre, Tra Vinh, Tien Giang, An Giang, Soc Trang, and Vinh Long. Traditional cattle feed elephant grass and natural grass is supplemented with agricultural by-products, which range from remnants of agricultural

production and food processing such as rice straw, peanut vines, cassava leaves, and sweet potato vines and tuber by-products. According to Preston *et al.* (2011), utilizing locally available feed resources for ruminant livestock production not only allows for the effective use of agricultural products and by-products, but it also aids in reducing waste produced in the process. Vinh Long province's agricultural cultivation is particularly noticeable for its extensive sweet potato growing area, mainly in Binh Tan district. After the tubers are harvested, a considerable amount of still-green vines and abundant sweet potato tuber by-products remain. These can be used as feed in livestock production, thereby enhancing farmers' economic efficiency (Mai Truong Hong Hanh and Ho Thanh Tham, 2022).

The use and preservation of agricultural by-products for animal feed hold significant value in contemporary farming. Utilizing these by-products as animal feed also aids in diminishing reliance on imported feed and results in monetary savings on feed purchases. Total mixed ration (TMR) is a farming method that mixes all crucial nutrients such as energy, protein, fiber, vitamins, and minerals into a consistent blend. The TMR offers animals an evenly balanced nutritional intake, boosting their growth and health. Included in this is the fermented total mixed ration (FTMR), a type of silage TMR, which is viewed as an efficient way to conserve high moisture agricultural by-products (Song *et al.*, 2023). Consequently, this study was undertaken to evaluate the impact of FTMR on the diet's nutrient digestibility and rumen fermentation parameters.

2. MATERIALS AND METHODS

2.1. Object, location and time

Three Fermented Total Mixed Rations (FTMR) formulas were carried out at the ruminant production laboratory of the Faculty of Animal Sciences, College of Agriculture, Can Tho University from June 2022 to September 2022.

2.2. Experimental design

The experiment to evaluate *in vitro* ruminal fermentation was arranged in a completely randomized design with 3 treatments, each treatment was repeated 4 times in 50 and 100ml glass bottles. The difference between the treatments was the mixing ratio of the feed sources across the 3 FTMR formulas shown in Table 1 with 14% CP and 2,400 kcal/kg DM.

Table 1. Ingredients of FTMR formulas

ITEM	FTMR1	FTMR2	FTMR3
Rice straw	3.0	4.0	5.0
Sweet potato vines	30.0	30.0	30.0
Sweet potato tubers	20.0	20.0	20.0
Ground corn	14.5	17.3	20.1
Rice bran	13.4	9.78	6.14
Copra meal	5.67	6.52	10.0
Extracted soybean	12.0	11.0	7.38
Urea	0.50	0.50	0.50
Mineral premix	0.40	0.40	0.40
Salt	0.50	0.50	0.50
Total	100	100	100

2.2.1. *In vitro* incubation

The ingredients in each FTMR formula were mixed according to the ratios shown in Table 1 before being placed in the incubation bottle. Buffer solution (medium) was prepared according to the method of Menke and Steingass (1988) with some minor adjustments as reported by Thanh *et al.* (2020).

Weigh 312.5mg DM of the substrate into a 50ml glass incubation bottle to determine CP digestibility and ruminal fermentation patterns (pH, NH₃-N, VFA), or 625mg DM of the substrate was weighed into a 100ml glass incubation bottle to determine the digestibility of OM, DM and NDF. The incubation bottles were covered with rubber stoppers and aluminum caps and then vacuumed (Rocker 400, Rocker, Taiwan). During continuous CO₂ slump, the ruminal fluid was mixed with a medium (ratio 1:4, by volume) prewarmed at 39°C in a water bath (WNB 45, Memmert, Germany). 25ml of the incubation solution was introduced into a 50 ml incubation bottle or 50ml of the incubation solution was

introduced into a 100ml incubation bottle. All incubation bottles were then incubated in an automatic shaking system (ISS-4075R, Jeitech, Korea) at 39°C and 120rpm.

2.2.2. Sampling, measurement and chemical analysis

The ruminal fermentation parameters were determined at 24h of incubation. The fermentation reaction was stopped by placing the incubation bottles in ice-cold water. The pH of the ruminal fluid was determined (HI5222, Hanna Instrument, USA) and the incubation solution was then centrifuged at 5000rpm for 15min, removing the residue, and the solution was stored in H₂SO₄ 1M at a ratio of 10: 1 until analysis for NH₃-N content, or continue to filter through a glass pipette lined with glass wool to obtain a sample for analysis of the VFA composition.

The chemical composition of the samples in the experiment included: dry matter (DM), organic matter (OM), Ash, crude protein (CP), crude fiber (CF), and ether extract (EE) was analyzed according to the method of AOAC (1990). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to the method of Van Soest *et al.* (1991). Analytical data are calculated and presented based on DM. The N-NH₃ content of the incubation solution was analyzed by the micro-Kjeldahl method (AOAC, 1990), while the concentration of the VFAs was analyzed by a gas chromatograph (GC) (Thermo Trace 1310 GC, Thermo Scientific, USA) connected with a flame ionization detector. For the determination of VFAs, the GC was set up as follows: The injector and probe were set at 220°C. 1µL of DDC was injected with a 10:1 split ratio into the column (30m×0.25mm×0.25µm) polarized molten silica capillary (Cat. No: 24107, Supelco, Sigma-Aldrich, MO, USA) with a carrier gas stream (1ml/min) of pure nitrogen (99.999%). The combustion chamber was kept at 80°C for 1min, increased by 20°C/min until reaching 180°C, kept for 1 min, finally increased by 10°C/min until 200°C was reached, then kept for 14 minutes. The VFAs

were determined by comparing retention times with those of external standard VFAs, including acetic, propionic, butyric, valeric, iso-butyric, and iso-valeric acids (Sigma-Aldrich, MO, USA).

2.3. Statistical analysis

The data were statistically analyzed with the General Linear Model procedure for completely randomized designs using Minitab Release 16.1 (Minitab, 2010). Tukey pair-wise comparisons were used to determine differences between treatment means (P<0.05). The statistical model was $Y_{ij} = \mu + t_i + e_{ij}$, with Y_{ij} : is the dependent variable, μ : the overall mean, t_i : the effect of the treatments, and e_{ij} : the random residual error.

3. RESULTS AND DISCUSSIONS

3.1. Chemical composition of the ingredients

Table 2 shows that the NDF content of rice straw (76.5%) was higher than that of sweet potato vines (42.0%). Research results tended to be lower than that published by Chau Minh Tuan and Nguyen Binh Truong (2019) on sweet potato vines of 48.7% NDF, but consistent with the report of Don *et al.* (2020) on the NDF value of rice straw was 66.3-73.2%. Extracted soybean has a higher CP value than copra meal, rice bran, and ground corn, which had 42.2, 18.8, 13.9, and 8.02% CP, respectively. CP value of ground corn was consistent with the report of Nguyen Ngoc Duc An Nhu *et al.* (2016) was 8.48%.

Table 2. Chemical composition of ingredients

Ingredients, %	DM	OM	CP	CF	NDF	ADF	EE
Rice straw	91.4	86.9	5.74	38.2	76.5	39.6	1.55
Sweet potato vines	23.7	88.2	11.5	29.6	42.0	32.5	4.19
Sweet potato tubers	26.5	96.4	3.69	3.81	10.2	7.18	0.60
Ground corn	87.5	97.8	8.02	1.94	36.5	2.22	2.35
Rice bran	87.9	92.8	13.9	4.83	29.7	6.42	12.3
Copra meal	87.0	91.2	18.8	16.3	28.6	23.7	7.07
Extracted soybean	86.5	91.9	42.2	4.63	12.2	8.42	10.4

3.2. Nutrient digestibility of FTMR

The variation in nutrient digestibility showed no significant difference (P>0.05) in the cases of DM, OM, and NDF. However, the

CP digestibility was statistically significant ($P < 0.05$) between the treatments. The DM digestibility at 24 hours progressively climbs from 53.5% in FTMR1 to 56.0% in FTMR2, and further to 57.1% in FTMR3. However, at 72h incubation, the DM digestibility of 3 treatments ranged 65.6-61.7%. The results of this study were consistent with the *in vitro* results of Dung *et al.* (2014) where DM digestibility increased gradually from 24 to 48h. At 24h, CP digestibility of FTMR1 (50.8%) was statistically significant ($P < 0.05$) compared with FTMR3 (62.9%) but not different from FTMR2 (54.7%). At 24h, the NDF digestibility was not statistically significant between the 3 treatments, ranging from 84.0 to 84.6%. This value had a similar trend at 72h, 86.5, 86.3 and 86.3% for FTMR1, FTMR2, and FTMR3 respectively. However, this result was higher than that reported by Lamba *et al.* (2019) ranging 29.8-47.1% because of variations in CP sources in the dietary formulation. Overall, no notable statistical variation was observed in the digestibility of DM, OM, and NDF among the treatments during the 72h incubation (Inc), but CP digestibility was higher in FTMR3 at 24h incubation.

Table 3. Nutrient digestibility (%) over time

Item	Inc., h	FTMR1	FTMR2	FTMR3	P	SEM
DM	24	53.5	56.0	57.1	0.635	3.01
	48	61.9	58.8	61.4	0.499	2.28
	72	65.6	60.3	61.7	0.585	3.91
OM	24	60.3	59.5	62.6	0.152	1.34
	48	65.3	63.6	64.1	0.763	1.77
	72	69.7	67.1	69.1	0.196	1.07
CP	24	50.8 ^b	54.7 ^{ab}	62.9 ^a	0.028	2.34
	48	65.1 ^a	57.3 ^{ab}	57.0 ^b	0.033	1.83
	72	57.6	54.5	58.9	0.793	4.60
NDF	24	84.0	84.3	84.6	0.811	0.63
	48	86.1	85.6	86.4	0.796	0.88
	72	86.5	86.3	86.3	0.920	0.50

3.3. Rumen fermentation parameters of the FTMR

Ruminal fermentation patterns at 0, 3 and 24h were not different ($P > 0.05$) between the treatments (Table 4). The pH value at 0 h ranged of 7.18-7.41 and tended to decrease

at 24h (6.71-6.83). According to Sousa *et al.* (2017), diet-affected rumen pH exhibited differences that appeared only after 3h of feeding. $\text{NH}_3\text{-N}$ concentration at 0h was not significantly different ($P < 0.05$) between FTMR1, FTMR2 and FTMR3 treatments, 32.9 to 37.1 and 36.4, respectively. At 24h, $\text{NH}_3\text{-N}$ values were higher than 0h, but there was no difference between FTMR1, FTMR2 and FTMR3 treatments of 50.8, 56.0 and 55.3 mg/dl, respectively. The change in $\text{NH}_3\text{-N}$ concentration is a good indicator of the nitrogen status of the rumen fluid (Joomjantha and Wanapat, 2008). Protein sources from feed will be broken down by proteolytic bacteria with the help of protease enzymes into peptides, then hydrolyzed to amino acids, and amino acids will undergo deamination to become ammonia (Sari *et al.*, 2018). Values of VFAs at 0h ranged 58.3-58.4mM and tended to increase up to 24h, but the difference was not statistically significant between FTMR1, FTMR2 and FTMR3 treatments of 68.8 mM, 69.3 mM and 68.8 mM, respectively. In a low-fiber diet, the population of fiber-degrading microorganisms decreased, so lactic acid increased along with VFA, causing the pH to decrease (Lee *et al.*, 2019). Thus, the pH value tended to decrease over the incubation time from 0 to 24h, but $\text{NH}_3\text{-N}$ and VFAs were opposite and this was an explanation for the decrease of pH.

Table 4. Ruminal fermentation patterns

Item	Inc, h	FTMR1	FTMR2	FTMR3	P	SEM
pH	0	7.18	7.41	7.25	0.462	0.13
	3	7.28	7.12	7.01	0.295	0.12
	24	6.71	6.83	6.81	0.474	0.07
N-NH_3 , mg/dl	0	32.9	37.1	36.4	0.670	3.48
	3	39.9	40.6	35.4	0.369	2.70
	24	50.8	56.0	55.3	0.195	2.03
VFAs, mM	0	58.4	58.3	58.3	0.972	0.15
	3	58.4	58.7	58.3	0.578	0.31
	24	68.8	69.3	68.8	0.993	3.47

3.4. Individual proportions of ruminal volatile fatty acids between FTMR

Ruminal fermentation patterns at 0, 3 and 24h were not different ($P > 0.05$) between

the treatments (Table 4). The pH value at 0h ranged from 7.18 to 7.41 and tended to decrease at 24h (6.71-6.83). According to Sousa *et al.* (2017), diet-affected rumen pH exhibited differences that appeared only after 3h of feeding. $\text{NH}_3\text{-N}$ concentration at 0h was not significantly different ($P < 0.05$) between FTMR1, FTMR2 and FTMR3 treatments, 32.9 to 37.1 and 36.4, respectively. At 24h, $\text{NH}_3\text{-N}$ values were higher than 0 h, but there was no difference between FTMR1, FTMR2 and FTMR3 treatments of 50.8, 56.0 and 55.3 mg/dl, respectively. The change in $\text{NH}_3\text{-N}$ concentration is a good indicator of the nitrogen status of the rumen fluid (Joomjantha and Wanapat, 2008). Protein sources from feed will be broken down by proteolytic bacteria with the help of protease enzymes into peptides, then hydrolyzed to amino acids, and amino acids will undergo deamination to become ammonia (Sari *et al.*, 2018). Values of VFAs at 0h ranged 58.3-58.4mM and tended to increase up to 24h, but the difference was not statistically significant between FTMR1, FTMR2 and FTMR3 treatments of 68.8, 69.3 and 68.8mM, respectively. In a low-fiber diet, the population of fiber-degrading microorganisms decreased, so lactic acid increased along with VFA, causing the pH to decrease (Lee *et al.*, 2019). Thus, the pH value tended to decrease over the incubation time from 0 to 24h, but $\text{NH}_3\text{-N}$ and VFAs were opposite and this was an explanation for the decrease of pH.

The volatile fatty acid components, namely acetic, propionic, and butyric, did not demonstrate any statistical significance in their values across the treatments at time intervals of 0, 3 and 24h. The percentage of acetic acid was 62.2% at 0h and ranged from 62.1 to 62.3% at 3h. At the 24h incubation, the percentages were 61.6, 61.6 and 61.1% respectively for the FTMR1, FTMR2, and FTMR3 treatments. The propionic acid did not fluctuate from 0 to 3h in the range of 20.0% but tended to increase at 24h to 22.2, 22.0 and 22.2% for FTMR1,

FTMR2 and FTMR3 treatments, respectively. Similarly, butyric acid remained stable at 0 and 3h at 10.0%, but butyric acid at 24h of FTMR1, FTMR2 and FTMR3 treatments was 9.13, 9.38 and 9.70%, respectively. The study results were similar to that reported by Mbiriri *et al.* (2012) on the analytical results of acetic (31.0-33.5), n-butyrate (4.77 to 6.76) and propionic (9.88-10.8) values. Differences in component VFA values may depend on the formulation and ingredients in the studies or the proportion of roughage in the diet because the carbohydrates contained in the feed are still dominated by structural carbohydrates. Well-developed microorganisms are cellulose and hemicellulose-degrading bacteria (Sari *et al.*, 2018). Thus, the incubation time from 0 to 3h did not have large changes in acetic, butyric and propionic, but this change occurred at 24h.

Table 5. Proportions of ruminal volatile fatty acids

Inc, h	Item	FTMR1	FTMR2	FTMR3	P	SEM
0	Acetic	62.2	62.2	62.2	0.900	0.06
	Propionic	20.0	20.0	20.0	0.820	0.03
	Iso-butyric	1.95	1.95	1.95	0.768	0.01
	Butyric	10.0	10.0	10.0	0.940	0.02
	Iso-valeric	2.89	2.89	2.89	0.939	0.01
	Valeric	2.93	2.93	2.93	0.951	0.01
3	Acetic	62.2	62.3	62.1	0.545	0.13
	Propionic	20.0	20.0	20.0	0.481	0.05
	Iso-butyric	1.94	1.93	1.95	0.729	0.01
	Butyric	10.0	10.0	10.0	0.561	0.05
	Iso-valeric	2.88	2.87	2.89	0.585	0.01
	Valeric	2.92	2.91	2.93	0.576	0.01
24	Acetic	61.6	61.6	61.1	0.626	0.39
	Propionic	22.2	22.0	22.2	0.970	0.67
	Iso-butyric	1.74	1.73	1.73	0.989	0.07
	Butyric	9.13	9.38	9.70	0.682	0.45
	Iso-valeric	2.55	2.55	2.55	0.999	0.11
	Valeric	2.73	2.73	2.71	0.970	0.08

4. CONCLUSIONS

The incorporation of sweet potato vine and sweet potato tuber by-products, along with other feed sources in the FTMR diet, demonstrated consistent nutrient digestibility (excluding CP digestibility) and ruminal fermentation patterns throughout the incubation periods.

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VOLUNTARY FEED INTAKE AND GROWTH PERFORMANCE OF GROWING GOATS AND NUTRIENT DIGESTIBILITY OF IPIL-IPIL AND NAPIER GRASS IN DIFFERENT FEEDING RATIOS

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ABSTRACT

Feeding proportions play a crucial role in determining animal performance. This study evaluated the effects of different feeding ratios of *Ipil-ipil* and Napier grass on the feed intake, growth performance, and nutrient digestibility in goats. Twelve does were assigned to four treatments which consists of the following feeding ratios: T1 (100% *Ipil-ipil*), T2 (90% *Ipil-ipil* and 10% Napier grass), T3 (80% *Ipil-ipil* and 20% Napier grass), and T4 (70% *Ipil-ipil* and 30% Napier grass). The results showed that the inclusion of Napier grass did not significantly ($P>0.05$) affect nutrient intake. Although no statistically significant differences ($P>0.05$) were observed in growth performance indicators among the treatments, the groups receiving a combination of *Ipil-ipil* and Napier grass showed slightly better ADG, BWG, and FCR compared to the control group. Furthermore, the combinations of *Ipil-ipil* and Napier grass significantly improved ($P<0.05$) Organic Matter Digestibility (OMD) and Crude Fiber Digestibility (CFD) compared to the control group. The highest OMD and CFD were observed in the treatment with a ratio of 70:30 of the two forages. Therefore, incorporating a mixed forage diet of *Ipil-ipil* and Napier grass can enhance the nutritional value of goats' feed, improving FI, growth performance, and nutrient digestibility.

Keywords: *Apparent nutrient digestibility, grass, growth, legumes, voluntary feed intake.*

TÓM TẮT

Ảnh hưởng của tỷ lệ bình linh và cỏ Voi trong khẩu phần lên lượng ăn, tiêu hóa dưỡng chất và năng suất sinh trưởng của dê

Tỷ lệ thức ăn đóng một vai trò quan trọng trong việc xác định năng suất của vật nuôi. Nghiên cứu được thực hiện nhằm đánh giá ảnh hưởng của các tỷ lệ *Ipil-ipil* (bình linh) và cỏ Voi trong khẩu phần lên lượng ăn, năng suất sinh trưởng và sự tiêu hoá dưỡng chất của dê. 12 con dê cái được bố trí vào một thí nghiệm với 4 nghiệm thức (NT), bao gồm các tỷ lệ cho ăn như sau: 100% bình linh, đối chứng (T1), 90% bình linh + 10% cỏ Voi (T2), 80% bình linh + 20% cỏ Voi (T3), và 70% bình linh và 30% cỏ Voi (T4). Kết quả cho thấy lượng ăn không bị ảnh hưởng ($P>0,05$) bởi các mức bổ sung cỏ Voi trong khẩu phần. Mặc dù không có sự khác biệt ($P>0,05$) về chỉ tiêu sinh trưởng giữa các NT, nhưng dê được cho ăn kết hợp bình linh và cỏ Voi cho kết quả tốt hơn về tăng khối lượng, khối lượng cơ thể và hệ số chuyển hoá thức ăn khi so với dê chỉ được cho ăn bình linh. Thêm vào đó, dê được cho ăn kết hợp bình linh và cỏ Voi đã cải thiện đáng kể ($P<0,05$) tỷ lệ tiêu hoá OM và CF so với dê ăn khẩu phần đối chứng, tỷ lệ tiêu hoá OM và CF cao nhất ở NT có tỷ lệ 70:30. Do đó, kết hợp bình linh và cỏ Voi trong khẩu phần có thể cải thiện giá trị dinh dưỡng của thức ăn cho dê, tăng lượng ăn vào, năng suất sinh trưởng và tỷ lệ tiêu hoá dưỡng chất.

Từ khóa: *Cỏ, cây họ đậu, lượng ăn, tăng trưởng, tỷ lệ tiêu hoá biểu kiến.*

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1. INTRODUCTION

One of the most common problems in the Philippines today is the unbalanced nutritional value of goats' production to promote good health. Grazing small ruminants are the most likely species to suffer undernutrition, affecting growth performance due to insufficient nutrients through the forage they graze upon (Fernández-Rivera *et al.*, 2005). Lack of proper feeding and rationing information can also contribute to this (Devendra, 1991). Because of these issues, practical management, like feeding grasses and legumes, is one way to improve the production of animals.

Forage plants are vital resources that can be efficiently converted to animal protein and energy sources. Napier grass is one of the forages considered a primary feed source for livestock animals in tropical and subtropical countries, along with other native grasses (Rahman *et al.*, 2006). *Pennisetum purpureum* or commonly known as Napier grass or elephant grass is a tall type of leafy grass with a height of 3-5m. It is utilized as livestock feed in the tropics and is harvested through the cut-and-carry system. *Leucaena leucocephala* (*Leucaena leucocephala*), locally known in the Philippines as *Ipil-Ipil* is also classified as forage. *Leucaena leucocephala* is rich in protein, a feed source that can help the animals meet their nutritional requirements (Szyszka *et al.*, 1983). *Leucaena leucocephala* is known for its resistance to drought, persistence, adaptability to tropical conditions, and re-growth. Moreover, it is considered one of the leguminous forages containing a high amount of nutrients and is also very palatable to ruminant animals (Jones, 1979).

Fewer studies have analyzed the growth performance digestibility of a mixed forage diet made up of grasses and legumes available in a tropical climate like the Philippines (Abdulzara *et al.*, 1996; Aregheore, 2002). The complete feed for ruminants has a balanced ratio of grass to legume portions. Feeding imbalance feed rations may lead to

lower productivity. Hence, proper feeding requirements intended for ruminant animals must be with a combination of grass and legumes. The present study evaluated the voluntary feed intake, growth performance of goats, and nutrient digestibility of *Leucaena leucocephala* or *Ipil-ipil* and Napier grass (*Pennisetum purpureum*) in different feeding ratios.

2. MATERIALS AND METHODS

2.1. Animals and management

Twelve does of 2-3 years with approximately 22-24kg were used in the study. The animals were raised on a private farm in Pagahan, Initao, Misamis Oriental. Animals were placed in cages with their individual space requirements (2m²) per animal; the goats were distributed in three replications under intensive management. Before the experiment, cages were cleaned and disinfected using chlorine, powdered detergent soap, and clean water. Cages were dried for a few days, and subsequently, experimental animals were transferred to designated cages.

Napier grass (*Pennisetum purpureum*) and *Ipil-ipil* (*Leucaena leucocephala*) were collected early morning and late afternoon through the cut-and-carry system at Pagahan, Initao, Misamis Oriental, and nearby municipalities within the region. The *Ipil-ipil* and Napier grass was chopped manually using a chopping knife with a size of approximately 3.8cm length. Then the designated ration was separately prepared. For analysis of feeds, feed samples were harvested randomly in a particular area of the study location site. The feed samples were air-dried for 4-5 days to proceed with the analytical method.

The study consisted of four treatments, such as T1, composed of 100% *Ipil-ipil* (control). T2 was 90% of *Ipil-ipil* and 10% Napier grass, T3 is 80% *Ipil-ipil* and 20% Napier grass, and T4 is 70% *Ipil-ipil* 30% Napier grass. The control (T1) was made up of 100% *Ipil-ipil* because, conventionally, this is the farmer's practice.

Dried samples were ground using a hammer mill and allow to pass a 1mm mesh sieve for the uniformity of the sample particle size in preparation for proximate analysis according to the procedure described by AOAC (1990). Feeds were analyzed in dry matter, organic matter, crude protein, and fiber by the Department of Agriculture (DA) Region 10, in Cagayan de Oro City, Philippines.

Table 1. Proximate analysis of experimental diets

Proximate Analysis	Treatments			
	T1	T2	T3	T4
DM (%)	41	40.6	38	36.3
OM (%)	40.86	40.43	37.82	36.11
CP (%)	20.02	16.99	16.61	15.29
CF (CF%)	14.48	14.31	18.93	24.26

Goats were fed three times a day, at 7:30am, noon, and 4:30pm, respectively; the required quantity (dry matter basis) was given to each goat each day, divided equally into the three feeding portions. During feeding, the feeders were properly cleaned by washing them with water to avoid contamination before adding a new set of feeds. Water was always provided upon feeding to prevent animals from dehydration. The amount given was formulated according to the goat’s dry matter requirement, which is 3% per body weight (Rashid, nd). The goats were fed three

times daily to ensure animal selectivity and voluntary feed intake, and to account for 15% of the total amount of feed provided as leftovers.

2.2. Experimental Design and Data Collection

The treatments were replicated three times, and each Replicate consisted of one experimental doe. The animals were randomly distributed in a Completely Randomized Design (CRD). The nutrient feed intake was monitored daily and was determined by subtracting the leftover from the amount of feed offered. Growth performance was monitored every week using a digital weighing scale for weight measurement and was measured using kg as the unit. The collected data for growth performance served as the basis for the computation of the feed conversion ratio (FCR), average daily gain (ADG), and body weight gain (BWG).

For the digestibility data, fecal matter samples were collected using the net. The net was installed under the flooring of the experimental layout to hold the fecal matter. The apparent digestibility represents by the difference between the amount of feed ingested and the amount appearing in feces. It includes endogenous sources of the same chemical composition. It does not account for methane loss and heat loss (Ajman *et al.*, 2003).

Table 2. Daily feeding basis according to dry matter content

Treatment	Rations	<i>Ipil-ipil</i>	Napier grass	Daily Feeding
1	100% <i>Ipil-ipil</i>	3.410 kg	0	3.410 kg
2	90:10 <i>Ipil-ipil</i> and Napier grass	3.096 kg	0.344 kg	3.440 kg
3	80:20 <i>Ipil-ipil</i> and Napier grass	2.944 kg	0.736 kg	3.680 kg
4	70:30 <i>Ipil-ipil</i> and Napier grass	2.695 kg	1.155 kg	3.850 kg

2.3. Statistical analysis

Statistical analysis of the data was performed using SPSS version 21. ANOVA and the Tukey Post Hoc Test were used to statistically assess the mean of the growth performance and digestibility parameters. The significance level was considered at P<0.05.

3. RESULTS AND DISCUSSION

3.1. Feed Intake

The findings from this study present an interesting discussion on the feed intake of goats when subjected to varying proportions of *Ipil-ipil* (*Leucaena leucocephala*) and Napier grass in their diet. The feed intake was assessed in terms of Dry Matter Intake (DMI),

Organic Matter Intake (OMI), Crude Protein Intake (CPI), and Crude Fiber Intake (CFI).

Dry Matter Intake (DMI) showed a slight increase as the proportion of Napier grass in the diet increased, though the variations were not statistically significant. This suggests that increasing Napier grass intake does not necessarily lead to a significant increase in the consumption of feed (dry matter) by goats. This is consistent with the findings of Patra (2010) who reported that altering the ratio of legumes and grasses in ruminants' diets might not substantially affect their overall feed consumption. Organic Matter Intake (OMI) initially increased when a small amount of Napier grass was added (in T2) and then gradually decreased as the proportion of Napier grass increased further. This could suggest that although a small inclusion of grass might enhance feed consumption, a higher proportion may not be as appealing to the goats, causing a slight drop in intake. However, the variations observed across the treatments were not statistically significant. Crude Protein Intake (CPI) showed a similar trend to OMI. This is not surprising given that *Ipil-ipil*, being a legume, is generally higher in protein content compared to grasses like Napier (Gebregiorgis *et al.*, 2008). However, as with the other measures, these variations were not statistically significant, suggesting that goats were able to maintain their protein intake fairly constant despite the changes in the proportions of the two feeds. The Crude Fiber Intake (CFI) progressively increased with the increasing proportion of Napier grass in the diet. Napier grass is known to have a high fiber content so an increase in fiber intake was expected as its proportion in the diet increased. Again, these variations were not statistically significant.

According to the study by Niderkorn and Baumunt (2009), there is a higher feed intake when legumes are supplemented with grass the feeding the animals with legumes alone. This is because the soluble feed components are quickly broken down, which increases the

rate of particle disintegration and the passage of food through the rumen, increasing the intake of feed (Moseley and Jones, 1984). The least amount of nutrient feed intake was observed in T1 (100% *Ipil-ipil*). *Ipil-ipil*, or *Leucaena leucocephala* is a forage known for its high protein content. It can be attributed to the high concentration of nutrients, which means that once the protein and energy requirement is obtained, a specific hormone sends the signal to the brain, causing the animal to stop feeding (Institute of Medicine, 2015).

Table 3. Feed intake of goats fed with different feeding ratios of *Ipil-ipil* and Napier grass* (kg)

Treat	DMI	OMI	CPI	CFI
T1	1.29±0.14 ^a	1.09±0.25 ^a	0.43±0.12 ^a	0.43±0.20 ^a
T2	1.33±0.62 ^a	1.31±0.56 ^a	0.55±0.10 ^a	0.47±0.12 ^a
T3	1.35±0.03 ^a	1.24±0.13 ^a	0.55±0.11 ^a	0.62±0.21 ^a
T4	1.35±0.87 ^a	1.19±0.17 ^a	0.50±0.17 ^a	0.80±0.31 ^a

* Superscripts of the same letter in the column means not significant at $P>0.05$

3.2. Growth Performance

The mean values of the growth performance indicators in goats fed with different treatments (as shown in Table 4) provide valuable insights into the effects of the *Ipil-ipil* and Napier grass combination on the goats' growth. The treatment groups that received a combination of *Ipil-ipil* and Napier grass demonstrated better Average Daily Gain (ADG), Body Weight Gain (BWG), and Feed Conversion Ratio (FCR) performance compared to the control group (T1) that received *Ipil-ipil* alone. However, despite these observed trends, no statistically significant differences were found among the treatments ($P>0.05$). These findings are in line with the study by Phimpachanhvongsod and Ledin (2002) who reported that goats fed a combination of grass (Guinea grass) and legumes (*Gliricidia*) exhibited greater body weight gain compared to those fed only *Gliricidia*. This suggests that incorporating a combination of different feed sources, such as a mixture of energy/fiber and protein

sources, can positively impact the growth performance of animals. Similarly, Thang (2010) emphasized the importance of utilizing a combination of energy/fiber and protein feed sources to enhance animal performance and achieve better weight gain. This aligns with the present study's findings, as the combination of *Ipil-ipil* and Napier grass likely provided a balanced nutritional profile, enabling the goats to achieve comparable growth performance across the treatments.

Furthermore, Waghorn *et al.* (1989) highlighted the significance of considering both protein and energy sources in meeting the nutritional requirements of animals. This reinforces the notion that a combination of feed sources, as seen in the *Ipil-ipil* and Napier grass treatments, can contribute to satisfying the nutritional needs of goats, leading to improved growth.

While the current study did not yield statistically significant differences in growth performance indicators among the treatments, the consistent trend of improved performance in the treatment groups receiving a combination of *Ipil-ipil* and Napier grass suggests that the inclusion of diverse feed sources can have a positive impact on goat growth.

Table 4. Growth performance of goats fed with different feeding ratios of *Ipil-ipil* and Napier grass

Treat	ADG (kg)	BWG (kg)*	FCR
T1	0.11±0.04 ^a	2.10±0.79 ^a	11.73±1.42 ^a
T2	0.15±0.05 ^a	3.07±0.99 ^a	8.87±1.21 ^a
T3	0.14±0.02 ^a	2.73±0.42 ^a	9.64±1.13 ^a
T4	0.13±0.02 ^a	2.63±0.42 ^a	10.38±1.54 ^a

3.3. Nutrient Digestibility

The nutrient digestibility in Table 5 shows significant differences ($P < 0.05$) in OM, CP, and CF digestibility among treatments. Organic matter (OM) digestibility was higher in the combinations of *Ipil-ipil* and Napier grass treatment groups. It is significantly higher in T4 (70:30). Crude protein (CP) digestibility was observed higher in less and without the

combination of *Ipil-ipil* and Napier grass. The combination of *Ipil-ipil* and Napier grass was most tolerable in the digestion of crude fiber compared to sole *Ipil-ipil*.

The highest OMD and CFD were observed in T4 (70:30) grass and legume combination. *Leucaena leucocephala*, also known as *ipil-ipil*, is higher in protein and nutrients than most grasses, but it contains less energy content. The rumen bacteria in ruminants use fermentation to break down inferior grass and hay straw into energy, but they need protein to do so (Buxton and Russel, 1988). These rumen microbes are responsible for the rumen's degradation of organic feed substances. As such, feeding leguminous feedstuffs with grasses provides a balance in the rumen, thus, providing rumen microbes the essential environment for their growth and activity (Haddad, 2000).

Another key determinant in the digestibility of crude fiber is the lignin content found in forage. Lignin, which is often considered to be a lower-quality component within forage, has a negative impact on the nutritional value of plant fiber. According to Gonzalo (2017), grasses, despite having a higher fiber content than legumes, paradoxically contain less lignin. Lignin, however, creates a significant hurdle to fiber digestibility. No matter the length of time the fiber remains in the rumen, the existence of lignin inhibits certain portions of the fiber from being fully digested. This barrier to digestion can be linked to the high concentration of lignin located within the middle lamella and the primary wall of thick-walled cells (Buxton and Readfearn, 1997).

Crude protein refers to the total amount of protein in the feed. The highest CP digestibility was at the grass and legume ratio of 80:20. The rapid digestion of the soluble portion of legumes and a greater rate of particle breakdown and passage to the rumen can be seen with the association of grass and legumes, thus, resulting in better digestibility in terms of crude protein

in ruminant animals. Grass and legumes improve digestion when inadequate forage is augmented with a high nitrogen

concentration that encourages microbial activity, increasing nutrient absorption (Niderkorn and Baumont, 2009).

Table 5. Nutrient digestibility of *Ipil-ipil* and Napier grass at different feeding ratios

	Treatments	T1	T2	T3	T4	P-value
DM	DMD (%)	76.06±2.45	78.74±26.14	71.91±4.62	72.45±1.82	0.220
	Nutrient Absorbed (g)	981.18±26.74	1048.58±69.99	970.79±48.21	978.08±43.15	0.120
OM	OMD (%)	55.77±2.25 ^b	56.23±4.19 ^b	56.27±2.31 ^b	63.12±2.42 ^a	0.030
	Nutrient Absorbed (g)	607.90±20.99 ^b	736.19±32.17 ^a	697.75±33.82 ^a	751.13±41.57 ^a	<0.001
CP	CPD (%)	63.95±4.43	62.59±2.45	64.16±2.27	60.72±1.74	0.090
	Nutrient Absorbed (g)	274.96±6.13 ^b	344.25±7.34 ^a	352.88±6.76 ^a	303.60±5.80 ^{ab}	<0.001
CF	CFD (%)	71.46±2.9 ^b	77.10±6.6 ^{ab}	76.85±3.8 ^{ab}	83.10±1.0 ^b	0.037*
	Nutrient Absorbed (g)	307.28±13.46 ^c	362.37±8.54 ^c	476.47±14.89 ^b	664.80±36.20 ^a	<0.001

4. CONCLUSION

The study concluded that a mixed forage diet, specifically combining grass and legumes, improves the nutritional value of goats' feed, enhancing their feed intake, growth performance, and nutrient digestibility. It emphasizes the importance of balancing protein and energy sources to satisfy the nutritional needs of the animals effectively. Furthermore, the findings reveal that a diet consisting of *Leucaena leucocephala* (*Ipil-ipil*) and Napier grass (*Pennisetum purpureum*) in varying proportions can provide a balanced diet for goats, offering implications for improving small ruminant nutrition in tropical climates like the Philippines. Further studies may focus on optimizing the feeding ratios of grass and legume combinations for different goat breeds and production systems.

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EFFECTS OF FEEDING FREQUENCY ON FEEDING BEHAVIOR, FEED EFFICIENCY, WEIGHT GAIN AND ECONOMIC RETURNS OF GROWING CROSSBRED RABBITS

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ABSTRACT

This study aimed to find the optimal feeding frequency for the performance of growth crossbred rabbits. The experiment included 24 post-weaning growth crossbred rabbits with an average weight of 473 g/per and was also arranged in a completely randomized design with 4 treatments, and 3 replications. Two rabbits including 1 male and 1 female were in one experimental unit (a cage). Four treatments were different feeding times/day: T1 (feed 1 time/day), T2 (feed 2 times/day), T3 (feed 3 times/day), and T4 (feed 4 times/day). The experiment was divided into three stages: the first stage (30-60 days old), the second stage (60-90 days old), and the third stage (90-120 days old). In the second week of each stage, the feeding behaviors, faeces, and urine were recorded for 2 days continued. The results showed that the total feeding times were an average of 22-31 times/day and the feeding intake time lasted 3-4 mins/time during the different three stages. Rabbits have an increased tendency to feed intake more in the evening than in the morning. When feeding twice a day (T2), there were higher results in terms of WG ($P>0.05$), final weight ($P>0.05$), FCR ($P>0.05$), and the highest economic returns compared to other treatments.

Keywords: Daily weight gain, feeding behavior, growth rabbits.

TÓM TẮT

Ảnh hưởng số lần cho ăn lên tập tính ăn, khả năng sử dụng thức ăn, tăng khối lượng và hiệu quả kinh tế của thỏ lai sinh trưởng

Nghiên cứu này nhằm tìm ra tần suất cho ăn tối ưu trên năng suất của thỏ lai tăng trưởng. Thí nghiệm gồm 24 thỏ lai sinh trưởng sau cai sữa có khối lượng trung bình 473 g/con và cũng được bố trí theo thể thức hoàn toàn ngẫu nhiên với 4 nghiệm thức, 3 lần lặp lại. Mỗi đơn vị thí nghiệm bao gồm hai con thỏ: 1 đực và 1 cái ở trong cùng một chuồng. Bốn nghiệm thức là bốn lần cho ăn khác nhau trong một ngày: T1 (cho ăn 1 lần/ngày), T2 (cho ăn 2 lần/ngày), T3 (cho ăn 3 lần/ngày) và T4 (cho ăn 4 lần/ngày). Thí nghiệm được chia làm 3 giai đoạn: giai đoạn 1 (30-60 ngày tuổi), giai đoạn 2 (60-90 ngày tuổi) và giai đoạn 3 (90-120 ngày tuổi). Trong tuần thứ hai của mỗi giai đoạn, các tập tính ăn, phân và nước tiểu được ghi lại trong 2 ngày liên tục. Kết quả cho thấy tổng số lần ăn trung bình là 22-31 lần/ngày và thời gian ăn kéo dài 3-4 phút/lần trong 3 giai đoạn khác nhau. Thỏ có xu hướng ăn nhiều hơn vào buổi tối so với buổi sáng. Khi cho ăn ngày 2 lần (T2) cho kết quả về tăng trọng ($P>0,05$), khối lượng cuối cùng ($P>0,05$), FCR ($P>0,05$) và hiệu quả kinh tế cao nhất so với các nghiệm thức khác.

Từ khóa: Tăng khối lượng, tập tính thỏ, thỏ sinh trưởng.

1. INTRODUCTION

The Mekong Delta is known as an area with a suitable climate for rabbit husbandry

development. There are available and diverse plants as well as agricultural-industrial by-products as cheap feed sources for rabbits (Chau and Thu, 2014). O'Meara *et al.* (1992) reported rabbit meat has great nutritional value, due to its high protein content (20-21%), low fat (4-5%), and low cholesterol (45 mg/kg), it is recommended for people patients with cardiovascular disease can use (Hu and Willett, 2002).

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If weaning occurs between 28 and 35 days of age, feeding the young with a specific diet, different from the female's, seems an effective solution. Otherwise, it is necessary to find a compromise between the needs of the litter and the doe. Early weaning (<26 days) could also be a promising way to provide adequate feeding for the young as soon as they begin to eat solid food (Gidenne and Forthun-Lamothe, 2016).

Gabinaud *et al.* (2016) evaluated the effects of feed composition provided to weaned rabbits before weaning on feed intake, composition and maturation of the cecal microbiota in kits suggesting that feed composition at the onset of forage in weaned rabbits plays a role in promoting microbiota maturation through regulation of feed intake. According to Oliveira *et al.* (2012) reported feed restriction would be more economically efficient in terms of broiler and slaughtered rabbits. According to Birolo *et al.* (2016), feed restriction did not affect health status, nutrient digestibility, FCR, and slaughter performance, but improved FCR and reduce excretion.

In Vietnam, there have been many studies on nutrition in growing rabbits. However, there have been no studies on the eating behavior, eating time, and resting time of rabbits. The study effect of feeding frequency on feeding behavior, feed conversation ratio, weight gain, and economic returns of growth crossbred rabbits was carried out with the following objectives: to find the optimal feeding frequency for post-weaning performance as well as survey the feeding habits of rabbits. From there, the results are recommended to farmers to contribute to the development of rabbit farming and increase income.

2. MATERIALS AND METHODS

2.1. Location, time, and animals

The experiment consisted of 24 post-weaning rabbits (including 12 males and 12 females). Rabbits were fully vaccinated against parasitic diseases, hemolytic failure, and respiratory diseases. The experiment

was carried out at the experimental farm in Thoi Hoa ward, O Mon district, Can Tho City, Vietnam. The chemical analysis of feed, feces, and urine was done in the laboratories of the Faculty of Animal Sciences, College of Agriculture, Can Tho University. The implementation of this study was from August to November 2022.

2.2. Experiment design

The experiment was arranged in a completely randomized design with 4 treatments and 3 replications. Two rabbits including 1 male and 1 female were in one experimental unit (a cage). Four treatments were different feeding times/day: T1 (feed 1 time/day), T2 (2 times/day), T3 (3 times/day), and T4 (4 times/day). The experiment was divided into three stages: the first stage (30-60 days old), the second stage (60-90 days old), and the third stage (90-120 days old).

Commercial pellet feed was supplied many times a day followed by treatments. Drinking water bottles were provided throughout the experiment. Commercial pellet feed was given and was offered 1, 2, 3, and 4 times a day at 6:00, 12:00, 18:00, and 24:00h. The refusals were weighed daily in the morning to calculate the feed and drinking intake.

The feeds and refusals were taken for analysis of DM, OM, CP, NDF, ADF, and ash following procedures of AOAC (1990), Van Soest *et al.* (1991), Robertson and Van Soest (1981). The metabolizable energy (ME) values of feeds were calculated according to the formula proposed by Maertens *et al.* (2002).

Rabbits were weighed individually every week. The monitoring indicators included: initial live weight, final live weight, feed intake, the daily amount of feces and urine, feeding times/day, and eating time (g/time).

2.3. Statistical analysis

The data is preliminarily processed on an Excel spreadsheet, processed, and analyzed according to the GLM of the Minitab 16 program (Minitab, 2014). Compare the

differences between the tests by the Tukey method of the Minitab 16 program (2014).

3. RESULTS AND DISCUSSION

3.1. Chemical composition of feed ingredients

Table 1. The chemical composition of feed used in the experiment

Feed	DM	OM	CP	EE	NDF	ADF	CF	Ash	ME, MJ/kgDM
Commercial pellet feed	90.8	92.9	14.8	4.60	44.5	27.6	16.9	7.10	9.98

* DM: dry matter, OM: organic matter, CP: crude protein, EE: Ether extract, NDF: Neutral detergent fiber, ME: Metabolism energy (Maertens *et al.*, 2002)

Table 1 showed the chemical composition and energy value of the feed used in the experiment. Commercial pellet feed in the experiment had 90.8% DM and 14.8% CP, respectively.

3.2. Indicators in the first stage

Generally, all indicators recorded in Table 2 were not significant among all treatments ($P>0.05$). The total feeding times ranged from 30.3-32.2 times/day in the first period, this result was lower than Prud'hon *et al.* (1975) that the total feeding times were 39 times in the sixth week old. The total eating time increased gradually from T1 to T3 (93.6-102.6 mins/day) and fall at T4 (99.3 mins/day). The total feed intake increased gradually from T1 to T3 (56.8-58.2 g/day) and drop at T4 (53.4 g/day). This result was lower than Prud'hon *et al.* (1975) that the total feeding intake was 98 g/day/per at the sixth week.

Table 2. Feeding frequency, ET, DFI of 1st stage

Item	Treatments				P/SEM
	T1	T2	T3	T4	
TFT, times/day	31.3	32.2	31.5	30.3	0.872/1.57
TET, min/day	93.6	94.4	102.6	99.3	0.687/6.00
TFI, g/day	56.8	57.6	58.2	53.4	0.781/3.53
TAET, time, min	3.01	2.96	3.28	3.29	0.484/0.19
AFI, g/times	1.82	1.80	1.85	1.77	0.926/0.09
FIR morning, %	49.1	44.2	39.5	42.2	0.591/5.05
FIR evening, %	50.9	55.8	60.5	57.8	0.591/5.05
ETR morning, %	49.2	46.3	39.1	41.9	0.571/5.44
ETR evening, %	50.8	53.7	60.9	58.4	0.571/5.44
Faeces, g/day	47.8	42.7	62.3	52.7	0.294/7.24
Urine, g/day	46.8	86.4	62.7	79.6	0.317/15.9

In the first period, the feed intake and eating time rate in the morning were lower than in the evening ($P>0.05$). This result was

suitable with Orengo and Gidenne (2007) reported that a higher pellet intake was observed during the night period (18:00-09:00h) whatever the age. Faeces and urine were not different in significance ($P>0.05$).

Table 3. Feed and nutrients intake in first stage

Item (g/day)	Treatments				P	SEM
	T1	T2	T3	T4		
TFI, g/day	62.3	65.3	69.6	64.1	0.304	2.74
TDW, ml/day	152.8 ^b	163.2 ^{ab}	171.9 ^a	160.5 ^{ab}	0.003	3.15
Total						
DM	56.6	59.3	63.2	58.2	0.304	2.49
OM	52.6	55.1	58.7	54.0	0.304	2.31
CP	8.37	8.77	9.35	8.61	0.304	0.37
NDF	25.2	26.4	28.1	25.9	0.304	1.11
ADF	15.6	16.4	17.4	16.1	0.304	0.67
CF	9.56	10.0	10.7	9.83	0.304	0.42
ME, MJ	0.57	0.59	0.63	0.58	0.304	0.03

* Mean values with letters a, b in the same row are significantly different ($P<0.05$)

Table 3 showed the results of the feed and nutrient intake of the experimental in the second week of the first stage. The total feed intake increased gradually from T1 to T3 (62.3-69.6 g/day) and fall slightly at T4 (64.1 g/day), however, this difference did not significantly ($P>0.05$). These results were lower than the report of Gidenne *et al.* (2010) that the total feed intake was 100-120 g/day at the 5-7 weeks old period. Similarly, the total drinking water had a gradual growth tendency from T1 to T3 (152.8-171.9 ml/day) and fall slightly at T4 (160.5 ml/day) and it had a significant difference ($P<0.05$). This result was higher than the highest total drinking water intake of Prud'hon *et al.* (1975) was 153 ml/day in the sixth-week-old rabbits.

DM, OM, CP, EE, NDF, ADF, Ash, and ME have a steadily increased tendency from T1 to T3 and fall slightly at T4, and this difference was not significant ($P>0.05$).

Table 4. Growth performance in first stage

Item	Treatments				P	SEM
	T1	T2	T3	T4		
ILW, g	449	497	479	449	0.273	20.11
FLW, g	1221	1295	1297	1235	0.546	46.59
DWG, g/day	27.6	28.5	29.2	28.1	0.925	1.749
FCR	2.08	2.08	2.19	2.11	0.883	0.108

Table 4 showed the growth performance of the experiment in the first stage. The initial and final live weight had no difference between all treatments ($P>0.05$). Daily weight gain and FCR increased from T1 to T3 and fall at T4 ($P>0.05$). FCR in the first stage ranged from 2.08-2.19, this result was lower than the result of Lebas and Gidenne (2005) that the FCR of rabbits in a 5-7 week-old period was 2.2-2.4.

3.3. Indicators in the second stage

Generally, all indicators recorded (Table 5) were not significant between all treatments ($P>0.05$) in the second stage. The total feeding times in this stage were the same at 22.2-22.8 times/day, this result was lower than Prud'hon *et al.* (1975) that the total feeding times were 40 times/day in the 12th week old. The total eating time decreased gradually from T1 to T3 (96.1- 81.0 mins/day) and rose slightly at T4 (88.2 mins/day). The total feed intake ranged from 94.7- 96.8 g/day. This result was lower than Prud'hon *et al.* (1975) that the total feed intake was 194 g/day/per at the 12th week-old. In this period, the feed intake and eating time rate in the morning were lower than in the evening ($P>0.05$). This result was suitable with Orengo and Gidenne (2007) reported that a higher pellet intake was observed during the night period (18:00-09:00h) whatever the age. Faeces and urine were not different in significance ($P>0.05$).

Table 5. Feeding frequency, ET, DFI of 2nd stage

Item	Treatments				P/SEM
	T1	T2	T3	T4	
TFT, times/day	22.2	22.8	22.2	22.5	0.995/2.06
TET, mins/day	96.1	88.1	81.0	88.2	0.685/8.68
TFI, g/day	95.2	94.7	96.8	95.2	0.999/9.36
TAET, min	4.33	3.88	3.82	3.96	0.773/0.38
AFI, g/times	4.30	4.14	4.44	4.20	0.724/0.20
FIR morning, %	30.8	38.8	27.3	36.7	0.220/4.19
FIR evening, %	69.2	61.2	72.7	63.3	0.220/4.19
ETR morning, %	32.5	40.2	29.4	36.5	0.310/4.14
ETR evening %	67.5	59.8	70.6	63.6	0.310/4.15
Faeces, g/day	50.8	61.7	64.7	57.0	0.711/8.85
Urine, g/day	65.0	46.7	75.5	43.8	0.080/9.36

Table 6 showed the results of feed and nutrient intake of the experimental in the second week of the second stage of the experiment. The total feed intake increased gradually from T1 to T3 (84.9-95.4 g/day) and fall slightly at T4 (91.0 g/day) and this difference did significantly ($P<0.05$). These results were lower than Gidenne *et al.* (2010) in that the total feed intake was 140-170 g/day at the 7-10 weeks-old period. However, the total drinking water was not different obviously ($P>0.05$) and ranged from 206.0-213.8 ml/day. This result was lower than the highest total drinking water intake of Prud'hon *et al.* (1975) was 320 ml/day for the 12th-week-old rabbits.

Table 6. Feed and nutrient intake in 2nd stage

Item (g/day)	Treatments				P	SEM
	T1	T2	T3	T4		
TFI, g/day	84.9 ^b	85.7 ^{ab}	95.4 ^a	91.0 ^{ab}	0.037	2.66
TDW, ml/day	206.0	209.5	210.8	213.8	0.412	3.206
Total						
DM	77.1 ^b	77.9 ^{ab}	86.7 ^a	82.6 ^{ab}	0.037	2.41
OM	71.6 ^b	72.3 ^{ab}	80.5 ^a	76.8 ^{ab}	0.037	2.24
CP	11.4 ^b	11.5 ^{ab}	12.8 ^a	12.2 ^{ab}	0.037	0.36
NDF	34.3 ^b	34.6 ^{ab}	38.6 ^a	36.8 ^{ab}	0.037	1.07
ADF	21.3 ^b	21.5 ^{ab}	23.9 ^a	22.8 ^{ab}	0.037	0.67
CF	13.0 ^b	13.2 ^{ab}	14.7 ^a	13.4 ^{ab}	0.037	0.41
ME, MJ	0.77 ^b	0.78 ^{ab}	0.87 ^a	0.83 ^{ab}	0.037	0.02

DM, OM, CP, EE, NDF, ADF, Ash, and ME have a steadily increased tendency from T1 to T3 and fall slightly at T4, and this difference was significant ($P<0.05$). Specifically, DM,

OM, CP, EE, NDF, ADF, CF, and Ash values gave the best result at T3 and the lowest at T1 were 86.7, 80.5, 12.8, 3.99, 38.6, 23.9, 14.7, and 6.15 g/per/day compared to 77.1, 71.6, 11.4, 3.55, 34.3, 21.3, 13.0, and 5.47 g/per/day, respectively. Similarly, ME got the highest at T3 (0.87 MJ/per/day) and the lowest at T1 (0.77 MJ/per/day).

Table 7 showed the growth performance of the experiment in the second stage. The initial and final live weight increased from T1 to T3 and fall at T4 (P>0.05). Daily weight gain and FCR improved and got a higher performance at T2 with 22.4 g/per/day and 3.49, respectively.

Table 7. Growth performance in 2nd stage

Item	Treatments				P	SEM
	T1	T2	T3	T4		
ILW, g	1221	1295	1297	1235	0.546	46.60
FLW, g	1839	1923	1947	1885	0.565	56.40
DWG, g/day	22.1	22.4	23.2	23.2	0.744	0.896
FCR	3.54	3.49	3.74	3.57	0.642	0.141

3.4. Indicators in the third stage

Table 8 showed the total feeding times, eating time, and feed intake in the third stage (90-120 days old) of the experiment. Generally, all indicators recorded in Table 8 were not significant between all treatments (P>0.05) in the third stage (90-120 days old) of the experiment. The total feeding times decreased gradually from T1 to T4 (29.0-22.0 times/day) in this period. The total eating time ranged from 68.5-90.7 mins/day. The total feed intake decreased gradually from T1 to T4 (92.8-73.0 g/day). This result was lower than Prud'hon *et al.* (1975) that the total feed intake was 160 g/day/per at the 18th week-old. In this period, the feed intake and eating time rate in the morning were lower than in the evening (P>0.05). This result was suitable with Bellier *et al.* (1995) showed that over 60% of the solid feed (excluding soft faeces meals) is consumed in the dark period for a domestic rabbit submitted to a 12L/12D light schedule. Faeces and urine were not different in significance (P>0.05).

Table 8. Feeding frequency, ET and DFI 3rd stage

Item	Treatments				P-value/SEM
	T1	T2	T3	T4	
TFT, times/day	29.0	26.8	23.0	22.0	0.055/1.890
TET, mins/day	83.7	90.7	71.8	68.5	0.251/8.482
TFL, g/day	92.8	84.8	75.7	73.0	0.212/7.066
TAET, mins	2.89	3.34	3.16	3.09	0.554/0.215
AFI, g/times	3.20	3.16	3.32	3.28	0.878/0.155
FIR morning, %	42.0	32.2	35.0	39.2	0.225/3.458
FIR evening, %	58.0	67.8	64.9	60.8	0.225/3.458
ETR morning, %	41.3	29.6	35.4	41.8	0.070/3.453
ETR evening, %	58.7	70.4	64.6	58.2	0.070/3.453
Faeces, g/day	67.9	53.8	65.3	58.8	0.520/7.219
Urine, g/day	61.1	68.1	50.3	65.1	0.822/14.06

Table 9 showed the results of feed and nutrient intake of the experimental in the second week of the third stage. The total feed intake decreased gradually from T1 to T4 (105.6-91.4 g/day), however, this difference did not significantly (P>0.05). These results were lower than the report of Prud'hon *et al.* (1975) that the total feed intake was 160 g/day for the 18th-week-old rabbits. Similarly, the total drinking water was similar between all treatments (P>0.05) and ranged from 199-206.5 ml/day. This result was lower than the average drinking water intake of Prud'hon *et al.* (1975) was 297 ml/day for the 18th-week-old rabbits. DM, OM, CP, EE, NDF, ADF, Ash, and ME have a steadily decreased tendency from T1 to T4, and this difference was not significant (P>0.05).

Table 9. Feed and nutrient intake in 3rd stage

Item (g/per/day)	Treatments				P	SEM
	T1	T2	T3	T4		
TFI, g/day	105.6	98.1	96.3	91.4	0.287	5.07
TDW, ml/day	202.6	201.6	206.5	199.0	0.757	4.98
Total						
DM	95.9	89.0	87.4	83.0	0.287	4.600
OM	89.1	82.7	81.2	77.1	0.287	4.273
CP	14.2	13.2	12.9	12.3	0.287	0.681
NDF	42.7	39.6	38.9	36.9	0.287	2.047
ADF	26.5	24.6	24.1	22.9	0.287	1.270
CF	16.2	15.1	14.8	14.0	0.287	0.777
ME, MJ	0.96	0.89	0.87	0.83	0.287	0.046

Table 10 showed the growth performance of the experiment in the third stage. The

initial and final live weight had no difference between all treatments ($P>0.05$) in this period. Daily weight gain got highest at T1 (19.9 g/per/day) and FCR was improved at T1 (4.85). This difference was not significant ($P>0.05$).

Table 10. Growth performance in 3rd stage

Item	Treatments				P	SEM
	T1	T2	T3	T4		
ILW, g	1,839	1,923	1,947	1,885	0.565	56.40
FLW, g	2,396	2,459	2,448	2,334	0.757	90.89
DWG, g/day	19.9	19.1	17.9	16.0	0.532	1.948
FCR	4.85	4.90	5.08	5.68	0.619	0.491

3.5. Comparison of indicators between 3 stages

Table 11 showed a comparison through the three stages (S) of the experiment. Generally, almost indicators recorded in Table 11 were significant between three stages ($P<0.05$) otherwise faeces and urine. The total feeding times (TFI) got the highest value at S1 (30-60 days old), followed by S3 (90-120 days old) and last one was S2 (60-90 days old), and the total eating times (TFT) ranged of 22.4-31.3 times/day. The total eating time (TET) decreased gradually from S1 to S3 (97.5-78.7 mins/day). Contrarily, the TFI increased steadily from S1 to S3 (65.3-97.8 g/day). Total average eating time (TAET) and average feed intake (TAFI) got higher results at S2 were 4.00min and 4.27 g/times, respectively.

In three periods, FIR and ETR in the morning (%) had great results at S1 were 43.7

and 44.2%, respectively. Besides, FIR and ETR in the evening (%) got higher results at S2 were 66.6 and 65.4%, respectively. WG had a decreased tendency from S1 (28.4 g/per/day) to S3 (18.2 g/per/day). Similarly, FCR was improved significantly from S1 (2.12) to S3 (5.13). This result was lower than the study of Gidenne *et al.* (2010) that FCR was 2.2-2.4 at a 5-7 week-old period.

Table 11. Indicators between 3 stages

Item	Stages			P-value/SEM
	S1	S2	S3	
TFT (times/day)	31.3 ^a	22.4 ^b	25.2 ^b	0.001/0.94
TET (mins/day)	97.5 ^a	88.3 ^{ab}	78.7 ^b	0.004/3.85
TFI (g/day)	65.3 ^c	89.3 ^b	97.8 ^a	0.001/1.92
TAET (mins)	3.13 ^b	4.00 ^a	3.12 ^b	0.001/0.13
TAFI (g/times)	1.81 ^c	4.27 ^a	3.24 ^b	0.001/0.07
FIRmorning (%)	43.7 ^a	33.4 ^b	37.1 ^{ab}	0.004/2.14
FIRevening (%)	56.3 ^b	66.6 ^a	62.9 ^{ab}	0.004/2.14
ETRMorning (%)	44.2 ^a	34.6 ^b	37.0 ^{ab}	0.010/2.21
ETRevening (%)	55.9 ^b	65.4 ^a	62.9 ^{ab}	0.010/2.21
Faeces (g/day)	51.4	58.6	61.5	0.144/3.69
Urine (g/day)	68.9	57.8	61.2	0.471/6.52
DWG (g/day)	28.4 ^a	22.7 ^b	18.2 ^c	0.001/0.79
FCR	2.12 ^c	3.58 ^b	5.13 ^a	0.001/0.15

3.6. Growth performance and economic efficiency

Table 12 showed the growth performance and economic efficiency of experimental rabbits during 3 periods. From this table, the total cost was the same as all treatments and the final income got highest at T2 (53,600 VND/per). FCR was improved at T2 (3.25) compared to other treatments.

Table 12. Growth performance and economic efficiency of experimental rabbits during 3 stages

Item	Treatments				P-value	SEM
	T1	T2	T3	T4		
Initial live weight (g)	448.5	496.7	479.2	448.8	0.273	20.1
Final live weight (g)	2396	2459	2448	2334	0.757	90.9
Daily weight gain (g/per/day)	23.2	23.4	23.4	22.4	0.923	1.15
Feed (g/per/day)	84.3	83.0	87.1	82.2	0.614	2.75
FCR	3.30	3.25	3.39	3.36	0.792	0.11
Economic returns of experiment rabbits, VND						
Feed cost	79,300	78,100	81,900	77,300		
Breed cost	60,000	60,000	60,000	60,000		
Veterinary medicine	5,000	5,000	5,000	5,000		
Total cost	144,300	143,100	146,900	142,300		
Money for selling rabbits	191,700	196,700	195,800	186,700		
Income	47,400	53,600	48,900	44,400		

* Price of broiler rabbits: 80,000 VND/kg

4. CONCLUSION

From the results of this study, rabbits had feeding times from 22-31 times/day, each time lasting 3-4 mins. Rabbits had a tendency that more eating in the evening. When supplying commercial pellet feed twice/day gave higher results in daily weight gain, final live weight, FCR, and economic returns compared to other treatments.

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EFFECTS OF VITAMIN E SUPPLEMENTARY LEVELS IN DIETS ON THE REPRODUCTIVE PERFORMANCE OF CROSSBRED (NEWZEALAND WHITE X LOCAL) RABBITS IN VIETNAM

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ABSTRACT

An experiment was conducted to evaluate the effect of different levels of vitamin E (α -Tocopherol) supplements in the diets of crossbred (NZWhite x Local) rabbits on their reproductive performance in the Mekong Delta. The experiment was carried out in a completely randomized design, consisting of four treatments with eight replicates each. Each experimental unit was a rabbit doe aged between 5-6 months, having an average body weight of 2693 \pm 40.3 g. The treatments comprised of different levels of vitamin E supplementation at 0, 40, 80, and 120 mg/kg DM feed, namely treatments E0, E40, E80, and E120, respectively. The experiment was carried out on 2 parities of rabbits. The results indicated that vitamin E supplementation at 80 mg/kg DM feed tended to improve reproductive parameters in parity 2 compared with parity 1 in terms of the number of alive newborn kits per litter, the number of weaned kits, and no statistically significant differences were found between treatments in terms of dead embryos, litter weight at birth/litter; weaned kits weight/litter and economic efficiency improvement. However, the milk yield, the alive newborn kits per litter, and the weaned kits per litter decreased at E120.

Keywords: Crossbred rabbit, heat stress, reproductive performances, vitamin E.

TÓM TẮT

Ảnh hưởng các mức độ bổ sung vitamin E (α -tocopherol) trong khẩu phần lên năng suất sinh sản của thỏ lai NZ

Nghiên cứu này nhằm đánh giá ảnh hưởng của việc bổ sung vitamin E ở các mức độ khác nhau trong khẩu phần đến năng suất sinh sản của thỏ trong điều kiện nuôi dưỡng ở Đồng bằng sông Cửu Long (ĐBSCL). Thí nghiệm được bố trí hoàn toàn ngẫu nhiên với 4 nghiệm thức, 8 lần lặp lại mỗi đơn vị thí nghiệm là một thỏ cái sinh sản lai địa phương, 5-6 tháng tuổi có khối lượng trung bình 2693 \pm 40,3 g, với 4 nghiệm thức E0, E40, E80 và E120 tương ứng với các mức bổ sung vitamin E ở 0, 40, 80 and 120 mg/kg DM thức ăn. Thí nghiệm được thực hiện qua 2 lứa đẻ ở thỏ. Kết quả nghiên cứu chỉ ra rằng bổ sung vitamin E ở 80 mg/kg DM vào khẩu phần có xu hướng cải thiện các chỉ tiêu sinh sản ở lứa 2 so với lứa 1 cụ thể về số con sơ sinh sống/ổ, số con cai sữa và không nhận thấy dấu hiệu phôi chết, khối lượng sơ sinh/ổ, khối lượng cai sữa/ổ và cải thiện về hiệu quả kinh tế. Bổ sung vitamin E ở 120 mg/kg DM có dấu hiệu giảm về lượng sữa thỏ mẹ, số con sơ sinh sống và số con cai sữa.

Từ khoá: Stress nhiệt, năng suất sinh sản, thỏ lai, vitamin E.

1. INTRODUCTION

In Vietnam, rabbit husbandry has the potential to develop rapidly in the future, particularly in the Mekong Delta of Vietnam.

This is due to the abundance of vegetation, agro-industry by-products, and affordable livestock costs. However, rabbits are very sensitive to temperature, and high temperatures can lead to stress, adversely affecting reproductive and growth performance, reducing meat quality, and increasing mortality rates (Marai *et al.*, 2008; Hassan *et al.*, 2016). Heat stress also reduces milk yields for breastfeeding does

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by increasing corticosteroid levels, reducing LH and FSH secretion, and affecting ovarian development and ovulation (Chatterjee, 2009; Arabameri and Bandegi, 2017).

Vitamin E was the primary natural antioxidant that can eliminate the generation of free radicals through a non-enzymatic defense system. It was considered an excellent antioxidant that represents the first line of defense against lipid peroxidation and free radical scavenging in cell membranes (Traber and Atkinson 2007; Dalolio *et al.*, 2015; Amaneh *et al.*, 2017). Vitamin E was thought to play an important role in increasing blood levels of free FSH and LH by minimizing the binding of the hormone to specific plasma proteins (Yoxussif *et al.*, 1989), leading to better ovarian activity (Ahmed *et al.*, 2000). According to Salem and Yasmin (2014), the injection of 20 IU of vitamin E improved the conception rate of rabbits. However, a study that added 0.3 mg/kg of selenium and 40 mg/kg of vitamin E to the feed (Shaibu, 2016) showed no effect on the pregnancy rate but did affect the litter weight at birth of kits (g/kit) and the litter weight at birth of the litter. In addition, vitamin E supplementation at 80 mg/kg feed improved litter weight at weaning (Abdel-Khalek *et al.*, 2008). Although many research results from around the world show the positive effects of vitamin E on rabbits, no studies have been conducted on the climate conditions of Vietnam, especially the Mekong Delta. Therefore, the objective of this experiment was to evaluate the effect and determine the optimal level of vitamin E supplementation in the diet on the reproductive performance of crossbred rabbits.

2. MATERIALS AND METHODS

2.1 Animals and management

The experiment comprised of 32 crossbred rabbit does (New Zealand White x local) aged 6 months, with an average weight of $2,693 \pm 40.3$ g. The rabbits were fully vaccinated against parasitic and respiratory diseases. The experiment was conducted at the experimental

farm located in Thoi Hoa ward, O Mon district, Can Tho City, and the laboratory E205 of the Faculty of Animal Sciences at the College of Agriculture, Can Tho University. The study was conducted from June to November 2022.

2.2. Experimental design and data collection

The experiment was arranged in a completely randomized design with four treatments and eight replications. The four treatments consisted of four levels of vitamin E added to the diet at 0, 40, 80, and 120 mg/kg DM. Each experimental unit consisted of crossbred rabbit does (New Zealand White x local) equipped with an automatic nipple drinker system. The experiment was conducted over the course of two litters. The ingredients and chemical composition of the trial diet are shown in Table 1.

Table 1. Ingredients and chemical composition of diet

Feed, g/head/day	Treats, mg vitE/kgDM feed			
	E0	E40	E80	E120
Commercial feed	30	30	30	30
Soybean extraction meal	30	30	30	30
Soya waste	200	200	200	200
Vitamin E, mg	0	4	8	12
<i>Pennisetum Purpureum</i>	300	300	300	300
% CP	21.0	21.0	21.0	21.0
ME, MJ/kg of dry matter	10.5	10.5	10.5	10.5

Note: E0, E40, E80, E120: were the levels of vitamin E in the diet, CP: Crude protein, ME: Metabolizable energy.

The feed used in the experiment included *Pennisetum purpureum*, soya bean extraction meal, soya waste, commercial local feed, and vitamin E (α -tocopherol). The rabbits were fed three times a day at 8:00, 13:00, and 18:00pm. The feed was weighed before feeding, and the refusal was collected and reweighed the next morning to calculate feed intake. Feed samples were taken to analyze the nutrient composition, which allowed for calculating the amount of nutrients ingested during the experiment. The does were kept individually

in separate cages, while the bucks in similar reproductive performance were used for mating. During the pregnancy and lactation periods, allowances were increased by 20% in the first week of pregnancy, 30% in the second week, 40% in the third and fourth weeks, and for the four weeks of lactation. The breeding service was performed after weaning. The newborn kits were weighed daily before and after milk consumption. Weights of rabbit kits at birth and weaning, as well as daily milk yields, were measured. The rabbit does were weighed weekly from mating until weaning of their kits, and their weight gains were calculated.

The feed offers and refusals were analyzed for DM, OM, CP, EE, NDF, ADF, and ash using the procedures outlined in AOAC (1990), Van Soest *et al.* (1991), and Robertson and Van Soest (1981). The metabolizable energy (ME) values of the feeds were calculated according to the formula proposed by Cheeke (1987) and Maertens *et al.* (2002) as follows: $ME = DE * [0.995 - 0.0048 * (DCP / DE)]$, where $DCP = (\%CP \times TLTH \ CP)$ and $DE = 13.932 - 0.196 * CF$. Measurements taken included daily feed and nutrient intakes for each litter, alive litter size at birth, litter weight at birth, litter

size and weight at weaning, dead embryos, rate of live kits from birth to weaning, milk yield of the doe.

2.3. Statistical analysis

The data were analyzed using the General Linear Model of the Minitab 13.21 program (Minitab, 2016). To determine the significance of pairwise comparisons, a Tukey method was performed. Significance was declared at $P < 0.05$.

3. RESULTS AND DISCUSSION

The CP content of the soya bean extraction meal was the highest at 45.5%, followed by soya waste at 21.5%. Commercial feed had a CP content of 14.3%, while *Pennisetum purpureum* had the lowest CP content at 8.8%. On the other hand, *Pennisetum purpureum* had the highest NDF content at 67.5%, while soybean extraction meal had the lowest at 23.7%. This suggests that the diet was carefully constructed, considering the different nutritional requirements of the experimental animals and economic efficiency. Soybean extraction meal and soya waste were mainly used to supplement the diet's CP, while *Pennisetum Purpureum* was the principal source of fiber.

Table 2. Chemical composition of feed (%DM basis except for DM which is on fresh basis)

Feeds	DM	OM	CP	EE	NDF	ADF	Ash	ME, MJ/kg DM
Soya waste	13.1	96.2	21.5	9.23	32.4	19.3	3.84	12.3
Soybean extraction meal	86.9	94.1	45.5	3.90	23.7	16.2	5.94	12.9
Commercial feed	90.8	92.0	14.3	3.70	41.8	24.8	8.00	10.7
<i>Pennisetum Purpureum</i>	12.4	86.9	8.80	2.65	67.5	40.9	13.1	7.04

Table 3 showed that the average nutrient intake during pregnancy and lactation in the first litter. Generally, there was no significant difference ($P > 0.05$) in nutrient intake between all treatments. However, there was a tendency to decrease from E0 to E40 and increase at E120. The average intake of DM was between 116-125 g/head/day, which was 100-102g higher than Du Thanh Hang and Le Tran Tinh Quyen (2012) results and lower than the results of Nguyen Thi Kim Dong (2009). The CP intake ranged from 26.2-27.2g, which was lower than the experimental results of Pham Thi Cam Nhung and Nguyen Van Thu (2021) whose CP ranged from 28.6-30.0 g/head/day and higher than Nguyen Van Dat *et al.* (2015) experimental results where CP ranged from 15.48-27.51 g/head/day.

Results from Table 4 indicated that alive litter size at birth in litter 1 did not differ significantly ($P > 0.05$), with the highest value observed in E40 (6.88 kits/litter). This finding was consistent

with Truong Thanh Trung and Nguyen Binh Truong (2020) on doe rabbits, which reported a range of 5.00-7.00 kits/litter, and higher than Shaibu (2016) of 3.67-5.33 kits/litter. The number of dead embryos ranged from 0.13 to 0.62 embryos, and there was no significant difference between treatments ($P>0.05$). This result was lower than that of Truong Thanh Trung and Nguyen Binh Truong (2020), which reported a range of 0.00-1.00 embryos. Salem and Yasmin (2014) attributed dead embryos to oxidative reactions, which could arise from the surrounding environment, such as oxygen consumption, metal cations, and spermatozoa (Goto *et al.*, 1993; Alvarez *et al.*, 1996). Based on the results of Table 3, nutrient intake in E40 was the lowest, and the total dead embryo and alive litter size at birth were the highest. Therefore, the increase in dead embryos may be influenced by nutritional factors.

Table 4. Reproductive performance of doe rabbits in the first parity

Item	Treatments				±SE/P
	E0	E40	E80	E120	
The alive litter size at birth, kits/litter	5.88	6.88	6.75	5.88	0.768/0.685
Dead embryo	0.25	0.62	0.13	0.25	0.281/0.626
The litter weight at birth, g/litter	56.4	54.3	53.5	52.8	2.463/0.751
The litter size at weaning, kits/litter	5.25	6.13	6.00	5.00	0.600/0.515
The litter weight at weaning, g/litter	1829	1914	2060	1746	110.3/0.242
Milk yield of doe, g/day	94.6	94.7	97.9	83.0	5.746/ 0.291
The of kits live from birth to weaning, %	92.1	89.1	91.9	89.1	4.453/0.932

The litter weight at birth with the best results in E40 was 361g, with no significant difference observed between treatments ($P>0.05$). The litter size at weaning ranged from 5.00-6.13 kits/litter across treatments, with the highest in treatment E40 and the lowest in treatment E120 ($P>0.05$). The rate of kits that survived from birth to weaning ranged from 89.1 to 92.1% ($P>0.05$), with an average of 90.6%, higher than that reported by Zeweil and Elgindy (2016) of 82.1%. According to Lopez *et al.* (2004), the allowable mortality rate was between 5 and 20.7%. The litter weight at weaning did not show a significant difference ($P>0.05$). The litter weight at weaning reached

Table 3. Nutrient intake of rabbit doe during pregnancy and lactation period in the first litter

Item, g/head/day	Treatments				±SE/P
	E0	E40	E80	E1200	
Dry matter					
Soya waste	28.6	27.4	28,5	29.0	0.614/0.296
Soybean extract meal	29.0	28.3	28,8	29.1	0.595/0.756
Commercial feed	35.8	35.8	35,8	35.8	0.045/0.503
<i>Pennisetum Purpureum</i>	31.5	26.2	23,0	29.5	2.297/0.069
Vitamin E, mg	0.00	4.80	9.60	14.4	-/-
Total					
DM	125	118	116	124	2.295/0.124
OM	115	109	107	114	2.627/0.131
CP	27.2	26.2	26.4	27.2	0.503/0.331
EE	5.91	5.63	5.68	5.90	0.115/0.205
NDF	52.3	48.2	46.6	51.2	1.703/0.087
ADF	32.0	29.5	28.5	31.3	1.037/0.088
Ash	9.80	9.03	8.68	9.57	0.327/0.086
ME,MJ/head/day	1.33	1.27	1.27	1.32	0.026/0.183

the highest value in E80 and the lowest in E120, ranging from 1746-2060g.

The milk yield of does show no significant difference ($P>0.05$), with the highest yield in treatment E80 and the lowest in treatment E120, ranging from 97.9 to 83.0 g/day. This result aligns with Pham Thi Cam Nhung and Nguyen Van Thu (2021) findings, where milk yield ranged from 85.2 to 94 g/day, and was 52.1-72 g/day higher than Truong Thanh Trung and Nguyen Binh Truong (2020) results, which spanned from 52.1 to 72.3 g/day. Overall, dietary vitamin E supplementation enhanced the reproductive performance parameters (Ismail *et al.*, 1992). Although the indicators

showed no significant difference ($P>0.05$), there was a noticeable trend of improvement in the litter size at weaning for E40, as well as the milk yield of does and the litter size at weaning for E80.

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Table 5 shows the average nutrient intake during pregnancy and lactation in the second litter. When compared with the highest nutrient intake of the first litter in E0, the nutrient intake in (Dalolio *et al.*, 2015); increased in treatments supplemented with vitamin E, with the highest intake observed in E80. In particular, dry matter content in soya waste and soya bean extraction meal reached the highest recorded value in E80 at 30.5 and 30.4 g/head/day, respectively, which were significantly different ($P<0.05$). This finding was consistent with the studies conducted by Abdel-Khalek *et al.* (2008) and El-Moniem *et al.* (2016), which reported increased nutrient intake when vitamin E was added to the rabbit's diet. There was no significant difference in the amount of DM and CP intake ($P>0.05$), with DM ranging from 116-119 g/head/day, which was higher than that reported by Truong Thanh Trung and Nguyen Binh Truong (2020) at 89-108 g/head/day. The highest CP in E80 was 26.0-27.4 g/head/day, which was higher than the value reported by

Zeweil and Elgindy (2016) ranged from 20.08 to 20.20 g/head/day.

Table 5. Nutrient intake of rabbit doe during pregnancy and lactation period in the second litter

Item, g/head/day	Treatments				±SE/P
	E0	E40	E80	E120	
Dry matter					
Soya waste	27.9 ^b	29.6 ^{ab}	30.5 ^a	29.1 ^{ab}	0.507/0.012
Soybean extract meal	27.8 ^b	29.2 ^{ab}	30.4 ^a	29.1 ^{ab}	0.670/0.050
Commercial feed	35.8	35.9	36.2	35.8	0.110/0.078
<i>Pennisetum Purpureum</i>	24.8	23.8	20.5	24.4	1.932/0.396
Vitamin E, mg	0.00	4.80	9.600	14.4	-/-
Total					
DM	116	119	118	118	2.201/0.885
OM	108	110	109	110	1.958/0.853
CP	26.0	26.9	27.4	26.8	0.420/0.136
EE	5.63	5.81	5.87	5.78	0.086/0.255
NDF	47.3	47.6	46.0	47.8	1.336/0.798
ADF	28.9	29.1	28.2	29.2	0.811/0.809
Ash	8.83	8.85	8.55	8.90	0.257/0.771
ME,MJ/head/day	1.26	1.29	1.3	1.29	0.019/0.491

* The numbers with different superscript letters in the same row were significantly different ($P<0.05$)

The results of the reproductive performance of the second litter, as shown in Table 6, indicate that the alive litter size at birth was the highest in the E80 with 6.63 kits/litter, and the lowest in the E120 with 5.43 kits/litter ($P>0.05$). These results were higher than those reported by Yassein *et al.* (2008) of 4.93-5.88 kits/litter and Hosny *et al.* (2020) of 3.5-6.15 kits/litter. The number of dead embryos improved compared to the first litter; the lowest was observed in the E40 and E80 treatments (0.00 embryo), while the highest was in the E120 treatment (0.50 embryo). Ismail *et al.* (1992) suggested that vitamin E may play an important role in reducing the incidence of dead embryos. The litter weight at birth was the highest in the E80 with 361 g/litter, and the lowest in the E120 with 309 g/litter. These results were higher than those reported by Truong Thanh Trung and Nguyen Binh Truong (2020) of 248-350 g/litter and much higher than the result of Gbore (2017)

ranged from 116.67 to 284.60 g/litter. The litter weight at birth in the E80 got a great result compared to other treatments. The litter at weaning reached the highest value in the E80 and the lowest in the E120 ($P>0.05$). The litter

size at weaning ranged from 5.00 to 6.25 kits/litter, which was consistent with Zeweil and Elgindy (2016) ranged from 4.25 to 6.83 kits/litter and higher than Kumar (2005) who reported a range of 3.54-4.48 kits/litter.

Table 6. Reproductive performance of doe rabbits in the second litter

Item	Treatments				±SE/P
	E0	E4	E80	E120	
The alive litter size at birth, kits/litter	5.86	6.38	6.63	5.43	0.689/0.619
Dead embryo	0.25	0.00	0.00	0.50	0.206/0.278
The litter weight at birth, g/litter	357	336	361	309	30.90/0.629
The litter size at weaning, kits/litter	5.29	5.87	6.25	5.00	0.566/0.410
The litter weight at weaning, g/litter	2021	1962	2158	1942	132.1/0.657
Milk yield of doe, g/day	92.7	87.1	93.8	91.0	4.454/0.732
The rate of kits live from birth to weaning, %	92.0	94.4	96.6	93.4	4.595/0.912

The litter weight at weaning ranged from 1942 to 2158 g/litter, which was higher than Shaibu (2016) of 733-1960 g/litter. The rate of kit survival from birth to weaning was significantly improved compared to the first litter ($P>0.05$), with E80 reaching the highest value of 96.6%, which was higher than the control treatment of 92% and the remaining vitamin E supplementation treatments. This result was also higher than the range of 85-92.7% reported by Kumar (2005). The highest milk yield of the doe was 93.8g in E80, while the lowest was 87.1g in E40 ($P>0.05$). This result was consistent with the study by Nguyen Thi Kim Dong (2009), which reported a range of 75.4-96.3 g/day and significantly higher than the range of 57-73.9 g per day reported by Truong Thanh Trung and Nguyen Binh Truong (2020). In general, dietary vitamin E supplementation improved reproductive performance in rabbits. Although most of the indicators were not statistically significant ($P>0.05$), there was an improvement in indicators such as the alive litter size at birth, the litter weight at weaning, dead embryo, the litter weight at weaning, milk yield of doe, and the rate of kit survival from birth to weaning ratio, which reached high values in E80. This finding was consistent with the study by Abdel-Khalek *et al.* (2008), which reported the results on the litter weight at weaning and

higher milk yield of doe in E80 compared to E40 and E160.

Table 7. Reproductive performance among two litters

Item	Litter 1	Litter 2	±SE/P
Litter size at birth, kits	6.34	6.10	0.44/0.539
Dead embryo	0.31	0.19	0.16/0.442
Litter weight at birth, g	330	341	22.8/0.649
Litter size at weaning, kits	5.63	5.60	0.35/0.949
Litter weight at weaning, g	1887	2021	86.9/0.136
Milk yield of doe, g/day	92.6	91.2	3.51/0.689

Table 7 showed that comparison of the reproductive performance among two litters. In this table, the litter weight at birth, litter size at weaning, litter weight at weaning, and dead embryo were improved in the second litter compared to the first one. Although milk yield of doe, alive litter size at birth, and litter size at weaning was lower in litter 2 (91.2g, 6.10 kits/litter, and 5.06 kits/litter, respectively) than in litter 1 (92.6g, 6.34 kits/litter, and 5.63 kits/litter, respectively), the difference was not significant ($P>0.05$). However, the improvement of weight gains indicators such as the litter weight at birth, litter weight at weaning, and dead embryo ($P>0.05$) showed that the doe's milk yield was improved. McDowell (1989) suggests that dietary vitamin E supplementation can increase litter size at

weaning and reduce pre-weaning mortality of rabbits, possibly due to the amount of vitamin E absorbed in rabbit milk (Ghaly, 1988, Hassanien *et al.*, 1995), which improves the growth rate and survival rate of rabbits. Abdel-Khalek *et al.* (2008) analyzed the quality of rabbit milk when adding vitamins E and C to the diet, which showed a positive trend of polyunsaturated fatty acids.

Table 8. Economic returns of two litters (VND)

Item	E0	E40	E80	E120
Feed cost	74,641	74,559	75,374	75,933
Medicine cost	10,000	10,000	10,000	10,000
Total cost	84,641	84,559	85,374	85,933
Income	395,250	450,000	459,375	375,000
Economy return	310,609	365,440	374,001	289,067

Note: Cost was 75,000 VND/weaned rabbit, 1,000 VND/kg Soya waste, 15,000 VND/kg soya bean extraction meal, 500 VND/kg *Pennisetum purpureum*, vitamin E 500,000 VND/kg, 23,000VND=1 USD

Based on the mean economic efficiency table for two litters, it showed that the highest total expense of 85,933VND was recorded in E120, while the highest total expense for treatment was in E80, amounting to 459,375VND. However, upon deduction of the costs for feed, breeding, and medicine, E80 gave the highest profit of 374,001VND.

4. CONCLUSIONS

By supplementing the diet of local crossbred rabbits with 80 mg/kg DM of vitamin E, several parameters improved significantly, including the alive litter size at birth, litter weight at birth, litter size at weaning, litter weight at weaning, dead embryo rate, and the rate of kits live from birth to weaning, which increased to 96.6%, leading to improved economic benefits. However, supplementing with 120 mg/kg DM of vitamin E resulted in increased milk yield of the doe and litter size at weaning, but low economic efficiency.

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EFFECTS OF DIFFERENT LEVELS OF GARLIC POWDER (*ALLIUM SATIVUM*) ON GROWTH, CARCASS TRAITS AND DRIP LOSS OF ROSS 308 BROILER CHICKENS

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ABSTRACT

A study was done to determine the effects of different levels of garlic powder on growth, carcass yields, and meat quality in Ross 308 broiler chickens. The experiment was randomly designed into 5 dietary treatments as responded to 5 different levels of garlic powder at 0, 0.5, 1.0, 1.5 and 2%. There were three replicates with 30 birds per replicate. At 35 days of age, eight birds per treatment were slaughtered. The results showed that supplementation of garlic powder in the broiler diets reduced the infection rates of coccidiosis, Gumboro, and ORT diseases as well as the percentage of bird mortality compared to the control ($P < 0.05$). In addition, the live weight and daily weight gain of birds at 35 days old and the average feed consumption ratio were significantly higher on all supplemented garlic powder diets in comparison to the control diet. Moreover, chicks fed diets containing 0.5-1.5% garlic powder had significantly higher than control ($P < 0.05$) on carcass traits in terms of body weight before slaughter, after bled out, after plucking feather and carcass weight, where BT1.5 was highest in comparison with control and BT2.0. Similarly, breast and thigh yields of BT1.0 and BT1.5 were significantly higher than control and BT2 ($P < 0.05$). Moreover, driploss of the breast at 12 and 24h after slaughter was lowest on BT0.5 versus highest control ($P < 0.05$). From the above results, it is recommended that 0.5 or 1.5% garlic powder should be added to the diet of Ross 308 broiler chickens for improvement of growth, high carcass yield, and lowest driploss.

Keywords: Broiler, ADG, FCR, carcass traits, garlic powder, driploss.

TÓM TẮT

Ảnh hưởng của bột tỏi lên khả năng sinh trưởng, năng suất và chất lượng thân thịt gà Ross308

Đề tài được thực hiện nhằm xác định ảnh hưởng của việc bổ sung bột tỏi vào khẩu phần lên khả năng sinh trưởng, năng suất và chất lượng thân thịt ở gà Ross 308. Thí nghiệm được bố trí hoàn toàn ngẫu nhiên với 5 NT tương ứng với 5 mức bổ sung bột tỏi 0; 0,5; 1,0; 1,5 và 2% trong khẩu phần với tên gọi lần lượt là ĐC; BT0.5; BT1.0; BT1.5 và BT2.0. Mỗi NT được lặp lại 3 lần, mỗi lần lặp lại là 30 con gà. Ở 35 ngày tuổi, 8 gà thịt ở mỗi NT được mổ khảo sát. Kết quả phân tích cho thấy bổ sung bột tỏi làm giảm tỉ lệ bệnh cầu trùng, Gumboro, ORT và tỉ lệ hao hụt so với ĐC ($P < 0,05$). Ngoài ra, khối lượng, tăng khối lượng, hệ số chuyển hóa thức ăn toàn kì cao so với ĐC ở giai đoạn 35 ngày tuổi ($P < 0,05$). Bên cạnh đó, khẩu phần có bổ sung 0,5-1,5% bột tỏi có các chỉ tiêu về khối lượng sống, khối lượng sau cắt tiết, khối lượng sau nhổ lông và khối lượng thân thịt của gà cao hơn có ý nghĩa về mặt thống kê so với ĐC ($P < 0,05$), trong đó BT1.5 cao nhất so với ĐC và BT2.0. Tương tự, khối lượng thịt ức và đùi ở BT1.0 và BT1.5 cao hơn đáng kể so với ĐC và BT2.0 ($P < 0,05$). Độ rỉ dịch của thịt ức gà ở thời điểm 12 giờ và 24 giờ sau khi giết mổ thấp nhất ở BT0.5 và cao nhất ở ĐC ($P < 0,05$). Từ các kết quả trên đề nghị nên bổ sung bột tỏi ở 0,5 hoặc 1,5% vào khẩu phần ăn của gà thịt Ross 308 để cải thiện năng suất sinh trưởng, thân thịt cao và độ rỉ dịch thấp nhất.

Từ khóa: Bột tỏi, độ rỉ dịch, đặc điểm thân thịt, tăng trọng, hệ số chuyển hóa thức ăn, gà thịt.

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1. INTRODUCTION

The widespread use of antibiotics in animal feed helps to prevent pathogens and promote weight gain thereby increasing animal performance, but at the same time, it also has side effects such as making bacteria resistant to drugs and drug residues in livestock products (Issa and Omar, 2012). Therefore, the search for substitutes for antibiotics of natural origin is of great interest to researchers. Garlic is one of the medicinal herbs that contains antibacterial active components such as allin, diallyl trisulfide, and allicin (Amagase *et al.*, 2001) that help animals not only against some pathogens but also promote the activity of beneficial bacteria in the gastrointestinal tract thereby improving carcass performance (Puvača *et al.*, 2014). Some authors suggested that garlic essential oil has the ability to enhance digestion, balance the intestinal microbial ecosystem, and stimulate the secretion of endogenous digestive enzymes thereby improving the growth performance of chickens (Lovkova *et al.*, 2001; Williams and Losa, 2001; Salah, 2012). The experimental results of Milošević *et al.* (2013), Pistová (2016) and Ali (2016) suggested that garlic powder should be supplemented at 1.5-3% in Ross 308 broilers, and Choi *et al.* (2010) concluded that adding more than 4.5% had harmful effects on the growth of animals.

On that basis, this study was conducted to collect more information on the effect of garlic powder supplementation on growth, carcass performance, and meat quality of Ross 308 chickens.

2. MATERIALS AND METHODS

2.1. Animals and management

A total of 450 Ross 308 chicks at 1 day old were fully vaccinated against infectious diseases and dewormed before the experiment started.

Feed used for chickens in three stages respectively as follows: 1-14 days of age with CP value of 21% and ME value of 3,000 kcal/

kg; 15-28 days old, feed contains 19% CP and 3,050 kcal/kg ME, and 29 days of age to slaughter with CP of 18% and ME of 3,150 kcal/kg. Garlic powder in the form of an ivory white powder has a characteristic strong smell of garlic and was purchased from Vianco Joint venture enterprise, address: 451/5 Nguyen Trai, Ward 7, District 5, HCM city.

The study was conducted at Long Binh Livestock Company Limited located in Thuan An hamlet, Song Ray, Trang Bom, Dong Nai. Chicks imported at 1-day old were vaccinated with Newcastle, Gumboro, and Infectious Bronchitis vaccines according to disease vaccination protocols that are strictly implemented in accordance with the regulations of Long Binh Livestock Company, in order to reduce the risk of disease in chickens. All chickens during the experiment were cared for and brought up under the same conditions, differing only in supplementary garlic powder, feeding time was divided into 3 sessions. In the morning, weighed the excess feed at 6:30 am, cleaned the feeder, turn the litter layer, and then fed the chickens with the amount of feed equal to 30% of daily feed intake, and the rest was eaten at 15:00pm (20%), 20:00pm (50%).

Temperature and humidity were measured and recorded daily at 6:30, 14:00, and 18:00. Chicken was allowed to drink water freely.

2.2. Experimental design and data collection

The experiment was arranged in a completely randomized design consisting of 5 treatments corresponding to 5 diets with garlic powder (BT) supplementation ratio as follows: (1) for control (control) chickens were fed the baseline diet (KPCS) without garlic powder; (2) BT0.5 included KPCS with additional 5g BT/kg feed; (3) BT1.0 included KPCS with additional 10g BT/kg feed; (4) BT1.5 included KPCS with additional 15g BT/kg feed; and (5) BT2.0 including KPCS supplemented with 20g BT/kg feed. The experiment was repeated 3 times with a total of 15 treatment units, each

treatment being 30 animals arranged at 8 days of age with the same initial weight. The total experimental animals were 450 birds.

The chickens were weighed at the beginning (7 days of age) and every 7 days of the experiment. Feed and leftovers were weighed and recorded the next morning. Health status, and loss rate (%) of chicken flocks were observed and recorded with signs and symptoms of infected birds, record the number of diseased, dead and discarded chickens through the weeks of age.

The carcass traits and meat quality were analyzed by selecting broiler chickens from the treatments at 35 days of age. The total number of birds for slaughter was 40 (5 treatments x 8 replicates). Experimental chickens were cut off feed 12h before slaughter, only water was given. Chickens for the experiment were selected with a weight close to the average weight. Weighed the chickens again after each stage of cutting blood, plucking feathers, and removing the internal organs to record the data. All experimental chickens were slaughtered at Thuan Truong Poultry Slaughterhouse with a closed slaughter process, with modern equipment, strict quality control, and quality control from receiving chickens to packaging. Products store comprehensive information on all export and import orders, ensuring easy product traceability while adhering to food safety and hygiene standards outlined in No: 05/2015/YTHCM-XNCB.

Thighs and breasts were separated for meat quality. The breast meat was used to determine the driploss, cut a piece of breast meat without fat, of equal weight, then weighed the cut meat, then used a hook to hang old the meat and put it in a plastic bag (be careful not to put it in a plastic bag). Tied the bags with a string, then hang them in the refrigerator at 4°C and stored for 12 and 24h. Weighed samples before and after storage by electronic balance to calculate driploss percentage.

The performance parameters of the

experimental chickens were evaluated according to the method of Bui *et al.* (2011) such as liveweight, daily weight gain, feed consumption and feed conversion ratio. The recorded carcass parameters of chickens included live weight, carcass weight, breast weight, thigh weight, weight of internal organs and their proportions.

2.3. Statistical analysis

The collected raw data were recorded and processed by Microsoft Excel software, then statistically processed by Minitab Version 16 software according to the GLM-ANOVA model. The mean values were compared using the Tukey method with 95% confidence intervals. Infection rates of coccidiosis, Gumboro, ORT diseases and mortality rate of chickens in this experiment were statistically processed by Chi-Square test (Minitab Version 16). The temperature and humidity data were processed using descriptive statistics.

3. RESULTS AND DISCUSSION

3.1. Temperature, humidity and health status of the experimental chickens

The temperature and humidity inside the barn of experimental chickens are shown in Table 1. The lowest temperature at 6:30am was in the range of 24.8-27°C, the highest temperature at 14:30pm was 31-35°C and the average temperature ranged of 25.4-30.6°C. The recorded results showed that the temperature in the house was quite high, which can be detrimental to the growth and development of experimental chickens. As observed during the experiment when the temperature was above 30°C, the chickens drank a lot of water, ate less, were easily excited, and opened their mouth to breathe. The results in Table 1 showed that the lowest humidity at 6:30am was in the range of 74%, the highest humidity ranged of 80-94.7%, and the average humidity ranged of 72-89.7%. The collected humidity data revealed that the house had higher humidity levels compared to other studies, indicating that

the optimal humidity range for the growth and development of chickens falls between 60 and 80%. The moisture levels recorded in the

present study may have detrimental effects on the health of experimental chickens.

Table 1. Temperature (°C) and humidity (%) in the experimental house

Factor	Period (day)	Minimum			Average			Maximum		
		6:30	14:30	18:00	6:30	14:30	18:00	6:30	14:30	18:00
Temperature (°C)	8-14	27	29	28	29	29.7	29.5	30	35	31.7
	15-21	26.5	30	27	27	30.9	29	27.5	34	31
	22-28	24.8	29	26	25.4	30.6	27	27.5	31	28.8
	29-35	25	29	25	26	30	26	27.7	31.5	27
Humidity (%)	8-14	74	59	71.6	79.5	76	75.2	86.8	79.3	80
	15-21	77.8	56.8	69.5	89	73	80	92.2	75.3	85
	22-28	79	70	75	89.7	72	78.8	94.7	79.1	92.8
	29-35	82	70.9	75	88.8	78	84	92.3	84.7	91.77

Table 2. Prevalence of coccidiosis, Gumboro and ORT

Name of diseases	Treats	Infection rate, %	P	Symptom characteristics
Coccidiosis	Cont	64	0.001	diarrhea, wheezing, wet breast
	BT0.5	78		
	BT1.0	74		
	BT1.5	55		
	BT2.0	83		
Gumboro	Cont	14.8	0.001	ruffled feathers, lethargy, anorexia, diarrhea with white, gray and green colors, some died
	BT0.5	6.8		
	BT1.0	10		
	BT1.5	7.8		
	BT2.0	4.4		
ORT	Cont	6.8	0.016	cough, difficulty breathing, runny nose, high fever, lethargy, anorexia, mouth open as if inhaling air and then died
	BT0.5	8.1		
	BT1.0	12.5		
	BT1.5	11.2		
	BT2.0	5.5		

The health status of experimental chickens presented in Table 2 showed that chickens were infected with coccidiosis at a relatively high rate, especially in the period of 8-11 days old. The lowest infection rate was on BT1.5 (55%) compared with the control group (64%) and BT0.5 (78%), BT1.0 (74%), BT2.0 (83%) ($P < 0.01$). In addition, in the period of 14-24 days of age, the chickens showed signs of Gumboro infection and the highest rate of Gumboro disease in experimental chickens was in the control group, accounting for 14.5% compared with the treatments supplemented with garlic powder (4.4-10%) ($P < 0.01$).

Moreover, at 30 days of age, experimental chickens showed signs of ORT disease, and the infection rate in the additional garlic powder (8.1-12.5%) was higher than that of the control group (6.8%), except that the treatment with BT2.0 had an infection rate of 5.5%.

The results showed that the addition of garlic powder, especially BT1.5, helped to reduce the infection rate of coccidiosis and Gumboro diseases in the experimental chickens compared with control and other supplementation levels. Studies have shown that the biologically active substances of garlic powder such as allicin, a very powerful natural antibiotic, stronger than penicillin, have the ability to inhibit many gram-negative and gram-positive bacteria such as *Staphylococcus*, *Streptococcus*, *Salmonella*, *V. cholerae*, *B. dysenteriae*, *Mycobacterium tuberculosis* (Vo Ha, 2008), and it is possible that this bioactive ingredient has worked to make the prevalence of Gumboro disease in experimental chickens supplemented with garlic powder less than in control. In addition, the compound of alliin, diallylsulfides and allicin present in garlic have antibacterial, antiviral, antiparasitic and antifungal properties (Raesi *et al.*, 2010). Chickens were infected with ORT, a respiratory disease, and the humidity observed in this experiment made the chickens more susceptible. Amagase *et al.* (2001) reported that the medicinal effect of garlic improved

intestinal disorders, increased abdominal muscle tone, and treated intestinal worms as well as respiratory infections in chickens.

Mortality of experimental chickens (Table 3) was significantly different between treatments ($P<0.05$), in which the loss rate of the control accounted for 6.67%, the highest compared with the treatments supplemented with garlic powder, ranging of 1.11-5.56%. The increased mortality in the control group can be attributed to the presence of infected chickens affected by Gumboro disease and ORT. This result also shows that garlic powder has an effect on the immunity of experimental chickens, helping to reduce mortality compared to the control. This is consistent

with the findings of Onibi *et al.* (2009) reported that garlic supplementation reduced mortality from 1.67 to 3.33%. However, this loss rate was still higher than that given by Long Binh company (3%).

Table 3. Mortality rate (%) of the experimental chickens

Treatments	Mortality rate, %	P
Control	6.67	
BT0.5	5.56	
BT1.0	4.44	0.011
BT1.5	1.11	
BT2.0	3.33	

3.2. Growth performance of Ross 308 broiler chickens

Table 4. Growth performance of the experimental chickens

Parameters	Treatments					SEM	P	
	Control	BT0.5	BT1.0	BT1.5	BT2.0			
BW, g/bird/week	1-7	213.4	208.3	211.5	207.5	208.7	1.30	0.057
	8-14	493.5	503	496.2	496.2	506.5	5.13	0.397
	15-21	1060.7	1102	1071.7	1086.3	1039.7	24.27	0.467
	22-28	1646.7	1690	1743.3	1700	1610	37.91	0.200
	29-35	2173.3 ^{ab}	2350.0 ^{ab}	2383.3 ^{ab}	2530.0 ^a	2143.3 ^b	80.75	0.037
Cumulative WG, g/bird/day	8-14	40.01	42.01	40.67	41.24	42.54	0.81	0.255
	8-21	60.52	63.79	61.44	62.77	59.35	1.73	0.437
	8-28	68.25	70.53	72.95	71.07	66.73	1.81	0.201
	8-35	70.00 ^{ab}	76.47 ^{ab}	77.57 ^{ab}	82.95 ^a	69.09 ^b	2.87	0.034
ADG, g/bird/day	8-14	40.01	42.01	40.67	41.24	42.54	0.81	0.255
	15-21	81.03	85.57	82.21	84.31	76.17	3.46	0.405
	22-28	83.71	84.00	95.95	87.67	81.48	4.17	0.192
	29-35	75.2	94.3	91.4	118.6	76.2	9.94	0.065
	8-35	71.9	73.49	76.14	77.59	70.88	7.38	0.778
Feed intake, g/bird/day	8-14	58.85	60.34	59.85	59.59	58.14	1.36	0.808
	15-21	94.33	96.93	97.46	95.70	136.88	18.89	0.653
	22-28	141.17	140.88	144.86	141.84	132.97	2.69	0.094
	29-35	147.96	167.69	169.68	170.08	160.28	6.37	0.148
	8-35	110.58	116.46	117.96	116.80	111.34	2.23	0.127
FCR, g feed/g wg	8-14	1.47	1.44	1.47	1.45	1.37	0.04	0.439
	15-21	1.17	1.13	1.19	1.14	1.80	0.23	0.277
	22-28	1.7	1.69	1.51	1.62	1.64	0.08	0.508
	29-35	2.28	1.79	1.86	1.44	2.14	0.32	0.408
	8-35	1.6 ^a	1.5 ^{ab}	1.5 ^{ab}	1.4 ^b	1.6 ^a	0.04	0.05

Different letters within the same row represent significant differences ($P<0.05$)

The results in Table 4 show that the initial weight of experimental chickens was not statistically significant ($P>0.05$), which proved the equality in the treatment, avoiding the effect of affecting the experimental results. In addition, the live body weight and cumulative weight gain of chickens among treatments over the age periods of 8-14 days old, 15-21 days old, and 21-28 days old were not statistically significant ($P>0.05$). However, live body weight and cumulative weight gain of chicken from 29-35 days old were different and statistically significant among treatments ($P<0.05$), the highest on BT1.5 (82.95g) and lowest on BT2.0 (69.09g). The above results showed that the body weight and cumulative weight gain of the experimental chickens were improved in the diet with garlic powder added to 1-1.5 g/kg of feed. Lovkova *et al.* (2001), Williams and Losa (2001) suggested that garlic essential oil has the ability to enhance digestion, balance the intestinal microbial ecosystem, and stimulate the secretion of endogenous digestive enzymes thereby improving the growth performance of chickens. Research by Salah (2012) found that the bioactive ingredient of garlic powder increased the body weight of experimental chickens. In contrast, Onibi *et al.* (2009) found that garlic had no significant effect on chicken weight gain.

The results of Table 4 showed that the daily weight gain, feed consumption, and feed conversion ratio of chickens over weeks of age were not statistically significant among treatments ($P>0.05$). The statistically significant difference among treatments on feed conversion ratio was only found in the whole period of 8-35 days of age ($P<0.05$), in which BT1.5 (1.4g feed/g weight gain) was the lowest and the highest was BT2 (1.6g feed/g weight gain). This indicates a positive effect of the bioactive compounds in garlic powder in treating intestinal disorders and flatulence, thereby increasing the digestibility of the chickens and stimulating the chickens to eat more (Amagase *et al.*, 2001). Salah

(2012) suggested that the bioactive ingredient of garlic powder increased absorption in chickens. However, the results of Chowdhury *et al.* (2002) suggested that the addition of garlic powder at different levels to the diet had an insignificant effect on growth, feed consumption, and feed efficiency.

3.3. Carcass traits and meat quality

The slaughtered results (Table 5) showed that there was a statistically significant difference among treatments in terms of live weight, body weight, post-harvesting weight, carcass weight, and percentage after cutting, plucking and carcass rate ($P<0.05$). The treatments with garlic powder added at 0.5, 1.0, and 1.5 g/kg feed all achieved results on the above criteria higher than the control, except BT2.0. The reason may be that the bioactive ingredient, allicin, in garlic promotes the activity of the beneficial microflora of the intestine, which improves the digestion process and enhances the absorption of nutrients, thereby helping to improve weight gain (Pourali *et al.*, 2010) and due to increased protein intake at the cellular level (Ademola *et al.*, 2005; Ortserga *et al.*, 2008). The body weight of BT1.5 reached 2550 g while BT2.0 only reached 2150 g, which showed a positive effect on carcass quality at 1.5% garlic or less, and supplemented with 2 % affected the production parameters (Stanaćev *et al.*, 2011). Meanwhile, the experiment of Milošević *et al.* (2013), Pistová (2016) and Ali (2016) in the same chicken breed, Ross 308 broiler, suggested that garlic powder should be supplemented at 1.5-3%, and Choi *et al.* (2010) concluded that added more than 4.5% had harmful effects on the growth of animals.

The indicators of breast weight, breast meat weight, thigh weight, and thigh meat weight were statistically different among the experimental groups ($P<0.05$), in which the carcass weight was highest in BT1.5 and the lowest in control and BT2.0. The percentage of breasts, the percentage of thighs and the percentage of thigh meat were significantly

different ($P < 0.05$), BT1.0 gave higher than BT2.0. The explanation that the 2% garlic powder supplementation in this present study had lower carcass parameters compared with other garlic levels could be due to the fact that garlic contains 4.6% saponins and 1.2% flavonoids (in the dry matter), which are

substances that reduced performance in Ross 308 chickens (Otunola *et al.*, 2010; Stanačev *et al.*, 2012; Lukanov *et al.*, 2015). In contrast, some other results suggested that garlic powder had no effect on carcass traits and major organs (Javandel, 2008; Onibi *et al.*, 2009).

Table 5. Carcass traits of the experimental chickens

Parameters	Treatments					SEM	P
	Control	BT0.5	BT1.0	BT1.5	BT2.0		
Live weight (LW, g)	2200.0 ^{bc}	2362.5 ^b	2375 ^{ab}	2550.0 ^a	2150.0 ^c	43.661	0.01
BW after blood removed (g)	2079.9 ^{bc}	2254.4 ^{ab}	2253.8 ^{ab}	2427.4 ^a	2027.9 ^c	46.054	0.01
BW after blood removed (%)	94.5 ^a	95.4 ^a	94.9 ^a	95.2 ^a	94.3 ^a	0.271	0.048
BW after feather (g)	1999.4 ^{bc}	2173.3 ^{ab}	2172.6 ^{ab}	2348.8 ^a	1947.9 ^c	45.875	0.01
BW after feather (%)	90.8 ^{ab}	91.9 ^a	91.5 ^{ab}	92.1 ^a	90.6 ^b	0.316	0.006
Carcass weight (g)	1642.4 ^{bc}	1818.7 ^{ab}	1819.3 ^{ab}	1992.5 ^a	1592.6 ^c	45.978	0.01
Carcass (%)	74.5 ^{bc}	76.9 ^a	76.6 ^{ab}	78.1 ^a	74.0 ^c	0.579	0.01
Breast weight (g)	743.9 ^{bc}	832.1 ^{ab}	841.1 ^a	911.3 ^a	705.6 ^c	22.799	0.01
Breast (%)	45.24 ^b	45.75 ^{ab}	46.23 ^a	45.71 ^{ab}	44.30 ^c	0.186	0.01
Breast meat (%)	34.0 ^b	34.4 ^{ab}	34.7 ^a	34.4 ^{ab}	33.2 ^c	0.146	0.01
Breast meat (g)	558.9 ^{bc}	626.4 ^{ab}	631.6 ^a	685.3 ^a	529.4 ^c	17.147	0.01
Thigh weight (g)	581.5 ^{bc}	652.5 ^{ab}	657.5 ^a	713.6 ^a	551.3 ^c	17.756	0.01
Thigh (%)	35.4 ^b	35.9 ^{ab}	36.1 ^a	35.8 ^{ab}	34.6 ^c	0.143	0.01
Thigh meat weight (g)	351.4 ^{bc}	385.6 ^{ab}	386.4 ^{ab}	423.8 ^a	338.9 ^c	9.745	0.01
Thigh meat (%)	21.4	21.2	21.2	21.3	21.3	0.060	0.21
Thigh skin weight (g)	39.4 ^{bc}	43.6 ^{ab}	42.6 ^{bc}	48.5 ^a	37.8 ^c	1.209	0.09
Head weight (g)	45.9 ^b	49.6 ^{ab}	46.4 ^b	52.6 ^a	51.2 ^{ab}	1.452	0.007
Neck weight (g)	88.9	87.9	85.9	100.9	94.0	4.112	0.094
Wing weight (g)	231.3 ^b	250.1 ^{ab}	238.9 ^b	270.5 ^a	244.4 ^b	5.028	0.01
Leg weight (g)	77.6 ^{ab}	81.8 ^{ab}	75.3 ^b	85.1 ^a	83.8 ^a	1.895	0.003
Intestinal length (cm)	187.1	222.2	191.5	190.8	204.5	10.32	0.12
Cecum length (cm)	18.8	18.9	20.5	21.3	19.6	0.667	0.06
Abdominal fat (g)	33.3	38.6	31.1	30.3	27.0	3.511	0.22
Abdominal (%)	2.0	2.1	1.7	1.5	1.7	0.199	0.18
Gizzard weight (g)	48.6	59.5	59.5	60.4	53.8	3.536	0.11
Liver weight (g)	42.8	47.6	44.0	51.1	46.3	2.260	0.10
Heart weight (g)	8.8 ^c	12.1 ^{ab}	9.6 ^{bc}	13.1 ^a	11.5 ^{abc}	0.804	0.003

Head weight, wing weight, and leg weight were all statistically significant among treatments ($P < 0.05$). Overall, BT1.5 gave the best results compared to the other treatments (52.6, 270.5, 85.1g), in which neck weight was not statistically significant among treatments ($P > 0.05$). However, the experiment of Lukanov *et al.* (2015) suggested that garlic supplementation had an effect on wing weight. The percentage of thigh meat and

thigh skin in this study did not have statistical significance among the experimental groups ($P > 0.05$). In addition, the weight of internal organs such as gizzard, liver, and abdominal fat was not statistically significant among treatments ($P > 0.05$). The experimental results are consistent with the study of Stanačev *et al.* (2010) and Raeesi *et al.* (2010) reported that garlic powder had no significant effect on liver and heart weights. Raesi *et al.* (2010) and

Senthilkumar *et al.* (2015) showed that 1 and 3% garlic supplementation in broiler diets had no significant effect on digestive organs. The reason may be due to the presence of allicin in garlic, which is a powerful antibiotic and antioxidant (Sallam *et al.*, 2004; Bozin *et al.*, 2008; Brzóška *et al.*, 2015), in addition to sulfur-containing compounds helping to cope with free radical-induced liver damage and at the same time stimulate the growth of new liver cells (Amagase *et al.*, 2001; Tatara *et al.*, 2005). Moreover, in this study, there was a statistically

significant difference in heart weight among the treatments ($P < 0.05$), the highest at BT1.5 (13.1g), the current experimental result is consistent with the results of Milošević *et al.* (2012) reported that the addition of 1.5% garlic powder in the diet resulted in significantly greater heart weight compared with other treatments. Fayed *et al.* (2011) also agreed with the present study, while Lukanov *et al.* (2015) stated that heart mass was not affected by the amount of garlic added to the diet.

Table 6. Driploss of breast meat (%)

Time postmortem	Treatments					SEM	P
	Control	BT0.5	BT1.0	BT1.5	BT2.0		
12hrs	7.93 ^a	2.36 ^b	2.74 ^b	2.90 ^b	4.50 ^{ab}	0.875	0.01
24hrs	8.24 ^c	2.34 ^b	4.12 ^b	3.21 ^b	5.41 ^{ab}	0.886	0.01

The exudation of breast meat in Ross 308 chickens (Table 6) is shown that there was a statistically significant difference in breast meat driploss at 12 hours and 24h after slaughter ($P < 0.05$), in which BT0.5 had the lowest driploss (2.36 and 2.34%) and the highest in the control (7.93 and 8.24%). The reason may be that natural antioxidants combined with substances in animal feed have a synchronous effect in improving the stability and quality of chicken meat. According to Yin and Cheng (1998), Gorinstein *et al.* (2005), Safa *et al.* (2014) reported that garlic has several antioxidant compounds, mainly polyphenols such as flavonoids and sulfur-containing compounds that have stronger inhibitory effects on lipid oxidation. Therefore, under the effect of garlic powder, the driploss of breast meat of the added treatments was improved, contributing to reducing the loss rate of meat stalls during storage.

4. CONCLUSION

Adding garlic powder at 0.5 or 1.5% to the Ross 308 chicken diet had a positive effect on weight gain, feed conversion ratio as well as performance parameters and carcass quality.

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EFFECTS OF PREMIX-VITAMIN SUPPLEMENTATION IN DRINKING WATER ON EGG PERFORMANCE AND QUALITY OF ISA BROWN LAYING HENS FROM 44-52 WEEKS OF AGE

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ABSTRACT

The study was carried out to determine the effect of premix-vitamin products supplementation in drinking water continuously every day or at one-week intervals on the performance and egg quality of laying hens from 44-52 weeks of age. 3487 Isa Brown laying hens at 43 weeks of age were distributed in a completely randomized design experiment, with 5 treatments and 3 replicates, each replicate consisted of a line cage with 60 pens (3-4 birds/pen). data were collected over 8 weeks. Treatments used: 1/ĐC: Basal diet without any supplementation in drinking water (control); 2/VLT: Basal diet + Permavit every day; 3/VCT: Basal diet + Permavit at one-week intervals; 4/SLT: Basal diet + Permasol every week; 5/SCT: Basal diet + Permasol at one-week intervals. All supplements were added to drinking water at 1 g/litter. Results showed that no effect of all supplements on feed intake, egg weight, and feed conversion ratio ($P > 0.05$), but a slight improvement hen day production in VLT (89.95%), VCT (91.56%), SLT (87.1%), SCT (90.06%) compared with control (88.42%) ($P < 0.05$), especially in the supplemented treatments one week intervals had better egg performance than that in every day continuously supplement. The egg quality parameters such as egg shape index, yolk index, white index, and HU units were not significantly different in all the treatments, but there was an improvement in the yellow color (b) of the egg yolks of VLT (30.85), VCT (32.11), SLT (30.41), SCT (30.8) compared with ĐC (28.29). In conclusion, adding both Permavit and Permasol products to the drinking water for Isa Brown chickens at 44-52 weeks of age improved the hen day production and the yellow color (b) of the yolk. Supplementing at one-week intervals had better results than continuous daily supplementation of both products.

Keywords: *Permavit, permasol, premix-vitamin, hen day production, egg quality.*

TÓM TẮT

Ảnh hưởng của việc bổ sung chế phẩm premix- vitamin vào nước uống đến năng suất và chất lượng trứng gà chuyên trứng Isa Brown từ 44-52 tuần tuổi

Thí nghiệm được tiến hành để xác định việc bổ sung 2 chế phẩm bổ sung Premix-vitamin liên tục hay cách tuần vào nước uống lên năng suất và chất lượng trứng gà Isa Brown giai đoạn từ 44-52 tuần tuổi. Thí nghiệm được bố trí theo thể thức hoàn toàn ngẫu nhiên với 5 nghiệm thức (NT), mỗi NT được lập lại 3 lần, mỗi lần lập lại là 1 dãy tầng lồng gồm có 60 ô lồng (3-4 con/ô). Thời gian thu thập số liệu là 8 tuần với tổng số gà thực hiện là 3487 con. Các NT như sau: 1/Đối chứng (ĐC): Khẩu phần cơ sở (BD), không bổ sung chế phẩm vào nước uống; 2/VLT: BD+ Permavit uống liên tục; 3/VCT: BD+Permavit uống cách tuần; 4/SLT: BD+Permasol uống liên tục; 5/SCT: BD+Permasol uống cách tuần. Tất cả các chế phẩm bổ sung vào nước uống cùng liều lượng là 1g/lít nước uống. Kết quả cho thấy lượng thức ăn trung bình hàng ngày (FI) của gà, khối lượng trứng và hệ số chuyển hóa thức ăn (FCR) của gà ở các NT gần như không có sự khác biệt có ý nghĩa thống kê ($P > 0.05$), tuy nhiên tỷ lệ đẻ có cải thiện hơn ở các NT có bổ sung VLT (89.95%), VCT (91.56%), SLT (87,1%), SCT (90.06%) so với ĐC (88.42%), đặc biệt ở các NT bổ sung cách tuần có tỷ lệ đẻ cao hơn bổ sung liên tục. Các chỉ tiêu về chất lượng trứng như chỉ số hình dạng trứng, chỉ số lòng đỏ, lòng trắng, đơn vị

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HU đều không khác nhau so với ĐC, nhưng có sự cải thiện về màu vàng (b) của lòng đỏ trứng gà có bổ sung so VLT (30.85), VCT (32.11), SLT (30.41), SCT (30.8) so với ĐC (28.29). Nhìn chung khi bổ sung cả 2 sản phẩm Permavit và Permasol vào nước uống cho gà Isa Brown giai đoạn 44-52 tuần tuổi có khuynh hướng cải thiện được tỷ lệ đẻ và màu vàng của lòng đỏ. Bổ sung cách tuần cho hiệu quả tốt hơn so với bổ sung liên tục ở cả 2 sản phẩm.

Từ khóa: *Permavit, Permasol, premix-vitamin, năng suất trứng, chất lượng trứng.*

1. INTRODUCTION

Poultry production has an important role in Vietnam's agriculture, accounting for 512.6 million heads of total poultry production in the country, which chicken production occupying 409.5 million heads in early of the year 2021, an increase of around 7% compared with that the year 2020 (GSO, 2021). The productive value of poultry production ranks second in the livestock industry after pig production, in which laying hens is the most important sector of poultry production because egg production relatively occupies a high number of poultry production as food for humans. In the process of raising commercial laying hens, egg production will increase gradually from the beginning of laying to peak at the last 30-40 weeks of age, then stabilize and decrease when chickens reach over 50 weeks of age, and culling around 72-76 weeks of age (Haider and Babu, 2014). Therefore, how to slowly reduce performance in laying hens over 40 weeks of age, and how to supply every day during the laying stage or one-week intervals are questions for the farmers. Some studies have used some products such as vitamins or probiotics to supply chickens over 60 weeks of age (Nguyen Thi Thuy, 2019) which can partly improve the laying rate. There are many kinds of supplementation products in the market which can be supplemented for laying hens at the late stage of the laying circle. Recently, many studies used some kinds of premix-vitamin added to diets or drinking water for broilers, but not in laying hens. Therefore, this study was conducted using 2 kinds of premix-vitamin products (Permavit and Permasol) supplementation in drinking water every day or at one-week intervals, in order to evaluate the effects of supplements on egg production

and quality during the late stage of Isa Brown laying hens.

2. MATERIALS AND METHODS

2.1. Materials

The Permavit and Permasol products were provided by Vemedim Corporation Company, their main ingredients were shown in Table 2. The experiment was conducted at the Experimental farm of Vemedim Corporation Company in O Mon district, Can Tho City from November 2022 to Jan 2023.

2.2. Experimental design

A total of 3,487 Isa Brown laying hens at 43 weeks of age were housed in cages and received 17h of light per day. The chemical composition of the basal diet is shown in Table 1. The dietary treatments were given for 8 weeks (44-52 weeks old) period. Feed was supplied twice a day, 30% was fed at 08.00am and another 70% was given at 02.00pm, water was supplied *ad-libitum* in the drinking line. The study was arranged as a completely randomized design with 5 treatments and 3 replicates, each replicate consisted of a line cage with 60 pens (4 birds/pen).

Experimental treatments and diet

Table 1. Chemical composition of the basal diet

Chemical composition	Basal diet, %
DM	88.0
Crude protein	17.0
Ether extract	5.0
Ash	10.2
Crude fiber	5.0
Ca	3.2
P	0.8
NaCl	0.2
NFE	63.2
ME (MJ/kg feed, calculated)	11.3

The basal diet was formulated by the Experimental farm of Vemedim Corporation Company with the chemical composition presented in Table 1. Feed ingredients in the basal diet included: maize, broken rice, rice bran, fish meal, soya bean meal, amino acids, and premix- vitamins.

Table 2. Composition of supplemented products

DC	VLT	VCT
Vitamin A (min)	5,000,000IU	5,000,000IU
Vitamin D3 (min)	800,000IU	800,000IU
Vitamin E (min)	2,000IU	2,000IU
Vitamin K3 (min)	2,000mg	2,000mg
Vitamin B1 (min)	2,000mg	2,000mg
Vitamin B2 (min)	5,000mg	5,000mg
Vitamin B5 (min)	5,000mg	-
Vitamin B6 (min)	1,000mg	-
Vitamin B12 (min)	1,000mcg	1,000mcg
Folic acid (min)	400mg	400mg
Niacinamide	6,000mg	6,000mg
Methionine (min)	16,000mg	16,000mg
Natri sodium (min-max)	2,268-2,772mg	2,520mg
Kali sodium (min-max)	3,366-4,114mg	3,740mg
Iron (min-max)	1,926-2,354mg	2,140mg
Zinc (min-max)	117-143mg	130mg
Manganese (min-max)	126-154mg	140mg
Cobalt (min-max)	198-242mg	220mg

There were five treatments as follows:

1/DC: Basal diet (BD) without any supplementation in drinking water

2/ VLT: BD + Permavit in drinking water every day (1 g/litter)

3/ VCT: BD + Permavit in drinking water at one-week intervals (1 g/litter)

4/ SLT: BD + Permasol in drinking water every day (1 g/litter)

5/ SCT: BD + Permasol in drinking water at one-week intervals (1 g/litter)

Sampling and measurements

Egg performance: During the experimental period, the average feed intake, hen day production, and egg weight were recorded daily to compute the average daily egg production. The egg mass and feed conversion ratio were weekly calculated. Egg mass was

determined by calculating hen day production x egg weight. The feed conversion ratio was calculated by feed intake/egg mass (g/g).

Egg quality: At the 46, 48 and 50th weeks old, 60 eggs/treatment (20 from each replicate) were randomly collected for egg quality analysis. The egg shape index was determined by calculating (egg width/egg length) x 100 (Sandi *et al.*, 2013). After that, eggs were broken, and egg contents were poured onto a horizontal glass. Albumin, yolk, and shell weight were separated and weighed individually (Englmaierová *et al.*, 2014). Shell thickness was determined by calculating the mean of triplicate measurement from different sides of the shell (Güçlü *et al.*, 2008). Haugh unit (HU) was measured using the formula $HU=100 \times \log(H-1.7W^{0.37}+7.57)$ (Saleh, 2013). Yolk color was recorded using a colorimeter (Chromameter Minolta, CR-400 Head, DP-400/ Japan), which indicated degrees of lightness of a yolk sample (L), redness (a), and yellow-ness (b).

Chemical analysis: The chemical composition of basal feed was determined following the Association of Official Analytical Chemists methods (AOAC, 1990).

2.3. Statistical analysis

Collected data were analyzed by ANOVA using the GLM of Minitab Statistical Software Version 16. Tukey pair-wise comparisons were used to determine differences between treatment means at $P < 0.05$. The statistical model was $Y_{ij} = \mu + \alpha_i + e_{ij}$. Where: Y_{ij} was the egg performances or egg quality; μ was the overall mean averaged over all treatments; α_i was the effect of treatment; e_{ij} was the random error associated with treatment and replicate within the treatment.

3. RESULTS AND DISCUSSION

3.1. Egg performance and feed efficiency

The egg production performance of laying hens during the period between 44 and 52 weeks of age is presented in Table 3.

Table 3. Effect of premix-vitamin on egg performances and feed efficiency of laying hens 44-52 weeks age

Variables	DC	VLT	VCT	SLT	SCT	SEM	P
Feed intake, g/hen/day	111.64	111.18	112.49	110.83	112.09	1.13	0.95
Hen day production, %	88.42 ^b	89.95 ^{ab}	91.56 ^a	87.1 ^b	90.06 ^{ab}	0.82	0.03
Egg weight, g/egg	59.85	59.21	59.40	59.40	60.01	0.45	0.73
Egg mass, g egg/day	52.92	53.26	54.39	51.74	54.05	0.59	0.53
FCR, g feed/g egg	2.11	2.09	2.07	2.14	2.07	0.02	0.08

DC: Basal diet (BD) without any supplementation; VLT: BD+ 1g/lit Permavit everyday in drinking water; VCT: BD+ 1g/lit Permavit in drinking water one week intervals; SLT: BD+ 1g/lit Permasol everyday in drinking water; SCT: BD+ 1g/lit Permasol in drinking water one week intervals. Means within rows with different letters are different at P<0.05

There was no effect of supplementing premix-vitamin on the feed intake of the hens in all treatments, this result was consistent with research by Afshar *et al.* (2006) and Nobakht (2014), who found that mineral and vitamin premix supplementations in the diets did not affect the amount of feed consumed by the hens. In fact, in order to achieve high production, feed is the main factor that should be considered, in which vitamins and minerals have an additional dimension. They are required at adequate levels to enable the animal to efficiently utilize all other nutrients in the feed. Therefore, optimal nutrition occurs only when the hens are offered the correct mix of macro and micronutrients in the feed and are able to efficiently utilize those

nutrients for their reproduction and survival (Zang *et al.*, 2011).

Normally, vitamins and minerals are included in commercial feed (Sugiharto *et al.*, 2018), which are sometimes stored for a long time which may reduce the efficient utilization of vitamins. In addition, raising hens in hot weather can cause stress that in general increases mineral and vitamin mobilization from tissues and their excretion, which may exacerbate a marginal vitamin and mineral deficiency, which may lead to a series of health problems (Nobakht, 2014). Therefore, how to supply vitamins and minerals every day or at one-week intervals is considered.

Table 4. Effect of premix-vitamin on FI (g/day)

Age,w	DC	VLT	VCT	SLT	SCT	SEM	P
44	105.54	103.11	101.98	104.15	103.31	1.24	0.39
45	104.42	104.84	105.24	103.86	104.15	1.29	0.94
46	101.08	105.33	104.36	99.44	100.69	1.47	0.07
47	101.13 ^b	104.92 ^{ab}	105.62 ^{ab}	101.71 ^{ab}	106.51 ^a	1.09	0.02
48	120.62	117.82	122.20	120.11	123.27	1.34	0.12
49	118.86	119.27	118.59	118.17	120.29	1.43	0.86
50	120.73	117.40	117.69	119.60	119.01	1.92	0.73
51	120.74	116.76	116.25	119.62	119.50	2.16	0.54

Table 5. Effect of premix-vitamin supplements on hen day production (%) of the experimental laying hens

Age,w	DC	VLT	VCT	SLT	SCT	SEM	P
44	92.78	92.26	93.42	92.72	92.88	0.77	0.88
45	90.87	92.39	93.31	91.64	91.80	0.57	0.10
46	88.23 ^{abc}	89.50 ^{ab}	90.68 ^a	84.53 ^c	85.90 ^{bc}	0.98	0.01
47	82.36 ^{ab}	87.62 ^a	90.25 ^a	78.45 ^b	87.84 ^a	1.78	0.01
48	85.23 ^{ab}	88.84 ^{ab}	91.02 ^a	82.15 ^b	88.87 ^{ab}	1.79	0.04
49	83.5	87.08	88.17	83.94	87.97	1.67	0.30
50	92.15	90.93	92.81	91.59	92.79	0.70	0.33
51	92.21	90.98	92.79	91.81	92.41	0.93	0.70

Hen day production during the period between 44 and 52 weeks of age is presented in Table 5. Seidler (2003) reported that egg production of commercial laying hens often starts to slowly decrease after 40 weeks of age, but in this study the hen day production was not decreased during the period from 44-51 weeks of age. It can be explained that the reduction of egg production is quick or slow depending on the nutrition and management that laying hens received. These results demonstrated that because of supplemented products in the drinking water made slowly reducing of egg production after 40 weeks of age. The improvements in egg production with premix-vitamin in both supplementations every day or one-week intervals can be explained by enough vitamin supply in drinking water, when vitamins were included together with minerals and supply into drinking water may be more effective than supply in diets. Karimi *et al.* (2010) reported that adding supplements to drinking water was better than adding them to feed due to better solubility.

Table 6. Effect of premix-vitamin on EW (g/egg)

Age,w	DC	VLT	VCT	SLT	SCT	SEM	P
44	59.11	58.43	58.73	59.00	59.30	0.44	0.67
45	58.73	58.38	58.71	59.14	59.25	0.55	0.79
46	59.17	58.95	58.96	59.40	59.64	0.54	0.87
47	59.80	58.85	59.20	60.04	59.78	0.49	0.45
48	60.06	59.44	59.54	60.37	60.53	0.49	0.45
49	60.75	59.66	59.46	60.08	59.96	0.71	0.75
50	60.74	60.10	60.63	61.21	61.11	0.49	0.55
51	60.45	59.89	59.97	60.81	60.98	0.50	0.48

In addition, Nobakht (2014) explained that vitamin and mineral supplements for laying birds are indeed essential, as most vitamins cannot be synthesized by birds in sufficient quantities to meet physiological needs, they must be supplied from food (Zang *et al.*, 2011). A deficiency of some vitamins can cause a reduction in egg production and some egg quality indicators. For example, vitamin A deficiency reduces the laying rate, and vitamin D deficiency leads to thinner eggs and also reduces the laying rate. Vitamin B is important for laying hens, hens lose their appetite and die due to vitamin B1 or thiamine deficiency (Nobakht *et al.*, 2008).

There was no effect of treatments on egg weight, which agreed with research from Nobakht (2014), who showed that different levels of dietary mineral and vitamin premixes did not affect egg weight. And also there was no significant difference in FCR in all supplemented treatments compared to the control group (Table 7).

Table 7. Effect of premix-vitamin on FCR

Age,w	DC	VLT	VCT	SLT	SCT	SEM	P
44	1.92	1.91	1.86	1.90	1.88	0.02	0.11
45	1.96	1.94	1.92	1.92	1.92	0.01	0.11
46	1.94	2.00	1.95	1.98	1.97	0.03	0.74
47	2.05 ^{ab}	2.04 ^{ab}	1.98 ^b	2.17 ^a	2.03 ^{ab}	0.03	0.02
48	2.36 ^{ab}	2.23 ^b	2.26 ^{ab}	2.43 ^a	2.29 ^{ab}	0.04	0.03
49	2.34	2.30	2.26	2.34	2.28	0.07	0.48
50	2.16	2.15	2.09	2.13	2.10	0.02	0.12
51	2.17	2.14	2.09	2.14	2.12	0.03	0.34

3.2. Egg quality

Table 8. Effect of premix-vitamin on egg quality

Variable	DC	VLT	VCT	SLT	SCT	SEM	P
Egg weight, g	66.52	66.38	61.35	65.39	62.28	1.50	0.06
Egg shape index	76.37	76.90	77.52	78.43	76.96	0.94	0.60
Shell weight pec, %	12.93	12.80	13.52	13.15	12.14	0.47	0.36
Shell thickness, mm	0.36	0.35	0.36	0.36	0.35	0.01	0.97
Albumen weight proportion, %	60.74	62.58	60.46	60.06	58.08	1.43	0.32
Yolk weight pro, %	26.33	25.13	26.00	26.79	29.76	1.25	0.13
York index	0.43	0.42	0.41	0.42	0.35	0.02	0.17
Albumen index	0.09	0.08	0.09	0.09	0.09	0.01	0.75
Haugh unit (HU)	85.41	78.03	79.39	80.14	84.67	3.85	0.58
Yolk color							
L	52.57	53.35	53.04	5.24	53.91	0.76	0.80
a*	2.16	1.87	1.52	1.78	1.76	0.22	0.40
b*	28.29 ^b	30.85 ^{ab}	32.11 ^a	30.41 ^{ab}	30.80 ^{ab}	1.10	0.03

The shape index, egg part ratio, yolk and white index, and shell thickness were all similar in chickens with and without supplements. However, the yellow color of the yolk was increased in supplemented hens compared with control hens. Because both supplemented products contain high levels of vitamins A, D, and E, which in turn would increase the absorption of xanthophyll concentrations in feed (Nguyen Thi Thuy, 2019). This leads to an increase in the yellow color (b) of egg yolk in chickens supplemented with premix-vitamin.

4. CONCLUSIONS

The addition of premix-vitamin products (Permavit and Permasol) to the drinking water (1 g/l) of Isa Brown laying hens, between 44-52 weeks of age, resulted in improvements in both hen day production and the yellow color of the egg yolk when compared to the control group. Moreover, supplying the premix-vitamin products at one-week intervals yielded better performance than providing them on a daily basis.

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EFFECTS OF DIFFERENT DIETS ON GROWTH PERFORMANCE OF THAI CRICKETS

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ABSTRACT

The experiment was carried out to evaluate the effect of different commercial diets (CD) on the growth, and feed efficiency of Thai crickets, and their nutritional composition. The experiment was arranged in a completely randomized design into 3 treatments and 4 replicates with each repetition being 500 crickets at 7 days old. So, there was a total of 6000 crickets being used in this experiment and it was carried out for 6 weeks. The experimental treatments included Control (Con) used only fresh young cassava leaves; Diet 1: a CD1 contained 2-phase feedings of 18% CP with 3000 kcal ME/kg and 20% CP with 2800 kcal ME/kg for 7-21 and 22-49 days of age, respectively; and Diet 2: a CD2 contained 19% CP and 3150 kcal ME/kg; 21% CP and 3000 kcal ME/kg, respectively. The results showed that at 21, 42, and 49 days old, the body weight of crickets in Diet 2 was the highest, followed by Diet 1 and the lowest was in Con ($P < 0.05$). Similarly, Diet 2 had the highest ADG at 7-14, 15-21, 36-42, and 42-49 days of age and the lowest in Con. In contrast, Diet 2 had the lowest FI and FCR, and Con was the highest at 7-14 and 15-21 days of age. However, in the period of 29-35, 36-42, and 43-49 days old, Diet 2 had the highest FI and Con had the lowest mean. Diet 2 and Diet 1 were also the highest in terms of DM, OM, and EE content in cricket meat, in contrast, Con was highest in CP and Ash content.

Keywords: Cricket, FCR, weight gain, EE, protein.

TÓM TẮT

Ảnh hưởng của các khẩu phần thức ăn lên khả năng tăng trưởng của dế Thái

Thí nghiệm được thực hiện nhằm đánh giá của các khẩu phần thức ăn hỗn hợp (CD) đến khả năng tăng trưởng, hiệu quả sử dụng thức ăn và thành phần dưỡng chất của dế Thái. Thí nghiệm được bố trí theo thể thức hoàn toàn ngẫu nhiên với 3 nghiệm thức (NT) và 4 lần lặp lại, mỗi lần lặp lại là 500 con dế con ở 7 ngày tuổi. Tổng số dế sử dụng trong thí nghiệm là 6000 dế con và thí nghiệm được thực hiện trong 6 tuần. Các NT bao gồm đối chứng (Con): thức ăn là lá khoai mì tươi; Diet 1: CD1-1 với 18% CP và 3000 kcal ME/kg; và CD1-2 với 20% CP và 2800 kcal ME/kg; và Diet 2: CD2-1 với 19% CP và 3150 kcal ME/kg; CD2-2 với 21% CP và 2800 kcal ME/kg lần lượt cho giai đoạn 7-21 và 22-49 ngày tuổi. Kết quả cho thấy ở 21, 42 và 49 ngày tuổi, khối lượng dế ở Diet 2 là cao nhất, kế đến là Diet 1 và thấp nhất là Con. Tương tự, Diet 2 có tăng trọng cao nhất ở 7-14, 15-21, 36-42 và 42-49 ngày tuổi và thấp nhất ở Con. Ngược lại, Diet 2 có tiêu tốn thức ăn và hệ số chuyển hóa thức ăn thấp nhất, và Con cao nhất ở 7-14 và 15-21 ngày tuổi. Tuy nhiên, giai đoạn 29-35, 36-42 và 43-49 ngày tuổi, Diet 2 có TTTA cao nhất và Con là thấp nhất. Đồng thời, hàm lượng DM, OM và EE cao nhất ở Diet 2 và Diet 1, ngược lại, Con có hàm lượng CP và Ash cao nhất.

Keywords: dế đồng, tăng trọng, hệ số chuyển hóa thức ăn, EE, protein

1. INTRODUCTION

The current socio-economic situation

in Vietnam is increasingly developing, the population is increasing along with the developed economy, so the demand for food is higher, especially for meat, egg, and milk products (FAO, 2022). As a result, the number of animals and the number of livestock farms have increased (Paustian *et al.*, 2006), and have increasingly affected the global climate. Since

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then, an insect-based food industry, which has emerged and is growing rapidly in recent years, aims to provide food for humans and as part of a protein source to replace soy bean meal or fishmeal in animal feed and aqua feed (Van *et al.*, 2013). Recent studies by Udomsil *et al.* (2019) and Nowakowski *et al.* (2022) also showed that crickets and some insects have very high protein content (60-70% dry matter) with full amino acids and essential fatty acids such as omega 3, omega 6, this is an innovation according to modern livestock trends, which have a positive impact on the ecosystem and sustainability. Studies have determined that crickets are an environmentally friendly alternative (Oonincx *et al.*, 2010).

Cricket (*Gryllus bimaculatus*), called Thai cricket, is one of the species commonly raised in Vietnam. The need for sustainable large-scale commercial insect production has stimulated interest in developing artificial diets that include agricultural by-products (Nation, 2002). Feed is an important input in cricket production, crickets will continue to eat non-stop until their daily protein intake has been met (Hallett, 1995). Studies show that dietary feed and its quality affect the growth and development of crickets (Maklakov *et al.*, 2008; Tawes, 2014). The results of a recent study showed that crickets fed young cassava leaves gained weight and contained more essential amino acids than crickets fed mixed feed (Hakansson, 2018). Some other research results showed that the diet with 32% CP supplemented with lettuce improved body weight and weight gain of Thai crickets, while the feed with 25% protein supplemented with water spinach improved the amount of fat and protein contents in cricket meat compared to control fed only young cassava leaves and others (Khang *et al.*, 2020). The results of these studies show that there are differences not only in cricket growth rates but also in the nutritional value of cricket meat when fed diets with different nutrients. To date, there is no suggestion on the nutritional content of the feed for optimal cricket growth. Therefore,

this study was done to evaluate the effect of different commercial diets on the growth, feed efficiency of Thai crickets, and their nutritional composition.

2. MATERIALS AND METHODS

2.1. Animals and management

The experiment was conducted from December 17 to January 28, 2023, at the Experimental breeding farm of the College of Agriculture, Can Tho University, Campus II, 3/2 Street, Xuan Khanh ward, Ninh Kieu district, Can Tho city. All experimental samples were analyzed at the Laboratory of Animal Nutrition and Feed Technology of the Faculty of Animal Sciences.

Crickets originated from the Experimental breeding farm and were selected at 7 days old after hatching. Commercial feeds (CD) were purchased from 2 different feed manufacturers with different nutritional compositions for 7-21 and 22-49 days old, respectively. Diet 1 with CD1-1 had 18% CP and 3,000kcal ME/kg, and CD1-2 had 20% CP and 2,800kcal ME/kg; Diet 2 with CD2-1 had 19% CP and 3,150kcal ME/kg and CD2-2 had 21% CP and 3,000kcal ME/kg. Cassava leaves were grown and collected from the Experimental breeding farm.

The cricket house was designed according to an open barn system, with two corrugated iron roofs, the farm was divided into cricket-raising rooms and tool rooms for the rearing process. Each room has dimensions of 4x2.6m. The breeding cage was made of an iron frame with an area of 50x50cm covered with thick rubber, the lid was made of a wooden frame with a net to avoid predator animals such as *Hemidactylus frenatus*, *Dasia olivacea* from the outside attack or eat crickets. In each cell cage, egg cartons were layered to make room for crickets.

Analytical equipment and tools: Testo 435-1 temperature and humidity meter, Digital Scale electronic micro-electrolyte balance, Ohaus AX224 balance, Velp protein

distiller UDK 129, UUF Plus drying oven and tools, and other chemicals.

2.2. Experimental design and data collection

The experiment was arranged in a completely randomized design with 3 treatments with 4 replicates, each repetition was 500 crickets at 7 days old. The total number of crickets used in the experiment was 6,000 young crickets. The experimental treatments included Control used only fresh young cassava leaves; Diet 1: a CD1 contained 2-phase feedings of 18% CP with 3,000kcal ME/kg (CD1-1) and 20% CP with 2,800kcal ME/kg (CD1-2) for 7-21 and 22-49 days of age, respectively; and Diet 2: a CD2 contained 19% CP and 3,150kcal ME/kg (CD2-1), and 21% CP and 3,000kcal ME/kg (CD2-2), respectively.

The care procedure was performed in the same environment for all experimental cage plots. Humidity in the rearing cages was provided by spraying water 2 times per day and drinking water for crickets using a shallow dish with a few small pebbles. Crickets were fed twice daily at 8:00am and 15:00pm, the feed was provided *ad libitum*, and the number of feed offers and leftovers were recorded daily. The crickets were weighed and measured the body length and width at 7 days of age and repeated every 7 days, specifically on days 14, 21, 28, 35, 42, and 49 days, by random selection of 25 crickets at 5 positions per each cage plot (25x5x1 plot = 75 crickets). During the period of rearing, dead cricket carcasses and parts of cricket bodies were collected and recorded daily. The end of each rearing cage plot was defined by observing the wings appearing of crickets reached 95%. A tray with a moist mixture of coconut flakes and dust was offered to the cage for 5-7 days to allow the female crickets to lay eggs. The live weights of the birds were weighed at the beginning of the experiment and repeated at weekly intervals. Feed intake and feed refusal were recorded daily. The growth performance parameters such as weight gain, feed intake, and feed conversion

were determined according to the procedures of McDonald *et al.* (2011). Economic efficiency was calculated based on the feed cost and income from selling the crickets. Costs for caretakers and others were the same for all treatments and not involved.

At the end of the experiment, all crickets were harvested and together with commercial feeds and cassava leaves were analyzed the nutritional compositions according to the AOAC method (1990). Then, the DM, OM, Ash, CP, and EE of feeds and cricket meat were calculated and presented based on % dry matter. Dry matter (DM) was determined by drying at 105°C for 12hrs. Organic matter (OM) and total mineral matter (Ash) was determined by completely burning the sample's organic matter at 550°C for 3hrs. Crude protein (CP) was determined by the Kjeldahl method; crude lipids (EE) were determined by extraction in organic solvent ether using the Soxhlet system.

2.3. Statistical analysis

The collected data were processed by Microsoft Excel software and statistically processed by Minitab V16 software according to the One-way ANOVA model. To determine the significance level of the treatments by Tukey's method with a 95% confidence interval.

3. RESULTS AND DISCUSSION

3.1. Growth performances of the experimental crickets

Feed consumption of the experimental crickets during the period of 7-49 days in Table 1 showed that there were significant differences among treatments on daily feed consumption of each period of age ($P < 0.05$), highest feed consumption was on Control at the period of 7-14, 15-21 and 22-28 days old, however, Diet 2 was highest on feed consumption from 29-35 days old to 36-49 days old. Average feed consumption was not significantly different among treatments at the whole period of 7-49 days old ($P > 0.05$).

The BW of crickets by weeks of age in Table 1 showed that there was a statistically significant difference between the treatments on body weight at 21, 42, and 49 days of age, the highest body weight was in Diet 2 and the lowest was in the control group ($P < 0.05$). The body weight of crickets in Diet 1 and 2 was higher than that of the control at 14, 28, and 35 days old, but this difference was not statistically significant ($P > 0.05$).

Table 1. Growth performance of the crickets

Parameters/ Period, day	Treatments (Mean±SD)			P
	Control	Diet 1	Diet 2	
<i>Feed consumption (mg/head/day)</i>				
7-14	1.99 ^a ±0.27	0.42 ^b ±0.13	0.45 ^b ±0.13	0.00
15-21	2.39 ^a ±0.38	0.87 ^b ±0.126	1.15 ^b ±0.13	0.00
22-28	4.30 ^a ±1.50	1.70 ^b ±0.98	3.72 ^b ±0.48	0.02
29-35	6.91 ^b ±1.87	4.80 ^b ±0.66	8.62 ^a ±1.44	0.01
36-42	12.32 ^b ±1.85	14.72 ^b ±5.24	20.75 ^a ±2.87	0.02
43-49	15.22±2.41	32.31±13.25	31.43±9.36	0.05
7-49	5.94±1.04	7.01±2.37	8.47±1.62	0.18
<i>Body weight (mg/head)</i>				
7	0.33±0.05	0.30±0.03	0.33±0.05	0.63
14	1.18±0.11	1.44±0.20	1.45±0.20	0.09
21	3.20 ^b ± 0.08	4.06 ^{ab} ± 0.76	4.68 ^a ± 0.39	0.01
28	8.39±1.83	9.05±1.15	10.61±1.44	0.15
35	24.28±4.94	24.91±7.48	27.12±8.81	0.85
42	39.00± 5.02	56.98 ^b ± 7.27	69.96 ^a ± 3.90	0.00
49	69.60 ^b ± 6.33	101.34 ^a ± 15.51	122 ^a ± 10.14	0.00
<i>Average daily weight gain (mg/head/day)</i>				
7-14	0.122±0.01	0.164±0.02	0.161±0.02	0.05
15-21	0.289 ^b ±0.01	0.373 ^{ab} ±0.09	0.461 ^a ±0.08	0.02
22-28	0.741±0.27	0.714±0.07	0.847±0.24	0.65
29-35	2.269±0.61	2.266±1.06	2.358±1.17	0.99
36-42	2.104 ^b ±1.07	4.581 ^a ±0.11	6.12 ^a ±1.00	0.00
43-49	4.371 ^b ±0.73	6.34 ^{ab} ±2.05	7.434 ^a ±1.41	0.05
7-49	1.649 ^b ±0.15	2.406 ^a ±0.37	2.897 ^a ±0.24	0.00
<i>Feed conversion ratio</i>				
7-14	16.68 ^a ±3.73	2.57 ^b ±0.71	2.58 ^b ±0.48	0.00
15-21	8.29 ^a ±1.61	2.35 ^b ±0.54	2.60 ^b ±0.75	0.00
22-28	6.37±3.57	2.32±1.13	4.58±0.99	0.09
29-35	3.34±1.62	2.63±1.48	5.36±4.73	0.45
36-42	7.47±4.77	3.40±0.17	3.21±1.12	0.11
43-49	3.49±0.17	5.96±3.82	4.35±1.61	0.38
7-49	4.38±0.84	3.93±1.64	3.85±0.95	0.55

* Mean values with different letters in the same row are statistically different ($P < 0.05$)

Average daily weight gain (ADG) of crickets did not differ between treatments at 7-14, 22-28, 29-35 days old ($P > 0.05$). However, there was a statistically significant difference between the treatments on ADG at 15-21, 36-42, 43-49, and 7-49 days of age, the highest ADG was in Diet 2, next by Diet 1, and the lowest was in the control group ($P < 0.05$). In contrast, the control had the highest feed conversion ratio compared to the other treatments, and this statistically significant difference was found only at 7-14 and 15-21 days of age ($P < 0.05$).

Different nutrient diets, especially protein, carbohydrate, and fat content in insect diets can affect the survival rate, feed efficiency, and development time of crickets (Oonincx *et al.*, 2015). In this study, crickets fed either CD1 or CD2 gave the best weight gain and feed conversion ratio than the control. Comparing the effect of the different diets on FCR revealed that increasing the protein content of diets enhanced feed efficiency in converting feed to cricket body size. The significant effects of dietary treatment in this study concur with previous findings (Orinda *et al.*, 2017; Bawa *et al.*, 2020). These studies have also shown that house crickets grow very well on a 20-30% protein diet and thus explain the growth performance of house crickets on these diets. Kuo and Fisher (2022) reported that crickets need high crude protein content to provide high performance up to saturation. Bawa *et al.* (2020) found that crickets reared on PPF (22% protein) had 9% more mean body weight and length compared to BF (16% protein), as normally observed in insects in response to high- and low-protein diets (Orinda *et al.*, 2017). In this study, crickets grew 2 times faster than the control and higher than the results of these authors. The difference in the present study compared to that proposed by Fuah *et al.* (2015) and Megido *et al.* (2016) suggested the possibility of using young cassava leaves and brown rice flour as food for crickets. The reason for the difference between the results of this study may be the use of feed according

to each stage of the cricket's growth, while previous studies only used one type of feed during the development of crickets, or just used each ingredient.

3.2. Nutritional composition of the experimental feeds and cricket meat

The nutrient composition of the experimental feeds was shown in Table 2.

Table 2. Nutritional composition of feed (as %DM)

Feeds	DM	OM	CP	EE	Ash
Fresh cassava leaves	23.4	91.3	30.5	9.87	8.67
CD1-1	88.2	94.4	20.8	7.16	5.59
CD1-2	89.6	93.7	22.9	4.50	6.28
CD2-1	87.8	95.0	22.0	5.79	5.02
CD2-2	89.6	93.5	23.5	5.46	6.48

Table 1 showed that fresh young cassava leaves had higher crude protein (30.5%), EE (9.87%), and Ash (8.67%) compared to CD1-1, CD1-2, CD2-1 and CD2-2, respectively. CD1-1 had lower CP (20.8%) than CD1-2 (22.9%), CD2-1 (22%) and CD2-2 (23.5%). However, DM, OM, and CP of fresh cassava leaves contained 23.4, 91.3 and 30.5% were lower than the results of Hang *et al.* (2022) with values of 27.2, 94.6, and 31.4%, respectively. Meanwhile, the CP content of CD2-2 in this study was lower than that of the commercial feed used for crickets reported by Miech *et al.* (2016) with a value of 23.4%, but the EE value of CD2-2 was higher, respectively. In general, fresh young cassava leaves used in this study had a higher CP content than other commercial feeds and has been suggested by Fuah *et al.* (2015) and Megido *et al.* (2016) reported on the possibility of using young cassava leaves and brown rice flour as feed for crickets.

The nutritional composition of the experimental cricket meats at 49 days of age (as %DM) was presented in Table 3.

The results in Table 3 show that all the nutritional components in crickets in terms of DM, OM, CP, EE and Ash were significantly different between treatments ($P < 0.05$), Diet 1 and Diet 2 had the highest DM, OM, and EE content compared to Con. In contrast, Con had

the highest protein and mineral content. There have been many studies trying to scientifically prove the correlation between cricket's diet and the nutritional value of cricket meat. Crickets have been reported to be high-feed converters (Nakagaki and Defoliart, 1991; Finke, 2002; Wilkinson, 2011). The present study is consistent with previous research, as the quality of cricket meat is affected by the diet, especially the protein, carbohydrate, and fat content of the diet (Oonincx *et al.*, 2019). Some theories suggest that crickets will have a high protein and low-fat content when fed a high-protein, low-fat diet. The results in Table 2 showed that crickets had high protein and low fat on the control diet. Therefore, the results of previous research that high protein content in the diet leads to high protein and low-fat content in crickets are plausible (Oonincx *et al.*, 2015).

Table 3. Nutritional composition cricket meats

Nutrients	Treatments (\pm SEM)			P
	Control	Diet 1	Diet 2	
DM	26.16 ^b \pm 3.78	32.75 ^a \pm 2.36	32.35 ^a \pm 1.98	0.02
OM	94.77 ^b \pm 0.19	95.79 ^a \pm 0.35	95.68 ^a \pm 0.51	0.01
CP	69.69 ^a \pm 3.40	57.40 ^b \pm 1.61	58.47 ^b \pm 0.68	0.00
EE	13.72 ^b \pm 5.24	28.51 ^a \pm 5.26	30.67 ^a \pm 2.81	0.00
Ash	5.22 ^a \pm 0.19	4.21 ^b \pm 0.35	4.32 ^b \pm 0.51	0.01

3.3 Economic efficiency

Table 4. Economic efficiency (VND)

Criteria	Treatments		
	Control	Diet 1	Diet 2
Egg tray (kg/Tray)	0.6	0.6	0.6
Amount of feed used (g)	579.80	181.89	219.74
Feed price (VND)	0	17,000	16,500
Feed cost (VND)	0	3,100	3,700
Total weight of cricket (g)	378.65	566.96	515.28
Price of crickets (VND/kg)	100,000	100,000	100,000
Total sale of cricket (VND)	37,870	56,670	51,530
Income (VND)	37,870	53,570	47,800
Efficiency (%)	100	141.45	126.22

The economic efficiency of the experimental crickets (Table 4) showed that the profit expressed from high to low is 53,570; 47,800 and 37,870VND respectively for treatment Diet 1, 2, and Con. The study

showed that economic efficiency was affected by the productivity of crickets, when using CD crickets gave a higher yield than using fresh cassava leaves.

4. CONCLUSION

Diet 2 and 1 exhibited the highest cricket weight, ADG, and the lowest FCR. Additionally, crickets fed with Diet 2 and Diet 1 demonstrated higher DM, OM, and EE content, while Con had the highest CP and ash content. Notably, both Diet 2 and 1 yielded greater economic returns compared to Con.

Based on these findings, it is highly recommended to incorporate Diet 2 or 1 in cricket farming practices to enhance cricket productivity and improve overall economic efficiency.

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EFFECTS OF TURMERIC AND CINNAMON POWDER SUPPLEMENTATION ON GROWTH PERFORMANCE IN JAPANESE QUAIL

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ABSTRACT

The experiment was carried out to compare and evaluate the effect of turmeric (TP) and cinnamon (CiP) supplementation on the growth performance of Japanese quail from G0 to G1 generation. Generation G0 had a total of 200 quails at 1 day old arranged in a completely randomized design with 4 treatments and 5 replicates, 10 birds each. Generation G1 with a total of 160 bird quails at 1 day old was arranged to be the same as the G0 generation with 4 treatments and 5 replicates, 8 birds each. The experimental treatments were: control group using only the basic diet (KPCS) without supplementation, TP: KPCS supplemented with 0.1% turmeric powder, CiP: KPCS supplemented with 0.025% cinnamon powder, and TCP: KPCS added 0.1% turmeric combined with 0.025% cinnamon powder, respectively. The experimental results showed that the treatments supplemented with TP, CP, or a combination had an improvement in the growth of Japanese quail in both the G0 and G1 generations. In the G0 generation, there was a statistically significant difference among treatments in terms of body weight (BW) and feed conversion ratio (FCR) at 28 days of age ($P < 0.05$), TP with the highest BW and lowest FCR, and control was lowest BW and highest FCR. In the G1 generation, there was a statistically significant difference among treatments in terms of BW, average daily weight gain (ADG), and FCR of quail at 21 and 35 days of age ($P < 0.05$), TP had the highest BW, ADG, and the lowest FCR. The G1 generation, BW and ADG were statistically significantly higher than the G0 generation ($P < 0.05$). The results of the economic efficiency analysis showed that the additional treatments were more profitable than the control group, in which TP in both G0 and G1 generations had 15-12% higher profits.

Keywords: Japanese quail, turmeric powder, cinnamon powder, ADG, FCR.

TÓM TẮT

Ảnh hưởng của bổ sung bột nghệ và bột quế đến khả năng sinh trưởng của chim cút Nhật

Thí nghiệm được thực hiện nhằm so sánh và đánh giá ảnh hưởng của bổ sung bột nghệ (TP) và bột quế (CiP) đến năng suất tăng trưởng của chim cút Nhật từ thế hệ G0 đến G1. Thí nghiệm được thực hiện trên tổng số 200 chim cút con ở thế hệ G0 từ 1 ngày tuổi được bố trí theo thể thức hoàn toàn ngẫu nhiên với 4 nghiệm thức (NT) và 5 lặp lại với 10 cút con cho mỗi lần lặp lại; và 160 chim cút con ở thế hệ G1 từ 1 ngày tuổi được bố trí thí nghiệm tương tự thế hệ G0 với 4 NT và 5 lặp lại, 8 cút con cho mỗi lần lặp lại. Các NT lần lượt là đối chứng (ĐC) sử dụng khẩu phần cơ sở (KPCS) không có bổ sung, TP: KPCS có bổ sung 0,1% bột nghệ, CiP: KPCS có bổ sung 0,025% bột quế, TCP: KPCS có bổ sung 0,1% bột nghệ và 0,025% bột quế. Kết quả thí nghiệm cho thấy các NT có bổ sung TP, CiP hoặc kết hợp có sự cải thiện về tăng trưởng của cút Nhật ở cả thế hệ G0 và G1. Ở thế hệ G0, có sự khác biệt có ý nghĩa thống kê giữa các NT về khối lượng (KL) và hệ số chuyển hóa thức ăn (HSCHTA) giai đoạn 28 ngày tuổi ($P < 0,05$), TP có KL cao nhất và HSCHTA thấp nhất, và ĐC có KL thấp nhất và HSCHTA cao nhất; ở thế hệ G1, sự khác biệt có ý nghĩa thống kê giữa các NT về KL, tăng trọng tuyệt đối (TTTĐ) và HSCHTA của cút giai đoạn 21 và 35 ngày tuổi ($P < 0,05$), TP có KL và TTTĐ cao nhất, và HSCHT thấp nhất. Thế hệ G1, KL và TTTĐ cao hơn có ý nghĩa thống kê so với thế hệ G0 ($P < 0,05$). Kết quả phân tích hiệu quả kinh tế cho thấy các NT bổ sung TP và CiP có lợi nhuận cao hơn so với ĐC, trong đó BN ở G0 và G1 đều có lợi nhuận cao hơn 15-12%.

Từ khóa: Chim cút Nhật, bột nghệ, bột quế, tăng trọng, hệ số chuyển hóa thức ăn.

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1. INTRODUCTION

Turmeric powder (*Curcuma longa* L.) and cinnamon powder (*Cinnamomum verum*) are two precious herbs with many advantages, in particular, turmeric and cinnamon are also spices and medicines that are popular and widely used by humans (Ravindran, 2006). The main compound of turmeric is curcuminoid (Mukundan *et al.*, 1993), which has antibacterial and antioxidant properties (Gupta *et al.*, 2012), immune system booster (Araujo *et al.*, 2001), and anti-inflammatory response (Aggarwal *et al.*, 2003). Turmeric has the effect of treating respiratory diseases, gastritis, and bile duct inflammation. A published study reported that turmeric supplementation at 1 g/kg feed could reduce the Chronic Respiratory Disease infection rate, improve growth performance of Cobb500 broiler chickens and economic benefits to farmers (Nguyen Thi Kim Khang *et al.*, 2016). Moreover, recent studies suggested that supplementation of 0.15% turmeric powder to the diet of Noi laying hens or 0.1% turmeric powder to diet of Japanese laying quails improved reproductive performances of animals (Nguyen Thi Kim Khang *et al.*, 2020a, 2020b; Nguyen Thao Nguyen *et al.*, 2021). On the other hand, cinnamon mainly composed of cinnamaldehyde essential oil, which has antibacterial (Lee *et al.*, 2004) and antioxidant properties. Adding cinnamon powder to the diets of laying hens reduced serum lipids, cholesterol, and albumin (Hassan, 2016). Adding 0.15-0.25g of cinnamon powder to 1kg of feed reduced the incidence of CRD, and coccidiosis and at the same time limit the mortality and elimination rate of Cobb500 broilers (Nguyen Thi Kim Khang *et al.*, 2015).

There have been many studies evaluating the effectiveness of turmeric and cinnamon powder on performance as well as disease prevention in cattle and chickens, but studies on quail are limited. Therefore, this study aimed to compare and evaluate the effects of turmeric (TP) and cinnamon (CP)

supplementation on the growth performance of Japanese quail from G0 to G1 generation.

2. MATERIALS AND METHODS

2.1. Animals and management

The experiment was conducted at an experimental poultry farm of the Department of Animal Sciences located in Thuan Tien B hamlet, Thuan An commune, Binh Minh district, Vinh Long province, Vietnam. The Japanese quails originated in Ben Tre and Tien Giang provinces (Figure 1a). All experimental quails were disinfected and vaccinated against cholera and influenza.

Experimental quails were fed a basic feed which was a compound feed with crude protein and metabolizable energy content as shown in Table 1. Besides, turmeric and cinnamon powder (Figures 1b,1c) were added to quail diets from 15 days old according to different levels of each treatment. The cinnamon powder used in the experiment was a fine, brown, fragrant powder with 100% cinnamon composition, produced at 96 Street 6, Binh Trung Tay Ward, District 2, Ho Chi Minh City; Turmeric powder was a fine powder, orange-yellow color, fragrant, containing 0.3% curcumin, produced at 67 Nguyen Cu Street, Quarter 4, Thao Dien Ward, District 2, Ho Chi Minh City.

The quail house was an open type, with an area of 20x6x2.5m. The roof was covered with corrugated iron and the two sides of the barn were covered with canvas. Quails from 1-14 days old were raised in an incubator with dimensions of 100x50x30cm, which could raise 50 birds/cage, and used infrared lights to brood the bird quails. Quails from 15-35 days old were raised in rear quail cages designed with 2 floors with 5 cages/floor and the size of each cage was 50x40x30cm. Each floor of the quail housing system was lined with canvas to collect quail droppings, allowing for the raising of up to 10 quails per cage. The lowest cage floor was positioned 50cm above the ground.

Table 1. Nutritional composition of feed

Life stage of bird	CP (%)	ME (Kcal/kg)
At 1-14 days old	23	2,850
At 15-35 days old	20	2,750
At 36 days old	20	2,750

2.2. Experimental design and data collection

A total of 200 quails in the G0 generation from 1 day old were arranged in a completely randomized design with 4 treatments and 5 replicates with 10 quails each, and 160 quails in the G1 generation from 1 day old were arranged in a completely randomized design with 4 treatments and 5 replicates with 8 quails each. The experimental treatments included: control group using only the basic diet (KPCS) without supplementation, TP: KPCS supplemented with 0.1% turmeric powder, CiP: KPCS supplemented with 0.025% cinnamon powder, and TCP: KPCS added 0.1% turmeric combined with 0.025% cinnamon powder.

The incubation period from 1-14 days old: quails at 1 day old were raised in brooding cells. The baby quails were heated with infrared lights and observed the quail's behavior to adjust the brooding temperature by increasing or decreasing the number of lights. Every day, quail's drinking water was mixed with powder to keep warm, and changed the water twice a day at about 6:00am and 16:00pm.

Growth stage from 15 days old onwards: all quails were cared for and raised in the same conditions, only differing in adding turmeric and cinnamon powder. They were fed twice daily at around 7:00am (40% of daily

feed intake) and 15:00pm (60% of daily feed intake). Lighting time was 23h a day, in the morning took advantage of sunlight, and in the evening used 10W lights to illuminate.

Feed intake and leftovers were recorded daily. The health status and survival rate of quails were recorded during the experiment. The live weights of quails were weighed weekly to assess growth performance. The costs of care, electricity, water, and breeding in all treatments were the same, therefore economic efficiency was calculated based on the cost of feed and supplements; and proceeds from the sale of quail meat.

2.3. Statistical analysis

The collected raw data were recorded and processed by Microsoft Excel software, then statistically processed by Minitab Ver 16 software according to GLM. Mean values of significant differences among treatments were compared by Tukey's method with 95% confidence. The survival rate of quails in this experiment was statistically processed Chi-Square test.

3. RESULTS

3.1. Survival rate of quail 15-35 days old

The survival rate (%) of the experimental quails at 15-35 days old in 2 generations, G0 and G1, was presented in Table 2. The survival rate of quails between treatments was not statistically significant in the G0 and G1 generations ($P > 0.05$); however, the treatments with turmeric, cinnamon, or a mixture supplementation had a higher survival rate than the control.

Table 2. Survival rate (%) of the experimental quails at 15-35 days old (Mean±SD)

Generation	Items	Control	TP	CiP	TCP	Chi-Sq	P
G0	Total, bird	50	50	50	50		
	Survival rate, %	96±5.48	100±0	98±4.47	98±4.47	0.04	0.998
G1	Total, bird	40	40	40	40		
	Survival rate, %	97.5±5.59	100±0	97.5±5.59	100±0	0.03	0.999

3.2. Growth performance of G0 and G1 at 1-14 days old

Growth performance of Japanese quails at 1-14 days old generation G0 and G1 in Table 3 showed that the BW of quails at 7 and 14 days old had a statistically significant difference between the two generations G0 and G1 ($P < 0.05$) with BW at G1 (21.98 and 52.39 g/bird) higher than G0 (20.31 and 49.55 g/bird). Similarly, the ADG of G1 was also higher than that in G0 ($P < 0.05$). There was no difference between the G0 and G1 generations regarding feed consumption and FCR across age stages ($P > 0.05$).

Table 3. Growth performance G0 and G1

Paramet	Age	G0 (n=324)	G1 (n=332)	P
BW, bg	1d	7.71±0.52	7.86±0.21	0.385
	7d	20.31±0.75	21.98±0.97	0.002
	14d	49.55±1.58	52.39±1.64	0.003
ADWG g/b/d	1-7d	1.80±0.16	2.02±0.11	0.005
	8-14d	4.18±0.19	4.34±0.12	0.037
FI, g/b/d	1-7d	3.65±0.24	3.87±0.41	0.248
	8-14d	8.58±0.28	8.72±0.45	0.496
FCR g/g	1-7 d	2.04±0.14	1.92±0.19	0.201
	8-14d	2.06±0.13	2.01±0.13	0.459
	1-14d	2.05±0.08	1.98±0.09	0.146

Values with different letters in the same row have statistical significance $P < 0.05$

3.3. Growth performance in G0 and G1 at 15-35 days old

The results on the growth performance of Japanese quails in G0 generation at 15-35 days old are presented in Table 4. The body weight of the experimental quails at 15 days of age was the weight at the beginning of the growth experiment; therefore, the body weights were not statistically different, ensuring uniformity among treatments. Compared with the body weight of quails fed a diet without supplementation over weeks of age, the experimental quails with supplemented diets had a higher body weight, although not statistically significant among them ($P > 0.05$).

Similarly, average daily weight gain and feed intake among treatments across the age stages were also not statistically significant ($P > 0.05$). Feed conversion ratio at 15-21, 29-35, and 15-35 days old also had no statistical difference among treatments ($P > 0.05$). However, statistically significant differences among treatments in the BW and FCR were found at 28 and 22-28 days of age, respectively ($P < 0.05$), where TP had the highest BW (120.89 g/bird) and lowest FCR (3.16-3.26), while the control had the highest FCR (3.99) and lowest BW (111.28 g/bird).

Table 4. Growth performance G0 15-35 days old

Param	Cont	TP	CiP	TCP	SEM	P
<i>BW, g/b</i>						
15d	49.65	50.40	51.87	50.24	0.88	0.354
21d	84.19	86.53	84.00	83.30	1.09	0.214
28d	111.28 ^b	120.89 ^a	117.48 ^{ab}	116.77 ^{ab}	1.99	0.027
35d	143.54	154.00	150.93	147.35	2.64	0.066
<i>ADWG, g/b/d</i>						
15-21d	4.93	5.16	4.59	4.72	0.18	0.148
22-28d	3.87	4.91	4.78	4.78	0.28	0.060
29-35d	4.61	4.73	4.78	4.37	0.37	0.866
15-35d	4.47	4.93	4.71	4.62	0.12	0.090
<i>FI, g feed/b/d</i>						
15-21d	11.66	12.04	11.59	11.63	0.30	0.712
22-28d	15.41	15.40	15.18	15.39	0.47	0.981
29-35d	17.93	16.93	17.34	17.26	0.44	0.459
15-35d	15.00	14.79	14.70	14.76	0.27	0.878
<i>FCR, g feed/g WG</i>						
15-21d	2.37	2.36	2.54	2.47	0.11	0.609
22-28d	3.99 ^a	3.16 ^b	3.23 ^b	3.26 ^b	0.16	0.006
29-35d	3.98	3.67	3.84	4.02	0.39	0.919
15-35d	3.36	3.01	3.12	3.20	0.10	0.112

The growth performance indicators of the experimental quails in the G1 generation in Table 5 showed that the BW of quails at 21 and 35 days of age had statistically significant differences among treatments ($P < 0.05$), the highest in TP (88.81 and 154.85 g/bird) and the lowest in control (83.68 and 147.45 g/bird). Similarly, there was a statistically significant difference among treatments in terms of ADG and FCR recorded at 15-21 and 15-35 days of age ($P < 0.05$), TP had the highest ADG, the lowest FCR compared to the Cont with the lowest ADG, and the highest FCR.

Table 5. Growth performance G1 at 15-35 days old

Param	Cont	TP	CiP	TCP	SEM	P
<i>BW, g</i>						
15d	52.33	52.81	52.59	52.17	0.84	0.949
21d	83.68 ^b	88.81 ^a	86.77 ^{ab}	85.73 ^{ab}	0.98	0.015
28d	116.31	123.09	119.82	119.15	1.80	0.106
35d	147.45 ^b	154.85 ^a	151.80 ^{ab}	149.95 ^{ab}	1.76	0.054
<i>ADWG, g/b/d</i>						
15-21d	4.48 ^b	5.14 ^a	4.88 ^{ab}	4.79 ^{ab}	0.13	0.018
22-28d	4.66	4.90	4.72	4.77	0.19	0.844
29-35d	4.45	4.54	4.57	4.40	0.18	0.896
15-35d	4.53 ^b	4.86 ^a	4.72 ^{ab}	4.66 ^{ab}	0.07	0.019
<i>FI, g/b/d</i>						
15-21d	11.20	11.15	11.58	11.59	0.26	0.513
22-28d	15.79	16.16	15.92	15.80	0.43	0.924
29-35d	18.43	18.19	18.31	17.90	0.26	0.521
15-35d	15.14	15.17	15.27	15.10	0.22	0.957
<i>FCR, g feed/g wg</i>						
15-21d	2.51 ^a	2.18 ^b	2.38 ^{ab}	2.43 ^{ab}	0.08	0.049
22-28d	3.42	3.30	3.39	3.31	0.12	0.880
29-35d	4.16	4.01	4.04	4.12	0.16	0.907
15-35d	3.34 ^a	3.12 ^b	3.23 ^{ab}	3.24 ^{ab}	0.05	0.030

Table 5. Economic profits of the experimental quails in the G0 and G1 generations at 15-35 days old

Parameters	Units	Treatments			
		Control	TP	CP	TCP
<i>Generation G0</i>					
Initial number of quail	Bird	50	50	50	50
Final number of quail	Bird	48	50	49	49
Total feed consumption	Kg	15.12	15.53	15.13	15.19
Feed cost	VND/kg	10,800	11,069	10,863	11,132
Total feed cost	VND	163,296	171,896	164,309	169,066
Total LW of quail	Kg	6.89	7.70	7.40	7.22
Selling price of quail meat	VND/kg	75,000	75,000	75,000	75,000
Total sales of quail meat	VND	516,744	577,500	554,668	541,511
Total income	VND	353,448	405,604	390,359	372,445
Profit efficiency	time	2.16	2.36	2.37	2.20
	%	100	115	110	105
<i>Generation G1</i>					
Initial number of quail	Bird	40	40	40	40
Final number of quail	Bird	38	40	39	40
Total feed consumption	Kg	12.08	12.74	12.51	12.68
Feed cost	VND/kg	10,800	11,069	10,863	11,132
Total feed cost	VND	130,483	141,050	135,848	141,192
Total LW of quail	kg	5.60	6.19	5.92	6.00
Selling price of quail meat	VND/kg	75,000	75,000	75,000	75,000
Total sales of quail meat	VND	420,233	464,550	444,015	449,850
Total income	VND	289,750	323,500	308,167	308,658
Profit efficiency	Time	2.22	2.29	2.27	2.19
	%	100	112	106	107

4. DISCUSSIONS

Turmeric or cinnamon powder, herbs of plant origin, are used as potential additives to replace antibiotics in animal production to improve growth performance as well as reproduction in livestock. Turmeric powder

3.4. Economic efficiency

The economic results presented in Table 5 demonstrate that the treatments incorporating turmeric, cinnamon, or a combination of both resulted in higher incomes compared to the control group in both the G0 (5-15%) and G1 (6-12%) generations. Specifically, the diet containing turmeric powder at a concentration of 0.1% yielded the highest profit across both experimental generations. If we considered the income after deducting the cost of the DC as 100%, then the economic efficiency of N in the G0 and G1 generations was 115 and 112%.

or cinnamon powder both contain biologically active substances such as curcuminoids, essential oils, polysaccharides, and peptides, of which essential oils and curcuminoids are considered the main compounds in turmeric (Abbas, 2009), while cinnamon powder with essential oils cinnamaldehyde, has

antibacterial (Lee *et al.*, 2004) and antioxidant properties (Faix *et al.*, 2009). Furthermore, Lal (2012) reported that turmeric contains many molecular components, categorized into numerous groups that exhibit diverse biological activities. Among these components, 20 molecules have antibiotic properties, 12 are known to possess cancer-preventive effects, and 12 act as antioxidants. These findings highlight the potential benefits of turmeric for promoting growth and reproduction in poultry. The present results on the survival rate of Japanese quails in the G0 and G1 generations showed that the survival rate was improved in quails fed diets supplemented with turmeric or cinnamon powder or a mixture of turmeric and cinnamon compared to the control. Moreover, the survival rate of quails in this study was higher than that published by Bui Huu Doan (2010), this can be explained by the biologically active compounds of turmeric, especially curcuminoid, which help to increase immunity, antioxidant, anti-inflammation, anti-bacteria (Araujo *et al.*, 2001; Aggarwal *et al.*, 2003; Gupta *et al.*, 2012) and in some studies showed the antibacterial effect of turmeric compounds against *Staphylococcus*, *Salmonella paratyphi*, *Mycobacterium tuberculosis*, *Trichophyton gupreum*, and *Streptococcus* (Do Huy Bich *et al.*, 2006). On the other hand, cinnamon with antimicrobial, antioxidant, and cholesterol-lowering activities, has the ability to prevent many types of bacteria, molds, and viruses (Lee *et al.*, 2004; Hassan *et al.*, 2004).

In addition, the results of this research on growth performance of experimental quails in G0 and G1 generations showed that treatments supplemented with turmeric and cinnamon either alone or in combination had improved growth performance in experimental quails. In the G0 generation, the body weight of 28-day-old quail was higher in TP, CiP, and TCP compared to the control group. Similarly, the feed conversion ratio at 22-28 days of age was also improved in TP, CiP, and TCP when compared to the control. In the G1 generation,

TP exhibited the highest body weight of quail at 21 and 35 days old, followed by CiP and TCP, while the control group had the lowest body weight. Therefore, the average daily weight gains at 15-21 and 15-35 days of age of quails fed the diet supplemented with 0.1% turmeric powder was also higher than the unsupplemented diet. Thereby, increasing the efficiency of feed used at the period of 15-21 and 15-35 days of age compared to the control. Al-Sulta and Gameel (2004) suggested that adding turmeric or cinnamon powder or their combination to poultry diets would increase metabolism. In previous studies, when adding yellow turmeric powder to the broiler diet helped chickens grow faster, have heavier weight gain, lower feed conversion ratio (Al-Jaleel, 2012), and have bacteria resistance (Gowda *et al.*, 2009). Research results on Cobb broilers showed that there was an improvement in chicken weight gain compared to the control group when adding turmeric or cinnamon to the diet (Nguyen Thi Kim Khang *et al.*, 2015, 2016). Thus, adding turmeric powder, cinnamon powder or combination to the experimental quail's diet improved their growth performance, especially the diet supplemented with 0.1% turmeric powder. Moreover, these growth performance results showed that the addition of turmeric powder or cinnamon powder or turmeric powder combined with cinnamon powder improved economic efficiency in meat quail production.

5. CONCLUSION

Adding only turmeric powder, cinnamon powder, or a combination in the G0 and G1 generations all had an improvement in growth compared to the control, in which the body weight of quail at 28 days old was highest in turmeric powder (120.89 g/offspring) and FCR at 22-28 days of age were improved in all the supplemented treatments compared with control. Besides, improved economic efficiency by 10-15% when adding 0.1% turmeric powder to quail's diet. It is

recommended to add 0.1% turmeric powder to the diet of Japanese quails to improve their growth performance and economic efficiency for farmers.

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EFFECTS OF ANTIMICROBIAL PEPTIDES SUPPLEMENTATION ON COMMERCIAL PIG PRODUCTION EFFICIENCY

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ABSTRACT

The study evaluated antibacterial peptides (AMPs) extracted from *Citrobacter braakii* to replace antibiotics in pig production. The work consisted of 2 main experiments: Experiment 1: Evaluation of the inhibitory ability of AMPs against *E. coli* ATCC 25922 and three *E. coli* strains isolated from diarrhea pig feces by the method of minimum inhibitory concentration (MIC). Experiment 2 was carried out on 500 commercial pigs from 70 days to 130 days old, pigs were randomly divided into 2 treatments: experimental (with the addition of 0.5g AMPs/kg of feed and without antibiotics in feed) and control (add antibiotics in feed). Experimental results show that in experiment 1 the MIC for *E. coli* ATCC 25922 was 25 µg/ml, for 3 strains of *E. coli* in the field, the MIC was 12,5 µg/ml. In experiment 2, the diarrhea rate of pigs in the control group was 30% and the experimental group was 20% ($P<0.05$); the weight of pigs in the control and experimental groups was 100 and 101kg, respectively ($P>0.05$); feed efficiency was 2.15 and 2.14kg feed/kg weight gain, respectively ($P>0.05$). However, the average cost of veterinary medicine per pig in the experimental groups was 27,000VND less than that of the control groups.

Keywords: Antibacterial peptides, *Citrobacter braakii*, diarrhea, *E. coli*, feed efficiency.

TÓM TẮT

Tác dụng của bổ sung peptide kháng khuẩn đối với hiệu quả chăn nuôi lợn thương phẩm

Nghiên cứu được thực hiện để đánh giá các peptide kháng khuẩn (AMP) chiết xuất từ *Citrobacter braakii* để thay thế kháng sinh trong chăn nuôi lợn. Nghiên cứu bao gồm 2 thí nghiệm (TN): 1) Đánh giá khả năng ức chế của AMPs chống lại *E. coli* ATCC 25922 và ba chủng *E. coli* được phân lập từ phân lợn tiêu chảy bằng phương pháp nồng độ ức chế tối thiểu (MIC); 2) được thực hiện trên 500 con lợn thương phẩm từ 70 ngày tuổi đến 130 ngày tuổi, lợn được chia ngẫu nhiên thành 2 nghiệm thức: bổ sung 0,5g AMPs/kg thức ăn và không có kháng sinh trong thức ăn và đối chứng (ĐC) thêm kháng sinh vào thức ăn. Kết quả cho thấy: tại TN1, MIC đối với *E. coli* ATCC 25922 là 25 µg/ml, đối với 3 chủng *E. coli* còn lại, MIC là 12,5 µg/ml. Ở TN2, tỷ lệ tiêu chảy của lợn trong nhóm ĐC là 30% và nhóm TN là 20% ($P<0,05$); khối lượng của lợn trong nhóm ĐC và TN lần lượt là 100 và 101 kg ($P>0,05$); hiệu suất thức ăn lần lượt là 2,15 và 2,14kg thức ăn/kg khối lượng ($P>0,05$). Tuy nhiên, chi phí thuốc thú y trung bình cho mỗi con lợn ở các nhóm TN thấp hơn 27.000 đồng so với nhóm ĐC.

Từ khóa: Peptide kháng khuẩn, *Citrobacter braakii*, tiêu chảy, *E. coli*, hiệu quả tiêu thụ thức ăn.

1. INTRODUCTION

Antibiotics have been used in the swine industry for over 50 years to improve growth and prevent infectious diseases. However, overuse of antibiotics has created antibiotic-resistant strains of bacteria and created

products containing antibiotic residues that affect human health (Diez, 2007). As a result, a global trend has emerged towards restricting the inclusion of antibiotics in swine diets as a routine means of growth promotion. In response, a considerable amount of research has been focused on developing alternatives to antibiotics to maintain swine performance and health (Xiao *et al.*, 2015).

Antimicrobial peptides (AMPs) are among the most widely researched alternatives to

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conventional antibiotics. AMPs are potent, broad-spectrum antibiotics that have been demonstrated to kill gram-negative and gram-positive bacteria, mycobacteria, viruses, fungi, and even transformed or cancerous cells while having no effect on the cells of treated animals (Reddy *et al.*, 2004). In recent years, studies on AMPs and their applications have become one of the hot spots in the areas of agricultural science, biology, medicine, and physiology as well as having potential applications in medicine and the food industry (Xiao *et al.*, 2015).

Supplementation with various antimicrobial peptides has been reported to positively affect performance, nutrient digestibility, intestinal microflora, intestinal morphology, and immune function in pigs (Wang *et al.*, 2011; Yoon *et al.*, 2014; Tang *et al.*, 2009). Yoon *et al.* (2014) tested AMPs to replace the antibiotic avilamycin in weaned piglets and the results after 28 days showed that pigs fed diets supplemented with AMPs improved performance, nutrient digestibility, gut morphology, and reduced microbiome pathogenic bacteria. Experimental results on pigs showed that AMPs increased weight at slaughter by 13.3% (Xiao *et al.*, 2015).

So far, in Vietnam, there has not been any research on using AMPs for livestock in general and pig production in particular. This study was conducted with the objective: to evaluate the ability of AMPs to inhibit bacteria in some virulent *E. coli* strains isolated from diarrhea pig feces and the effect of AMPs supplementation on the efficiency of commercial pig production.

2. MATERIALS AND METHODS

2.1. Materials

The study was conducted from Jun to Oct 2022 at the Microbiology Laboratory, Faculty of Animal Science and Veterinary Medicine, Tay Nguyen Uni., and a pig farm in Gia Lai province.

AMPs extracted from *Citrobacter braakii*; *E. coli* ATCC 25922; Three virulent *E. coli* strains isolated from diarrhea pig feces (*E. coli* strain 1, *E. coli* strain 2, and *E. coli* strain 3); Commercial pigs from 70 to 130 days old.

2.2. Methods

2.2.1. Evaluation of the inhibitory ability of AMPs against *E. coli* ATCC 25922 and three *E. coli* strains isolated from diarrhea pig feces

The test bacteria were first inoculated on LB plates and cultured at 35-37°C for 16-24h. A single colony on the plate was picked and inoculated into 50ml of sterile LB liquid medium, and cultured on a shaker (speed of 200rpm) at a culture temperature of 37°C for 10-12h. The cultured bacterial solution was diluted to a physiological saline solution of 0.090-0.10 (OD600) for use.

Prepare 800ml of LB liquid medium. Weigh 5.120g of AMPs sample and dissolve it in LB medium (ultrasound until completely dissolved) to bring the volume to 100ml. Transfer the solution after constant volume to a centrifuge tube and centrifuge at a speed of 5000-10000rpm for 5min. The centrifuged supernatant is transferred to a 250ml Erlenmeyer flask (beaker), number 1. Take another 12 Erlenmeyer flasks/250ml (beakers), and add 50ml LB liquid culture medium to each bottle, numbered 2, 3, ...13 in sequence. Take 10ml of each bottle and insert it into a test tube and mark. Plug the prepared test tube, sterilize it at 121°C for 15min, and cool to room temperature.

Inoculate 1ml of diluted bacterial solution (1% inoculation volume) into each test tube, cover the stopper, and culture by slanting and shaking.

Temperature 37°C, rotation speed 170rpm, cultured 4-5hrs, first observe the positive control should have bacterial growth, the negative control should have no bacterial growth, and then observe the test sample, visible bacterial growth is turbid, no bacterial growth is clear, and the lowest concentration of clear test tube is judged as

minimum inhibitory concentrations (MIC). Each bacterial strain was replicated 3 times.

2.2.2. Effect of AMPs supplementation on the efficiency of commercial pig production

Experimental pigs were raised in a closed barn with ventilation fans, the temperature in the barn was maintained at 23-25°C. In total, 500 pigs were divided equally into 10 cages (50m²), and in each cage selected 5 animals whose weight is close to the average of the herd to number the ears. Randomly divided 10 cages into 2 treatments: Control (Pigs ate the farm’s basic diet supplemented with antibiotics), and Experiment (Pigs ate the farm’s basic diet supplemented with AMPs). The experimental time is 60 days. The experimental scheme is shown in Table 1.

Table 1. Effect of *A. fistulosum* L. powder on ND antibody titer of chickens

	Control	Experiment
Pigs (head)	250	250
Cages	10	10
Feed	Farm’s basic diet	Farm’s basic diet
Colistin 10%	200g/ton of feed	0
AMPs	0	500 g/ton of feed

Note: All breeding conditions during the experiment were the same

Diarrhea rate: Daily monitoring of pig feces status to identify diarrheal syndrome in pigs. Pigs have diarrhea when stools are thin, without mold.

Monitor growth: Pigs were weighed at the beginning of the experiment, after 1 month, and at the end of the experiment (Weigh only pigs with ear numbers). Weigh each individual in the morning before feeding with a clock scale.

Feed conversion ratio (FCR): Record the amount of feed for each stage in each barn to calculate FCR of each barn.

Estimating economic efficiency: Comparing the cost difference to raise a pig between treatments, the costs incurred include feed, drugs for disease prevention and treatment, and experimental products added to the feed.

Other costs such as breeding stock, electricity, water, and care workers are the same.

2.3. Statistical analysis

Experimental data were analyzed by Minitab 16.2 software (Minitab, 2010). Compare mean values using Student’s test (T-test), and compare proportions using Chi-square (χ^2).

3. RESULTS AND DISCUSSION

3.1. Evaluation of the inhibitory ability of AMPs against *E. coli* ATCC 25922 and three *E. coli* strains isolated from diarrhea pig feces

After 3 experiments, the minimum inhibitory concentration (MIC) for *E. coli* ATCC 25922 was 25.0, higher than that of 1, 2 and 3 strains of *E. coli* isolated (12.5) from diarrhea pig feces.

3.2. Effect of AMPs supplementation on the efficiency of commercial pig production

The experimental results showed that there was a significant difference in the incidence of diarrhea syndrome between the two treatments (P<0.05). Pigs raised with diets supplemented with AMPs significantly reduce the incidence of diarrhea syndrome (Table 2).

Table 2. The incidence of diarrhea syndrome

Treatments	n	Pigs with diarrhea	Rate (%)
Control	250	125	50.00
Experiment	250	76	30.40

At the time of the experiment, the difference in the weight of pigs in the treatments was not statistically significant (P>0.05). Thus, using AMPs to substitute antibiotics in feed does not affect the growth of pigs.

Table 3. Effects of AMPs on growth (Mean±SD, kg)

Expt. time	Control	Experiment	P-value
0-30 days	30.50±3.84	31.25±1.41	0.36
30-60 days	54.67±5.49	54.54±2.71	0.91
0-60 day	87.00±6.90	87.75±2.52	0.62

Supplementing with AMPs so that antibiotic substitution in the feed does not

affect the FCR of pigs ($P>0.05$). In this study, the FCR throughout the experiment was relatively low, ranging from 2.14 to 2.15kg feed/kg WG.

Table 4. Effect AMPs on FCR (Mean±SD, kg/kg)

Expt. time	Control	Experiment	P-value
0-30 days	1.59±0.06	1.53±0.10	0.36
30-60 days	2.32±0.10	2.20±0.20	0.91
0-60 days	2.15±0.14	2.14±0.09	0.62

In this experiment, the costs of feed, breeding stock, electricity, water, and care workers were the same. The cost difference is mainly antibiotics, AMPs, and veterinary medicine. The results in table 6 show that when adding AMPs to the feed, the cost is reduced by about 27,000 VND/head compared to adding antibiotics to the feed.

Table 5. Cost difference between the two treats

Item cost	Control	Experiment
Vaccine	88,000	88,000
Veterinary medicine	107,000	73,000
Colistin 10%	18,000	-
AMPs	-	25,000
Total	213,000	186,000
Difference	27,000	

3.3. Discussion

The antibacterial activity of AMPs has been demonstrated by many studies (Diez-Gonzalez, 2007; Xiao *et al.*, 2015; Cheung-Lee *et al.*, 2019). Cheung-Lee *et al.* (2019) detected that Citrocin (a peptide of the bacterium *Citrobacter braakii*) has the ability to inhibit *E.coli*, *Salmonella*, *Citrobacter*, *Pseudomonas*. However, its strongest activity was against enterohemorrhagic *E. coli* (EHEC) O157:H7 TUV93-0 with a MIC of 16 µm. Guilhelmelli *et al.* (2013) explained the antibacterial activity of AMPs by two properties: firstly, AMPs have membrane activity, the AMPs insert directly into the lipid core of the target membrane to form transmembrane pores to form channels for leakage of ions and possibly larger molecules across the membrane. Second, AMPs have intracellular activity by directly

entering the microbial cell, thereby preventing DNA and protein synthesis. Studies by Cutler *et al.* (2007) and Tang *et al.* (2009) showed that AMPs supplementation reduced the incidence of diarrhea in weaned piglets through antibacterial effects, modulating immune function, and improving Fe absorption. Tang *et al.* (2012) and Yoon *et al.* (2014) also showed that AMPs supplementation to piglets significantly increased growth performance. Yoon *et al.* (2014) found that pig diets supplemented with AMPs showed an increase in the apparent total tract digestibility of dry matter, crude protein, and gross energy. So, previous studies have shown that AMPs have activity against bacterial species including *E. coli*, and adding AMPs to the feed rations increases growth and feed efficiency for piglets. The results of our study showed that AMPs were active against *E. coli* ATCC 25922 and three strains of *E. coli* isolated from diarrhea pigs with MIC from 12.5 to 25.0 µg/ml. Adding AMPs to the diet reduced the incidence of diarrhea, but the effect on weight gain and feed conversion ratio was not significant. The reason for this may be that in this experiment we conducted on pigs, which were relatively large, so the digestive system was fully developed, so the addition of AMPs did not affect the growth process and the feed conversion ratio.

4. CONCLUSION

AMPs extracted from *Citrobacter braakii* inhibited some virulent *E.coli* strains isolated from diarrheal swine feces, with MICs ranging from 12.5 to 25 µg/ml. Adding AMPs to the feed did not affect the growth performance and FCR of pigs at 70 to 130 days of age, but reduced the rate of diarrhea, thereby saving the cost of veterinary drugs to treat the disease.

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EFFECT OF INCREASING CONCENTRATE LEVEL IN THE DIET ON INTAKE, DIGESTIBILITY AND RUMINAL FERMENTATION IN NON-LACTATING GOATS

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ABSTRACT

This study aimed to investigate the effects of increasing concentrate levels in the diet on intake, digestibility, ruminal fermentation and nitrogen retention in non-lactating goats. The repeated measures study used 3, 12-month-old, non-lactating crossbred Saanen goats (σ^7 Saanen \times ϕ Bach Thao) weighing 30.2 ± 1.25 kg. Each experimental period lasted 15 days. Goats received a diet consisting of 75:25 forage [F]: concentrate [C] from d1 to d15 (C25) followed by 55:45 F:C (C45) from day 16 to day 30, and lastly a 35:65 F:C from day 31 to day 45 (C65). The concentrate was mixed at the farm and contained 18.5% crude protein. Samples were collected during the last 4 days of each period. Statistical analysis was performed using PROC MIXED with goat as random effect, while F: G ratio was the fixed effect. The results showed that increasing the level of concentrate in the diet linearly increased DM intake ($P<0.01$) as well as ruminal concentrations of $\text{NH}_3\text{-N}$ ($P<0.05$) and total volatile fatty acids ($P<0.01$) after 3h feeding. Moreover, nitrogen retention tended to increase linearly ($P=0.085$), but nutrient digestibility showed a linear decrease ($P<0.05$, except EE) with increasing rates of concentrate in the diet. Overall, these results demonstrated that concentrate containing 18.5% CP is more effective in increasing intake and ruminal fermentation in goats; however, a high level of concentrate leads to reduce nutrient digestibility. Thus, a level of 45% concentrate containing 18.5% CP in the diet might be suitable for optimizing intake, ruminal fermentation, and digestibility in non-lactating goats.

Keywords: Concentrate, digestibility, intake, non-lactating goats, ruminal fermentation.

TÓM TẮT

Ảnh hưởng của mức độ thức ăn hỗn hợp trong khẩu phần lên lượng ăn, tỷ lệ tiêu hóa và lên men dạ cỏ của dê cái giai đoạn không cho sữa

Thí nghiệm được tiến hành nhằm đánh giá ảnh hưởng của việc tăng mức độ thức ăn hỗn hợp (TAHH) trong khẩu phần lên lượng ăn, tỷ lệ tiêu hoá, lên men dạ cỏ và tích lũy nitơ của dê cái giai đoạn không cho sữa. Ba con dê sữa lai Saanen (σ^7 Saanen \times ϕ Bách Thao), 12 tháng tuổi, có khối lượng $30,2\pm 1,25$ kg, trong giai đoạn khô sữa được bố trí vào một nghiên cứu dọc (Longitudinal study). Mỗi giai đoạn thí nghiệm bao gồm 15 ngày với 11 ngày thích nghi và 4 ngày lấy mẫu. Dê được ăn khẩu phần bao gồm 75:25 cỏ Voi: TAHH (C25) từ ngày 1 đến 15, tiếp theo là 55:45 cỏ Voi: TAHH (C45) từ ngày 16 đến ngày 30, và cuối cùng là 35:65 cỏ Voi: TAHH từ ngày 31 đến ngày 45 (C65). Thức ăn hỗn hợp tự phối trộn tại trại và có hàm lượng đạm thô là 18.5%. Số liệu thí nghiệm được xử lý theo mô hình PROC MIXED với dê là ảnh hưởng ngẫu nhiên và tỷ lệ thức ăn hỗn hợp trong khẩu phần là ảnh hưởng số định. Kết quả cho thấy DM ăn vào tăng tịnh tiến ($P<0,01$) khi tăng TAHH trong khẩu phần. Thêm vào đó, việc tăng TAHH trong khẩu phần cũng tăng tịnh tiến hàm lượng $\text{NH}_3\text{-N}$ ($P<0.05$) và acid béo bay hơi tổng số ($P<0,01$) trong dịch dạ cỏ sau 3 giờ cho ăn. Nitơ tích lũy cũng có xu hướng tăng tịnh tiến ($P=0.085$), nhưng tỷ lệ tiêu hoá dưỡng chất (trừ béo thô)

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giảm tịnh tiến ($P < 0,05$) khi TAHH tăng trong khẩu phần. Như vậy, TAHH với hàm lượng 18,5% CP có hiệu quả cao trong việc tăng lượng ăn và thông số dạ cỏ của dê, tuy nhiên mức độ cao TAHH làm giảm tỷ lệ tiêu hoá các dưỡng chất. Do đó, 45% TAHH có hàm lượng 18,5% CP là phù hợp trong khẩu phần của dê cái giai đoạn không cho sữa để tối ưu lượng ăn, thông số lên men dạ cỏ và tỷ lệ tiêu hoá dưỡng chất.

Từ khóa: *Dê càn sữa, lên men dạ cỏ, lượng ăn, thức ăn hỗn hợp, tiêu hóa.*

1. INTRODUCTION

In recent years, the demand for livestock products in Vietnam has increased rapidly (average +15.5%/year), especially after the outbreak of African swine fever, and goat farming has received more and more attention. Goats have high adaptability to different environments, hot climates and tolerance to water deprivation; meanwhile, they are enabled efficient utilization of low-quality feeds to produce healthy milk and meat for human consumption (Darcan and Silanikove, 2018). They can consume a variety of feed such as fresh grasses, tree leaves and agricultural by-products. However, feeding only these kinds of feeds may not meet the nutrient requirement of animals even during the non-lactating period, thus it is necessary to supply them with concentrate. Thanh (2021) found that feeding an unbalanced level of concentrate in the diet can have an adverse effect on nutrient digestibility and milk quality in goats. A study to find out a suitable ratio of concentrate to forage in goat diet is necessary. This study aimed to investigate the effect of different concentrate levels on feed intake, digestibility and ruminal fermentation in non-lactating goats.

2. MATERIALS AND METHODS

2.1. Site and duration of study

This study was conducted from February to May 2022 at the College of Rural Development and Laboratory of Ruminant Production Techniques, Faculty of Animal Sciences, College of Agriculture, Can Tho University, Viet Nam.

2.2. Animal and experimental design and diet

Three female crossbred Saanen goats (♂Saanen × ♀Bach Thao), 12 months old, 30.2 ± 1.25 kg of body weight, were used in a Longitudinal study. Each experimental period lasted for 15 days including 11 days for adjustment, followed by 4 days for sample collection. Each experimental period lasted 15 days. Goats received a diet consisting of 75:25 forage [F]:concentrate [C] from day 1 to 15 (C25) followed by 55:45 F:C (C45) from day 16 to 30, and lastly a 35:65 F:C from day 31 to 45 (C65). The goats were kept in individual cages (1.2×0.6×1.2m, L×W×H) and had free access to fresh water. Diets were offered twice a day at 08:00am and 17:00pm. An 18.5%CP concentrate was weekly mixed and daily offered to animals as fixed amount before elephant grass was fed *ad libitum*.

2.3. Sampling and measurements

Daily dry matter intakes of feeds were calculated by the difference from offered to refused feeds during the experiment and correcting for the DM content of each dietary component. Feed samples were collected, pooled, and stored at -20°C until further analysis of chemical composition. From day 12 to 14, total feces were collected to calculate nutrient digestibility following the method of McDonald *et al.* (2002). At the same time, a 5l bucket containing 50ml of 10% H_2SO_4 to keep the final pH below 3 (Pathoummalangsy and Preston, 2008) was placed under each cage for total urine collection. After recording the weight, 20% proportions of 24h feces were dried in a forced-air oven at 60°C for 48h, milled through a 1-mm mesh and stored for later chemical analysis. On day 15 of each period, ruminal fluid samples were collected at 0 and 3h post morning feeding using a 100ml hand glass syringe. The ruminal fluid

was immediately determined pH value using a digital pH meter. The samples were then filtrated through a clean double layer of cotton cloth and the liquid fraction was analyzed NH₃-N concentration and total volatile fatty acids (VFA).

2.4. Chemical analysis

Feed and fecal samples were analyzed dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE) and total Ash using the standard methods of AOAC (1990). Neutral detergent fiber (NDF) in these samples was analyzed using the methods described by Van Soest *et al.* (1991). All chemical components were expressed on a DM basis. Ruminal pH was determined by a pH meter (HI5222, Hana Instruments, US). Ruminal NH₃-N concentration was analyzed using the Kjeldahl methods (AOAC, 1990). Total VFA concentration was determined following the method of Barnett and Reid (1957).

2.5. Statistical analysis

The MIXED procedure of SAS version 9.4 (SAS Institute Inc., Cary, NC, USA) was used to analyze the current data. The fixed effect in the model was diet, and the random effect was the goat. Compound symmetry (CS) was the most appropriate covariate structure used for analysis. The degrees of freedom were corrected using the Kenward-Roger (KR) method, which yields more precise and efficient estimates of the fixed effects in experiments with small sample sizes. The trend of ratio responses was performed by orthogonal polynomials. Differences were considered statistically significant when $P \leq 0.05$ and considered a trend when $0.05 < P \leq 0.10$.

3. RESULTS AND RESULTS

3.1. Chemical compositions of feeds used in the experiment

It can be seen from Table 1 that the DM content of elephant grass is relatively high (17.2%) similar to the result of Hai (2019), 17.1%. The concentrations of OM (87.1%) and

EE (2.34%) were higher than those in the study of Hai (2019). CP content of elephant grass was 11.5% higher than the measurement of Thanh (2020) which was 10.4%. The content of NDF (60.9%) in the study was 67.1% lower than that in the study Hai (2019). The content of CP in the concentrate (18.4%) was similar to which in the experimental design (18.5%). The chemical compositions of the current concentrate and elephant grass revealed that they were suitable to fully meet the nutrient requirements for goats. Some chemical compositions of elephant grass used in this study differed from previous studies may be effected by factors geographical conditions, cultivation methods, harvest time, and growth stage.

Table 1. Feed ingredient, chemical composition

Feed	Chemical compositions, %					
	DM	Ash	OM	CP	NDF	EE
Concentrate	89.4	7.95	92.1	18.4	28.2	4.11
Elephant grass	17.2	12.9	87.1	11.5	60.9	2.34

3.2. Feed intake

Table 2. Feed and nutrient intake

Item	Diet			SEM	P	Contrast ²	
	C25	C45	C65			L	Q
<i>Feed intake, g DM/day</i>							
Concentrate	173 ^c	368 ^b	680 ^a	25.3	<0.001	<0.001	0.056
Elephant grass	602 ^a	453 ^b	382 ^c	30.8	0.005	0.002	0.222
<i>Ratio of feed intake, % DM</i>							
Concentrate	22.4 ^c	44.8 ^b	64.0 ^a	0.36	<0.001	<0.001	0.007
Elephant grass	77.6 ^a	55.2 ^b	36.0 ^c	0.36	<0.001	<0.001	0.007
<i>Nutrient intake, g/day</i>							
DM	775 ^b	822 ^b	1,062 ^a	47.0	0.007	0.004	0.076
OM	692 ^c	735 ^b	949 ^a	41.9	0.007	0.004	0.077
CP	91.6 ^c	115 ^b	163 ^a	6.69	<0.001	<0.001	0.107
NDF	422	377	410	23.5	0.258	0.649	0.130
EE	25.3 ^b	27.5 ^b	33.0 ^a	1.54	0.016	0.007	0.270

Feed intake is an important factor affecting livestock weight gain, in which DM requirements, feed quality (nutrients and digestibility), and palatability are important factors affecting feed intake. Table 2 shows that total DM intake linearly increased ($P < 0.01$) when increasing the level of concentrate in the diets,

reaching the highest value in the C65 diet (1,062g DM/day) and lowest in the C25 diet (775g DM/day). When the level of concentrate increased gradually, CP intake also increased linearly ($P<0.001$), from 91.6 g/day in C25 diet to 163 g/day C65 diet. Intake of NDF was not affected by different levels of concentrate in the diet ($P<0.05$).

3.3. Nutrient digestibility

Digestibility of nutrients, except EE, was quadratically decreased ($P<0.05$: Table 3) when increasing the level of concentrate in the diet. DM digestibility was highest in C45 (72.9%) and lowest in C65 (60.9%). C45 had the highest CP digestibility (77.6%) and the lowest one was detected in C65 (67.0%). The digestibility of CP in the current study was in agreement with Hang *et al.* (2020) who reported a 77.1-84.0% CP digestibility when goats were fed 50-70% CP in the diet. NDF digestibility linearly decreased ($P<0.01$) when increasing the level of concentrate in the diet, the highest in C25 (71.9%) and the lowest in C65 (52.4%). In contrast, increasing the level of concentrate in the diet led to linearly increased digested CP ($P<0.01$) and EE ($P<0.05$). Similar results were detected in the study of Hang *et al.* (2020).

Table 3. Total-tract digestibility

Item	Diet ¹			SEM	P	Contrast ²	
	C25	C45	C65			L	Q
<i>Digestibility, %</i>							
DM	71.5 ^a	72.9 ^a	60.9 ^b	1.47	0.002	0.002	0.006
OM	73.1 ^a	74.9 ^a	64.5 ^b	1.43	0.004	0.004	0.008
CP	75.0 ^{ab}	77.6 ^a	67.0 ^b	2.06	0.015	0.018	0.021
NDF	71.9 ^a	68.2 ^a	52.4 ^b	2.45	0.003	0.001	0.046
EE	73.4	74.7	74.7	2.25	0.818	0.597	0.774
<i>Digested nutrient, g</i>							
DM	554	601	647	36.9	0.151	0.066	0.996
OM	506	552	613	32.9	0.075	0.031	0.822
CP	68.7 ^c	89.6 ^b	109 ^a	5.47	0.005	0.002	0.909
NDF	305	259	216	25.6	0.060	0.025	0.935
EE	18.6 ^b	20.5 ^b	24.7 ^a	1.02	0.009	0.004	0.251

3.4. Ruminal fermentation patterns

Table 4 shows that ruminal pH value was linearly decreased ($P<0.05$) when increasing

the level of concentrate in the diets. Ruminal pH in this study (6.60-7.33) was consistent with the recommendations of Van Soest (1994) who reported that ruminal pH values between 6 and 7 were optimal for bacterial growth and a pH value lower than 6.2 would have a negative effect on fermentability of ruminal microorganisms. The reason for the neutral characteristic of ruminal pH has been previously explained by many researchers as the buffering effect of the salivary glands secreted during feeding and rumination, the buffering solution provided by saliva has sodium bicarbonate and sodium phosphate.

Table 4. Ruminal fermentation patterns

Item	Diet ¹			SEM	P	Contrast ²	
	C25	C45	C65			L	Q
<i>pH</i>							
0 h	7.33	7.12	6.93	0.12	0.063	0.026	0.912
3 h	7.09 ^a	6.80 ^{ab}	6.60 ^b	0.13	0.043	0.018	0.810
<i>NH₃-N (mg/dl)</i>							
0 h	17.3	20.1	21.5	1.55	0.118	0.053	0.629
3 h	14.5 ^b	17.7 ^{ab}	21.9 ^a	1.78	0.035	0.014	0.778
<i>VFA (mM)</i>							
0 h	27.8 ^c	30.6 ^b	47.7 ^a	4.49	0.022	0.011	0.139
3 h	42.3 ^b	51.3 ^b	70.8 ^a	3.87	0.004	0.002	0.194

At 3 h post-feeding, the highest ($P<0.05$) ruminal NH₃-N value was detected in the C65 goats (21.9 mg/dl), followed by C45 goats (17.7 mg/dl) and the lowest in C25 goats (14.5 mg/dl; Table 4). This result was consistent with the conclusion of Preston and Leng (1987), the suitable NH₃-N concentration in the rumen was between 5 and 25 mg/dl, NH₃-N in the rumen includes proteins, peptides, amino acids and other soluble nitrogen materials. Feeding concentrate at 65% in the diet promoted an increase in ruminal N-NH₃ concentration, favoring the growth of ruminal microorganisms, high protein synthesis and providing valuable protein for ruminants (Thu, 2003). Ruminal VFA concentration at 0h had the lowest ($P<0.05$) value in C25 goats (27.8mM) and highest in C65 goats (47.7mM). Ruminal VFA concentration at 3h was linearly increased ($P<0.01$) when increasing the level of concentrate in the diets. The ruminal VFA

levels observed in the current study (27.8-70.8mM) were consistent with the findings reported by Hang *et al.* (2020) (49.1-75.1mM).

3.5. Nitrogen balance

It is shown from Table 5 that the increasing rate of concentrate in the diets linearly increased ($P<0.001$) nitrogen intake but also linearly increased ($P<0.05$) the amount of N excreted in feces and urine. Retention nitrogen tended to increase linearly ($P=0.085$) from C25 (4.49 g/day) to C65 (8.01 g/day).

Table 5. Nitrogen retention

g N/day	Diet ¹			SEM	P	Contrast ¹	
	C25	C45	C65			L	Q
Intake	14.6 ^a	18.5 ^b	26.1 ^c	1.07	0.001	<0.001	0.107
Feces	3.65 ^a	4.13 ^a	8.64 ^b	0.58	0.002	0.001	0.016
Urine	6.51	6.93	9.49	1.04	0.087	0.046	0.300
Retention	4.49	7.41	8.01	1.54	0.161	0.085	0.432

4. CONCLUSION

Combine data suggest that concentrate is more effective in increasing intake and ruminal fermentation; however, a high level of concentrate can reduce nutrient digestibility. Thus, a level of 45% concentrate containing 18.5% CP in the diet might be suitable for optimizing intake, ruminal fermentation and digestibility in non-lactating goats.

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EFFECT OF SUPPLEMENTATION RATES OF FERMENTED TOTAL MIXED RATION (FTMR) FROM JACKFRUIT BY-PRODUCTS ON THE GROWTH OF CROSSBRED BOER GOATS

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ABSTRACT

The experiment was conducted from March 2023 to June 2023 at a livestock farm in Phung Hiep district, Hau Giang province. It aimed to evaluate the impact of Fermented Total Mixed Ration (FTMR), mixed from young jackfruit, on feed nutrient consumption and weight gain in crossbred Boer goats. Twelve male Boer crossbred goats (Boer×BachThao), aged 3-4 months and weighing 22.17±3.84kg, were used in the study. The experiment was arranged using a completely randomized design, which involved four treatments incorporating four different levels of FTMR addition: 0, 25, 50, and 75%, specifically designated as 0FTMR, 25FTMR, 50FTMR, and 75FTMR. Each treatment was replicated three times, with every replication involving a male goat. The FTMR formula included the following ingredients: young jackfruit, ground corn, rice bran, extracted soybean, copra meal, urea, mineral premix, and salt. The results showed that feed intake, ether extract (EE), crude protein (CP) intakes and total intake (%DM/body weight) were significantly different ($P<0.05$). The FTMR intake increased from 156.48 to 508.69g DM/head/day and the Elephant grass decreased from 417.41 to 162.18g DM/head/day among the FTMR-supplemented treatments ($P<0.05$). The intake of young jackfruit was highest at 0FTMR (314.13g DM/head/day) and lowest at 25FTMR (78.24g DM/head/day) ($P<0.05$). Dry matter (DM) and EE intakes ranged from 573.89 to 670.87 g/head/day and 16.24 to 30.29 g/head/day, respectively ($P>0.05$). The CP intake increased with the rate of FTMR addition, the highest at 75FTMR (86.02 g/head/day) and lowest at 25FTMR (49.70 g/head/day), but 0FTMR had a CP value of 51.41g DM/head/day ($P<0.05$). The intake (%DM/body weight) in the 0FTMR, 25FTMR, 50FTMR treatments, and 75FTMR reached 2.58, 2.53, 2.63 and 2.88%. The metabolizable energy (ME) intake (MJ/head/day) improved for 50FTMR (5.81 MJ) and 75FTMR (6.42 MJ) compared to 0FTMR (5.47 MJ), but it decreased for 25FTMR (5.10 MJ). The live weight gains (LWG) (g/head/day) of the goats were 88.89; 122.22; 116.67; and 105.56 for 0FTMR, 25FTMR, 50FTMR and 75FTMR treatments, respectively ($P<0.05$). Feed conversion ratio (FCR) was improved with the addition of FTMR at 25% (4.69kg DM/kg body weight) and 50% (5.58kg DM/kg body weight) compared with 0% FTMR (6.80kg DM/kg body weight) ($P>0.05$). The findings suggest that adding 25% FTMR to the diet enhances the nutritional value, weight gain, and FCR of crossbred Boer goats.

Keywords: Fermented total mixed ration (FTMR), jackfruit by-products, silage, crossbred Boer goats.

TÓM TẮT

Ảnh hưởng của tỷ lệ bổ sung khẩu phần hỗn hợp hoàn chỉnh được lên men (FTMR) từ phụ phẩm mít lên tăng trưởng của dê Boer lai

Thí nghiệm được thực hiện từ tháng 3/2023 đến tháng 6/2023 tại trại chăn nuôi thuộc huyện Phụng Hiệp, tỉnh Hậu Giang. Thí nghiệm nhằm mục đích đánh giá ảnh hưởng của khẩu phần hỗn hợp hoàn chỉnh lên men (FTMR), được phối trộn từ mít non, đến khả năng tiêu hóa chất dinh dưỡng và tăng khối lượng của dê Boer lai. Mười hai con dê lai Boer đực lai (Boer×BachThao), 3-4 tháng tuổi và khối lượng 22.17±3.84kg được sử dụng trong nghiên cứu. Thí nghiệm được thiết kế theo kiểu hoàn toàn ngẫu nhiên, bao gồm bốn nghiệm thức (NT) là bốn mức bổ sung FTMR khác

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nhau: 0, 25, 50 và 75%, tương ứng với kí hiệu các NT 0FTMR, 25FTMR, 50FTMR và 75FTMR. Mỗi NT được lặp lại ba lần, mỗi lần lặp lại là một con dê đực. Công thức FTMR bao gồm: mít non, bắp nghiền, cám gạo, đậu nành ly trích, bánh dầu dừa, urê, hỗn hợp khoáng và muối. Kết quả cho thấy khối lượng thức ăn tiêu thụ, béo thô (EE), protein thô (CP) ăn vào và tổng lượng ăn vào (%DM/khối lượng cơ thể) khác biệt có ý nghĩa thống kê ($P < 0.05$). FTMR ăn vào tăng từ 156,48 lên 508,69 gDM/con/ngày và cỏ Voi ăn vào giảm từ 417,41 xuống 162,18 gDM/con/ngày ở các NT cho ăn FTMR ($P < 0.05$). Khối lượng mít non ăn vào cao nhất ở 0FTMR (314,13 gDM/con/ngày) và thấp nhất ở 25FTMR (78,24 gDM/con/ngày) ($P < 0,05$). Vật chất khô (DM) và EE ăn vào lần lượt dao động từ 573,89 đến 670,87 gDM/con/ngày và 16,24 đến 30,29 gDM/con/ngày ($P > 0.05$). CP ăn vào tăng theo tỷ lệ bổ sung FTMR, cao nhất ở 75FTMR (86,02 gDM/con/ngày) và thấp nhất ở 25FTMR (49,70 gDM/con/ngày), nhưng NT 0FTMR có CP ăn vào là 51,41 gDM/con/ngày ($P < 0,05$). Khối lượng thức ăn ăn vào (%DM/trọng lượng cơ thể) ở các NT 0FTMR, 25FTMR, 50FTMR và 75FTMR lần lượt là 2,58; 2,53; 2,63 và 2,88%. Năng lượng trao đổi (ME) tiêu thụ (MJ/con/ngày) được cải thiện ở NT 50FTMR (5.81 MJ) và 75FTMR (6.42 MJ) so với 0FTMR (5.47 MJ), nhưng giảm đối với 25FTMR (5,10 MJ). Tăng khối trọng (g/con/ngày) của dê là 88,89; 122,22; 116,67 và 105,56 tương ứng với NT 0FTMR, 25FTMR, 50FTMR và 75FTMR ($P < 0,05$). Hệ số chuyển hóa thức ăn (FCR) đã được cải thiện với việc bổ sung FTMR ở mức 25% (4,69 kgDM/kg khối lượng cơ thể) và 50% (5,58 kgDM/kg khối lượng cơ thể) so với 0% FTMR (6,80 kgDM/kg khối lượng cơ thể) ($P > 0,05$). Kết quả cho thấy việc bổ sung 25% FTMR vào khẩu phần giúp nâng cao giá trị dinh dưỡng, tăng khối lượng và FCR của dê Boer lai.

Từ khóa: Khẩu phần hỗn hợp hoàn chỉnh được lên men (FTMR), phụ phẩm mít, ủ chua, dê Boer lai.

1. INTRODUCTION

The practice of combining livestock farming with fruit tree cultivation, particularly jackfruit, is quite prevalent in the Mekong Delta. This model leverages local by-products, ensuring a self-reliant supply of feed to decrease livestock expenses and minimize environmental pollution. Among ruminants, goats exhibit a high capacity to adapt to diverse new feed sources (Shaheen *et al.*, 2020). In goat farming, numerous agricultural and manufacturing by-products can be utilized to cut costs and enhance profitability. Common examples of such by-products include jackfruit waste, sweet potato vines, peanut vines, corn stalks, and rice bran. Jackfruit, particularly young and unqualified jackfruit, are abundant during cultivation and serve as an excellent feed for goats, mitigating the risk of high feed costs. Ho Thanh Tham (2017) found significant potential in using jackfruit waste in animal feed. Thanh *et al.* (2021) reported a Crude protein (CP) content of 10.3% in young jackfruit (%DM). If properly preserved and processed, these by-products can alleviate feed shortages in livestock farming.

When it comes to goat feed preparation, the Total Mixed Ration (TMR) is a well-balanced diet consisting of a calculated mix of roughage and concentrate that can fulfill livestock nutritional requirements. To preserve TMR, the Fermented Total Mixed Ration (FTMR) offers an innovative and sustainable method for goat farming, especially when it's based on agricultural by-products. FTMR can enhance nutrients, reduce costs, promote environmental sustainability, improve digestibility, and benefit animal health. A study by Pakpahan and Restiani (2019) revealed that goats fed a 50% forage and 50% FTMR diet exhibited a higher average weight gain than those fed a 100% forage diet. Therefore, this study aims to assess the impact of FTMR derived from young jackfruit on feed nutrient consumption and weight gain in crossbred Boer goats.

2. MATERIALS AND METHODS

2.1. Animal, location and time

The experiment was carried out on 12 (Boer×BT) crossbred Boer goats, 3-4 months old, weighing 22.17 ± 3.84 kg. Goat pens are

divided into individual cells, each with separate feeding and drinking troughs. Goats are selected to ensure the same condition, health, breed and weight. They were vaccinated against Pasteurellosis and Foot-and-mouth disease and were treated for internal and external parasites. The experiment took place from Mar to Jun 2023 at a livestock farm in Phung Hiep district, Hau Giang province.

2.2. Experimental design

The experiment was arranged in a completely randomized design with 4 treatments and 3 replications. The four treatments were four diets as follows:

0FTMR: 50% jackfruit + 50% Elephant grass

25FTMR: 25% FTMR + 75% Elephant grass

50FTMR: 50% FTMR + 50% Elephant grass

75FTMR: 75% FTMR + 25% Elephant grass

The FTMR formula used in the experiment is shown in Table 1. The different levels of FTMR, The farmer’s feed were determined based on the total expected intake of 3.28% of goat weight/day (in DM), according to the experimental results of the experimental results of Nguyen Dong Hai (2008), Ngo Tien Dung *et al.* (2005) and Hango *et al.* (2007). Mineral premix is a product of ANOVA Joint Venture Company Limited.

Table 1. Ingredients for mixing FTMR

Ingredient	Percentage (%DM)
Young jackfruit	50
Ground corn	5
Rice bran	22.5
Extracted soybean	5
Copra meal	16
Urea	0.5
Mineral premix	0.5
Salt	0.5

Preparation of FTMR and goat management: Young jackfruits are harvested as raw materials and subsequently processed to remove skin imperfections such as damage, discoloration,

or mold. These jackfruits are machine-sliced to a thickness of approximately 5mm. They are then placed into fermentation bags for FTMR processing, with each bag capable of holding 40kg of the mixed material. The raw materials are weighed and uniformly spread over a plastic tarpaulin. Ingredients are proportioned according to %DM in the formula. The mixing follows a particular sequence where lower ratio raw materials are mixed in first and then spread evenly over the material surface and thoroughly mixed. Bon Silage Forte, a silage additive with a concentration of 1.25×10^{11} CFU/g of lactic acid bacteria (Lactosan GmbH & Co. KG, Austria), is utilized. A solution of 0.2g of Bon Silage Forte in 5ml of water is sprayed evenly over every 100kg of the material. The mixture is then layer by layer, each 10-20cm thick, tightly packed into the fermentation bag, ensuring minimal air space and the bag is securely tied shut. The bags are stored in a dry place and feeding to the goats begins after 3-5 days.

Goats are fed the FTMR at 7:30am and 1:00pm, and the farmer’s feed, which consists of elephant grass and unprocessed fresh young jackfruit, is given at 10:00am and 4:00pm. The total feeding experiment spans 90 days with a 10-day adjustment period for the goats to get used to the new feed.

2.3. Sampling, measurement and chemical analysis

Feed intake: record daily feed intake (intake and refusal) per individual goat and nutrient intake. Feed refusal was weighed and determined at 6:00 am the following morning.

Average weight gain (g/head/day): goats were weighed on days of 31, 61, and 91 in the morning before feeding. Use a 100kg scale (Nhon Hoa, error 100g) to determine the weight of goats.

Feed conversion ratio (FCR) = Feed intake (kg DM/day)/Live weight gain (kg/day)

The chemical composition of experimental feed was sampled monthly such as: dry matter (DM), crude protein (CP), total ash, organic matter (OM), crude fat (EE), crude fiber (CF) was analyzed according to AOAC (1990). Acid detergent fiber (ADF), and neutral detergent fiber (NDF) was determined according to the procedure of Van Soest *et al.* (1991).

2.4. Data analysis

The data of the experiment were preliminarily processed on Microsoft Excel 2016 software, then analyzed for variance (ANOVA) according to the GLM on Minitab Release 16.1 software (Minitab, 2010). When there is a difference between the mean values of the treatments, the Tukey test will be used to determine the difference between each treatment pair ($P < 0.05$). The statistical model used is $Y_{ijk} = \mu + t_i + e_{ij}$ with Y_{ij} : the research criterion, μ : an overall mean effect, t_i : the effect of the treatments, and e_{ij} : the random error.

3. RESULTS AND DISCUSSIONS

3.1. Chemical composition of the ingredients

The DM and CP contents of FTMR in the experiment were 26.16% and 14.89%, respectively, lower than those of Lam Phuoc Thanh *et al.* (2021) on the combined diet of concentrate feed, Elephant grass and young jackfruit fruit was 45.80% DM and the value was 12.5% higher than CP of the same study. Elephant grass used in the experiment had DM and CP of 12.73 and 6.32%, lower than those announced by Truong Thanh Trung and Nguyen Binh Truong (2020) of 18.5 and 10.5%. NDF content of Elephant grass (69.27%) was similar to the above study (69.4%). The young jackfruit in the experiment had CP and NDF contents of 10.72 and 41.07% respectively, equivalent to the findings of Lam Phuoc Thanh *et al.* (2021), which recorded levels of 10.30 and 42.80%.

Table 2. Chemical composition of FTMR and mixing materials

Ingredient	DM (%)	DM (%)							ME (MJ/kg)
		Ash	OM	EE	CP	CF	ADF	NDF	
FTMR	26.16	13.39	86.61	5.34	14.89	21.47	19.88	43.42	9.91
Elephant grass	12.73	7.76	92.24	1.92	6.32	41.13	40.47	69.27	8.51
Young jackfruit	20.05	4.71	95.29	3.46	10.72	25.56	25.72	41.07	9.81
Rice bran	88.80	9.95	90.05	8.55	11.20	22.60	19.90	33.40	9.43
Ground corn	87.52	2.23	97.85	2.35	8.02	1.94	2.22	36.52	10.37
Extracted soybean	88.00	7.90	92.10	0.38	39.60	20.10	28.70	16.70	10.00
Copra meal	95.80	8.17	91.83	9.89	18.80	7.70	11.20	53.00	13.11

3.2. Feed intake and nutrient intakes

A significant difference was observed in the feed obtained between the treatments ($P < 0.05$). The increase in dietary FTMR from 0 to 508.69g DM/head/day corresponds to a range from 0FTMR to 75FTMR. However, the consumption of Elephant grass gradually decreased in the FTMR-supplemented treatments. The highest consumption of young jackfruit fruit was observed in the 0FTMR treatment (314.13g DM/head/day), whereas the lowest consumption was seen in the 25FTMR treatment (78.24g DM/head/

day). This distinction was influenced by the experimental formulas.

Nutrient consumption in the trials was not significantly different ($P > 0.05$) in DM, OM, CF, ADF, NDF, and ME, except for EE, CP, and %DM/body weight. The DM intake of 573.9-670.8 g/head/day was higher than Nguyen Binh Truong 's study (2018) on Saanen goats with water spinach supplement (443-584 g/head/day). The EE consumption increased steadily (16.24-30.29 g/head/day), aligning with an increase in dietary FTMR. This was lesser than the results reported by

Le Van Phong and Nguyen Van Thu (2018) on Bach Thao goats with cabbage by-product supplement (26.9-39.7 g/head/day). CP intake was highest in 75FTMR (86.02 g/head/day) and lowest in 25FTMR (49.70 g/head/day). These results were lower than the study on male crossbred goats (Jumnapari×Saanen) which used Velvet bean hay (*Mucuna pruriens*) to replace Elephant grass by Ngo Thi Thuy *et al.* (2016) (53.23-97.76 g/head/day). However, these results were higher than the study of Lam Phuoc Thanh and Pham Truong Thoai Kha (2018) who added oil to a Saanen×BachThao diet (49.35-60.45 g/head/day). Similarly, they were higher than the study by Truong Thanh

Trung and Nguyen Binh Truong (2020) on Bach Thao goats with monosodium glutamate (MSG) production by-products supplement (Vedafeed-CMS) (67.5-69.3 g/head/day). The intake of NDF gradually decreased from 25FTMR to 75FTMR (357.11-333.24 g/head/day), and ADF showed similar results (200.05-166.75 g/head/day). These experimental results were higher than the study of Thanh Trung and Nguyen Binh Truong (2020) when goat diets were supplemented with 4% Vedafeed-CMS (239 g/head/day and 150 g/head/day). TMR treatment (78.24g DM/head/day), due to the impact of experimental formulas.

Table 3. Feed intake and nutrient intakes

Item	Treatment				SEM	P	
	0FTMR	25FTMR	50FTMR	75FTMR			
Feed intake, g DM/head/day	FTMR	0 ^d	156.48 ^c	331.05 ^b	508.69 ^a	26.90	<0.001
	Young jackfruit	314.13 ^a	78.24 ^c	165.53 ^{bc}	254.35 ^{ab}	20.28	<0.001
	Elephant grass	280.49 ^{ab}	417.41 ^a	296.86 ^{ab}	162.18 ^b	35.72	0.007
Nutrient intake, g DM/head/day	DM	594.63	573.89	627.91	670.87	64.18	0.73
	OM	558.12	520.60	560.61	590.22	58.13	0.86
	EE	16.24 ^b	16.37 ^b	23.38 ^{ab}	30.29 ^a	2.15	0.01
	CP	51.41 ^b	49.70 ^b	68.08 ^{ab}	86.02 ^a	6.37	0.01
	CF	195.65	205.27	193.17	175.91	20.92	0.80
	ADF	194.32	200.05	185.96	166.75	20.36	0.69
	NDF	323.31	357.11	349.40	333.24	36.73	0.91
DM/BW, %	2.58 ^c	2.53 ^d	2.63 ^b	2.88 ^a	0.00	<0.001	
ME, MJ/ head/day	5.47	5.10	5.81	6.42	0.59	0.48	

* Mean values between treatments with different letters are statistically significant (P<0.05)

The amount of DM/BW was highest in 75FTMR (2.88%) and lowest in 25FTMR (2.53%) (P<0.05), equivalent to the report of Le Van Phong and Nguyen Van Thu (2018) on average-weight Bach Thao goats 24.3 kg is 2.64-2.80%. ME intake was not significantly different between the treatments of 5.10, 5.81, and 6.42 MJ/head/day, corresponding to the FTMR increase of 25FTMR, 50FTMR, and 75FTMR, respectively. However, 0FTMR (5.47 MJ/head/day) was higher than 25FTMR (5.10 MJ/head/day), which may have affected the amount of jackfruit in the diet. This result is higher than the study of Nguyen Binh Truong (2018) when using a supplement of water

spinach on Saanen goats at 4.04-5.45 MJ/head/day and the study by Truong Thanh Trung and Nguyen Binh Truong (2020) with ME levels ranged of 4.10-4.18 MJ/head/day. The increase in the proportion of FTMR in the diet did not change the amount of DM and OM consumed between the treatments, but the ME increased gradually when increasing the level of FTMR in the diet, and CP intake improved significantly.

3.3. Live weight and live weight gain

The initial and final weights of the four treatments were not significantly different. There was a notable statistical difference in the

LWG (g/head/day) of goats across treatments ($P < 0.05$). The LWG were 88.89, 122.22, 116.67, and 105.56, corresponding to 0FTMR, 25FTMR, 50FTMR and 75FTMR treatments. These results, from treatments supplemented with FTMR, were higher than the LWG observed in Boer x Bach Thao goats at 80.4g (Nguyen Binh Truong *et al.*, 2018), crossbred Bach Thao goats at 80.20 g (Nguyen Thi Thu Hong and Duong Nguyen Khang, 2017), and Bach Thao male goats at 110 g/head/day (Do

Thi Thanh Van and Nguyen Van Thu, 2018). The FCR of goats in the experiment tended to improve when the diet was supplemented with FTMR, but when the amount of FTMR was 75% in the diet (75FTMR), this value was equivalent to 0FTMR (6.42 and 6.80kg DM/kg BW). The FCR in 25FTMR (4.69kg DM/kg BW) was less than that observed in the research of Nguyen Thi Kim Dong and Nguyen Van Thu (2018) on Bach Thao goats fed with Elephant grass diets (6.2 and 6.8kg DM/kg BW).

Table 4. Live weight and live weight gain of goats

Item	Treatment				SEM	P
	0FTMR	25FTMR	50FTMR	75FTMR		
Initial weight, kg/con	22.00	21.33	23.00	22.33	2.36	0.97
Final weight, kg/con	27.33	28.67	30.00	28.67	2.12	0.85
LWG, g/head/day	88.9 ^b	122.2 ^a	116.7 ^{ab}	105.6 ^{ab}	6.80	0.04
FCR, kg DM/kg BW	6.80	4.69	5.58	6.42	0.87	0.38

4. CONCLUSIONS

For optimal results, it is recommended to add 25% FTMR to the diets of growing goats. This addition of FTMR helps enhance protein consumption, ME, LWG, and FCR.

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COLLEGE OF AGRICULTURE - 55 YEARS OF CONSTRUCTION AND CONTINUOUS DEVELOPMENT

The College of Agriculture (CoA) was founded in 1968. It is one of the largest colleges of CTU and was awarded the Labour Hero Medal by the Government in 2000. As of November 2022, CoA has a total number of 198 staff, 115 of whom are full-time lecturers

with 100% postgraduate qualifications. CoA is the training unit that has the highest number of professors and associate professors in CTU. CoA consists of 07 academic faculties, 03 supporting units (including the Centre for Agricultural Service)



Figure 1. College of Agriculture



Figure 2. CTU Hi-Tech Building

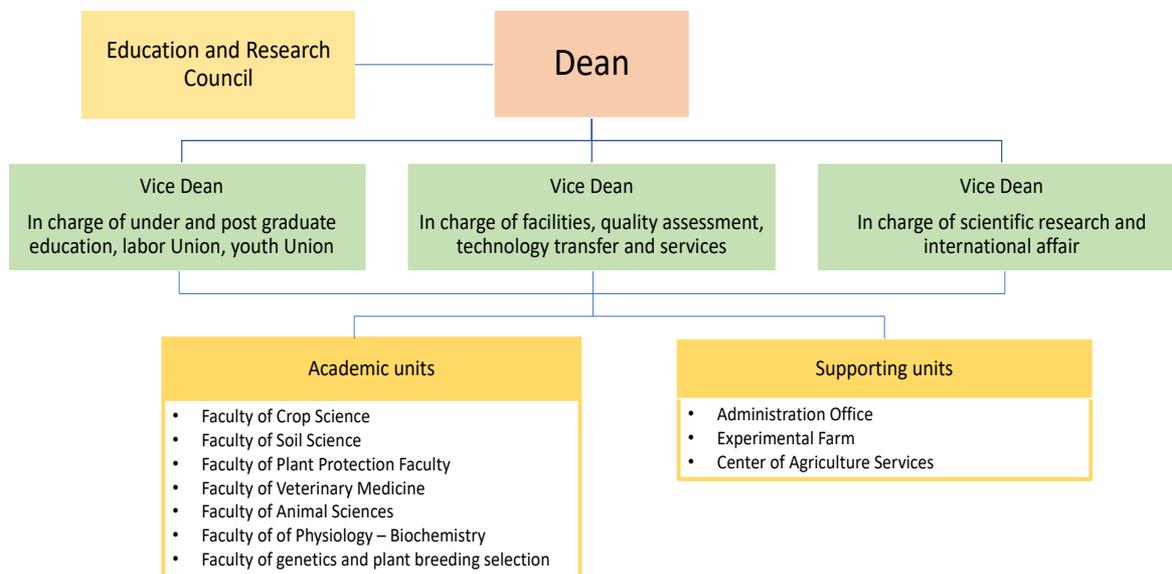


Fig 3. The organisational structure of CoA

As a part of CTU, CoA has also set up its vision and mission in order to conduct the duties of an academic unit in meeting the mission and vision of the University as a whole.

Mission: CoA strives to be the leading institution in education, research and technology transfer, making significant contributions to the development of high-quality human resources and the advancements of science and technology to cater for the national and international socio-economic development.

Vision: The CoA (of CTU) is one of the leading institutions in education, research and

technology transfer in agriculture of Vietnam and the world in 2030.

CoA's achievements

• **Training activities**

CoA offers educational programmes leading to several different degrees in various fields and levels. As of November 2022, CoA has offered 13 undergraduate programmes (including 01 high-quality programme), 10 master's programmes (2 programmes instructed in English), and 6 doctoral programmes as shown in Table 1 and Table 2 shows the number of students by training level.

Table 1. Programmes offered by CoA

No.	Programme levels	Quantity	Programme name:
1	Doctoral	6	Animal Science, Crop Science, Food Technology, Pathology and Treatment of Animals, Plant Protection, Soil Science
2	Master	7	Animal Science, Crop Science, Food Technology Veterinary Medicine, Plant Genetics and Breeding, Plant Protection, Postharvest Technology, Soil Science, Climate change and sustainable tropical
3	Undergraduate (Bachelor)	10	Agronomy Applied Biology Horticulture and Landscape Design Animal Science Crop Science High Technology Agriculture Plant Protection Veterinary Medicine Veterinary Pharmacy Soil Management and Fertilizer Technology

Table 2. Number of CoA students by training level from 2018 to 2021

No.	Programme	Number of students			
		2018	2019	2020	2021
1	Bachelor's				
	Regular programmes	5,527	5,504	5,195	5,250
	High-quality programmes	38	58	87	167
2	Master's	117	97	179	114
3	Doctoral	5	4	5	20

- **Scientific Research Activities**

CoA collaborates on scientific research and education effectively with various partners, including Ghent University and KU Leuven (Belgium), Tokyo University of Agriculture and Technology (Japan), Kyoto Institute of Technology (Japan), Charles Sturt University (Australia), Copenhagen University (Denmark), Prince of Songkla University (Thailand), Suranaree University of Technology (Thailand), and others. Several research projects have been successfully completed. Efforts are being made to increase the CoA's training scale, improve the quality of its academic staff, upgrade the facilities, and search for more collaboration opportunities. Furthermore, CoA collaborates with various agricultural partners to train students with agricultural professional skills and to develop agricultural products. It also develops extensive collaboration with many provinces and cities in Vietnam in applying scientific research findings in agricultural production and technology transfer. CoA's research strengths include transferring new technology methods and agricultural products.

- **Quality Assurance**

CoA has paid much attention to improving and developing the quality of education and training programmes towards the national standards and the trend toward regional and international integration. The CoA's Quality Assurance Team was established in 2014 in studying and providing consultancy for

the Dean's board in quality assurance and training improvement as well as to deploy the quality assurance activities of the unit. Recognising the importance of self-assessment in QA and programme improvement, CoA has continuously conducted self-assessment for each training programme. Ten undergraduate programmes of CoA were internally assessed from 2009 to 2018. They are Food Technology (2009), Animal Science (2010), Plant Protection (2012), Soil Science (2013), Agronomy (2015), Food Technology, Crop Science and Veterinary Medicine (2018), Horticulture and Landscape Design (2019 CoA programmes, Food Technology and Crop Science in 2021 and Plant Protection in 2022, were successfully assessed according to AUN-QA standards and certified in May 2022 and December 2022.

In terms of academic reputation, the Agriculture and Forestry group of CTU is ranked 1st in Vietnam. In 2022, according to the QS World University Rankings by Subject, the University of Agriculture and Forestry (UAF) was ranked among the top 301-350 universities in the world for the field of Agriculture and Forestry. All of these aspects represent initial achievements of CTU in general and of CoA in particular in the journey toward regional and international integration.