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ANIMAL GENETICS AND BREEDING

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SCIENTIFIC NEWS

EFFECT OF PROLACTIN, NEUROPEPTIDE Y GENE POLYMORPHISM ON EGG YIELD OF HAC PHONG FEMALE LINE CHICKEN BREED UP TO 38 WEEKS OF AGE

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ABSTRACT

The aim of this study was to investigate the association of Prolatin *(PRL/Indel)* and Neuropeptide Y (*NPY/Dra*I) gene polymorphism with egg production trait of Hac Phong (HP) female line chicken breed. A total of 675 individual HP chickens (295 and 380 chickens for the first and second generations) was examed. The results showed that polymorphism of these genes significantly impacted on the egg production at 38 weeks of age. The group of hens bearing the DD genotype at *PRL/Indel* locus had a significant higher number of eggs up to 38 weeks of age as compared to group of hens bearing II genotype (54.7 eggs and 49.6 eggs; P<0.05) for over two generations examined. Similarly, the group of hens bearing BB genotype of *NPY/Dra*I locus had a significant higher egg numbers than other groups of hens bearing AA or AB genotype over two generations. Combination effect of polymorphism of the *PRL/Indel* and *NPY/Dra*I loci was performed and results showed that the egg yield up to 38 weeks of age of the group hens bearing DD-BB (59.5 eggs) or ID-BB (56.4 eggs) genotype was higher than other genotypes. Polymorphisms at the 24bp *PRL/indel* or *NPY/Dra*I position could be used for molecular-assisted selection to improve egg production in Hac Phong chickens, requiring further research.

Keywords: *Hac Phong chicken, Prolactin gene, Neuropeptide Y gene, genetic polymorphism.*

1. INTRODUCTION

Prolactin (PRL) and Neuropeptide Y (NPY) have been described as two genes significantly related to reproductive ability in poultry. Prolactin (PRL) is a polypeptide hormone primarily synthesized in the pituitary gland of vertebrates, significantly influencing reproductive characteristics, particularly egg production traits (Purwantini *et al*., 2020). PRL regulates differences in egg production by controlling the development process of follicles, egg formation, and can be applied to support selection processes to create new breeds (Rohmah *et al*., 2022). Neuropeptide Y (NPY) is one of the most abundant peptides in the chicken brain, acting as a neurotransmitter that plays a key role in regulating feed intake and body weight regulation. NPY has been demonstrated as a gene that stimulates appetite in chickens, thereby influencing the supply of nutrients and energy consumption for reproductive performance, especially egg production traits (Rastidout *et al*., 2019; Sartsoongnoen *et al*., 2021). Additionally, NPY is also associated with brooding behavior and nurturing of chicks in hens (Kamkrathok *et al*., 2021). The Hac Phong chicken is a valuable genetic resource and the selection program has been carrying out in order to establish a male line with high body weight and a female line with high egg productivity by the VIGOVA Poultry Research and Development Center-Institute of Animal Sciences for Southern Vietnam (VIGOVA Center-IASVN). In fact, local chicken breeds like the Hac Phong chicken often have low laying rates and low egg productivity due to the interruption of the reproductive period caused by brooding behavior (Rohmah *et al*., 2022). In recent years, the selection and breeding of

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specialized poultry lines with high productivity has been successfully carried out by the VIGOVA Center, but mainly based on phenotypic values (body weight, egg productivity, etc). Therefore, this study aimed to evaluate the influence of polymorphism of PRL and/or NPY gene on the reproductive ability of HP chickens, the basic data can be used as the molecular markers to support the selection program for improving the genetic progress of the Hac Phong chickens combined with marker assisted selection.

2. MATERIALS AND METHODS

2.1. Materials and location

Birds and samples: The study was conducted on Hac Phong chickens over 2 generations (G), blood samples were collected individualy at the end of 38wks old.

Location: The Hac Phong chickens were raised at the VIGOVA Center-IASVN. Genetic polymorphism analysis of the first generation chickens (G1) was conducted at the Biotechnology Laboratory-IASVN, the second generation (G2) at the Laboratory of Animal Embryonic Technology-Institute of Biotechnology for Environment and Faculty of Biological Sciences, Nong Lam University, Ho Chi Minh City.

2.2. Methods

2.2.1. Female line seletion for high egg yield through two generations

Hac Phong chicken breed was selected to create the female line after two generations with the target such as accumulated egg yield upto 38 weeks of age, consistent body weight at 8 and 18 weeks of age.

In G1, individual chickens with an average egg production higher than the flock average and carrying positive PRL, NPY genotypes was selected as a replacement flock for G2. In G2, individual chickens were selected to produce the next generation based on estimated breeding value (EBV) of egg production higher than the mean EBV of the flock at 38 weeks of age and positive PRL, NPY genotypes.

2.2.2. Egg yield determination

Egg laying was observed daily and recorded upto 38weeks of age. A total of 675 individual hens (295 for G1, 380 for G2) with complete data recorded were collected for blood sampling for genetic analysis.

2.2.3. PRL and NPY genotype

The target fragment of each gene was successfull amplified and identified the genotyes by PCR-RFLP and has been reported in previous studies (Hoang Tuan Thanh *et al*., 2023a; 2023b).

2.2.4. Feeding and management

The chickens were raised according to the feeding and management procedure of VIGOVA Center. The starter and grower stage (0-20w) of birds were raised under on ground confined condition, the laying stage were raised in individual cage. The nutrition requirement were provided depending on the physiological stages as follows:

Table 1. Nutrition requirement for female line

Items	Weeks of age		
	0-8w	9-20w	>20w
CP, %	19.5-20.0	14.5-15.0	17.0-17.5
ME, kcal/kg	2,900-3,000	2,700	2,750
CF (max), %	5.0	7.0	5.0
Calcium, %	0.7-1.7	0.7-1.7	3.0-4.5
Phosphorus, %	0.6-1.1	0.6-1.1	0.5-1.1
Lysine (min), %	1	0.8	0.90
Met+Cys (min), %	0.7	0.6	0.7

2.3. Data analysis

Data were subjected to analyse by Least Squares Mean using Minitab 16 software.

3. RESULTS AND DISCUSSION

3.1. Association of PRL polymorphism on egg yield upto 38w of age of 2 generations

As reported in the previous study (Hoang Tuan Thanh *et al.,* 2023a), polymorphism at the *PRL/Indel* locus in the promoter region of Hac Phong chickens with 2 alleles I (Insertion) and D (Deletion) and three genotypes (DD/ID and II) were identified.

Table 2. Effect *PRL/Indel* genotype to egg yield 38w

Generation	Genotype	n (hen)	LSM±SEM (egg)	Max (egg)	Min (egg)
G1	II	4	49.8±4.2	58	47
	ID	37	50.6±1.6	72	39
	DD	254	54.0±0.8	77	39
G2	II	6	49.3±3.0	53	50
	ID	28	52.8±1.5	68	43
	DD	346	55.2±0.6	77	39
G1 and G2	II	10	49.6^{a}±2.5	58	47
	ID	65	51.4^{ab}±1.1	72	39
	DD	600	54.7^{b}±0.5	77	39

Within the same column and generation, data with the superscript letters differs (P<0.05).

Data from table 2 indicate that the chickens with the DD genotype had higher egg numbers up to 38 weeks of age than II or ID genotype in the first and second generations (54.0 vs 49.8 or 50.6 eggs in the 1st generation, 55.2 vs 49.3 or 52.8 eggs in the 2nd generation, respectively). In the DD genotype, the number of eggs value up to 38 weeks was improved by 1.2 eggs in the second generation compared to the first generation. The accumulative data through the two selected generations, it can be elucidated that the average of egg yield at 38 weeks of age was significant higher in the DD genotype group than in the II or ID genotype group (54.7 vs 49.6 or 51.4 eggs, respectively). Significant differences were only found between the DD and II genotypes.

A *PRL/indel* polymorphism in Tellichery native chickens has been reported (Manoharan *et al*., 2021) and it indicated the II genotype had a positive effect on egg production, which is conversed trend with our current study. On the other hand, the other research in BC1 chickens was reported that the polymorphism at PRL/Indel did not affect on egg production (Kilatsih, 2021), however, the other site of polymorphism pf PRL on the exon 5 was reported to have the positive effect on egg production in IPB-D1 chickens (Rohmah *et al*., 2022).

3.2. The association of NPY/DraI polymorphism with egg yield upto 38ws of age after two generations of selection

Previous study has confirmed that the *NPY/DraI* polymorphism in the promoter region was recognized with 2 alleles (A and B) and three genotypes (AA, AB and BB), in which B allele and BB genotype are dominant in the surveyed Hac Phong chicken population (Hoang Tuan Thanh *et al*., 2023b). The effect of *NPY/Dra*I polymorphism on egg yield at 38 weeks of age was evaluated and presented in table 3.

Table 3. Effect *NPY/Dra*I genotypes to egg yield 38w

Generation	Genotype	n (hen)	LSM±SEM (egg)	Max (egg)	Min (egg)
G1	AA	15	48.5^{a}±2.6	55	43
	AB	110	50.0^{a}±1.6	65	39
	BB	170	55.9^{b}±1.5	77	40
G2	AA	22	49.2^{a}±1.8	55	47
	AB	150	50.5^{a}±1.2	65	39
	BB	208	57.6^{b}±1.2	77	45
G1 and G2	AA	37	48.8^{a}±1.5	55	43
	AB	260	50.2^{a}±1.0	65	39
	BB	378	56.7^{b}±0.9	77	40

Data from table 3 showed that a positive effect of the BB genotype on egg number up to 38 weeks of age was found. The group of hens with BB genotype had higher egg numbers in each generation or overall generation compared to the AA or AB genotypes (55.9 vs 48.5 or 50.0eggs in the 1st generation; 57.6 vs 49.2 or 50.5eggs in the 2nd generation or 56.7 vs 48.8 or 50.2eggs in overall population examined).

Rastidout *et al*. (2019) reported that the *NPY/Dra*I polymorphism and its association with reproductive traits in Iranian Turkeys were identified. In the white Iraqi local chickens, the polymorphism of *NPY/Dra*I was also recognized with two genotypes (DD and II). The weight of the first egg, body weight at sexual maturity, was significantly higher in hens carrying the DD than in hens carrying

the II genotype of the Neuropeptide Y gene (Al-Zubaidi *et al*., 2023).

3.3. Effect of *PRL/Indel* and *NPY/Dra*I polymorphism on egg yield upto 38wks old

As the above data indicate the effect of genetic polymorphism of each gene on egg yield until 38 weeks of age. The PRL gene impacts on egg yield through regulation of laying and nesting behaviour (Manoharan *et al*., 2021; Rohmah *et al*., 2022). While, the NPY gene contributes to maturity age, body weight at first egg laying, the first egg weight which resulted to improve reproductive performances of birds (Rastidout *et al*., 2019). The combined effect of the two *PRL/Indel* and *NPY/Dra*I polymorphism was evaluated and presented in Table 4.

Table 4. Effect *PRL/Indel* and *NPY/Dra*I on egg 38w

Genotype	hen	Egg yield upto 38wks (egg)
ID-AA	1	43.0
II-AB	2	47.5
ID-AB	22	49.7
II-AA	2	50.0
DD-AA	34	51.5
DD-AB	236	53.0
II-BB	6	53.3
ID-BB	42	56.4
DD-BB	330	59.5

Data from table 4 indicate that there were 9 combined genotypes between PRL/Indel and NPY/DraI were obtained in overall generation of chicken examed. In which, a group of chicken bearing DD-BB genotype had the highest egg yield at 3 weeks of age (59.5 egg/hen), followed by lower in ID-BB (56.4 egg/hen). Interstingly, only two combined genotypes (DD-BB and ID-BB) had egg yield at 38 weeks of age higher than the average mean of examed herd and can be considered as molecular markers to support selection for improving egg production.

4. CONCLUSION

The polymorphism of PRL/*Indel* and *NPY/Dra*I had a positive effect on egg yield of Hac Phong chicken through two generations of selection. The female chickens bearing DD (*PRL/Indel*) or BB (*NPY/Dra*I) genotype had higher egg yield at 38 weeks of age. A group of chickens bearing DD-BB combined genotype had higher potential egg yield in the population examined. Polymorphism at the 24bp *PRL/indel* or *NPY/Dra*I site can be used for molecular-aided selection to improve egg production in Hac Phong chickens, requiring more in-depth study

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GENERATIONS AND HEN WEIGHTS AFFECTING TO THE REPRODUCTIVE OF NOI CROSSBRED LAYING HENS

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ABSTRACT

The objective of this study was to evaluate the effect of two selected generations and the weight of Noi crossbred laying hens on their reproductive performances. The experiment was carried out on 60 Noi laying hens and 1,625 eggs contained 977 eggs from generation G0 and 648 eggs from G1. In addition, the weight of Noi crossbred laying hens was divided into 2 groups, 1.15-1.55 and 1.60-1.90kg, respectively. The results showed that the final weight and average hatching time on the G0 generation (1.99kg and 21.36 days) were significantly higher than G1 (1.74kg and 20.38 days). Moreover, egg weight, percentage of eggs with embryos, and hatching rate, as well as the survival rate of chicks in G1, were all very significantly higher than in G0, while the two groups of hen weights were not found to be statistically significant differences. From the above research results, it can be suggested that the selected laying hens in the G1 generation improve the egg weight, the rate of eggs with embryos and hatchability, and the weight and survival rate of chicks after hatch.

Keywords: *Generations, hen weight, reproductive performances of Noi crossbred laying hens.*

1. INTRODUCTION

Noi chicken is a local chicken breed popularly raised in the Mekong Delta provinces due to its many advantages such as high disease resistance, yellow skin, firm, delicious meat, low fat, thick and well-fitted breast, good taste... However, the Noi chicken breed also has some disadvantages such as slow growth, low fertility, and a lot of cross-breeding.

Research results by Chau Thanh Vu (2018) showed that the first egg-laying age of hens was 178 days, the body weight (BW) of laying hens was 1.68kg, egg weight was 46.1g, egg yield was 100 eggs/hen/year, embryo egg rate was 84.3% and hatching rate was 72.1%. This result was higher compared to the survey results of households raising domestic chickens using traditional methods, hens incubate and raise chicks themselves, egg yield was about 40-50 eggs/hen/year and hatching rate was about 70-80% (Nguyen Van Quyen, 2010).

Nguyen Thi Kim Khang *et al.* (2020) published initial results on the maturity and egg productivity of Noi crossbred hens at the age of 18-26 weeks. Recent results of selective research on the Noi chicken breed show initial improvements in weight and meat yield of this chicken breed in the G1 compared to the G0 generation (Nguyen Thi Kim Khang *et al.*, 2022). However, there are still not many published results on the reproductive performance and hatching ability of the Noi crossbred. Therefore, this study was conducted to evaluate the effects of intergenerational selection and hen weight on the hatching rate and survival rate of chicks.

2. MATERIALS AND METHODS

2.1. Animals and management

The experiment was conducted on 1,625 eggs with a total of 60 Noi crossbred hens at 20-30 weeks of age in generation G0 and G1; and chicks. All experimental crossbred hens were fully vaccinated against infectious diseases and dewormed before the experiment started.

The feed provided for experimental chickens was 2,700 kcal/kg ME and 17% CP.

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2.2. Experimental design and data collection

A total of 1,625 chicken eggs were collected from 60 Noi crossbred hens with 30 hens in each generation G0 and G1. Besides, hens were arranged into two weight groups: 1.15-1.55 and 1.60-1.90kg. All chicken eggs must reach a weight of 37g or more before being incubated. Noi crossbred hens were raised in individual cages and were mated directly with roosters at a ratio of 1:3.

Chickens were fed twice a day, fed 33.3% of the diet at 8 am and 66.7% of the diet at 14.00PM. Water was available for free access. Feeders and drinkers were cleaned daily.

The experimental chickens were weighed at the beginning (20 weeks of age) and at the end (30 weeks of age) of the experiment. G0 and G1 generation chicken eggs were collected from the chicken farm in Vinh Long to the laboratory at Can Tho University. All eggs were left to stabilize for 1 day at room temperature before being put into the incubator.

Egg selection: Eggs were weighed and weight recorded (g), eggs weighing 37g or more were selected for incubation. Small eggs (weighing less than 37g), eggs with cracked or uneven shells, eggs that were too thick as well as too thin and rough, deformed eggs, too long or too short were all classified as rejected eggs.

Eggs were incubated using an automatic incubator (Life 1056, Vietnam). The incubator was cleaned and inspected before eggs are put into the incubator. The temperature of the incubator should be stable at 37°C, to balance the temperature inside the chicken embryo and create the best conditions for the embryo to hatch and develop. Pour water into the water tank.

Egg cleaning: eggs were cleaned by wiping with a soft towel to remove stains, blood and feces remaining on the surface of the egg shell before sterilizing the eggs.

Sterilization stage: eggs are placed in the egg tray and placed in the sterilization chamber with a UV light bulb, exposure time was 30 minutes and transferred to the incubator. Eggs were placed in trays to be put into the incubator, so the big end of the chicken egg must face up, not the other way around.

All selected eggs were put into the incubator and the egg turning mode was turned on, the eggs will be turned once every 30 minutes. Embryo eggs were examined on the 7^{th} day after incubation using a 45W LED lamp. Inspection of eggs with dead embryos was conducted on the the 18th day after incubation; Eggs were re-examined to remove dead eggs, which are eggs with a black spot stuck to the shell; to avoid generating CO_2 and CH_3 gases that affect the quality of egg incubation.

The hatching rate was recorded based on the number of hatched and unhatched eggs, and the number and weight of newly hatched chicks were also recorded. Chicks with deformities, exposed navels, etc. were recorded. The hatching date of the eggs was also recorded. After hatching, the chicks were transferred to the chicken farm in Vinh Long, raised in a brooding cage measuring 98x50x50cm and 50cm above the ground and the chicks were cared for and monitored for survival rate after 3 days.

All pre-incubation eggs and newly hatched chicks were weighed. In addition, other indicators were recorded such as the total number of eggs collected, total number of eggs incubated, total number of eggs culled, eggs with embryos, eggs with dead embryos, total number of chicks hatched, total number of chicks hatched with defects, the total number of chicks alive after hatching and the days from incubation to hatching for 2 generations.

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 One-way ANOVA

model. The mean values were compared using the Tukey method with 95% confidence intervals. The hatching rate of egg and survival rate, … of chicks were treated by Chi-Square test (Minitab Version 16).

3. RESULTS AND DISCUSSION

3.1. Effect of chicken generation on incubation criteria and survival rate of chicks

The results of table 1 showed that the incubation criteria of Noi crossbred hens show that the weight of hens at the beginning between G0 (1.56kg) and G1 (1.51kg) was not statistically different (P>0.05), but the final hen weight in G0 (1.99kg) was statistically significantly higher than in G1 (1.74kg, P<0.05). Similarly, the hatching time of eggs in G0 (21.36 days) was longer than in G1 (20.38 days, P<0.05). Although no statistically significant differences were found between G0 and G1 in the total number of eggs incubated/total number of eggs collected, culled egg rate, total number of eggs with embryos/total number of eggs collected, total number of eggs hatched/total number of eggs with embryos and the dead embryos/total number of eggs with embryos. However, these indicators tended to be higher in G1 than in G0, except for the rate of culled eggs and the rate of dead embryos (P>0.05).

The laying weight of both generations G0 (1.56 kg/hen) and G1 (1.51 kg/hen) in this study was lower than the research results of Chau Thanh Vu (2018), the weight of Noi hens was 1.68kg. In addition, the average hatching time of Noi chicken eggs in the current research results is similar to that reported by Nguyen Van Quyen (2010) which was 21.5 days. However, in the G1 generation, the hatching time of eggs started from day 19 to day 21, while in the G0 generation, the hatching time started from day 21 to day 22. This difference may be due to the influence of selection, environmental temperature and humidity,... The experimental result on the ratio between eggs hatched and eggs with embryos of both generations G0 and G1 (77.88 and 81.86%) were lower than that reported by Nguyen Van Quyen and Vo Van Son (2008) on Noi laying hens with the hatching rate was 93.84%. Research results by Chau Thanh Vu (2018) showed that the hatching rate of Noi chickens after 12 months of laying was 85.3%. The difference in hatching rate between experiments may be due to differences in rearing conditions, artificial or self-incubating methods of mother hens, the mating ratio of male/female, direct mating or artificial insemination, age of hens,... The results of this experiment show that there is an improvement due to genetic selection in the percentage of eggs with embryos and the hatching rate of Noi chickens.

Table 1. Incubation criteria of Noi hens between two generations

Parameters	Generations		P
	G0 (n=30)	**G1** (n=30)	
Initial weight of hen, kg	1.56±0.14	1.51±0.20	0.246
Final weight of hen, kg	1.99±0.15	1.74±0.29	0.000
Total number of eggs collected, egg	977	648	-
Total number of eggs incubated, egg	826	614	-
Total number of eggs incubated /Total number of eggs collected, %	85.31±17.65	91.53±18.30	0.194
Total number of eggs culled/Total number of eggs collected, %	14.66±17.65	8.47±18.80	0.194
Total number of eggs with embryos /Total number of eggs collected, %	43.65±22.67	47.01±23.67	0.576
Total number of eggs with embryos/Total number of eggs incubated, %	52.10±24.82	52.23±23.37	0.983
Total number of eggs hatched, egg	322	275	-
Total number of egg hatched/total number of eggs with embryos, %	77.88±19.44	81.86±19.32	0.429
Dead embryos/ total number of eggs with embryos, %	12.35±11.05	8.06±8.41	0.096
Average hatching time, day	21.36±0.48	20.38±0.69	0.001
Hatching rate on day 19, %	-	11.66	-
Hatching rate on day 20, %	-	38.33	-
Hatching rate on day 21, %	63.95	50	-
Hatching rate on day 22,%	36.04	-	-

The results in table 2 showed that there was no statistically significant difference between two generations in terms of egg weight (P>0.05). However, the weight of 1-day-old chicks, the survival rate of chicks after 3 days, and the mortality of chickens that died + deformed/total eggs hatched in G0 were statistically significantly lower at 30.34g, 96.52%, and 4.67% compared to G1 with 32.33g, 100%, and 0%, respectively (P<0.05). The result of the current experiment on the weight of 1-day-old chicks in G1 was higher than the previous results published by Nguyen Van Quyen (2009), Le Thi Hoa (2013), and Chau Thanh Vu (2018) on the weight of chicks (31.9g).

Table 2. Survival of chicks between two generations

Parameters	G0	G1	P
Egg weight, g	42.91±3.05	44.07±3.41	0.170
Chick weight, g	30.34±3.10	32.33±3.06	0.020
Survival rate, %	96.52±7.51	100±0.00	0.014
Deformed, dead rate, %	4.67±10.16	0.00±0.00	0.015

3.2. Effect of Noi hen weights on some reproductive performance

The results in table 3 showed that the egg weight (42.91-44.07g) of the two groups of hen weight was not statistically different (P>0.05). Similarly, chick weight, percentage of eggs with embryos, percentage of hatched eggs/total number of eggs with embryos and percentage of chicks alive after 3 days had no statistically significant differences between the two groups of hen weight (P>0.05). Although these parameters were not statistically significant between the two hen weight groups, the egg weight of this study was higher than the egg weight recorded in Noi chickens at 29.51-38.1g (Nguyen Van Quyen, 2010), but lower than reported by Chau Thanh Vu (2018) with egg weight of 45.7-48.2g. The current experimental result showed that there was a Pearson correlation between hen weight and chick weight but no correlation with egg weight. However, the comparison of the hen weight between the current experiment and the previous experiment shows that the hens in the current experiment are much lower than the results of Chau Thanh Vu (2018).

Table 3. Survival of chicks by hen weight

Variable	**Hen weights** (kg, n=30)		
	1.15-1.55	**1.60-1.90**	**P**
Egg weight, g	43.56±3.24	43.42±3.33	0.867
Chick weight, g	31.57±2.64	30.90±3.67	0.417
∑embryo eggs/∑incubated eggs,%	54.34±24.87	49.99±23.11	0.486
∑hatched eggs/∑embryo eggs,%	80.32±19.22	79.41±19.74	0.857
Survival rate, %	98.32±4.20	98.20±6.72	0.936

4. CONCLUSION

The G1 generation had a significant improvement in hatching time, chick weight and survival rate of chicks after hatching compared to the G0 generation. Meanwhile, hen weight did not affect hatching rate, egg weight, chick weight as well as their survival rate after hatching. Future studies need to evaluate the improvement of heritability of these criteria to select good Noi local chicken lines.

ACKNOWLEDGEMENTS

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PRODUCTION PERFORMANCE AND EGG QUALITY OF TAM HOANG CHICKENS AT 25-32 WEEKS OLD

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ABSTRACT

A total of 3.429 Tam Hoang breeder hensaged 25-32 weeks were used in this study to evaluate the production performance and egg quality at the present time - with the influence of new breeds. The birds were kept in a closed house, with the density of 0.33 m^2/bird and the male:female ratio of 1:8. The results showed that there were statistically significant differences ($P<0.05$) among the week ages for the production performance such as laying rate (49.93-60.81%), egg weight (51.3-54.98 g/egg), feed intake (143.24-176.09 g/bird/day), feed conversion ratio (2.79-3.24 g feed/g egg), percentage of double-yolked eggs (0.65-0.86%) and percentage of abnormal eggs (1.23-1.60%). The parameters of egg quality also showed that there were statistically significant differences ($P<0.001$) among the week ages in terms of yolk index (0.41-0.47), albumen index (0.01-0.08) and Haugh unit (71.90-89.27). These findings provide updated information about the production performance and egg quality of Tam Hoang breeder chickens, laying the foundation for further research and orienting chicken production.

Keywords: *Egg quality, Tam Hoang chickens, production performance, week ages.*

1. INTRODUCTION

In recent years, the poultry industry has encountered several challenges. The production cost increases unceasingly, while the market demand decreases, entailing animal backlogs and losses that may severely affect the poultry sector. As a result, many breeding facilities have reduced the flocks or temporarily stopped raising, causing farmers to struggle (General Statistics Office, 2021). However, in such circumstances, the animal production still maintains economic growth at a rate of 4-6%; and the production value increased from 20.35% to 25.2% compared to the values of other agricultural sectors. To obtain these achievements, it is worth mentioning the important contributions of science, technology and innovationwhich have improved the competitiveness of animal products in both domestic and international markets. For poultry, it is estimated that 29-30% of the value-added poultry products is generated owing to the scientific research and technological development. From then on, many different breeds of indigenous and exotic chickens have been selected and improved, playing a significant role in the socio-economic development of the country (Nguyen Duc Trong, 2023; Bui Huu Doan *et al*, 2024).

Tam Hoang is a breed of chicken with coloured featherand has been imported many times into our country from China. This is a dual-purpose poultry breed suitable for micro-climatic conditions of many regions as well as farming models/conditions in Vietnam. The contribution of Tam Hoang chickens to the breed structure is undeniable. Recently, many new Tam Hoang chicken lines have been introduced into our country via different ways, but there is a dearth of information on the characteristics of this breed. The aim of the current study was, therefore, to investigate the production performance and egg quality of Tam Hoang chickens in the early laying period.

2. MATERIALS AND METHODS

***Animal, time and location*:** A total of 3,429 Tam Hoang breeder hens aged 25-32 weeks old was used in this study from July to

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October 2024 on a Tam Hoang chicken farm in Ba Ria-Vung Tau province.

***Feeding and housing*:** The feed was mixed soybean meal, fish meal, broken rice, maize, rice bran, cassava, animal fat, vitamins and minerals. The chemical composition of the feed was crude protein (min) 21%, metabolizable energy (min) 2700 kcal/kg, crude fiber (max) 5%, calcium (min-max) 2.5-4.5%, total phosphorus (min-max) 0.5-2.0%, total lysine (min) 0.95%, methionine+total cysteine (min) 0.8% and moisture (max) 14%.

All birds, with a 1:8 ratio of male:female, kepting in a closed house (with temperature and humidity monitoring system) with a size of 14m×80m (0.33m^2 /bird), the floor was covered with a layer of rice husks. The system of automatic feeders, drinkers and nesting boxes were arranged vertically.

The birds were vaccinated against common diseases in the pullet stage, such as Marek (1 time), Gumboro (2 times), infectious bronchitis (2 times), arthritis (3 times), Coryza (2 times), avian influenza (3 times), pasteurellosis (1 time), and New Castle (3 times). There was also a program to provide an additional care for the chickens to prevent stress–related health problems.

***Parameters*:** The production traits included mortality (%), laying rate (%), egg weight (g/egg), daily feed intake (FI) , feed conversion ratio (FCR), percentage of double-yolked eggs (%) and percentage of abnormal eggs (%, typically classified as yolkless eggs and eggs with oddly shaped/soft/misshapen shells). The parameters of egg quality included egg yolk colour, eggshell thickness (mm), eggshell proportion (%), albumen proportion (%), egg yolk proportion (%), albumen/egg yolk, egg yolk index, albumen index, Haugh unit, and egg shape index. During the study, problems affecting the chickens' health were also observed and recorded.

***Statistical analysis*:** Data were processed using Excel software, and variance was analyzed using the general linear model (GLM) of Minitab 16. Differences between the means were compared using Tukey's test at a 5% significance level.

3. RESULTS AND DISCUSSION

3.1. Production performance

The results in table 1 showed statistically significant differences ($P<0.05$) among weeks of age in terms of laying rate, egg weight, FI, FCR, percentage of double-yolked eggs, and percentage of abnormal eggs. The chickens had *(i)* the lowest laying rate at the week 25 - the first stage after pullets. Then, it gradually increased in the following weeks, especially from the week 26 and reached the peak at the weeks 27, 28, and 32, *(ii)* Egg weight gradually increased from the week 27 and maintained at a stable level in the following weeks but reached the highest level at the week 32 of 54.98 g/egg, *(iii)* FI, FCR and percentage of double-yolked eggs also tended to gradually increase from 0.65 to 0.86%, similar to egg weight. However, this was an unexpected trend in poultry farming.

The FCR of Tam Hoang chickens in this study (2.79-3.24) was lower than the value of the F_1(Dong Tao x Luong Phuong) chickens, being 4.97 (Nguyen Van Duy *et al*., 2020), probably due to the difference in chicken strains. In addition, the egg weight of the F_1(Dong Tao x Luong Phuong) chickens at 38 weeks old was 52.48 g/egg (Nguyen Van Duy *et al*., 2020), similar to the value of Tam Hoang chickens in the period of 25-28 weeks old (52.16 g/egg) but lower than the value in the period of 29-32 weeks old (54.11 g/egg). There was probably a relationship between FI and egg weight although no correlation analysis was performed in this study; *(iv)* The percentage of abnormal eggs decreased with age, along with the maturation of the hens' reproductive system. In addition, the FI of the birds tended to increase, reflecting the continued growth after the pullet stage as well as the good health status of the flock.

Table 1. Production performance by weeks of age

Parameters	25	26	27	28	29	30	31	32	SEM	P
Mortality (%)	0.00	0.02	0.02	0.02	0.02	0.00	0.01	0.00	0.01	0.157
Laying rate (%)	49.93^{c}	56.48^{b}	60.30^{a}	60.81^{a}	58.79^{ab}	59.04^{ab}	59.37^{a}	60.23^{a}	0.61	0.000
Egg weight (g/egg)	51.30^{e}	51.63^{e}	52.44^{d}	53.26^{c}	53.33^{c}	53.76^{bc}	54.33^{ab}	54.98^{a}	0.16	0.000
FI (g/hen/day)	143.24^{c}	145.97^{c}	160.73^{b}	161.00^{b}	161.18^{b}	161.29^{b}	176.02^{a}	176.09^{a}	0.91	0.000
FCR (g feed/g eggs)	2.79^{c}	2.83^{c}	3.07^{b}	3.02^{b}	3.02^{b}	3.00^{b}	3.24^{a}	3.20^{a}	0.02	0.000
Double-yolked eggs (%)	0.65^{ab}	0.68^{ab}	0.67^{ab}	0.75^{ab}	0.64^{b}	0.72^{ab}	0.81^{ab}	0.86^{a}	0.05	0.032
Abnormal eggs (%)	1.60^{ab}	1.49^{ab}	1.51^{ab}	1.69^{a}	1.23^{b}	1.51^{ab}	1.57^{ab}	1.57^{ab}	0.09	0.034

Note: Means within a row followed by different superscripts are significantly different at 5% level (P<0.05).

The results in table 2 showed the increase in laying rate, egg weight, and FI of the birds over time (P<0.05). The FI gradually increased to meet the maintenance and production needs of the chickens, from 152.73 g/hen/day at 25-28ws to 168.90 g/hen/day at 29-32w. The laying rate was lower than 80.57-92.14% of Isa Brown chickens (Tran Thi Bich Ngoc *et al*., 2021) but higher than 46.43-43.21% of Van Linh chickens (Duong Thu Huong *et al*., 2024).

During the same period, the egg weight of Tam Hoang chickens (52.16-54.11 g/egg) was lower than the value of Isa Brown chickens (54.41-61.03 g/egg) (Tran Thi Bich Ngoc *et al*., 2021) but higher than that of Tau Vang chickens (35.89-47.36 g/egg) (Do Vo Anh Khoa, 2013), which was classified as small eggs (from over 50 to 55 g/egg) according to the size classification system of the national standard for chicken eggs (TCVN 1858:2018).

Table 2. Production performance by periods (weeks)

Parameters	25-28	29-32	SEM	P
Mortality (%)	0.02	0.01	0.00	0.074
Laying rate (%)	56.88^{b}	59.36^{a}	0.65	0.009
Egg weight (g/egg)	52.16^{b}	54.11^{a}	0.15	0.000
FI (g/hen/day)	152.73^{b}	168.90^{a}	1.53	0.000
FCR (g feed/g eggs)	2.93^{b}	3.12^{a}	0.02	0.000
Double-yolked eggs (%)	0.69	0.76	0.03	0.074
Abnormal eggs (%)	1.57	1.47	0.05	0.135

3.2. Egg quality

The results in Table 3 showed that most of the quality parameters were not significant different among weeks of age, except for the egg yolk index, albumen index, and Haugh unit (P<0, 05). These values also decreased with age period (Table 4). The average values of egg yolk index, albumen index, and Haugh unit were higher at 25-28 weeks of age than at 29-32 weeks of age. In overall, the Haugh unit varied in the range of 76.54-83.32 and higher than values of the fresh egg standard (>72) (TCVN 1858:2018).

Table 3. Egg quality by weeks of age

Parameters	25	26	27	28	29	30	31	32	SEM	P
Egg yolk colour	13.33	13,11	13.00	13.00	13.56	13.78	13.22	13.44	0.28	0.444
Eggshell thickness (mm)	0.35	0.33	0.35	0.34	0.34	**0.33**	0.34	0.34	0.01	0.702
Eggshell proportion (%)	11.64	11.34	11.68	11.65	11,13	11.76	11.36	11.38	0.23	0.492
Albumen proportion (%)	59.64	59.54	60.04	57,60	58.97	59.35	59.55	58.67	0.82	0.546
Egg yolk proportion (%)	27.64	28.13	27.23	29.52	28.59	28.06	27.72	28.70	0.72	0.437
Albumen: Egg yolk	2.17	2.13	2.21	1.98	2.09	2.14	2.16	2.05	0.08	0.520
Egg yolk index	0.45^{ab}	0.47^{a}	0.43^{bc}	0.45^{ab}	0.44^{abc}	0.45^{ab}	0.41^{c}	0.42^{bc}	0.01	0.000
Albumen index	0.08^{ab}	0.10^{a}	0.08^{ab}	0.08^{ab}	0.08^{ab}	0.08^{ab}	0.06^{b}	0.07^{b}	0.01	0.000
Haugh unit	81.03^{abc}	89.27^{a}	82.97^{ab}	79.99^{bcd}	80.10^{bcd}	80.39^{abcd}	71.90^{d}	73.77^{cd}	2.04	0.000
Shape index	77.14	78.21	76.83	77.32	76.80	79.92	78.13	76,72	1.16	0.523

Accordingly, the three parameters of egg yolk index, albumen index, and Haugh unit were indicators of the egg freshness which was governed by the laying stage (as shown in Table 4) and storage time, although most parameters of egg quality were largely dependent on the feed composition and breed factors (Bui Huu Doan *et al*, 2011). In this study, the egg yolk index (0.47), albumin index (0.47), and Haugh unit (89.27) of the

birds showed the highest value at 26 weeks of age, being significantly different compared to the values at other time points (P<0.05).

Table 4: Egg quality by periods (weeks old)

Parameters	25-28	29-32	SEM	P
Egg yolk colour	13,11	13.50	0.14	0.047
Eggshell thickness, mm	0.34	0.34	0.00	0.459
Eggshell proportion, %	11.58	11.41	0.11	0.295
Albumen proportion, %	59.20	59.13	0.41	0.905
Egg yolk proportion, %	28.13	28,27	0.36	0.792
Albumen: Egg yolk	2.12	2.11	0.04	0.842
Egg yolk index	0.45 ^{a}	0.43 ^{b}	0.00	0.001
Albumen index	0.09 ^{a}	0.07 ^{b}	0.00	0.000
Haugh unit	83.32 ^{a}	76.54 ^{b}	1.16	0.000
Shape index	77.38	77.89	0.58	0.529

The values in table 5 showed that small eggs had lower eggshell thickness (0.33mm), albumen proportion (57.17%), and albumen:egg yolk ratio (1.91) than the medium (Med) and large eggs (P<0.05). However, the yolk proportion accounted for the highest proportion (30.02%) in the small eggs.

Table 5. Egg quality by egg weight classification (g)

Parameters	Big 58-69	Med 54-57	Small 43-53	SEM	P
Egg yolk colour	13.25	13.21	13.21	0.17	0.544
Eggshell thickness,mm	0.35^{a}	0.34^{ab}	0.33^{c}	0.00	0.017
Eggshell rate, %	11.44	11.41	11.63	0.14	0.464
Albumen rate, %	60.66^{a}	59.67^{a}	57.17^{b}	0.40	0.000
Egg yolk rate, %	26.83^{b}	27.74^{b}	30.02^{a}	0.35	0.000
Albumen:Egg yolk	2.27^{a}	2.16^{a}	1.91^{b}	0.04	0.000
Egg yolk index	0.44	0.44	0.44	0.01	0.950
Albumen index	0.08	0.08	0.07	0.00	0.491
Haugh unit	79.98	80.34	79.46	1.59	0.926
Shape index	77.19	77,78	77.94	0.71	0.734

Eggshell thickness was related to the strength and degree of shell damage during egg packaging, transport, incubation, storageand and also affected the hatchability and quality of the chicks. Normally, eggshell thickness varied between 0.2-0.6 mm, preferably >0.32 mm. The average eggshell thickness of Tam Hoang chickens at the ages of 25 and 32 weeks in this study ranged from 0.34 to 0.35 mm, similar to the value of Ri chickens (Hoan *et al*., 2016) but higher than that of commercial Isa Brown chickens (Tran Thi Bich Ngoc *et al*., 2021). In general, it could be seen that the egg quality traits of Tam Hoang chickens were within the recommended standards for chicken eggs.

3. CONCLUSION

Tam Hoang chickens at the age of 25-32 weeks had the laying rate of 49.93-60.81%, egg weight of 51.3-54.98 g/egg, FI of 143.24-176.09 g/hen/day and FCR of 2.79-3.24 g feed/g eggs. Some parameters of egg quality (yolk index 0.41-0.47; albumen index 0.06-0.10 and Haugh unit 71.90-89.27) were significantly different among weeks of age, but all were within the acceptable standards for chicken eggs.

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GROWTH PERFORMANCE AND CARCASS TRAITS OF LANDRACE AND YORKSHIRE GILTS RAISED AT KBANG FARM, GIA LAI PROVINCE

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ABSTRACT

The aim of this research was to estimate growth rate and carcass traits of Landrace and Yorkshire gilts at Kbang breeding farm, Mavin Group Joint Stock Company in Gia Lai province, South Vietnam, from July 2022 to June 2023. A total of 324 gilts (125 Landrace and 199 Yorkshire) were recorded from the birth to the end of the fattening period (190.19±15.36 days). Body weight at birth (BWB), at the end of fattening period (BWF) were individually recorded. Backfat thickness (BFT), depth of longissimus muscle depth (LMD), longissimus muscle area (LMA), intramuscular fat content (IMF) and lean meat percentage (LMP) were predicted using ultrasound. For Landrace and Yorkshire gilts respectively, BWF (127.67 and 126.11kg), ADG (661.45 and 654.41g/day), LMP (58.66 and 59.11%) and IMF (2.65 and 2.66%) were similar between the two breeds (P>0.05). LMD (59.02 and 57.18mm) and LMA (55.66 and 54.15cm^2) of Landrace were higher than those of Yorkshire (P<0.05). The same trend (P<0.1) was observed for BFT (13.93 and 13.11mm). Relationships between traits were the same tendency for both breeds.

Keywords: *Correlation, growth rate, swine, ultrasound.*

1. INTRODUCTION

In Vietnam, breeding program in pig farms is applied pyramid model, including great-grandparents (GGP), grandparents (GP) and parents (PS). GGP and GP are pure breeds such as Yorkshire, Landrace, Duroc and Piétrain. In the period from 2017 to 2022, 240 breeding establishments, including public and private produced locally about 89% GGP and GP pigs (Livestock Department, 2024). Landrace and Yorkshire pigs had been using popular as female lines under industrial conditions. The reproductive performance of these females lines was reported in Luu Van Thang *et al.* (2021), Nguyen Thi Hong Nhung *et al.* (2020a) and Ha Xuan Bo and Do Duc Luc (2020) and Doan Phuong Thuy *et al.* (2015). While productive performance and carcass traits also published in studies of Santiago *et al.* (2021), Nguyen Thi Hong Nhung *et al.* (2020b), Alam *et al.* (2021), and Luu Van Trang *et al.* (2019). Mavin Farm, originating from the Swine Breeding Department of the Mavin Group Joint Stock Company, currently operates five nucleus pig breeding centers with over 4,000 breeding pigs (Mavin, 2024). Therefore, investigating the performance of these breeding animals is important. This research aims to estimate the growth rate and carcass traits of Landrace and Yorkshire gilts at a breeding farm belonging to the Mavin Group Joint Stock Company.

2. MATERIALS AND METHODS

2.1. Experiment design

The experiment was conducted at Kbang farm in Gia Lai province, South Vietnam, from July 2022 to June 2023. A total of 324 gilts (125 Landrace and 199 Yorkshire) were recorded from the birth to the end of the fattening period (190.19±15.36 days).

At birth, each piglet was identified by ear tattoo and body weight (BWB) was recorded. At the end of fattening period, the bodyweight (BWF) was individually recorded by the electronic scale Kelba (Australia). At the same time, ultrasound images were taken 6 cm from the dorsal midline at the last ribs using Exago

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ultrasound device with L3130B probe (IMV, France). Two types of images were scanned: (1) longitudinal was taken parallel to the dorsal midline for estimating back fat thickness (BFT, mm), depth of *longissimus* muscle (LMD, mm) and intramuscular fat content (IMF, %); and (2) transverse was taken perpendicular to the midline for predicting area of *longissimus* muscle (LMA, cm^2). These traits predicted by using Biosoft Toolbox II for Swine from ultrasound images. Lean meat percentage (LMP) was predicted from BFT and LMD using a regression equation recommended by Ministère des Classes Moyennes et de l'Agriculture (1999): $Y=59.902386-1.060750X_1+0.2229324X_2$; *where Y=LMP (%), X_1=BFT (including skin), X_2=LMD. ADG (g/day) was calculated by BWB, BWF, age (AGE, day).*

The feed was supplied by Mavin Austfeed Joint Stock Company. The nutrition information according to periods is presented in Table 1. Pigs had free access to water by nipple drinkers and were fed *ad libitum*.

Table 1. Nutrition feed information according to periods

Period	ME (Kcal/kg)	Protein (%)	Fiber max (%)	Ca (%)	P (%)	Lysin (%)
7-days age to weaning	3300-3400	20.0-21.0	-	0.6-1.2	0.5-1.0	1.4-1.5
From weaning to 15 kg	3300-3400	19.0-20.0	-	0.6-1.2	0.5-1.0	1.35-1.40
From 15 to 60 kg	3100-3200	18.5-19.5	6.00	0.6-1.2	0.5-1.0	1.1-1.2
From 60 to the end of fattening	3100-3200	16.5-17.5	6.00	0.6-1.2	0.5-1.0	1.05-1.1

2.2. Data analysis

The data were analysed with the statistical model $Y_{ij} = \mu + B_i + A_j + \varepsilon_{ij}$, *where Y_{ij} = study trait, μ = overall mean, B_i = breed effect (i=2, Landrace or Yorkshire), A_j = covariate effect (age of gilts) and ε_{ij} = random error*. Number of observations (n), least square mean (LSM), standard error (SE) and coefficient of determination (R^2, %) are presented in the result session. The Pearson correlation coefficient (r) between studied traits was calculated. Statistical significance level was preset at $P<0.05$ while a trend was confirmed at $0.05 \le P < 0.1$. All data were performed SAS® OnDemand for Academics.

3. RESULTS AND DISCUSSION

3.1. Performance of gilts

The effects of breed as fixed factor and age at the fattening period as covariate are presented in table 2. The BWB, LMD and LMA were significantly different between two breeds ($P<0.05$). For BFT, the trend of difference between breeds was observed ($P=0.0533$). While the age affected almost traits ($P<0.05$) except IMF ($P=0.1098$). The growth performance and carcass traits according to breeds are shown in Table 3.

Table 2. Effect of Breed and Age on performance of Landrace and Yorkshire gilts

Variable	Breed	Age	R^2 (%)
BWB	0.0114	-	1.97
BWF	0.3857	<.0001	37.70
ADG	0.4505	0.0290	1.53
BFT	0.0533	<.0001	12.06
LMD	0.0045	<.0001	9.58
LMP	0.3082	<.0001	6.96
LMA	0.0154	0.0031	30.82
IMF	0.8783	0.1098	2.05

R^2 = coefficient of determination, values in the table are probability

Table 3. Growth performance of gilts

Variable	Landrace			Yorkshire		
	n	LSM±	SE	n	LSM±	SE
BWB (kg)	125	1.64^a	±0.03	199	1.55^b	±0.02
BWF (kg)	125	127.67	±1.40	199	126.11	±1.11
ADG (g/ day)	125	661.45	±7.27	199	654.41	±5.75
BFT (mm)	125	13.93^A	±0.33	199	13.11^B	±0.26
LMD (mm)	125	59.02^a	±0.50	199	57.18^b	±0.40
LMP (%)	125	58.66	±0.34	199	59.11	±0.27
LMA (cm^2)	125	55.66^a	±0.48	199	54.15^b	±0.38
IMF (%)	46	2.65	±0.09	84	2.66	±0.06

Within a row, LSM followed by different letters are significantly different (P<0.05 for small letters and P<0.10 for capital letter)

BWB of Landrace was higher than that of Yorkshire (P=0.0114). BWF and ADG were similar between Landrace and Yorkshire (P>0.05). The results of table 3 show that, LMD and LMA of Landrace were higher than those of Yorkshire (P<0.05). The same trend was observed for BFT of Landrace (13.93mm) and Yorkshire (13.11mm).

ADG was significantly between Landrace and Yorkshire while BFT and LMD were similar (Lưu Văn Tráng *et al.*, 2019). This tendency was observed inversely in comparison with our study. This difference might be related to BWF in two studies. In a study of Nguyen Thi Hong Nhung *et al.* (2020b), ADG, BFT and LMP were different between Landrace and Yorkshire from French genetic resources. These authors also confirmed that IMF did not different between breeds. This result was consistent with our study. The difference was not observed between breeds for ADG and BFT (Doan Phuong Thuy *et al.*, 2016; Zebua *et al.*, 2017), which was consistent with our results.

3.2. Phenotypic correlation between economic traits

Table 4. Phenotypic correlation between traits of Landrace and Yorkshire gilts

Variable	BWF	ADG	BFT	LMD	LMA	LMP	IMF	AGE
BWF		0.85 <.0001 125	0.74 <.0001 125	0.51 <.0001 125	0.42 <.0001 125	-0.61 <.0001 125	0.25 0.0932 46	0.66 <.0001 125
ADG	0.86 <.0001 199		0.63 <.0001 125	0.42 <.0001 125	0.43 <.0001 125	-0.53 <.0001 125	0.12 0.4083 46	0.18 0.0501 125
BFT	0.67 <.0001 199	0.65 <.0001 199		0.37 <.0001 125	0.27 0.0024 125	-0.94 <.0001 125	0.44 0.0022 46	0.48 <.0001 125
LMD	0.52 <.0001 199	0.47 <.0001 199	0.29 <.0001 199		0.88 <.0001 125	-0.04 0.6467 125	0.03 0.8384 46	0.34 0.0001 125
LMA	0.48 <.0001 198	0.49 <.0001 198	0.31 <.0001 198	0.84 <.0001 198		0.02 0.7875 125	0.11 0.4468 46	0.16 0.0662 125
LMP	-0.52 <.0001 199	-0.52 <.0001 199	-0.95 <.0001 199	0.03 0.629 199	-0.04 0.5474 198		-0.42 0.0035 46	-0.39 <.0001 125
IMF	0.40 0.0002 83	0.42 <.0001 83	0.61 <.0001 83	0.04 0.7359 83	0.05 0.6767 83	-0.59 <.0001 83		0.27 0.0717 46
AGE	0.59 <.0001 199	0.09 0.201 199	0.26 0.0002 199	0.26 0.0002 199	0.16 0.0209 198	-0.18 0.0096 199	0.08 0.4773 83	

Values above and below diagonal are for Landrace and Yorkshire respectively; In each cell, first, second and third are correlation coefficient, p-value and number of observations, respectively

Relationship between traits were the same tendency for both Landrace and Yorkshire. A positive correlation between BWF, ADG and BFT, LMD, LMA was observed (0,42 to 0.74). It means that increasing BWF and ADG lead to increasing BFT, LMD, LMD. Inversely, LMP was negatively correlated with BWF (-0.52 to -0.61) and ADG (-0.52 to -0.53). Relationship between IMF and BWF, ADG did not observe (P>0.05). AGE was positively correlated with BFT (0.26 to 0.48), LMD (0.26 to 0.34), inversely negatively correlated with LMP (-0.39). Increasing AGE lead to decreasing LMP. BFT was strongly correlated with LMP (P<0.01) and high negative correlation (-0.94 to -0.95). However, the correlation did not exist between LMP and LMD, LMA (P>0.05).

Negative correlation between LMP and ADG, BFT was observed for Landrace and Yorkshire; while positively correlated with LMA (Klimas and Klimienė, 2009, Lopez *et al.*, 2018). There were no relationships between AGE and BFT for Yorkshire (Dube *et al.*, 2013). The same trendency was observed in our study. Genetic relationship between growth and carcass traits in Large White pigs. However, strong correlation between AGE and ADG (Dube *et al.*, 2013), no relationship between LMP, LMA (Dube *et al.*, 2013) and ADG, LMP (Luu Van Trang *et al.*, 2019) were found. It was contrary in comparison to our study where the relationship between these traits was not observed. The opposite between two these studies could be explained by AGE in these studies (134.64 to 190.19 days). BWF was strongly correlated with BFT, LMD and LMP (Klimas and Klimienė, 2009); which was similar to our results. Hoa *et al.* (2021) concluded that increasing BFT decreased LMP and LMA.

4. CONCLUSIONS

BWF, ADG, LMP and IMF were similar between the two breeds, while LMD and LMA of Landrace were higher than those of Yorkshire. The same trend was observed for BFT). Relationships between traits were the same tendency for both breeds.

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EFFECTS OF A POLYMORPHIC SITE IN MYOSTATIN GENE ON GROWTH PERFORMANCE OF BEEF CATTLE

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ABSTRACT

This study investigates the effects of a polymorphic site (*MSTN_Dra*I) in the myostatin gene on the growth performance of beef cattle. Using 240 F_2 crossbred cattle of 8 groups, the research assessed growth traits across different genotypes (AA, AB, BB) of the MSTN gene. The results showed that at the day of 360, animals with the AB genotype had an average body weight of 286.3kg, those with the AA genotype had an average body weight of 282.3kg, and those with the BB genotype had an average body weight of 271.1kg. Average daily gains from birth to 360 days were 712, 702 and 660 g/day for AB, AA, and BB genotypes. The homozygous (AA) and heterozygous (AB) genotypes showed the best outcomes in cattle weight at 360 days and average weight gain. The high frequency of allele A (0.81) suggests that phenotypic selection has influenced the genotype distribution within the herd. Cattle with superior genotypes exhibit better growth performance; therefore, breeding selection based on the *MSTN_Dra*I marker is expected to lead to further improvements in this trait.

Keywords: *Beef cattle, myostatin gene, single nucleotide polymorphism, growth.*

1. INTRODUCTION

To meet the growing demand for animal products, it's important to improve the growth traits of livestock (Hua *et al.*, 2009). Breeders use traditional selection methods based on an animal's physical traits to achieve this, but there's a new approach called marker-assisted selection that can help improve genetic traits.

Myostatin (*MSTN*) is a protein that limits muscle growth (Lu *et al.*, 2007; Beyer *et al.*, 2013). Research has shown that animals lacking myostatin have significantly larger muscles (McPherron *et al.*, 1997). Certain cattle breeds exhibit a "double muscling" phenotype, characterized by an increased number of normal-sized muscle fibers, resulting in bigger muscles with deeper grooves. This phenotype is linked to six specific mutations in the *MSTN* gene that render it nonfunctional (Karim *et al.*, 2000). Both cattle with one and two copies of these mutations (homogeneous and heterogeneous individuals, respectively) display increased muscle mass, higher birth weight, and faster growth rate (Casa *et al.*, 2004). *MSTN* mutations have been found in various species, including dogs (Mosher *et al.*, 2007), sheep (Bozhilova-Sakova *et al.*, 2022), cattle (Grobet *et al.*, 1997), pigs (Stinckens *et al.*, 2008), and even one human (Schuelke *et al.*, 2004).

Understanding these genetic variations can potentially enhance cattle growth and carcass traits. The *MSTN* gene demonstrates significant promise for improving cattle performance, especially in local populations that have not undergone intensive selection. However, limited information exists regarding the application of the *MSTN* gene in crossbred cattle, necessitating further investigation.

2. MATERIALS AND METHODS

2.1. Animals

The experiment was conducted on 240 F_2 beef cattle across eight different breed groups, with equal distribution by sex. The F_1(BBB×BR), F_1(CH×BR), F_1(BBB×RA) and F_1(CH×RA) female cattle were artificially inseminated with semen of BBB and

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Charolais bulls. These cattle were raised under household farming conditions in four districts: Chau Thanh, Cho Gao, Go Cong Tay, and Tan Phu Dong in Tien Giang province. The breed of the F_1 cattle was determined based on physical characteristics, and information from the owners, and local veterinary officials.

The animals were raised using a confinement system with additional feeding provided in the barn. The supplemental feed consisted of concentrate and rice bran, with roughage feed based on natural grass, elephant grass, and locally available by-products. The cattle were vaccinated against various diseases, including hemorrhagic septicemia, foot-and-mouth disease, and lumpy skin disease. They had free access to clean water.

2.2. Collected parameters

The weight of newborn calves was determined using a dial scale. At subsequent stages (90, 180, 270 and 360 days old), the cattle were measured for chest circumference and diagonal body length to estimate their weight using the following formula: W=88.4×(heart girth, m)^2×body length (m).

For genotyping, 240 blood samples were collected from the cattle's ears and stored in EDTA K2/K3 HTM tubes at -20°C. DNA was extracted from the blood samples using the TopPURE® Viral DNA/RNA extraction kit (HI-172) from ABT Company. The *MSTN* gene was genotyped using primers and the restriction enzyme *Dra*I, as referenced from Zhang *et al.* (2007).

2.3. Statistical analysis

The data were analyzed using the General Linear Model (Minitab ver16.0), incorporating factors that influence cattle weight gain into the model: $Y_{ijklm}=\mu+B_i+S_j+G_k+D_l+\varepsilon_{ijklm}$. *Where, Y_{ijklmn}: the observation of the trait, μ: the least square mean, B_i: the effect of breed, S_j: the effect of sex, G_j: the effect of genotype at DraI locus, D_l: the effect of the district where cattle were raised, ε_{ijklm} is the residual effect.*

3. RESULTS AND DISCUSSIONS

3.1. Growth performance of different crossbred cattle

The data presented in table 1 indicate a statistically significant variance in the cumulative growth of F_2 crossbred calves throughout the survey period ($P<0.05$). Calves born from cows incorporating the BBB breed in the crossbred formula exhibited higher birth weights compared to others, a trend that persisted until the calves reached 360 days of age. The weights of the animals at birth and at 360 days of age were positively correlated with the proportion of BBB in the crossbred formula. Conversely, crossbreeds with Charolais demonstrated lower birth weights and weights at 360 days of age, corresponding to the proportion of Charolais in the hybrid formula.

According to research conducted by Nguyen Thi Nguyet and Bui Dai Phong (2015), the F_1(BBB×LS) reached a weight of 326.4kg at 360 days old. For F_2 calves, research by Nguyen Thi Nguyet *et al.* (2020) indicated that F_2 calves BBB×F_1(BBB×LS) achieved a birth weight of 30.6kg and 202.6kg at 180 days. In addition, Tran Bich Phuong *et al.* (2021) documented that F_2 calves BBB×F_1(BBB×LS) weighed 198.90kg at 180 days old and reached 356.05kg at 360 days old. Based on the results of our study, the body weight of F_2 crossbred calves at the same age is generally lower than that reported in previous domestic and international studies. This discrepancy is likely due to the data being collected at the household level, where feed and raising conditions varied from farm to farm.

The growth rates of the cow population in the study demonstrated significant differences between crossbreed combinations in most stages, except for the period from 180 to 270 days old, as illustrated in table 1. Our findings indicate that calves in crossbreeds

with BBB cows exhibited higher average growth rates from birth to 360 days old in each specific stage. Moreover, our analysis reveals a general trend of higher growth rates in the early stages from birth to 180 days of age (averaging 700 g/head/day for hybrid combinations) and a subsequent decrease in the period from 180 days to 360 days of age (averaging 690 g/head/day for hybrid combinations).

Table 1. Body weight (kg) **and average daily gain** (g/head/day) **of different crossbred cattle breeds**

Breed	Breed	At birth	90 days	180 days	270 days	360 days
BW (kg)	BBB×(BBB×BR)	30.3^{a} ±0.68	95.2^{ab}±2.8	159.3±3.2	218.0^{abc}±3.2	294.0^{a}±4.1
	CH×(BBB×BR)	31.1^{a} ±0.69	99.5^{a}±2.8	160.9±3.2	224.0^{a}±2.4	290.1^{ab}±4.2
	BBB×(CH×BR)	29.1^{ab}±0.68	93.6^{ab}±2.8	157.3±3.2	216.4^{abc}±2.3	285.7^{ab}±4.1
	CH×(CH×BR)	27.8^{b} ±0.69	77.6^{d}±2.8	151.2±3.2	209.9^{c}±2.4	261.3^{c}±4.2
	BBB×(BBB×RA)	30.8^{a} ±0.66	94.2^{ab}±2.7	159.6±3.1	219.8^{ab}±2.3	290.0^{ab}±4.0
	CH×(BBB×RA)	30.4^{a} ±0.57	86.8^{bcd}±2.3	156.1±2.7	211.6^{bc}±2.0	274.4^{bc}±3.5
	BBB×(CH×RA)	30.3^{a} ±0.65	88.1^{bc}±2.8	150.8±3.1	213.5^{bc}±2.3	280.5^{ab}±4.0
	CH×(CH×RA)	29.4^{ab}±0.62	82.2^{cd}±2.5	150.0±2.9	209.8^{c}±2.1	264.4^{c}±3.7
	P	0.001	0.001	0.030	0.001	0.001
		Birth-90d	**90-180d**	**180-270d**	**270-360d**	**Birth-360d**
ADG (g/head/day)	BBB×(BBB×BR)	721^{ab}±30	712^{ab}±38	653±36	845^{a}±40	733^{a}±11
	CH×(BBB×BR)	760^{a}±31	683^{b}±38	701±37	734^{ab}±40	720^{ab}±11
	BBB×(CH×BR)	717^{ab}±30	707^{ab}±37	657±36	770^{a}±40	713^{ab}±11
	CH×(CH×BR)	554^{c}±31	818^{a}±38	652±37	571^{c}±40	649^{c}±11
	BBB×(BBB×RA)	704^{ab}±30	727^{ab}±37	669±35	780^{a}±39	720^{ab}±11
	CH×(BBB×RA)	626^{bc}±26	771^{ab}±32	616±31	697^{abc}±34	678^{bc}±10
	BBB×(CH×RA)	643^{bc}±29	696^{ab}±36	697±35	744^{a}±38	695^{ab}±11
	CH×(CH×RA)	587^{c}±28	775^{ab}±34	642±33	607^{bc}±36	653^{c}±10
	P	0.001	0.023	0.535	0.001	0.001

BR: Brahman, RA: Red Angus, BBB: Belgian Blue, CH: Charolais.

Within a column, means followed by different letters are significantly (P<0.05).

3.2. DNA amplification, polymorphism, and allele frequencies

The coding region of the *MSTN* gene was successfully amplified through PCR, yielding a DNA fragment measuring 1,346 base pairs. This result confirmed the high specificity of the amplified fragment, which was subsequently submitted for phenotype determination using the RFLP technique. The polymorphism of MSTN-*Dra*I was observed following enzyme digestion of the products. This polymorphic site of MSTN-*Dra*I is attributed to a T/A transversion at position -371 (relative to the ATG start codon), creating a recognition site for the *Dra*I restriction enzyme (Crisa *et al*., 2003). Enzyme digestion of the *MSTN* promoter PCR fragment with *Dra*I produced fragments of 505, 415, and 325bp for phenotype AA; 505, 350 and 325bp for phenotype BB; and 505, 415, 350, and 325bp for phenotype AB (Figure 1).

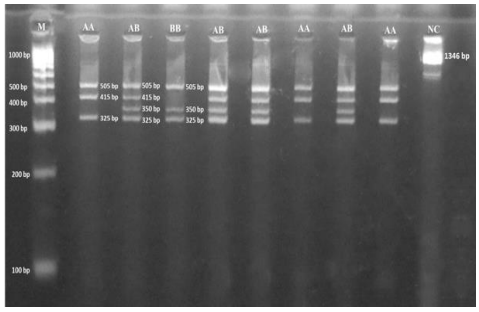

Figure 1. Agarose gel electrophoresis (3%) **of PCR fragment of *MSTN* gene digested with *Dra*I**

M is the DNA HyperLadder™ 100bp marker, NC is the negative control

Across all bovine breeds, the frequency of allele A (0.81) was found to be predominant. Genotype frequencies were 0.67, 0.29, and 0.04 for AA, AB, and BB, respectively (Table 2). Notably, only 10 cattle were found to exhibit the homozygous phenotype BB. Observed (Ho) and expected (He) heterozygosity for *MSTN-Dra*I were 0.29

and 0.30, respectively. It was observed that the genotype frequencies of *MSTN* in the bovine populations did not follow the Hardy-Weinberg equilibrium. Specifically, the chi-square value for this population (0.65) fell below 3.84 (the chi-square value at 1 degree of freedom and P-value of 0.05), leading to the rejection of the hypothesis that the observed and expected values are equivalent.

Table 2. Allele and genotype frequencies at *MSTN_Dra*I locus in 240 F2 beef cattle

Genotype	Number	Genotype frequencies		Allele frequencies		Ho	He	X^2
		Observed	Expected	A	B			
AA	160	0.67	0.66					
AB	70	0.29	0.30	0.81	0.19	0.29	0.30	0.65
BB	10	0.04	0.04					

Note: He = expected heterozygosity; Ho = observed heterozygosis; $\chi 2$ = Hardy–Weinberg equilibrium; not significant at α 5% (χ^2 obs ≤ 3.84); n=240 heads.

Previous investigations utilized the *Dra*I restriction enzyme to analyze polymorphism frequency within the *MSTN* gene across various bovine breeds (Table 3). Significant inter-breed heterogeneity was observed in genotype frequencies. The AA genotype was the most prevalent across most breeds, with an average frequency of 0.79±0.21, followed by the AB genotype with an average frequency of 0.16±0.11. The BB genotype exhibited the lowest frequency, averaging 0.05±0.14. Reflecting this genotype pattern, the frequency of allele A (0.87±0.17) was significantly higher compared to allele B (0.13±0.17). The elevated standard error values highlight the substantial variation in *MSTN-Dra*I genotype and allele frequencies across the studied breeds.

Table 3. Polymorphism of *MSTN* gene in different cattle breeds

Breed	Allele frequency		Genotype frequency			References
	A	B	AA	AB	BB	
Belgian Blue	0.21	0.79	0.03	0.34	0.62	Crisa *et al.* (2003)
Brown Swiss	0.85	0.15	0.77	0.15	0.08	
Chianina	0.86	0.14	0.73	0.28	0.00	
Holstein	0.92	0.08	0.84	0.16	0.00	
Limousine	0.95	0.05	0.90	0.10	0.00	
Marchigiana	0.85	0.15	0.73	0.24	0.04	
Italian Red Pied	0.95	0.05	0.91	0.09	0.00	
Piedmontese	0.94	0.06	0.88	0.12	0.00	
Romagnola	0.86	0.14	0.71	0.29	0.00	
Nanyang	0.95	0.05	0.91	0.09	0.00	Zhang *et al.* (2007)
Qinchuan	0.97	0.03	0.94	0.06	0.00	
Jiaxian	0.96	0.04	0.93	0.05	0.02	
Holstein	0.97	0.03	0.95	0.05	0.00	Han *et al.* (2012)
Jeju Black	0.78	0.22	0.63	0.31	0.06	
Hanwoo	0.88	0.12	0.77	0.21	0.02	
Holstein	1.00	0.00	1.00	0.00	0.00	Nasr *et al.* (2016)
Holstein	0.97	0.03	0.94	0.06	0.00	Fadhil & Zülkadir (2020)
Brown Swiss	0.88	0.12	0.76	0.23	0.00	
Cross-breeds	0.81	0.19	0.67	0.29	0.04	Current study
Mean	0.87±0.17	0.13±0.17	0.79±0.21	0.16±0.11	0.05±0.14	

The study identified breed-specific patterns in genotype and allele frequencies. Although the overall results generally aligned with expected trends, the cattle population under study exhibited distinct patterns in these frequencies. This suggests potential breed-specific selection pressures or unique genetic backgrounds. Further investigation could explore the underlying reasons for these breed-specific patterns and determine if they are associated with particular cattle breeds or functional traits.

Additionally, the study could examine the potential impact of *MSTN_Dra*I polymorphisms on cattle breeding programs aimed at enhancing muscle growth or other economically important traits.

3.3. Association *MSTN* gene with growth traits

Table 4 presents the impact of the *MSTN* gene on the growth traits of cattle, evaluating body weight at various stages and daily weight gain over specific intervals for three different genotypes. Significant differences in body weight were observed at 90, 180, and 360 days, with genotype AB generally exhibiting higher weights than BB, while AA displayed intermediate values. For daily weight gain, significant differences were noted from birth to 90 days and from birth to 360 days, with genotype AB consistently showing higher gains. However, the study included only ten animals with the BB genotype, complicating direct comparisons of growth traits among genotypes. The results suggest that phenotypic selection in natural populations may have affected the ratio of BB cattle due to their lower growth rate.

Table 4. Effects of *MSTN* gene on BW,WG
(LSM±SE)

Age	AA	AB	BB	P
At birth	29.8±0.27	29.8±0.38	30.1±0.98	0.943
90d	92.4^{ab}±1.09	93.5^{a}±1.57	83.1^{b}±4.01	0.049
180d	160.2^{a}±1.3	158.6^{ab}±1.8	148.9^{b}±4.6	0.048
270d	217.9±0.9	217.6±1.3	210.7±3.4	0.112
360d	282.3^{ab}±1.6	286.3^{a}±2.3	271.1^{ab}±5.9	0.038
Birth-90d	695^{a}±12	708^{a}±17	589^{b}±44	0.038
90-180d	754±15	723±21	731±54	0.414
180-270d	641±14	656±20	687±52	0.588
270-360d	720±15	764±22	671±57	0.117
Birth-360d	702^{ab}±5	712^{a}±6	669^{b}±16	0.032

Within a row, means followed by different letters are significantly (P<0.05)

The *MSTN* gene acts as a negative regulator of skeletal muscle growth, protecting the muscle system (Crisa *et al.*, 2003; Agrawal *et al.*, 2017). Polymorphisms in the promoter region that reduce myostatin expression can lead to increased muscle mass and growth. The change in nucleotide of *MSTN_Dra*I locus might exhibit reduced myostatin activity, resulting in enhanced muscle development and higher growth rates compared to cattle with genotypes that do not reduce myostatin expression. Han *et al.* (2012) noted that *MSTN* influences carcass traits, with higher meat quality and fat color indexes in the heterozygous genotype. Furthermore, Zhang *et al.* (2007) found correlations between *MSTN* genotypes and growth traits in Nanyang cattle, with the AB genotype showing superior heart girth, heart girth index, withers height, and heart girth to body length ratio. Likewise, Allais *et al.* (2010) linked *MSTN* to meat and carcass quality in French cattle, with heterozygous genotypes having reduced fat and collagen and more tender meat. Additionally, Khasanah *et al.* (2016) observed *MSTN* polymorphisms affecting growth traits in Bali cattle, with one polymorphism leading to higher body weight at 365 days in the heterozygous genotype. Sarti *et al.* (2014) showed that the TT genotype (AA in this study) promoted double muscling in Marchigiana cattle. In the same trend, Nugroho *et al.* (2017) reported *MSTN* polymorphism affecting growth traits in Bali cattle, with the BB genotype excelling in most traits except chest circumference, where AB was superior. Finally, Fadhil and Zülkadir (2020) found the AB genotype correlated with higher final fattening body weights in Holstein cattle and the AA genotype linked to increased live weight gain in Brown Swiss cattle. In short, the diverse effects of the *MSTN* polymorphism across different cattle breeds highlight the potential of this genetic marker in optimizing growth traits and meat quality through targeted breeding programs.

4. CONCLUSIONS

During the observed period from birth to 360 days of age, the crossbreed combination incorporating BBB cows exhibited superior performance in both body weight and daily weight gain. Analysis of the genotype and allele frequency of the *MSTN-Dra*I polymorphism revealed a departure

from Hardy-Weinberg equilibrium within the studied cow population. Among the three genotypes examined, the homozygous (AA) and heterozygous (AB) genotypes demonstrated the most favorable outcomes in cattle weight at 360 days of age and average weight gain throughout the study. The high prevalence of these genotypes indicates that phenotypic selection has shaped the herd; consequently, incorporating this polymorphic site into the breeding program is anticipated to enhance the growth rate of beef cattle.

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EFFECT OF AGE AND LEVELS OF VITAMIN E SUPPLEMENTATION IN THE DIET ON SPERM QUALITY OF CROSSBRED BUCK RABBITS (NEW ZEALAND WHITE × LOCAL)

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ABSTRACT

The study was conducted to evaluate the effects of age and levels of vitamin E supplementation in the diet on sperm quality of crossbred buck rabbits (New Zealand white × Local) in the Mekong Delta. Twenty-four crossbred buck rabbits in 2 age groups, 5-6 months old (12 young bucks) and 9-10 months old (12 mature bucks) were supplemented with vitamin E in a diet of 0, 40, 80, and 120 mg/kgDM feed (E0, E40, E80, and E120), respectively. The experiment was carried out for 12 weeks and recorded the quality of buck rabbit sperm once a week. The experimental results recorded that buck rabbits with an age range of 9-10 months had a sperm concentration of 414×10^6/ml and the sperm motile rate 55.6% better than the bucks with an age range of 5-6 months with sperm concentration of 284×10^6/ml and sperm motile rate of 47.2% ($P<0.05$) in the first 5 weeks of the experiment. Diets supplemented with 40 mg of vitamin E had higher activity and live sperm ratio than those without and 120 mg of vitamin E supplementation ($P<0.05$). There was a significant improvement in sperm motile in the E40 test, which increased from 46.8% in the first 5-week period to 59.3% in the remaining 7-week period, while other tests tended to decrease. The experiment did not record an interaction between age and dietary vitamin E supplementation on rabbit sperm quality ($P>0.05$). The experimental results showed that buck rabbits aged 9-10 months and supplemented with 40mg of vitamin E were suitable for sperm extraction in the farming conditions of the Mekong Delta.

Keywords: *Crossbred buck rabbits, mature age, sperm quality, vitamin E.*

1. INTRODUCTION

In recent years, rabbit production in the Mekong Delta has been increasing concern for AI technique application in breeding. However, this technique research is limited. Buck rabbits are known as one the animals with lower sperm quality around $150\text{-}500\times10^6$ sperm/mL (Lebas *et al.,* 1997), especially the Mekong Delta raising condition leading to rabbits under heat stress for a long time (Hai, 2024) resulting in adverse effects in both growth performance and lower sperm characteristics. Stress could reduce testis function and negatively impact buck's sperm quality (O'Bryan *et al.,* 2000). Vitamin E (α-tocopherol) is an antioxidant substance and plays an important role in terms of reproductive functions, sperm membrane integrity protection, and maintaining sperm motility (Castellini *et al.,* 2007). Some research showed that vitamin E supplementation in the diet increased sperm volume and concentration of breed boars (Brzezinska-Slebodzinska *et al.,* 1995) and buck rabbits (Yousef *et al.,* 2003). Daramola *et al.* (2016) observed that a lack of vitamin E caused the detriment of reproductive function in mammal animals.

Besides, reducing adverse effects of heat stress, choosing the suitable age for breed bucks for semen collection is a crucial issue, New Zealand white (NZW) rabbits have a mature age for mating at 32 weeks old, however, young bucks at 20 weeks old start mating in some small-scale farms. Skinner (1967) reported that buck's spermatozoa at 119 days old were lower in both volume and alive rate. Recently, there has been no research for recommendation about the optimal age for mating and semen collection in crossbred rabbit breeds (NZW×Local) raised in the Mekong Delta condition, a

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combination of the supplementation of vitamin E in the diet of bucks on sperm characteristics improvement in this crossbred breed. Therefore, this study was investigated to determine an optimal age and vitamin E level supplementation for improving the semen quality of crossbred buck rabbits in the Mekong Delta raising condition.

2. MATERIALS AND METHODS

2.1. Materials

The experiment was arranged in a factorial design and consisted of 24 New Zealand white (NZW) crossbred buck rabbits (NZW×Local). The first factor is the levels of vitamin E supplementation in the diet of bucks corresponding to 0; 40; 80 and 120mg vitamin E/kgDM feed (E0, E40, E80, and E120), respectively. The second factor is two age range groups of bucks for semen collection, including 12 bucks at 5-6 months of age (young bucks), and 12 bucks at 9-10 months of age (mature bucks).

All bucks were raised for 12 weeks, and 2 age ranges were fed the same diet, however, differences in the levels of vitamin E supplementation. All animals were provided the diet with 18% CP and 11,5 MJ/kgDM ME, including the ration of all feed in DM were 30% soya waste, 22% soybean extraction meal, 26% molasses, and 22% *Brachiaria mutica*. The sperm of the experimental rabbits was collected and analyzed for semen quality weekly. All experimental feed and chemical compositions were presented in table 1.

Table 1. Chemical composition of feed (%DM)

Feed	DM	OM	CP	NDF	EE	Ash	ME
Soya waste	15.5	95.2	18.2	43.3	10.8	4.80	11.3
Soybean extract	90.4	90.1	42.2	28.7	3.56	9.90	10.4
Brachiaria mutica	18.4	87.9	11.5	58.5	4.98	12.1	8.49
Molasses	69.1	93.0	3.51	-	-	7.00	14.7

DM: dry matter, OM: organic matter, CP: crude protein, EE: Ether extract, NDF: Neutral detergent fiber, Ash: total mineral, ME: metabolizable energy.

2.2. Methods

All feed used in the experiment was in fresh form. Before starting the experimental diet, rabbits were fed the diet *ad libitum* for 1 week to monitor feed intake and determine the amount of dry matter (DM) required by rabbits. The diet experiment was calculated based on DM and the nutritional values were balanced with a stable of %CP and ME. The feeds and refusals were taken for analysis of DM, OM, CP, EE, NDF, ADF, and ash following the procedures of AOAC (1990). The metabolizable energy (ME) values of feeds were calculated according to:

ME (MJ/kgDM) = DE(0.995-0.048DCP/DE) (Maertens *et al.*, 2002), in which:

DE (MJ/kgDM)=14.9-0.22ADF+0.35EE (De Blas et al., 1992); DCP (%/DM)=-1.15+0.82CP-0.06ADF (Fernandez-Carmona et al., 2004); Where: DCP is digestible crude protein

Semen samples were collected from individual bucks using an artificial vagina (Ewuola *et al.*, 2014), made of a plastic cylinder with a rubber lining fixed around the rim to warm the water. The artificial vagina (AV) was pre-warmed in water at 50-55°C, ensuring a temperature of 40-42°C at the time of collection. The inner sleeve was lubricated with vaseline, and then a teaser doe was introduced to the buck's pen at the time of collection as the buck mounted the teaser, the AV was introduced, and the ejaculate was collected. Fresh semen samples were mixed into the medium at a ratio of 1:10 in solution (cold stored at 12-17^{0}C) and analyzed for sperm characteristics.

Semen was evaluated as described by Hafez and Hafez (2000). The ejaculate volume was determined by reading the volume directly from the calibrated collecting tube and the gel-free ejaculate volume recorded (if any). Ejaculate pH was determined immediately following collection using pH paper (SpezialIndikatorpapier pH 5.5-9.0, Macherey-Nagel, Germany). All semen characteristics analysis was conducted under a microscope with 400 times magnification according to WHO (2021). The concentration of spermatozoa in semen was determined by

haemocytometric counts, then the sample was diluted 1:4 with 5% $NaHCO_3$. C=N×D×50.000, *where C = concentration of spermatozoa (×10⁶/ml). N = number of spermatozoa and D - dilution factor = 20)*. The % of live spermatozoa was determined based on the number of sperm uncolor by stained smears with eosin 1% nigrosin 10% stained divided by the total counted sperm. Sperm motility (%) is the number of forward progressive motility sperms out of the total number of sperm in each microsphere, motility forms in terms of non-progressive motility and immotility were also calculated similarly. The number of motility and alive sperm per ejaculation was calculated based on the respective products of semen volume, sperm concentration, live sperm percentage, and motility spermatozoa rate according to Hai and Trung (2024).

2.3. Statistical analysis

The data is processed and analyzed according to the General Linear Model of the Minitab 16 program, and the differences between the tests by the Tukey method of the Minitab 16 program (2014). Comparison of an average between 2 experiment periods by T-test. Significance was declared at P<0.05.

3. RESULTS AND DISCUSSION

3.1. The effects of vitamin E and age of bucks on FI, nutrient intake, and ME

Feed intake was presented in table 2, these results indicated that there was no affected on feed intake of experiment rabbits by the levels of vitamin E in the diet (P>0.05). The average crude protein was in a range of 11.7-12.3 g/per/day, corresponding to 18% crude protein in dry matter per diet. The average ME was in the range of 11.3-11.5 MJ/kgDMI. EE intake was higher values at E80 and E40 (P>0.05), this higher amount contributed to an increase in the greater absorption of vitamin E, because vitamin E was fat-soluble. Generally, nutrient intakes did not differ in the vitamin E-supplemented group, this explained better evaluation on the effects of vitamin E on the sperm characteristics, as removal of nutrients impacts on semen quality. On the other side, the diet containing 18% CP and 11.5 MJ/kgDM ME was recommended as an optimal diet for semen collection bucks and gave the best result on spermatozoa characteristics (Ahemen *et al.*, 2013; Abdulrashid and Juniper, 2016; Hai, 2024).

Table 2. The results of feed intake, nutrient intake, and metabolizable energy of the experiment bucks

Factors		Feed intake, gDM					Nutrient intake, g						
Age	**Vit.E**	**SW**	**SEM**	**Mol**	**Bm**	**Vit E,mg**	**DM**	**OM**	**CP**	**NDF**	**EE**	**Ash**	**ME, MJ**
-	E0	19.9	13.9^{b}	18.0	13.2	0.00^{d}	65.1	59.9	11.7	21.7	3.31	5.20	0.75
-	E40	19.6	15.0^{a}	18.3	14.9	2.81^{c}	67.8	62.3	12.3	23.0	3.39	5.51	0.77
-	E80	19.7	14.5^{ab}	18.1	15.4	5.51^{b}	67.7	62.2	12.1	23.3	3.41	5.51	0.77
-	E120	19.6	14.0^{ab}	17.0	13.9	7.81^{a}	64.6	59.4	11.7	22.1	3.31	5.20	0.74
SEM		0.294	0.251	0.319	0.570	0.080	1.075	0.973	0.178	0.450	0.046	0.105	0.012
P		0.809	0.026	0.055	0.061	0.0001	0.097	0.102	0.066	0.077	0.265	0.064	0.112
Mature	-	18.7^{b}	13.6^{b}	16.9^{b}	13.6^{b}	3.78^{b}	62.8^{b}	57.8^{b}	11.3^{b}	21.3^{b}	3.18^{b}	5.08^{b}	0.72^{b}
Young	-	20.7^{a}	15.1^{a}	18.8^{a}	15.1^{a}	4.29^{a}	69.7^{a}	64.1^{a}	12.6^{a}	23.7^{a}	3.53^{a}	5.64^{a}	0.80^{a}
SEM		0.208	0.177	0.225	0.403	0.057	0.760	0.688	0.126	0.318	0.032	0.074	0.009
P		0.001	0.001	0.001	0.017	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
SEM		0.415	0.355	0.451	0.806	0.113	1.521	1.376	0.252	0.637	0.064	0.148	0.017
P (age×vit.E)		0.986	0.990	0.998	0.998	0.004	0.993	0.993	0.988	0.994	0.985	0.995	0.993

Mature: 9-10 months of age, Young: 5-6 months of age

Feed intake got the higher differences between the two age ranges of bucks. In general, the feed intake of young bucks was higher than mature bucks (P<0.05). This could be explained by the age-related nutrient requirement of animals. In mature bucks, the nutrients met mainly maintenance requirements and semen production, while pre-mature bucks were required for accumulated requirements on

body gain weight. Vogel *et al.* (2017) reported that the nutrient requirements of animals would be affected by age. CP intake required a lower amount and more nutrient digestibility in mature animals (Marín-García *et al.*, 2023). Similarly, Salisu and Iyeghe-Erakpotobor (2014) observed that the rabbits at 19 weeks old had a higher weight compared to 15 weeks old ones, otherwise less feed intake.

3.2. The effects of vitamin E and age of bucks on sperm characteristics

Overall evaluated results of sperm characteristics were presented in table 3. There was an increased tendency in sperm volume of experiment bucks followed by the rise of the levels of vitamin E (P>0.05), specifically from 0.62ml (E0) to 0.78ml (E120). Lebas *et al.* (1997) report that the sperm volume of bucks was in a range of 0.3-0.6l. The sperm composition and volume were influenced by the size of accessory glands (Campos *et al.*, 2014). The sperm concentration of this study was in a range of 248-428×10^6/ml, higher values in the supply group compared to a control group with 1.3-1.7 fold (P<0.05). There was a decreasing trend of C from E40 to E120 (P>0.05).

Table 3. Spermatozoa characteristics of bucks during the first 5 weeks

Age	Vit.E	Volume (ml)	pH	Con (×10^6/ml)	Motility (%)	Non-pro (%)	Immo (%)	Live SR (%)	MSV (×10^6)	LSV (×10^6)
-	E0	0.62	7.45	248^b	50.5	22.1	27.4^b	51.3	78.7^b	79.7^b
-	E40	0.64	6.90	428^a	46.8	12.0	41.2^a	51.6	126^{ab}	143^{ab}
-	E80	0.65	7.10	386^{ab}	58.4	15.8	25.9^b	50.9	136^a	118^{ab}
-	E120	0.78	6.80	333^{ab}	50.1	19.4	30.5^{ab}	56.7	134^a	152^a
SEM		0.050	0.159	41.29	3.229	2.842	2.892	3.370	12.85	16.57
P		0.145	0.051	0.039	0.114	0.103	0.007	0.586	0.020	0.030
Mature	-	0.68	7.10	414^a	55.6^a	17.5	26.9^b	54.9	150^a	149^a
Young	-	0.66	7.00	284^b	47.2^a	17.2	35.6^a	50.3	87.3^b	97.2^b
SEM		0.035	0.113	29.19	2.283	2.009	2.045	2.383	9.088	11.72
P		0.702	0.915	0.006	0.020	0.914	0.009	0.189	0.001	0.006
SEM		0.071	0.225	58.39	4.566	4.019	4.090	4.765	18.18	23.44
P (age×vit.E)		0.051	0.102	0.344	0.924	0.916	0.740	0.787	0.343	0.348

Con: concentration, Non-pro: Non-progression motility, Im-mo: immotility, Live SR: live sperm rate; MSV: motility sperm volume; LSV: live sperm volume.

The pH value of sperm was from 6.80 to 7.45 (P>0.05) and was in a normal range of sperm pH (Mohamed, 2021) of 6.38-7.95. pH value of sperm increased leading to the change in ion plasma balance resulting in cell fluid impacts, sperm membrane integrity, and the capacity of sperm's nutrient storage (Abdulrashid and Juniper, 2016). The lower pH was a result of glycolysis, and lactic acid production leading to inhibition of sperm motility (Gadea, 2003).

The sperm motility was lower at E120 and E40 corresponding to pH value (P>0.05), while the rate of immotility of sperm was also higher compared to other treatments (P<0.05). Thereby, the pH value of sperm influenced the capacity of sperm motility. Otherwise, Hai (2024) reported that the Mekong Delta raising condition recorded higher heat stress almost the whole year, due to the high temperature-humidity with THI in a range of 27.0-32.0. Heat stress increased the oxidant process leading to a decreasing in semen quality. Some research showed that vitamin E played an important role in oxidant reduction (National Institutes of Health, 2016; Sharaf *et al.*, 2019), however, the reduction of sperm characteristics during the first 5 weeks of the experiment (Table 3) could not conclude obviously to the positive effects of vitamin E on semen characteristics, spermatozoa motility, and the live sperm rate did not differ among all treatments (P>0.05). It may be the buck's spermatogenic epithelial cycle lasted for 43.6 days resulting in both vitamin E and nutrient impacts that may not yet have on semen quality in the first 5 weeks.

Table 4. Spermatozoa characteristics of bucks during the remaining 7 weeks

Age	Vit.E	Volume (ml)	pH	Con (×10⁶/ml)	Motility (%)	Non-pro (%)	Immo (%)	Live SR (%)	MSV (×10⁶)	LSV (×10⁶)
-	E0	0.61	6.92	395	45.0^{b}	14.1	40.9^{a}	56.2^{b}	109	137
-	E40	0.60	7.24	383	59.3^{a}	10.8	29.8^{b}	66.3^{a}	141	153
-	E80	0.70	7.06	350	52.2^{ab}	8.10	38.8^{a}	58.8^{ab}	126	144
-	E120	0.73	6.91	355	49.6^{b}	12.5	37.9^{ab}	54.8^{b}	128	144
SEM		0.034	0.141	46.89	1.833	1.432	2.041	2.437	19.50	23.02
P		0.043	0.356	0.884	0.001	0.109	0.008	0.020	0.735	0.970
Mature	-	0.66	7.07	401	55.6^{a}	11.2	33.1^{b}	59.4	146	156
Young	-	0.66	6.50	340	47.4^{b}	12.0	40.6^{a}	58.7	107	133
SEM		0.024	0.100	33.16	1.296	1.013	1.443	1.723	13.79	16.27
P		0.912	0.590	0.214	0.001	0.580	0.002	0.773	0.064	0.319
SEM		0.049	0.199	66.31	2.593	2.025	2.886	3.446	27.57	32.55
P (age×vit.E)		0.222	0.888	0.495	0.314	0.248	0.185	0.986	0.646	0.566

The sperm motility and live sperm rate were different compared to the initial period (the first 5 weeks) (P<0.05). The sperm volume was maintained at the higher tendency in the supply group, with the highest value at E120 and the lowest at E0 and E40 (P>0.05). The pH value changed specifically higher 7.0 at E40 and a lower 7.00 at E0 (P>0.05). The reduction of pH got the adverse effects of sperm motility combination in vitamin E helping in decreasing the oxidant process under heat stress resulting in maintaining sperm's pH value. The rise of the pH of sperm in the late period was considered a great signal of the effects of vitamin E on semen characteristics. These results were suitable with the rate of sperm motility and the percentage of live sperm, with the higher values at E40, and the lower value at E0 and E120 (P<0.05). As a result, supplementation of vitamin E at the level of 120 mg/kgDM did not have any improvement in the semen characteristics. Although, at the level of 80mg vitamin E/kgDM, it was stable in sperm quality, it was not better than 40 mg/kgDM. It could be concluded that the supplementation of vitamin E at the levels of 40 mg/kgDM feed had positive effects on spermatozoa characteristics of bucks in the Mekong Delta raising condition.

There was an obvious improvement in the second period compared to the first one in terms of sperm concentration, motility, motility sperm volume, and live sperm volume (P>0.05). The live sperm rate increased by around 6% in the second period compared to the initial period of the experiment (P<0.05).

Table 5. Semen characteristics in period (Mean±SE)

Items	1st period	2nd period	P
Volume, ml	0.67±0.03	0.66±0.02	0.794
pH	7.06±0.10	7.03±0.07	0.794
Concentration, ×10⁶/ml	349±27.3	371±22.3	0.569
Motility, %	51.4±1.85	51.5±1.62	0.964
Live rate, %	52.6^{b}±1.61	59.0^{a}±1.38	0.010
MSV, ×10⁶	119±10.1	126±9.70	0.424
LSV, ×10⁶	123±11.1	145±10.6	0.104

The significant differences in spermatozoa quality during 2 periods may be due to the age of bucks and sufficient time for vitamin E to have a positive effect on semen characteristics. Some previous research reported that the mature age of NZW started from the 18th week (Frame *et al.*, 1994). Maintaining the stable semen characteristics until the 32nd week-old, depending on raising conditions, ambient temperature, photoperiod, nutrients, and breeds led to sooner or later and lasted for a long time as well in this sexually mature. The breed used in this study was (NZW×Local), therefore, the mature age could be later, it could be explained that the semen quality of the young bucks in the initial period was lower. Fielding (1991) observed that local breeds, domestic breeds had later sexually mature time could be around 5 months old.

4. CONCLUSION

The supplementation of vitamin E at the level of 40 mg/kgDM in the diet was suitable for raising (NZW×Local) buck rabbits on semen collection, which got higher sperm motility and a greater live sperm rate for 12 weeks. To get higher semen characteristics, it should be chosen the bucks for semen collection in a range age of 9-10 months old.

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EFFECT OF YEAST FERMENTATION ON CHEMICAL COMPOSITION OF RICE BY-PRODUCTS

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ABSTRACT

A study was conducted to investigate the effect of fermentation process on the quality changes of broken rice (BR) and rice bran (RB), the two important by-products of the rice industry. The substrates were anaerobically fermented for 72h by adding 2% of baker's yeast *Saccharomyces cerevisiae* solution at two different moisture levels (50 and 70%) to make the four treatments: BR-50, BR-70, RB-50 and RB-70. Before and after fermentation, BR and RB were analyzed for proximate components, including dry matter (DM), ash, organic matter (OM), crude protein (CP), ether extract (EE) and crude fiber (CF). The results showed that yeast fermentation of BR and RB was most effective at 48h. It significantly increased the DM, OM and CP of the samples but decreased the ash, EE and CF contents (P<0,05), as compared to the value of initial subtrates. Furthermore, fermentation at 50% moisture level for 48h significantly increased (P<0,05) the protein content but 70% moisture level was a favourable condition to reduce the fat content. In conclusion, fermentation at 50% moisture content for 48h significantly improved the chemical composition of either BR or RB.

Keywords: *Broken rice, chemical composition, fermentation process, rice bran, Saccharomyces cerevisiae.*

1. INTRODUCTION

Broken rice (BR) and rice bran (RB) are the major by-products of the rice processing, accounting for 14% and 10%, respectively of the total grain weight (Moraes *et al.*, 2014). The two sources have great potential as feed ingredients for livestock and poultry. While BR has a high starch content, typically more than 80% dry matter (Loyda *et al.*, 2021), RB is more well known for its high level of dietary fiber, sterols and various antioxidants. Phytochemical analysis of RB revealed high levels of biomolecules, mainly tocopherol, polyphenols and vitamin E, which enable to prevent oxidative damage and reduce free radicals (Esa *et al.*, 2013). However, the utilization of these by-products in the food and feed industry is still limited due to the unbalanced nutrient content. The high fiber content and the presence of anti-nutritional factors negatively affect digestibility and in turn reduce the feed bioavailability. Moreover, the instability during storage also hinders the use of RB (Bodie *et al.*, 2019). Addressing these problems, different techniques such as processing and fermentation have been applied to maximize the utilization of BR and RB.

Saccharomyces cerevisiae, or baker's yeast, is a strain of fungi used in food manufacturing to make bakery or to ferment alcoholic beverage products (Blandino *et al.*, 2020; Onyema *et al.*, 2023). Currently, *S. cerevisiae* is used in the fermentation process to produce biofuel to improve the quality of the agro-industrial by-products. During the growth phase, yeast cell hydrolysates the nutrients in the substrate and produces various products such as organic acids, CO_2, ethanol and heat (Maicas, 2020). In addition, feeding animals with yeast cells or yeast fermented products may increase nutrient

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digestibility, modulate the immune system and promote animal growth. Yeast fermentation also contributes to flavour development (Shurson, 2018). Considering the positive effects of *Saccharomyces cerevisiae*, both BR and RB were fermented at different moisture levels. The chemical compositions of the two ingredients were evaluated before and after fermentation process to obbserve the changes in their nutritive value.

2. MATERIALS AND METHODS

Samples

Samples of the BR and RB were collected from a rice mill in Dong Nai province. *S. cerevisiae* was obtained from a commercial baker's yeast (Instant Success Dry Yeast, Lesaffre, France).

Fermentation process

Prior to fermentation, BR and RB, as two substrates to inoculate with *Saccharomyces cerevisiae*, were mixed with distilled water. The moisture contents of the mixtures were adjusted to 50 and 70% for either BR or RB to make the four treatments: BR-50, BR-70, RB-50 and RB-70.

About 1g of dry yeast powder was added to an activator solution, including 100ml of 10% glucose solution. The solution was placed at room temperature (27-30^{o}C). After 24h, 2% (v/w) of the activated yeast solution with cell density of about 10^8 CFU/ml was inoculated in the substrates. The mixture was placed in a tight plastic bag for fermentation at room temperature. After 24h, 48h and 72h, samples of fermented rice by-products were hot air-dried at 55°C to attain relevant moisture content of less than 12%. The dried fermented samples were then ground into powder using a blender.

Experimental parameters

Viable yeast population and pH: Viable count of yeast cells in the fermented slurry were done by plating on the MRS agar and pH values were determined using the portable pH meter (Horiba PH210, Japan).

Proximate composition: The substrates and fermented powder samples were subjected for analysis of DM, ash, OM, CP, EE and CF. All analyses were carried out in duplicate according to the procedure of Association of Official Analytical Chemist (AOAC, 2005).

Statistical analysis

Experimental data were statistically analyzed using the the general linear model (GLM) of Minitab 16 software. Means were compared using Tukey's test at a 5% significance level ($P<0.05$).

3. RESULTS AND DISCUSSION

The proximate composition of the substrates showed in table 1 was in agreement with values reported by other authors (Ahmed *et al.*, 2018; Islam *et al.*, 2022). Most of the values in RB were higher than in BR, with the exception of DM and OM.

Table 1. The proximate composition of the rice by-products (%)

Substrate	DM	Ash	OM	CP	EE	CF
BR	90.20±0.06	1.49±0.15	88.71±0.12	7.17±0.49	1.03±0.04	1.81±0.06
RB	88.46±0.10	8.55±0.47	79.91±0.49	11.45±0.10	13.97±0.10	7.23±0.39

Note: Data was showed as mean ± standard deviation

The results indicated that time has a statistically significant influence on pH throughout the fermentation process. There was a notable decreasing trend as the time increased from 0h to 48h of the fermentation (Figures 3 and 4). The slope of the pH curve during the fermentation process was comparable between pH 4-6 and 4-7, respectively with the BR and RB. However, the pH values started to increase after 48h with BR (Figure 3) or slightly decreased with RB (Figure 4). The changes in pH during fermentation can be explained by the complex interaction between yeast and

fermentation medium, resulting in acid production and a pH reduction. However, after 48h, the high acidic media (pH<5) may inhibit the yeast activity, leading to the rise of pH levels. According to Alsuhaim *et al.* (2012), the number of yeast cells exponentially increase and reach the peak at around 12h after fermentation. After that, the cells begin to grow slowly, which is announced as the stationary phase. Eventually, no growth occurs due to the high waste concentration or complete substrate consumption.

The yeast cells have the ability to metabolize and convert sugars to energy. The yeast population or yeast biomass is an important criterion to evaluate the ability of the yeast cells in the fermentation broth. In addition, yeast biomass is a rich source of protein, enzymes, peptides, amino acids, carbohydrates, vitamins B and trace minerals (Jach *et al.*, 2022). Therefore, the higher mass of yeast cells is attained, the more yield of protein is collected. Figures 1 and 2 showed an increasing trend in yeast population from 0h to 48h of the fermentation process, in which the highest number of yeast cells was recorded at 48h fermentation in the medium of 50% moisture BR (6.13×10^7 CFU/ml). However, after 48h, the yeast cell number decreases, in both BR and RB fermentation media, probably due to the low acidic condition. According to Cao *et al.* (2021), yeast fermentation faces difficulties in accessing and utilizing nutrients at lower pH condition, resulting in a lower fermentation rate and a decrease in the growth of yeast cells.

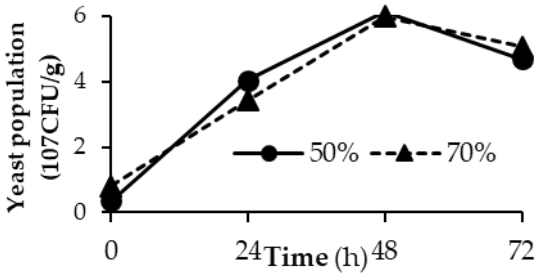

Figure 1. Changes of yeast population during 72h fermentation of BR

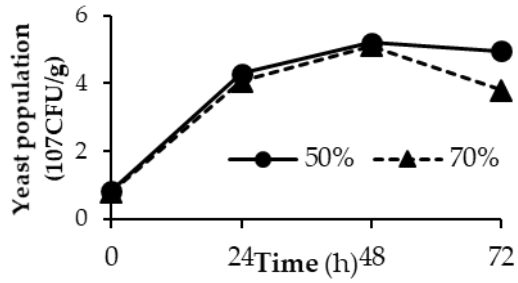

Figure 2. Changes of yeast population during 72h fermentation of RB

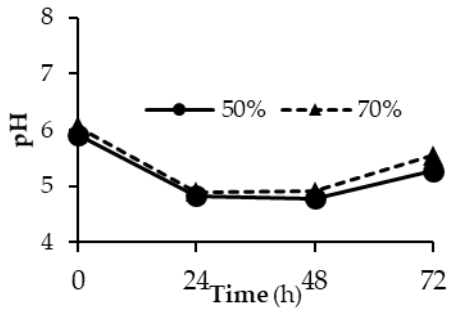

Figure 3. Changes of pH during 72h fermentation of BR

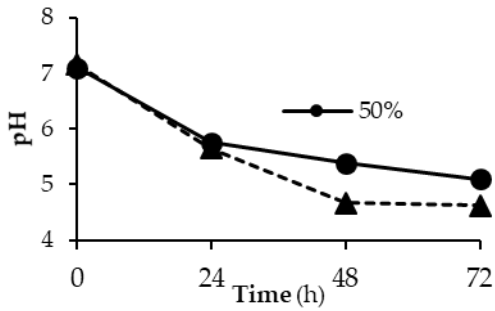

Figure 4. Changes of pH during 72h fermentation of RB

During the growing phase, yeast cells use the media nutrients and lead to changes in the composition of the substrates, particularly protein. Results in figure 5 revealed an increase in the CP content after 2 days of the BR fermentation, followed by a subsequent decrease in the following day. The protein contents significantly increased and reached 9.20 and 9.94% after 48h of fermentation, respectively at 50 and 70% moisture levels, compared to the beginning. However, the protein contents of RB samples

did not show any clear trends or patterns (Figure 6). Mohammady *et al.* (2023) reported that baker's yeast significantly increased the protein and reduced the fiber as well as lipid contents of wheat bran.

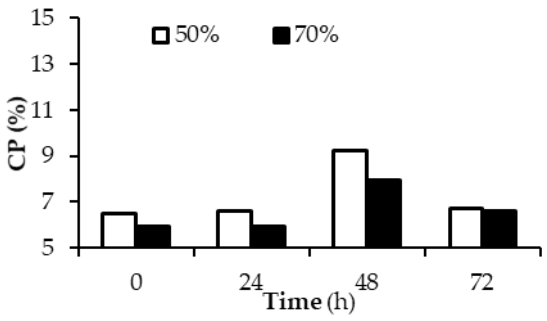

Figure 5. Changes of protein content during 72h fermentation of BR

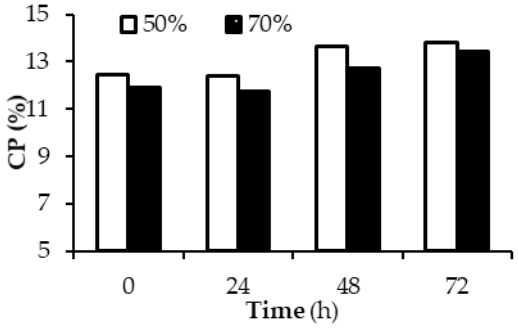

Figure 6. Changes of protein content during 72h fermentation of RB

From the above findings, fermented dried samples of BR and RB at 48h fermentation were selected to compare the differences in the chemical composition. Results of these variables as well as the changes in the composition of the samples compared to those of the substrates are showed in table 2.

Table 2. Chemical composition and changes in of rice by-products after 48h fermentation

Variable		BR-50	BR-70	RB-50	RB-70	SEM	P
Chemical composition (%)	Dry matter	91.10	91.25	91.03	90.86	0.44	0.938
	Ash	0.82^{b}	0.90^{b}	7.27^{a}	7.15^{a}	0.21	0.000
	Organic matter	90.28^{a}	90.34^{a}	83.76^{b}	83.71^{b}	0.44	0.000
	Crude protein	9.20^{c}	7.94^{d}	13.64^{a}	12.74^{b}	0.18	0.000
	Ether extract	0.65^{b}	0.41^{b}	12.23^{a}	11.72^{a}	0.15	0.000
	Crude fiber	0.98^{b}	0.82^{b}	5.50^{a}	5.23^{a}	0.24	0.000
Changes (%)	Dry matter	0.99^{b}	1.16^{b}	2.90^{a}	2.71^{a}	0.49	0.017
	Ash	-45.19^{c}	-39.49^{b}	-15.01^{a}	-16.37^{a}	8.05	0.028
	Organic matter	1.77^{b}	1.84^{b}	4.82^{a}	4.75^{a}	0.51	0.000
	Crude protein	28.36^{a}	10.67^{c}	19.08^{b}	11.28^{c}	1.95	0.000
	Ether extract	-36.89^{b}	-60.03^{c}	-12.45^{a}	-16.14^{a}	5.17	0.000
	Crude fiber	-46.13	-54.66	-23.96	-27.65	8.83	0.068

Note: BR-50; BR-70: BR samples with 50; 70% moisture content; RB-50; RB-70: RB samples with 50; 70% moisture content. Means within a row followed by different superscripts are significantly different at 5% level (P<0.05).

It can be seen that the DM contents were not significantly different among the treatments (P>0.05) while other values of ash, OM, CP, EE and CF were significantly different (P<0.05). The differences may be in relation to the values of the substrates. Also in table 2, it can be seen the differences in changes values among the experimental treatments. The higher changes in increased OM of RB samples would be related to the decreasing phytate-P levels after fermentation, as reported by Islam *et al.* (2022). For protein, the fermentation more significantly improved (P<0.05) the CP of the fermented BR-50 (28.36%), compared to other treatments. It can be noted that within 48h, there was an increase of 10.67-28.36% and 11.28-19.08% in CP, respectively for BR and RB. The decreases in the EE and CF contents were probably due to the use of the yeast cell for membrane synthesis. According to Hidayat *et al.* (2019), during fermentation, *S. cerevisiae* produces extracellular enzymes to decompose starch and dietary fiber as nutrients for its growth.

3. CONCLUSION

Yeast fermentation at 50% moisture level for 48h significantly increased the protein content but 70% moisture level was a favourable condition to reduce the fat content of either BR or RB. Further research is necessary to study the bioavailability of fermented products in non-ruminant animals.

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COMPARISON OF BANANA FLOWER POWDER AND SODIUM BICARBONATE SUPPLEMENTATION ON INTAKES, WEIGHT GAIN AND RUMINAL FUNCTION IN BOER CROSSBRED GOATS

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ABSTRACT

The use of native plants to improve rumen function is of interest to researchers and nutritionists because the use of additional chemicals in the diet can lead to residues in meat or milk. Plants with high mineral content have the potential to regulate rumen pH stability. Thus, this experiment aimed to compare the effects of banana flower powder (BFP) and sodium bicarbonate ($NaHCO_3$) supplementation on intakes, weight gain, ruminal function and blood electrolytes in Boer crossbred male goats. The experiment was conducted using a completely randomized design with three treatments including a control group (Control), supplementation with 4.5% banana flower powder (BF4.5), and supplementation with 4.5% $NaHCO_3$ (Na4.5), each replicated five times. The results show that dry matter intake, water intake and weight gain from Na4.5 were higher than those from BF4.5 and control groups ($P<0.05$). In addition, goats from Na4.5 and BF4.5 increased ruminal pH, whereas NH3-N level remained unchanged among treatments. There were no effects of BFP and $NaHCO_3$ supplementation on body weight and levels of plasma electrolytes ($P>0.05$). The results from this study indicated that BFP can repalce $NaHCO_3$ as rumen buffering agent in the diets of growing goats.

Key words: *Buffering agent, goats, plant mineral, production, ruminal function.*

1. INTRODUCTION

The production ability of goats and cattle depends on many factors in which nutrition is one of the important factors. In ruminants, the proper ruminal pH is usually maintained in the range of 6.5 to 7. Enemark (2008) reported that a decrease in ruminal pH below the optimal level reduces feed intake and growth of microorganisms, especially fibrous bacteria, thereby decreasing animal productivity. Sodium bicarbonate is commonly supplemented to ruminant diets as an agent to neutralize rumen pH and has become one of the standard practices in ruminant production in many countries around the world (Dijkstra *et al*., 2012). Previous studies found that $NaHCO_3$ supplementation improved ruminal function and feed intake in buffalo bulls (Sharif *et al*., 2010) and in dairy goat (Nguyen *et al*., 2020). However, at present the use of native plants to improve rumen function is of interest to researchers and nutritionists because the use of additionally chemicals in the diet can lead to residues in meat or milk. Plants with high mineral content have the potential to regulate rumen pH stability. Vietnam is one of tropical countries with large area of banana plantation and the flower of some kind banana do not use for human consumption due to bitter taste. Previous studies reported that BFP has potential as rumen buffering agent due to its high mineral content such as sodium, potassium and phosphorus (Ngamsaeng *et al*., 2006; Kang and Wanapat, 2013). Supplementation of BFP improved pH and fermentation efficiency in beef and dairy cattle (Kang *et al*., 2014, 2015). Additinally, Nguyen *et al.* (2021) found that growing goat fed with 4.5% BFP increased in ruminal pH and nutrient digestibility. Kang *et al.* (2015) reported that dairy cows supplement with BFP supplementation was the same buffering capacity as $NaHCO_3$. In general, BFP or $NaHCO_3$ supplementation improved ruminal function, nutrient digestibility and animal

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production in cattle, lambs and goats as reported by several studies. However, there is little information on the effects of BFP and $NaHCO_3$ supplementation on nutrient intakes and animal performance in growing crossbred goats. Therefore, the aims of present study were to compare the effects of BFP and $NaHCO_3$ supplementation on intakes, weight gain and blood electrolytes in Boer crossbred male goats aiming for better utilization of local resources.

2. MATERIALS AND METHODS

2.1. Experimental design

Fresh banana flowers were collected from a banana farm, chopped, and sun-dried for 3-4 consecutive days. The dried flowers were then ground using a mill equipped with a 2mm screen.

The experiment was conducted on 15 Boer crossbred male goats, aged 6-7 months, with an average body weight of 17.75±0.40kg. All animals were housed in individual metabolic cages measuring 1.2x0.7m with plastic floors for adaptation. The experimental design was completely randomized with three treatments including a control group (control), a group supplemented with 4.5% banana flower powder (BF4.5), and a group supplemented with 4.5% $NaHCO_3$ (Na4.5), with each treatment replicated five times. The experiment lasted for 7 weeks, comprising a 2-week adaptation period followed by a 5-week data collection period. The goats were fed experimental rations consisting of 70% corn silage and 30% concentrate, formulated as total mixed rations (TMR). The ingredients and chemical compositions of the rations are detailed in Table 1. The TMR was provided *ad libitum* twice daily at 07:00 and 14:00 hours. All goats had free access to water throughout the experiment.

The average temperatures recorded during the experiment at 07:00 hour and 09:00, 11:00, 13:00, 15:00, 17:00 and 19:00 hours were 27.88±0.11°C, 28.0±0.37°C, 29.75±0.56°C, 30.31±0.59°C, 29.50±0.26°C, 28.75±0.43°C and 28.25±0.43°C, respectively. Corresponding percentages of relative humidity at 07:00 hour and 09:00, 11:00, 13:00, 15:00, 17:00 and 19:00 hours were 81.50±1.13%, 79.25±0.22%, 74.00±1.75%, 71.63±3.01%, 75.25±3.31%, 75.75±2.05% and 78.63±0.96%, respectively. The temperature and humidity index (THI) was calculated based on the recommendations from Thammacharoen *et al.* (2020) as 69.42±0.09, 69.52±0.03, 70.95±0.96, 71.25±0.48, 70.74±0.21, 70.13±0.35 and 69.73±0.35, respectively.

2.2. Data collection and measurement

Feed offered and feed refusals were recorded daily each morning from the start to the end of experiment. Daily dry matter intake (DMI) was calculated as the difference between the feed offered and feed refusals on a dry matter basis. Samples of both the feed and refusals were collected daily throughout the experiment. These samples were divided into two parts, one half was immediately dried in an oven at 105°C until a constant weight was achieved to determine dry matter content, while the remaining samples were stored frozen at -20°C for subsequent chemical analysis. At the end of the experiment, all feed samples were thawed, thoroughly mixed, and subsamples were dried at 65°C overnight (approximately 12 hours) for later analysis. The chemical and mineral compositions of samples were analyzed as described by Nguyen et al. (2020) and presented in Table 1.

Water intake (WI) was measured daily throughout the experiment by subtracting the weight of water refused from the weight of water offered. The goats were weighed before the morning feeding at the start and end of the experiment to monitor changes in body weight.

Blood samples were collected from the jugular vein at 07:00 (before the morning feeding), and again at 11:00 and 15:00 at the end of the experiment. The samples were placed in lithium heparin tubes, kept on crushed ice, and centrifuged at 3,000rpm for

10min. Plasma samples were then immediately transported to the laboratory for electrolyte analysis. Plasma electrolytes were measured using an automatic analyzer (ST200 PRO, Sensa Core, India).

Table 1. Ingredients and chemical composition of diets

Items	Control	BF4.5	Na4.5
Ingredients (%)			
Corn silage	70.0	70.0	70.0
Rice bran	8.0	3.5	3.5
Corn meal	11.3	11.4	11.4
Soybean meal	7.8	7.7	7.7
Limestone	0.9	0.9	0.9
Molasses	2.0	2.0	2.0
Banana flower powder	0	4.5	0
$NaHCO_3$	0	0	4.5
Total	100	100	100
Chemical composition (%)			
DM	29.5	30.62	30.44
CP	16.2	16.3	16.01
EE	4.3	4.07	4.00
ADF	28.5	27.89	28.12
NDF	39.5	38.90	39.02
Na^+ (meq/100 g DM)	4.35	6.52	57.83
K^+ (meq/100 g DM)	42.82	48.11	40.30
Cl^- (meq/100 g DM)	14.38	11.88	15.63
S (meq/100 g DM)	15.71	12.86	15.43
DCAD[1] (meq/100 g DM)	17.08	29.90	67.07

[1]DCAD, in milliequivalents of (Na + K) – (Cl + S)/100 g of DM

Ruminal fluid samples were collected from the goats at the end of the experiment using a stomach tube connected to a syringe. Approximately 15 ml of rumen fluid was obtained before the morning feeding, and at 03:00 and 06:00hrs after the morning feeding. The ruminal pH was immediately measured using a pH meter (pH221, Lutron, Taipei, Taiwan). After that, the ruminal fluid samples were filtered through two layers of cheesecloth and preserved by adding 1ml of 6N HCl. The samples were then frozen at -20°C for later analysis. Ammonium nitrogen (NH_3-N) concentration was determined using the Kjeldahl method.

2.3. Statistical analysis

The data are presented as the mean ± SEM (Standard Error of the Mean). All data were analyzed using one-way ANOVA to determine the effects of the treatments. For pairwise comparisons, the Tukey post- hoc test was used to assess significance. Statistical significance was declared at $P<0.05$.

3. RESULTS

Dry matter intake (DMI) in the Na4.5 group was significantly higher than in the control and BF4.5 groups (Table 2, $P<0.05$), starting from the 3rd week to the 5th week of the experiment (Figure 1, $P<0.05$). The higher DMI in the Na4.5 group resulted in greater Na^+ intake compared to the BF4.5 and control groups (Table 2, $P<0.05$). Additionally, K^+ intake was higher in both the BF4.5 and Na4.5 groups compared to the control group (Table 2, $P<0.05$). In contrast, Cl^- intake did not differ significantly among the treatments (Table 2, $P>0.05$). Similar to DMI, water intake in the Na4.5 group was also significantly higher than in the control and BF4.5 groups (Table 2, $P<0.05$), starting from the 3rd week to the 5th week of the experiment (Figure 2, $P<0.05$). Supplementation with BFP and $NaHCO_3$ did not have a significant effect on body weight (Table 2, $P>0.05$). However, weight gain in the Na4.5 group was significantly greater than in the other treatment groups (Table 2, $P<0.05$).

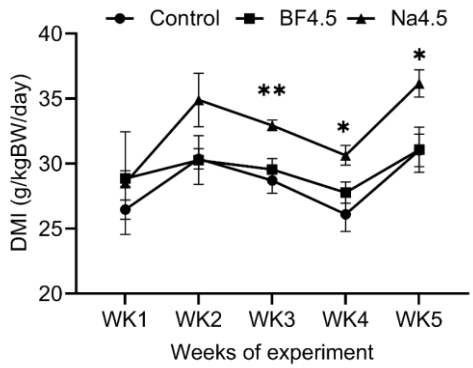

Figure 1. Effects of banana flower powder and $NaHCO_3$ supplementation on dry matter intake

*Control: without supplementation with 4.5% banana flower powder or 4.5% $NaHCO_3$; BF4.5: supplementation with 4.5% banana flower powder; Na4.5: supplementation with 4.5% $NaHCO_3$. *: $P<0.05$; **: $P<0.01$.*

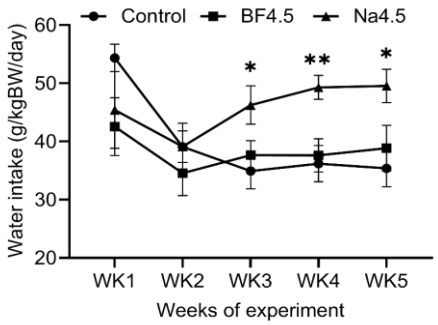

Figure 2. Effects of banana flower powder and $NaHCO_3$ supplementation on water intake

*Control: without supplementation with 4.5% banana flower powder or 4.5% $NaHCO_3$; BF4.5: supplementation with 4.5% banana flower powder; Na4.5: supplementation with 4.5% $NaHCO_3$. *: P<0.05; **: P<0.01.*

Ruminal pH was influenced by both BFP and $NaHCO_3$ supplementation, particularly before morning feeding and at 03:00 and 06:00 hrs after morning feeding in the present study. Ruminal pH in the Na4.5 and BF4.5 groups was higher than in the control group at 03:00 and 06:00 hrs after morning feeding (Table 3, P<0.05).

The concentrations of plasma electrolytes levels and CAD (Clinical Acid-Base Disorders) were similar among treatments at 07:00, 11:00, and 15:00 hrs, indicating no significant differences in these parameters across the different supplementation groups (Table 4, P>0.05).

Table 2. Effects of banana flower powder and $NaHCO_3$ on dry matter intake, mineral intake, water intake, body weight and weight gain in Boer crossbred male goats

Items	Control	BF4.5	Na4.5	SE	P
Dry matter intake (g/kgBW/day)	29.05^b	29.67^b	33.47^a	0.99	0.02
Na^+ intake (g/kgBW/day)	0.029^b	0.042^b	0.448^a	0.01	0.001
K^+ intake (g/kgBW/day)	0.494^b	0.567^a	0.555^{ab}	0.02	0.03
Cl^- intake (g/kgBW/day)	0.308	0.311	0.330	0.01	0.31
Cation and anion difference intake (g/kgBW/day)	0.215^c	0.297^b	0.673^a	0.01	0.001
Water intake (g/kgBW/day)	36.39^b	37.16^b	46.06^a	1.83	0.01
Initial body weight (kg/head)	17.42	17.88	17.96	0.74	0.86
Final body weight (kg/head)	19.55	20.32	21.08	0.64	0.28
Weight gain (g/head/day)	76.07^b	87.14^b	111.25^a	5.60	0.01

Mean values with different superscript within the same row are different at P<0.05

Control: without supplementation with 4.5% banana flower powder or 4.5% $NaHCO_3$; BF4.5: supplementation with 4.5% banana flower powder; Na4.5: supplementation with 4.5% $NaHCO_3$

Table 3. Effects of banana flower powder and $NaHCO_3$ on ruminal pH and NH_3-N in Boer crossbred male goats

Time	Control	BF4.5	Na4.5	SE	P
pH					
0 hour	6.90^b	7.01^{ab}	7.29^a	0.08	0.010
3 hours	6.68^c	6.88^b	7.07^a	0.04	0.001
6 hours	6.34^b	6.61^a	6.81^a	0.07	0.001
N-NH_3 (mg/dl)					
0 hour	18.48	21.92	21.50	1.32	0.18
3 hours	20.12	23.26	23.50	1.53	0.26
6 hours	17.50	21.58	20.50	1.42	0.15

$^{a,b,c:}$ Mean values with different superscript within the same row are different at P<0.05

Control: without supplementation with 4.5% banana flower powder or 4.5% $NaHCO_3$; BF4.5: supplementation with 4.5% banana flower powder; Na4.5: supplementation with 4.5% $NaHCO_3$

Table 4. Effects of banana flower powder and $NaHCO_3$ on plasma electrolytes concentration in Boer crossbred male goats

Items	Time	Control	BF4.5	Na4.5	SE	P
Na^+	07:00	142.23	143.88	143.73	0.52	0.08
K^+		5.21	5.54	5.63	0.21	0.37
Cl^-		103.50	104.08	104.55	0.52	0.40
CAD		43.93	45.34	44.81	0.69	0.37
Na^+	11:00	143.15	144.90	144.85	0.63	0.12
K^+		4.64	5.06	5.05	0.24	0.39
Cl^-		101.25	105.58	102.75	1.28	0.09
CAD		46.54	44.58	47.35	0.87	0.11
Na^+	15:00	145.40	145.08	145.20	0.45	0.88
K^+		5.07	5.10	5.20	0.18	0.87
Cl^-		104.20	104.38	103.18	0.53	0.26
CAD		46.27	45.79	47.22	0.54	0.21

Control: without supplementation with 4.5% banana flower powder or 4.5% $NaHCO_3$; BF4.5: supplementation with 4.5% banana flower powder; Na4.5: supplementation with 4.5% $NaHCO_3$

4. DISCUSSION

Dry matter intake from Na4.5 group was higher than those from control and BF4.5 groups. This increase may be attributed to improved ruminal function and nutrient digestibility when animal was supplemented with $NaHCO_3$ as suggested by previous studies in dairy animals (Nguyen *et al.,* 2020 ; West *et al*., 1992). However, Kang *et al.* (2015) reported that BFP and $NaHCO_3$ supplementation enhanced ruminal function and nutrient digestibility, but DMI remained unchanged. Similarly, Gonzales *et al*. (2008) found that beef cattle fed with 5% $NaHCO_3$ in diets did not affect on DMI. In Beetal bucks, Jamal *et al*. (2021) reported that bucks fed 15g/kg DM $NaHCO_3$ increased DMI and fiber digestibility by stabilizing the rumen environment. The DMI from this study indicated that Boer crossbred male goats increased DMI from $NaHCO_3$ supplementation and not from BFP supplementation in the ration.

The differences in mineral intake from this study may be due to the difference in mineral contents from experimental diets (high Na^+ from Na4.5 and K^+ from BF4.5 diets). Interestingly, higher Na^+ intake from Na4.5 group would increase WI from this study. But this did not happen for K^+ intake and WI was similar to between control and BF4.5 groups (Table 2, $P<0.05$). This could be because the K^+ intake was not sufficient to influence WI in the BF4.5 group. Nguyen *et al.* (2023) indicated that non-pregnant and non-lactating crossbred goats supplemented with $NaHCO_3$ and K_2CO_3 did not affect on WI. But, some studies suggested that higher WI from animal fed with $NaHCO_3$ (Nguyen *et al*., 2020) or higher WI with 1.0% saline water for lactating crossbred goats (Nguyen *et al*., 2018).

There was no effect of BFP and $NaHCO_3$ supplementation on body weight. But, weight gain from Na4.5 group was greater than those from other treatments. The higher weight gain observed in the Na4.5 group may be attributed to the greater DMI observed in this study, which is consistent with findings from a previous work in sheep (Enemark, 2008). However, Mandebvu and Galbraith (1999) found that sheep fed with 15 g $NaHCO_3$ did not affect on weight gain, whereas sheep consumed with 22.5g $NaHCO_3$/kg diet which decreased DMI and weight gain. They indicated that animal supplemented with high $NaHCO_3$ in diet which increased blood CO_2 and followed by increasing respiration rate and animal stayed in respiratory alkalosis. However, dairy or meat goats were supplemented with $NaHCO_3$ of 3.0-4.5% which improved DMI (Nguyen *et al*., 2018, 2020), even respiration rate increased (Nguyen *et al*., 2018). The results from this study indicated that goats consumed with 4.5% BFP did not affect on DMI and weight gain, whereas animal fed with 4.5% $NaHCO_3$ increased DMI and weight gain.

Ruminal pH from Na4.5 and BF4.5 was higher than those from control group at 03:00 and 06:00hr after morning feeding. Higher ruminal pH could cause either from increasing WI and solid passage rates or from neutralizing hydrogen ion from rumen (Rogers and Davis, 1982). In the present study, WI from Na4.5 was greater than those from BF4.5 and control groups. Additionally, Na4.5 and BF4.5 groups from this study also supplemented with either $NaHCO_3$ or BFP rich in Na^+ and K^+ levels. Thus they can contribute to the rumen buffer, followed by increasing ruminal pH (Nguyen *et al.,* 2020; Kang *et al*., 2015). Interestingly, ruminal pH from BF4.5 group was similar to those from Na4.5 group before morning feeding and at 06:00hrs after morning feeding. Accordingly, Nguyen *et al.* (2020) found that dairy goats supplemented with $NaHCO_3$ improved ruminal pH, similar to the findings of this study. Kang *et al.* (2014) also suggested that banana flower powder could be supplemented as a rumen buffering agent at 20-30 g/kg of DMI. The results from current

study show that NH_3-N levelswere similar among treatments before morning feeding and at 03:00 and 06:00hrs after morning feeding (Table 3, P>0.05). Previous studies reported that sodium bicarbonate supplementation increased ruminal pH and unchanged fermentation patterns in dairy cows (Roche *et al*., 2005) and dairy goats (Nguyen *et al*., 2020). Accordingly, Kang *et al.* (2014) observed that dairy steers fed with 30 g/kg DMI BFP did not affect on ruminal NH_3-N level. The results from the present study indicate that BFP and $NaHCO_3$ may have a similar influence on ruminal pH and NH_3-N levels. This study showed that the concentrations of plasma electrolytes levels and CAD were similar among treatments at 07:00, 11:00 and 15:00hrs and were within the normal ranges for healthy goats as mentioned by many authors (Tsukahara *et al*., 2016; Runa *et al*., 2020). In addition, Tsukahara *et al*. (2016) and Nguyen *et al*. (2022) found that growing goats drank saline water with high levels of Na+ and K^+, plasma electrolytes remained in normal range due to excretion via kidney route. In contrast, Nguyen *et al.* (2018) suggested that dairy goats fed with $NaHCO_3$ and K_2CO_3 did not affect plasma Na^+ level at 09:00hrs. But plasma Na^+ level from treatment group was higher than those from control group at 16:00hrs and as a result, CAD was higher at 16:00hrs. The present study shows that goats consumed with banana flowe powder or $NaHCO_3$ maintained plasma Na^+, K^+ and Cl^- concentration constant within the reference range.

4. CONCLUSIONS

The results from the current study indicated that banana flower powder and $NaHCO_3$ supplementation with 4.5% in diet have improved ruminal function. In addition, goats fed with $NaHCO_3$ have increased DMI, WI and weight gain, whereas BFP supplementation did not affect on DMI, WI and weight gain in growing goats. It is recommended that BFP can repalce $NaHCO_3$ as a rumen buffering agent in the diets of growing goats.

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EFFECT OF HERBAL PRODUCTS SUMPPLEMENTATION ON PRODUCTIVITY PERFORMANCES OF COMMERCIAL FROG PRODUCTION UNDER FIELD CONDITION

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ABSTRACT

The objective of this study was to initially evaluate the effect of herbal product supplementation in the ration on the performances of commercial frog production in field condition at Dong Thap province of Vietnam. Two trials have been carried out to evaluate the supplementation of Herb-1 (Herb-All™LIVER) and/or Herb-2 (Herb-All™PARA-X) at the farm condition with different scales. At the experimental scale, the results showed that the final weight (kg/cage) was the highest (P<0.05) in the Herb-1 group (109.73 kg/cage), followed by the control or combined Herb-1 and Herb-2 group (96.98 or 97.18 kg/cage, respectively) and the lowest value was found in the Herb-2 group (94.12 kg/cage). The weight gain was higher (P<0.05) in the Herb-1 group (101.22 kg/cage), with a similar value found in the control or combined Herb products (88.20 or 88.90 kg/cage), and the lowest value in the Herb-2 group (85.43 kg/cage). The FCR value was reduced by 10 or 17% in the combined Herb-1+Herb-2 group or Herb-1 group compared to the control group. Under practical conditions, the final weight in the control, Herb-1, Herb-2 or combined herbs group was 470.63, 544.60, 460.21 or 471.82 kg/cage, respectively. A significant difference (P<0.05) was found in Herb-1 or combined Herb-1+Herb-2 compared to other treatments. The FCR value was reduced by 15% in the Herb-1 group or by 8% in the combined Herb-1+Herb-2 group compared to the control group. Taken together, we conclude that Herb products could serve as novel natural feed additive in commercial frog cultivation as they can improve growth performance and feed efficiency of commercial frogs. However, the optimal concentrations and how to combine the two kinds of Herb products require further studies.

Keywords: *Amphibian, aquaculture, commercial frog culture, herbal, growth.*

1. INTRODUCTION

Freshwater aquaculture is one of the most important and fastest-growing food-producing industries worldwide, it plays an important role as an alternative source of cheap animal protein, and is also considered as an important sector that can create job opportunities for poor communities (Mbokane and Moyo, 2022). In Dong Thap province, frog cultivation is developing as an important sector in aquaculture. The regular model of culture is a cage in pond combined with Tra fish, rarely cage in ditch as Thailand's frog culture system (Kamatit *et al*., 2023), and intensive frog production system is generally applied by local farmers. In the current practical circumstance, frogs are normal grown in high density and may face many problems such as stress, limited space, inadequate diet, cannibalism, predators, and poor water quality (Zhang *et al*., 2015). Thus, good management of frog diet and health should be attained. Aquaculture growth is often linked to culture intensification, leading to overcrowding (high density) and poor water quality, facilitating the spread of pathogens and increasing disease outbreaks and mortality (Bondad-Reantaso *et al*., 2005). To avoid economic losses related to sanitary shortcomings, veterinary drugs are commonly used in aquaculture to prevent and treat disease outbreaks (Rico *et al*., 2013). The intensive use of synthetic drugs presents numerous disadvantages, for both the environment and public health. Intensive use

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of antibiotics has resulted in accumulation in muscle of commercialized animals (Cabello *et al*., 2006; Romero-Ormazabal *et al*., 2012) and the development of resistant bacteria strains (Miranda and Zemelman, 2002; Seyfried *et al*., 2010). Beside this, the use of antiparasitic drugs like trichlorfon or praziquantel in bath treatments is hazardous for animals and the environment and can also result in the development of resistance (Umeda *et al*., 2006; Forwood *et al*., 2013). This knowledge leads researchers to find novel feed additives obtained from plants or other natural products (Citarasu, 2010; Duzmic and Blache, 2012; Reverter *et al*., 2014). Herbal ingredients are natural ingredients derived from plants, which are containing the flavonoid, polyphenol/tannins, glucosides, alkaloid, etheric oils, saponin, biter substances, vitamin, protein and etc., for use in medicine. The results of scientific studies on the benefits of herbal ingredients have been widely carried out in fish cultivation and used as standard operating procedures for cultivation (Nurjanah *et al*., 2023). However, the use of herbs in frog culture has not been widely practiced, therefore, this study aimed to evaluate the effect of Herb products supplementation on the growth of commercial frog in the field condition.

2. MATERIALS AND METHODS

2.1. Animals and feeding

*Frog rearing conditions***:** Crossbred frogs with an initial weight of 4-5 g/froglet were obtained from a local supplier at My An Town, Thap Muoi District, Dong Thap province. They were randomly allocated in a cage (2×4m^2 or 2×4×1m^3) on an experimental scale or (4×10m^2; 4×10×1m^3) for practical conditions, a completely randomized design using 4 treatments and 3 replications. Each replication was about 2,000 froglets/cage in the experimental scale or 10,000 froglets/cage in the practical field. This investigation was performed during the hot season of Southern Vietnam (from Feb to June, 2024)

Feeding ration: The basal diets were obtained from the commercial feed company containing not less than 30% crude protein and 4% lipid. Two kinds of Herbs were used and added to the basal diets and mixed together by using a binder.

Ingredients of Herb All products: Herb-1 (Herb-All™LIVER): *Andrographis paniculata, Tinospora cordifolia* & Nut Fiber. Herb-2 (Herb-All™PARA-X): *Curcuma longa rhizome, Allium sativum* & Nut Fiber.

2.2. Experimental designs

2.2.1. Preliminary trial with experimental scale

In this trial, four treatments were set up and the frogs were raised in the cage with 2x4x1 m^3 including control group (basal diet without any herbal supplementation), treatment group with Herb-1 (basal diet with 0.02% of Herb-1), treatment group with Herb-2 (basal diet with 0.02% of Herb-2) and treatment group with combined Herb-1 and Herb-2 (basal diet with 0.02% of each Herb-1 and Herb-2).

2.2.2. Expanded trial with practiced condition scale

The experimental design was similar to the article 2.2.1, except the frogs were raised in a cage with (4×10×1 m^3).

2.2.3 Evaluation of growth parameters, feed conversion rate and economic efficacy

At the end of the treatment period, frogs were fasted for at least 12hrs. Growth parameters were evaluated by using following equations:

- Weight gain (WG) = final frog weight (kg/cage) – initial frog weight (kg/cage).
- Feed conversion ratio (FCR) = feed intake (kg/cage)/weight gain (kg/cage).
- Economic efficacy: Total costs consist of the fee for certain investments such as: buying froglets, commercial feed, labor and additive materials, herb

products. Total revenue was only the income by selling the frog at the end of trials, the unit was VND.

2.3. Data analysis

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

3. RESULTS AND DISCUSSION

3.1. Effect of Herb supplementation on productivity performances of commercial frog under the experimental scale

We initially started with the trial at experimental condition, collected data from the trial to analyze the effect of Herb products that supplemented at single or combined dose of each Herb on the growth parameters, the data were analyzed and presented in Table 1.

Table 1. Effect of Herb products on the growth of commercial frog production in under experimental scale

Parameters	Control	Herb-1 (0.02%)	Herb-2 (0.02%)	Herb-1+Herb-2 (0.02:0.02)
Initial weight (kg/cage)	8.78±0.04	8.52±0.22	8.68±0.18	8.28±0.04
Final weight (kg/cage)	96.98^{ab}±0.81	109.73^{b}±4.94	94.12^{a}±2.31	97.18^{ab}±1.24
Weight gain (kg/cage)	88.20^{a}±0.80	101.22^{b}±4.76	85.43^{a}±2.14	88.90^{a}±1.26
FCR (kg of Feed /kg weight gain)	1.60^{b}±0.06	1.40^{a}±0.02	1.65^{b}±0.06	1.59^{b}±0.07
FCR (compared to control; times)	1	0.83	1.01	0.90

Cage: 2m×4m. Within row, the mean with different superscript differs (P<0.05), data are presented as mean±SEM of 3 replicates

As shown in table 1, the initial weight of froglets per cage was 8.28 to 8.78 kg/cage (P>0.05), the final weight (kg/cage) was 109.73 kg/cage in Herb-1 group, then lower in Control or combined Herb-1 and Herb-2 group (96.98 or 97.18 kg/cage, respectively) and the lowest value was found in Herb-2 group (94.12 kg/cage), a significant difference was only found between Herb-1 and Herb-2 group (P<0.05). After 65 days of culture, the weight gain was higher (P<0.05) in Herb-1 group (101.22 kg/cage) and similar value was found in the control or combined Herb products (88.20 or 88.90 kg/cage), and the lower value was in the Herb-2 group (85.43 kg/cage). The FCR value was lowest in Herb-1 group (1.40) then higher in combined Herbs group (1.59) and highest in control or para group (1.60 or 1.65, respectively), a significant difference between Herb-1 and other groups was found. The FCR was reduced by 17% in the Herb-1 group or by 10% in the combined herbs group as compared to the control group.

The economic analysis was performed, and the results as shown in Table 2.

Table 2. Effect of Herb products on economic efficiency of commercial frog production under experimental scale

Parameters	Control	Herb-1 (0.02%)	Herb-2 (0.02%)	Herb-1+Herb-2 (0.02:0.02)
Total cost (VND/cage)	3,637,096.78	3,660,159.12	3,673,520.08	3.736.196,83
Total revenue (VND/cage)	4,461,233.33	5,047,733.33	4,329,366.67	4.470.433,33
Benefit (VND/cage)	824.136,55	1,387,574.21	655,846.58	734.236,51
Compared to control (times)	1	1.68	0.80	0.89

Cage: 2m×4m = 8m^2, data are presented as mean from three replicates

Data from table 2 indicated that the benefit was significant higher in Herb-1 group (VND 1,387,572.21) as compared to control (VND 824.136.55) or combined herbs group (VND 734.236,51) and the lowest benefit value was found in Herb-2 group (VND 655.846,58). Interestingly, the benefit value increased about 1.68 times when added to Herb-1 as compared to the control.

3.2. Effect of Herb supplementation on productivity performances of commercial frog under the field condition

In the practical field trial (40 m^2 per cage), four treatments were set up similar to the experimental scale, the data were collected and analyzed, the results of growth performances are presented in table 3.

Table 3. Effect of Herb products on the growth of commercial frog production under practical field

Parameters	Farm	Control	**Herb-1** (0.02%)	**Herb-2** (0.02%)	**Herb-1+Herb-2** (0.02:0.02)
Initial weight (kg/cage)	*Farm 1*	*39.93±0.48*	*39.77±0.64*	*39.49±0.46*	*39.60±0.55*
	Farm 2	*37.58±0.41*	*38.06±0.17*	*37.52±0.33*	*38.15±0.28*
	Overall	38.59±0.55	38.79±0.43	38.36±0.47	38.77±0.39
Final weight (kg/cage)	*Farm 1*	*474.96±31.21*	*549.50±5.04*	*468.07±14.70*	*511.17±10.44*
	Farm 2	*467.38^{ab}±13.82*	*540.93^{a}±14.98*	*454.33^{b}±9.28*	*510.16^{ab}±33.24*
	Overall	470.63^{bc}±14.00	544.60^{a}±8.41	460.21^{c}±7.95	510.59^{ab}±18.20
Weight gain (kg/cage)	*Farm 1*	*435.03±31.42*	*509.73±5.48*	*428.57±14.95*	*471.56±10.80*
	Farm 2	*429.80^{ab}±13.69*	*502.86^{a}±15.01*	*416.81^{b}±9.26*	*472.01^{ab}±33.41*
	Overall	432.04^{b}±13.99	505.81^{a}±8.40	421.85^{b}±7.88	471.82^{ab}±18.32
FCR (kg of Feed /kg weight gain)	*Farm 1*	*1.74±0.06*	*1.44±0.02*	*1.75±0.06*	*1.57±0.07*
	Farm 2	*1.59^{a}±0.04*	*1.37^{b}±0.03*	*1.67^{a}±0.03*	*1.47^{ab}±0.09*
	Overall	1.65^{a}±0.07	1.40^{b}±0.02	1.70^{a}±0.04	1.52^{ab}±0.06
FCR compared to control (times)	*Farm 1*	*1*	*0.83*	*1.01*	*0.90*
	Farm 2	*1*	*0.86*	*1.05*	*0.93*
	Overall	1	0.85	1.03	0.92

Cage: 4m×10m = 40m^2. Within the row, the mean with different superscripts differs (P<0.05), data are presented as Mean±SEM from three replicates in farm 1, four replicates in farm 2 and 7 replicates in overall.

Data in Table 3 indicated that the initial weight ranged 39.49 to 39.93 kg/cage in farm 1 and 37.52 to 38.79 kg/cage in farm 2 or 38.36 to 38.79 kg/cage in overall, no significant difference among treatments was observed in farm 1 or farm 2.

For final weight, the highest value was obtained in Herb-1 group of farm 1 and farm 2 (549.50 and 540.93 kg/cage, respectively), lower value in combined herbs group (511.15 and 510.16 kg/cage, respectively in farm 1 and 2), then lowest value was found in control (474.96 and 467.38 kg/cage) or Herb-2 (468.07 and 454.33 kg/cage) group in both farms. The trend of positive effect of Herb-1 on final weight was found (P=0.07) in farm 1, and a significant difference was found between Herb-1 group and Control or herb-2 groups (P<0.05) in farm 2. In overall, the final weight in control, Herb-1, Herb-2 or combined herbs was 470.63, 544.60, 460.21 or 510.59 kg/cage, a significant difference (P<0.05) was found in Herb-1 group or combined herbs as compared to other treatments.

For weight gain, the trend of data were similar to the final weight obtained in above that indicated in Table 3. Taking all data, we speculate that Herb-2 seems not effective in commercial frogs that are raised under an uncontrolled water environment.

The FCR value, in overall, was lowest in Herb-1 group (1.40), higher in combined Herb-1+Herb-2 group (1.52), and highest in control or Herb-2 group (1.65 or 1.70), a significant difference only found in Herb-1 as compared to control or Herb-2 group (P<0.05). In overall, the FCR value was reduced 15% in the Herb-1 group or 8% in combined Herb-1+Herb-2 group as compared to control group. In addition, the FCR value in all treatments seems to be high, it could be due to the trials carried out under the hottest conditions of Southern Vietnam, in parallel with many unknown factors that must be considered.

Several studies reported that the diets containing Herbs significantly increased final weight, weight gain, ADG and reduced FCR value as compared to those of the control

frogs (Kamatid *et al*., 2016) or fish (Reverter *et al*., 2014; Arief *et al*., 2015; Nurjanah *et al*., 2023) indicating that Herbs could be used as feed additive in aquatic feeds to promote productivity. The growth promotion observed in the current investigation could be attributed to the actions of alkaloids, flavonoids, phenolics or saponins, etc… contained in herb products used.

The economic analysis was performed, and the results are presented in Table 4.

Table 4. Effect of Herb products on economic efficiency of commercial frog production under practical field

Parameters	Farm	Control	Herb-1 (0.02%)	Herb-2 (0.02%)	Herb-1+Herb-2 (0.02:0.02)
Total cost (VND/cage)	*Farm 1*	*19.094.124,9*	*19.167.645,9*	*19.427.263,7*	*19.611.055,7*
	Farm 2	*18.004.533,0*	*18.172.200,0*	*18.258.264,7*	*18.153.229,2*
	Overall	18.549.328,9	18.669.923,0	18.842.825,3	18.882.142,4
Total revenue (VND/cage)	*Farm 1*	*21.848.313,3*	*25.277.000,0*	*21.531.066,7*	*23.513.666,7*
	Farm 2	*21.499.250,0*	*24.882.550,0*	*20.898.950,0*	*23.467.475,0*
	Overall	21.673.781,7	25.079.775,0	21.215.008,3	23.490.570,8
Benefit (VND/cage)	*Farm 1*	*2.754.188,4*	*6.109.354,1*	*2.103.803,0*	*3.902.611,0*
	Farm 2	*3.494.717,0*	*6.710.350,0*	*2.640.563,1*	*5.314.245,8*
	Overall	3.124.452,7	6.409.852,0	2.372.183,0	4.608.428,4
Compared to control (times)	*Farm 1*	*1.00*	*2.22*	*0.76*	*1.42*
	Farm 2	*1.00*	*1.92*	*0.76*	*1. 52*
	Overall	1.00	2.05	0.76	1.47

Data are presented as mean from three replicates in farm 1; four replicates in farm 2 and 7 replicates in overall.

Data from table 4 evidenced that the net profit improved 2.05 times added Herb-1 or 1.47 times when combined Herb-1 and Herb-2 in the ration as compared to the control group.

Additionally, the herbal dietary feed supplement method is the best traditional method and is also low-cost, nontoxic, and environmentally friendly (Zhang *et al*., 2015; Vijayaram *et al*., 2023). The herbal dietary feed supplement is an alternative way to enhance healthy commercial frog production in aquaculture.

4. CONCLUSION

Our current study is the first report in Vietnam to indicate that herbs could be used as a novel natural feed additive in commercial frog cultivation, as they can improve growth performance and feed efficiency of commercial frogs. The optimal concentrations and how to combine two kinds of Herb-All™ products require further studies.

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DETERMINATION OF HAEMATOLOGICAL PROFILES OF NATIVE PIGS RAISED UNDER CONFINED MANAGEMENT SYSTEM BASED ON AVAILABLE FEEDSTUFFS

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ABSTRACT

The objective of this study was to initially determine some blood haematological parameters of two groups of native pigs (unknown pedigree) are raised under a confined management system based on available feedstuffs. A total of 74 individual blood samples from two groups of pigs were collected, including 31 samples from wild crossbred (13 males and 18 females) and 43 native Binh Thuan (BT) black pig breed (8 males and 35 females) from the Ruminant Research and Development Center (RRDC). The samples were then analyzed for blood haematological parameters using the Mindray BC-2800 Vet haematology analyzer. The results showed that there were differences between three basic blood physiological indices between the two groups of pigs. The WBC (109/l) or PLT (109/l) values were significantly lower in the Binh Thuan black pig group than in the wild crossbred pig group (16.89 or 498.77 vs 21.02 or 604.77, P<0.05), while there was no significant difference in the red blood cell index (8.08 vs 7.97×1012g/l), P>0.05). Within the group of pigs, there was no difference in basic blood physiological indicators between female and male pigs in crossbred wild pigs. On the contrary, in the group of BT black pigs, there was a change in WBC or PLT indicators between male and female pigs (respectively 21.16 or 706.25 compared to 15.91 or 451.34, P<0.05). In conclusion, the haematological profiles of two native pig groups during the grower stage are determined and the variation of some critical parameter among breeds was found. Further research is needed to better understand the variability of porcine hematological profiles for further applications in diagnostic, health and production performance assessment.

Keywords: *Crossbred native pig, white blood cell, red blood cell, platelet cell, haematology.*

1. INTRODUCTION

Hematologic parameters are of great importance for clinicians and researchers when assessing the health status of both humans and animals (Zhang *et al*., 2022). Up to date, the measurement of haematological parameters in pigs was rarely performed as compared to pet animals. There are several reasons for this, such as the costs associated with labour and laboratory testing, especially due to the low economic value of an individual animal and the limited availability of reference intervals for different age categories in pigs required for correct interpretation of laboratory results (Thorn 2010; Cooper *et al*., 2014; Perri *et al*., 2017; Jezek *et al*., 2018). Since the ranges of most haematological parameters are quite wide, they vary depending on many factors, including diet, age, gender, physiological appearance, different husbandry techniques, biosecurity, season, restraint, sample collection techniques, time of sample transportation or preparation, and the type of the analyser used for haematological analysis (Thorn, 2010; Jezek *et al*., 2018).

There are many important reasons for determining haematological parameters in pigs. First, the assessment of these parameters can be used to establish a proper diagnosis and to assess not only the health status of individual pigs but also the health status of the entire herd (Perri *et al*., 2017;

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Jezek *et al*., 2018). In addition, assessment of haematological parameters can contribute to early identification of diseases or poor growth performances (Perri *et al*., 2017; Sanchez *et al*., 2019) and may be highly valuable in the treatment or prognosis of many diseases (Eze *et al*., 2010). Based on the assumption that many factors such as breed, age, nutrition, physiological or disease status have a significant effect on the haematological parameters of pigs' blood (Czech *et al*., 2017; Perri *et al*., 2017; Jezek *et al*., 2018; Sanchez *et al*., 2019; Oh *et al*., 2022), and all these factors must be considered when interpreting the results of haematological analysis. So, the aim of this study was to present the differences between basic haematological parameters of native pig blood depending on their breeds and sex under confined management condition and based on available feedstuffs.

2. MATERIALS AND METHODS

2.1. Animals and feeding

Two groups of native pigs that are raised at a farm of RRDC for conservation and development purposes were used in this study. A total of 74 individual samples were collected, including 31 samples from wild crossbred (13 males and 18 females) and 43 samples from native Binh Thuan (BT) black pig breed (8 males and 35 females). The grower stage of the animals were fed twice daily based mainly on the available materials such as casava waste, chopped banana stems, fresh vegetables and supplemented a little commercial feed as well as other supplements such as minerals.

Figure 1. Representative images of pigs examed: ***wild crossbred pig (A) and BT black pig***

2.2. Samples and heamatological analysis

2.2.1. Blood sample collection

Whole blood samples were collected from anterior vena cava and the animal was restrained by wire-noose snaires. Blood samples were collected in blood collection tube containing EDTA as anticoagulant after adequate restraint. Samples were performed about 2hrs after morning feeding, stored at 4°C on ice, and transported to the laboratory within 4hrs. The analysis was run immediately after the blood samples delivery to Lab.

2.2.2. Haematological analysis

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

2.3. Data analysis

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

3. RESULTS AND DISCUSSION

3.1. Haematological profile of different pig breeds

An automatic measurement analyzer of individual samples was performed, the data were analysed and presented in Table 1.

Table 1. Effect of breeds on haematological profile

Parameters	BT black pig (n=43)	Wild crossbred pig (n=32)	Ref. range
WBC (10^9/l)	16.89^a±0.84	21.02^b±1.08	11.0-22.0
RBC (10^{12}/l)	8.08±0.26	7.97±0.20	5.0-9.5
HGB (g/l)	128.51^b±4.56	112.52^a±3.68	99.0-165.0
HCT (%)	44.74±1.73	40.32±1.24	32.0-50.0
MCV (fl)	56.23^b±0.56	50.59^a±0.66	51.0-68.0
MCH (pG)	15.79^b±0.15	14.09^a±0.21	17.0-22.0
MCHC (g/l)	281.98^b±1.21	278.19^a±1.19	300.0-380.0
PLT (10^9/l)	498.77^a±41.46	604.77^b±43.59	200.0-700.0
MPV (fl)	7.77^b±0.11	7.28^a±0.11	6.0-12.0
PDW (%)	16.83^b±0.07	16.02^a±0.09	-
RDW (%)	17.82^a±0.20	20.18^b±0.45	14.0-19.0
PCT (%)	0.34^a±0.02	0.42^b±0.03	0.0-0.5

BT: Binh Thuan, within the same row (excluding Ref. range data) the value with different superscript letter differs (P<0.05).

Data from Table 1 showed that the WBC (10^9/l) value of grower BT black pig group was lower than that of the wild crossbred pig group (16.89 vs 21.02, P<0.05).

For RBC (10^{12}/l) values, they were similar in the BT black pig group and the wild crossbred pig group (8.08 vs 7.97, P>0.05). In depth analysis of the RBC characteristics showed that the HBG value was higher (P<0.05) in the BT back pig (128.51g/l) than in the wild crossbred pig (112.52 g/l). Similarly, the values of MCV (pg), MCH (g/l), MCHC (10^9/l) in the BT black pig group were also higher (P<0.05) than in the wild crossbred pig group (56.23, 15.79, 281.98 vs 50.59, 14.09, 278.19). The HCT (%) values were similar among pig groups (44.74 vs 40.32, P>0.05). The MCHC values were lower than the reference range, it could be due to the pig erythrocyte is highly susceptible to the stress when taking the sample without the use of anesthesia or hemolysis by hypotonic saline during analysis and are more fragile than that of other species. This explanation is also mentioned by Thorn *et al*. (2022).

For platelet parameters, significant lower PLT values were found in the group of the BT black pigs as compared to those in group of wild crossbred pigs (498.77 vs 604.77, P<0.05). Similarly, the percentages of PDW, RDW were significant higher (P<0.05) in the BT group than in the wild crossbred pig group (7.77, 16.83 vs 7.28, 16.02, respectively). In contrast , the percentages of RDW and PCT were lower (P<0.05) in the BT group than in the wild crossbred pig group (17.82 and 0.34 vs 20.18 and 0.42, respectively).

In the present study, the resuls indicated that the variation in haematological parameters was found at different genetic levels of pigs examined, the trend of the data was also reported by Himkar *et al.* (2020). Furthermore, several researches reported that the variations in blood parameters were also found between physiological status such as nursery piglets and sows (Zhang *et al*., 2022), peri-parturation of sows (Aparna *et al.,* 2023), different feeding systems (Eze *et al*., 2010; Albeni *et al*., 2018), health status (Buzzard *et al*., 2013) and provides fundamental data for monitoring pig health and productivity.

3.2. Haematological parameters of pigs examed according to sex

Clarified data according to breed and sex, the haematological parameters were obtained and presented in table 2a and 2b.

For BT black pig, the haematological profile was calculated and presented in table 2a.

Based on the data from table 2a, no significant differences were found in blood parameters between male and female pigs of BT black pigs, except for WBC and PLT. WBC

or PLT values were higher in the male group than the female group (21.16 or 706.25 vs 15.91 or 451.34, respectively; P<0.05). Furthermore, the blood parameters of wild crossed pigs by sex were calculated and presented in table 2b.

Table 2a. Haematological profile of pig by sex

Parameters	BT black pigs	
	Male (n=8)	**Female** (n=35)
WBC (10^9/l)	21.16[a]±2.06	15.91[b]±0.84
RBC (10^{12}/l)	9.02±0.57	7.86±0.28
HGB (g/l)	139.13±9.55	126.09±5.13
HCT (%)	48.98±3.35	43.77±1.97
MCV (fl)	54.30±0.62	56.67±0.65
MCH (pg)	15.35±0.16	15.89±0.18
MCHC (g/l)	283.38±1.48	281.74±1.41
PLT (10^9/l)	706.25[a]±73.80	451.34[b]±44.69
MPV (fl)	7.48±0.17	7.84±0.13
PDW (%)	16.49±0.17	16.90±0.08
RDW (%)	18.49±0.65	17.67±0.19
PCT (%)	0.49±0.05	0.31±0.02

Table 2b. Haematological profile of pig by sex

Parameters	Wild crossbred pigs	
	Male (n=13)	**Female** (n=18)
WBC (10^9/l)	20.72±1.42	21.23±1.58
RBC (10^{12}/l)	8.00±0.23	7.95±0.31
HGB (g/l)	111.00±3.66	113.61±5.84
HCT (%)	39.82±1.29	40.67±1.94
MCV (fl)	49.86±0.95	51.12±0.91
MCH (pg)	13.83±0.28	14.28±0.31
MCHC (g/l)	278.31±1.42	278.11±1.82
PLT (10^9/l)	604.54±73.94	604.94±54.65
MPV (fl)	7.18±0.15	7.35±0.16
PDW (%)	15.92±0.13	16.09±0.12
RDW (%)	20.93±0.67	19.63±0.58
PCT (%)	0.44±0.06	0.40±0.04

Interestingly, the data in table 2b indicated that no significant variation in blood parameters according to sex of wild crossbred pigs was found. From this result it can be confirmed that the variation of haematological profiles is affected by many factors as mention above.

Taken together, it should be noted that the blood samples were collected without the use of anesthesia can be stressful for pigs, which may influence the final results for the parameters that are sensitive to stress, especial in MCHC value. Thorn *et al*. (2022) also mentioned that stress during sampling is one of the largest sources of haematologic variation. Pigs are easily stressed through handling and restraint, and the stress response develops within 2 minutes, rapidly affecting the leukogram. Beside this, data on hematological profiles of pig lines are scarce, despite the increasing interest of this approach in research (Abeni *et al*., 2018), therefore, larger sample sizes, more pig species, different physiological stages will provide better results with regard to observing individual differences and precisely defining the range of reference values.

4. CONCLUSION

Under confined management condition and available feeding, blood parameters of two native pigs during the grower stage were determinated and variations of some critical parameters between breeds were found. More studies are required to better understand the variability of porcine hematological profiles for further applications in diagnostic, health as well as productivity performance assessment.

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PARTICIPANT OF FEMALE FARMERS ON BLACK SOLDIER FLY LARVAE PRODUCTION IN THE SOUTH OF VIETNAM

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ABSTRACT

Black soldier fly larvae (BSFL) production provides employment, economic support, and environmental benefits for rural families, particularly in rural areas who are landless. Women play a crucial role in BSFL rearing activities, often balancing these responsibilities with household tasks. However, their contributions in agriculture and related activities have been traditionally overlooked and undervalued. Our survey was conducted to examine the involvement of women in BSFL production in the Southern provinces of Viet Nam, specifically Tien Giang and Dong Nai. The study involved 100 women farmers engaged in BSFL farming in these two provinces. These women actively shared their experiences in small-scale production, displaying their enthusiasm for embracing this technology. They expressed their commitment not only to BSFL production in the fields, but also connected to manage organic waste at home. The data for the study was gathered through direct personal interviews. The participants included women from youth groups involved in waste management and BSFL production, as well as entrepreneurs and graduate students with an interest in rearing BSFL. The survey revealed that women were heavily involved in egg incubation (80%), feeding (75%), and caring for BSFL (72%), but less involvement in selling tasks (20%). Their participation in marketing, selection of BSF enterprises, accessing credit facilities, and record maintenance was relativelv low.

Keywords: *Black soldier fly larvae production, engagement, women, environmental benefit, management.*

1. INTRODUCTION

Black Soldier Fly (BSF) is an insect that can safely be reared (van Huis, 2013) because they are non-feeding adults, and require only water and non-transmission diseases. Larvae of BSF feed on a large variety of organic matter, including plant material (Hillaire *et al*., 2007). They are capable of converting large amounts of waste biomass that produced greenhouse gas and ammonia production (Oonincx *et al*., 2010) into their stored protein (≥40%) and fat (≥20%) (Barragan-Fonseca *et al*., 2017; Chia *et al*., 2020). Hence using larvae as a replacement protein source for fish will reduce dependence on commercial feed, it is estimated that the cost of 1kg feed with supplemented BSFL can be reduced by half compared to fully commercial feed. Based on the Official Dispatch No 2121/BTNMT-TCMT signed on April 26, 2022, the Ministry of Natural Resources and Environment allowed the BSF as edible insects production; the proposed solution for feed is to supply BSF larvae as a sustainable source of protein to livestock/and aquacultural farmers to raise rural farmers' income and improve farming practices; and use the BSF manure as organic fertilizer for crops.

Technology transfer is a method to transfer knowledge that links from the best result from the research or other experiences or good lessons learned to share with the community to improve productivity, increase the yield of animal/feed, reduce the input, good economic return and the production are safe for human health. To do these, the RTTC under financial support from the SIDA through the AgriFoSe2030 project has created the BSF project activities to do demonstrations in some provinces in

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Vietnam as Tien Giang, Long An and Dong Nai provinces.

Agricultural production in Vietnam relies largely on female workers. In rural areas, the gender difference in labor is significant with 63% of women working in the agriculture sector against 57% of men (FAO, 2019). Meanwhile, women often own small-area fields and cultivate self-subsistence crops. Hence, there is a need to support the female farmer by providing various vocational as well as specialized training programs for overall development in this area. It is vital to identify the problems of rural women on a priority base. Therefore, a project on the assessment of BSFL training needs of women in two provinces was surveyed. The objectives are (i) To study the profile attributes of the female farmers; (ii) To assess the BSFL training need in farming and non-farming areas of female farmers; and (iii) To identify the priority of BSFL training need as perceived by female.

2. MATERIALS AND METHODS

2.1. Location and times

The survey was conducted on farmers of Phu Hoi, Nhon Trach, Dong Nai province; Binh Nhi and Vinh Huu, Go Cong Tay, Tien Giang province, from February to May 2023.

2.2. Surveyal design

Total 04 villages of Dat Moi, Phu My 1, Phu My 2, Xom Ho of Phu Hoi, Nhon Trach, Dong Nai province; 03 villages of Binh Hoa Long, Binh Hoa Dong, Binh Dong Trung of Binh Nhi and 05 villages of Phu Quy, Binh An, Thanh Thoi, An Ninh, Hoa Binh of Vinh Huu, Go Cong Tay, Tien Giang province were purposively selected for the study. Eight women from each village were selected randomly to make a total sample of 100 respondents; 50 women from Nhon Trach district of Dong Nai province and 50 women from Go Cong Tay district of Tien Giang province. The data were collected by personal interview. The interview schedule was developed through discussion with experts, scientists of Nong Lam University, and local extension officers. The data were analyzed with appropriate statistical procedures. The respondents were asked to opine about BSFL training needs in various major areas of the field at three points quantum i.e., mostly needed, somewhat needed and not needed with a score of 3, 2 and 1 respectively. Based on the total BSFL training need score of all the respondents mean score for each major training area was worked out.

2.3. Data collection and explanation of framework

- Policies and programmes on (1) Animal production (2) Credit: interest rates, bank loans (3) Programmes on the application of technology and science on animal production (4) Market linkage policies: support to farmers in selling animal products, market information provision.

- Community factors on (1) Cultural factors: gender conceptions on the roles and capacity of men and women (2) Weather conditions and climate change (3) Local animal raise planning (4) System and quality of agricultural development services at communes.

- Household characteristics on (1) Household land scale (2) Family types: family with two spouses at home, family without husband at home/or no husband at home (migrant husband/working away/husband died/divorced) (3) Agricultural production forms: animal production and BSFL growing (4) Living standard: better-off, medium, poor (5) Decision making of female farmers (6) Gender-based labour division in agriculture production.

- Individual characteristics on (1) Education (2) Health (3) Marital status (4) Knowledge and skills of agriculture and technology.

- Animal/aquacultural production and BSFL growing by female farmers on (1) The capacity and opportunity to gain access to

programmes and policies: including access to loans, finance and training, participation in workshops, hiring and decisions about land usage (2) Market and animal house use opportunities (3) Decision making in the process of production (4) The extent of participation in BSFL production.

2.4. Data analysis

The data were subjected to analysis using Excel software.

3. RESULTS AND DISCUSSION

3.1. Research on gender issues in Vietnamese animal and BSFL production

The Vietnamese government has policies that aim to provide rural women with access to credit for animal or BSFL production. These policies support women in developing their economic status and sustainably reducing poverty through various credit channels. For example, the Women's Union provides increased support to poor and near-poor women by helping them access preferential credit sources from the Bank of Social Policy and microcredit projects managed directly by the Women's Union. Similarly, for wealthier households and farms, the Bank of Agriculture and Rural Development plays a significant role in providing credit for agriculture and rural development, thereby supporting women in these areas.

However, there have been some issues with credit policies for animal development. Firstly, the conditions for receiving a loan are tough, as credit institutions typically require clients to provide land or other assets as collateral for the loans. Secondly, the amount of credit available for animal-related activities is insufficient and does not meet the demand. As a result, farmers often rely on informal credit networks in rural areas. Lastly, there is a gender disparity in accessing and utilizing credit, with women having limited opportunities to access credit compared to men.

There were some troubles on how to manage the credit access from women. If women gain access to credit but cannot control it, it will be almost impossible for the use of the credit to create resulting changes in gender relations. The women need to possess qualities for controlling the loan such as the ability to do economic calculations, a degree of business knowledge and experience, skills in understanding loan application procedures and good communication skills, which poor women often lack.

Household economics is a typical form of agriculture in Vietnam, where gender-based division of labor and cultural norms are common in rural farming households. Female farmers play a crucial role in improving household economic performance, especially in meticulous and detailed livestock work, despite facing gender stereotypes that hinder their access to new techniques and methods in animal husbandry. Raising BSFL is seen as an important aspect of the sustainable circular agriculture model, offering high economic efficiency through low investment capital, small farming areas, and the utilization of leftover food and organic waste, which makes it suitable for creating livelihoods for women in rural areas, especially women in difficult economic circumstances. Raising BSFL has recently gained attention in Vietnam, but larval rearing techniques have not been standardized by competent authorities. Therefore, there is a need for cooperation between relevant stakeholders to devise appropriate technical procedures for practicing and designing effective agricultural extension programs. In new jobs of animal husbandry, male famer tend to dominate female famer, thereby limiting the latter's access to advanced technologies and their ability to contribute to development goals. Women have limited opportunities to participate in training seminars on scientific and technical knowledge, with only around 20% receiving guidance from agricultural extension officers and 4% learning from newspapers, radio, and television programs.

As a result, women have limited access to information and scientific achievements, which puts them at risk in the product marketing process.

3.2. Women in animal farming and BSFL growing: findings from research

The results are presented in Table 1 and 2. In areas where agricultural land cannot be expanded and farming activities have become more efficient thanks to mechanization and technology, farm families must seek non-agricultural employment. Typically, women manage household responsibilities and take care of domestic tasks, while also being involved in animal and BSFL production, childcare, and maintaining family and community relationships. On the other hand, men usually work outside the home to earn money for the family. In some regions, men's labor is considered more valuable than women's, so they are often given priority for work outside the household, such as in construction or local building projects.

Women participate in many stages of animal and BSFL farming because the jobs that are expected to be done by men often occur in a short period of the year such as using their extra time to assist their wives during a busy harvest time or when women need help with heavy work.

Table 1. The number of participation in BSF training course and farming in Dong Nai and Tien Giang provinces

Item	Dong Nai	Tien Giang	Total
Participation in training course			
Farmers in province	50	50	100
Female farmers in province	11	6	17
Participation in BSFL farming			
Female farmers in province awareness and knowledge BSFL farming	38	47	85
Female farmers in province worked and applied the BSFL raising	33	47	80
Female farmers in province applied the best BSFL technologies	25	35	60

Middle-aged women are often more engaged in caring for BSFLs and animal production than younger women because they have a higher likelihood of employment outside the home. This is attributed to their advanced education, diverse skill sets for new employment opportunities, and better overall health. Factories and industrial areas tend to attract a predominantly young labor force, including many female workers. If employment in agriculture is unstable and challenging, it may lead to young people leaving agriculture and their local community to seek opportunities in industrial zones. It leads to a shortage of qualified human resources that are healthy and receptive to adopting science and technology for agriculture. Establishing the black soldier fly model within a sustainable circular agriculture system, alongside appropriate policies, can help stabilize the lives of local young individuals who are primarily engaged in agriculture.

With households in which both husband and wife work in animal and BSFL farming, the division of labour usually follows the community's concept, based on the biological characteristics and social roles of women and men. The men often take on heavy or harmful jobs. The women are supposed to undertake the more gentle and meticulous work. This contributes to stereotypes that reduce the role of women in decision-making and management, and their abilities are not fully recognized.

In Table 2, presents labor division between husband and wife and children in animal and BSFL production. This model of labor division shows that women take on most of the activities in BSFL production but only get involved in some stages of animal production. The stages involving farming techniques are usually undertaken by men.

Table 2. Gender division of labour in animal and BSFL farming (%)

Activity	Men	Women	Children
Animal farming			
Putting up shelter/prepare pens	69	31	0
Preparing feed	78	22	0
Grazing/herding	77	16	7
Tethering	0	100	0
Feeding	33	67	0
Watering	22	78	0
Cleaning shelter/pens	13	78	9
Cleaning animal	14	77	9
Waste disposal	22	69	9
Gathering forage	62	33	5
Buying animal	87	13	0
Buying feed	100	0	0
Taking animal to market	78	22	0
Selling product (eggs, meat,...)	21	77	2
Average of all activities	*48.29*	*48.78*	*2.93*
BSFL farming			
Putting up shelter/prepare pens	40	60	0
Preparing feed	25	75	0
Management	28	72	0
Tethering	0	100	0
Egg incubation	20	80	0
Feeding	25	75	0
Providing water	22	78	0
Cleaning shelter/pens	22	74	4
Cleaning animal	22	74	4
Waste disposal	22	74	4
Gathering manures	46	50	4
Buying eggs for L incubation	20	80	0
Buying feed	10	90	0
Taking larvae to market	40	60	0
Selling product (larvae)	80	20	0
Average of all activities	*27.29*	*71.57*	*1.14*

The division of labor in animal and BSFL production is flexible, according to family circumstances. Overall, men and women are involved in animal husbandry activities in almost equal proportions, with 48.29% of women and 47.21% of men (Table 2). Women are primarily responsible for detailed techniques such as feeding, watering, tethering, cleaning pens, and selling eggs and meat, while men are more involved in heavy labor. Some women may be responsible for all farming activities, including injection of medicine to animal or transporting animals to market for sale. The division of labor also varies depending on the type of animal and its specific requirements. Men typically play dominant roles in other animal farming activities, while women can participate in many BSFL raising (74.43%). In the process of raising black soldier fly larvae (BSFL), women are most involved in egg incubation (80%), feeding (75%), and management (72%). These are important steps that affect the productivity of harvesting larvae and the effective development of the BSFL growing model. Therefore, it's essential to develop training courses that focus on various techniques of BSFL raising for women to achieve optimal productivity.

Table 3. Primary decision makers about issues relating to BSFL growing

Decision	Dong Nai	Tien Giang
BSFL eggs	Depends on the supplier and some cases are women	Husband knows which eggs aregood or bad for better yield
Egg incubation	Women	Both spouses
Feeding techniques (feed, nutritional supply, taking care, management)	Almost depends on the supplier, some cases are women based on their attendance in some training sessions.	Based on the men knowledge gained from their attendance in many training sessions.
Feed/Agriculturalby-products purchase	Women decide the production tools of the feed great value because of their knowledge	Women decide the production tools of the feed great value because of their knowledge
Loans for animal development	Husband	Both spouses
Sale of products	Husbands are better traders and usually the transporters for sales	Husbands have plenty of time tolearn the marketprices

3.3. Factors influencing women's participation in animal farming and BSFL growing

The shortage and fragmentation of land is a hindrance to animal mechanization and production development. The cultivated land of each household is small for farmers to produce animals. The conversion of land to animal uses when the new practice is not what is listed on official papers makes farmers feel insecure. Having a certificate of land use right for animal or BSFL raising will greatly prolong the period of land use. A long period of land use rights is security for farmers who are actively farming.

The development of technology and techniques contributes to the change in the division of labor between men and women (Table 4). Given the progress of technology, local people, especially women are better able to control their production and rely less on their husbands in animal production. The application of technology has a direct impact on the raising methods of farmers, especially women. If women are provided the knowledge and apply the correct technical process, women producing animals or BSFL can now spare time for other work and only need to oversee production.

Farmers' animal production greatly benefits from training for technology transfer. In the surveyed areas, training courses and workshops on animal science knowledge and techniques were organized through various channels. For instance, the district-level Agricultural Extension Department collaborated with the commune Women's Union and the commune Farmers' Association to operate agricultural extension courses. Additionally, local authorities, in partnership with veterinary medicine companies, provided guidance to farmers on livestock raising and promoted and sold medicine products. On average, five to six training classes were organized in each surveyed area per year. The content of the training courses focused on procedures and methods for caring for animals, as well as BSFL production. This included selecting excellent animals/BSFL, using appropriate feed and medicines, disease prevention, and producing higher quality meat, an attractive appearance, and delicious taste.

Table 4. Changes in women's and men's participation in animal production with the aid of machinery

	Previous	Present
Prepare the pens	Done by men and used by hand and small machines	Men's work with the large machines and new technologies
Animal raising	Primarily done by women Men helped with carrying animal to the farms by bikes or trucks.	Women are still work but it saves time because some machines are used so the works must be done quickly. Men help with carrying animal on the farms by using machines
Spraying bacteria for safety protection	Primarily done by men	Women can do it using machines
Cleaning	Done by women	Done by women
Harvesting	Done by both men and women	Done by both men and women. They use machines.
Carrying animal	Mainly done by men. It was a heavy task because farmers had to carry on bikes or small trucks	Hire a big tractor to carry
Animal plucking in slaughterhouse	Use simple machines	Use animal-plucking machines

3.4. Some major challenges to women's participation in the animal and BSFL production

Women play a critical role in labor as they are directly involved in agricultural activities. However, the income per capita of agricultural workers, especially women, remains very low. Women predominantly engage in most activities related to growing BSFL, which bring higher productivity and economic benefits, but are less recognized as they have less decision-making power than men.

The traditional farming methods based on personal experience and customs are still widely used by many households. Unfortunately, the collaboration between farmers, the government, scientists, and distributors in agriculture is limited. Women have limited access to training sessions where they can acquire the necessary scientific and technical knowledge, which hinders their ability to develop skills in these areas. Consequently, they face numerous risks during agricultural production.

The involvement of female farmers in decision-making and access to resources is lower compared to their male counterparts. In agricultural activities and projects, women often participate in work-related meetings, training, and tasks, but they have limited involvement in the management and supervision of agricultural work.

Therefore, priority and incentive policies for women should be promoted. This can include opening vocational training classes, creating more suitable job opportunities, increasing income for rural women, and helping them develop their full potential. Additionally, improved access to and ownership of land and credit for women should be established to enhance their role and control in the family and reduce gender stereotypes.

Raising black soldier flies can serve as a viable model for women working as household farmers, especially those facing challenging economic circumstances. Our information picture is through the process of transferring BSFL technology to farmers, focusing on women, which appears to be quite similar to typical livestock activities. However, black soldier fly farming offers a promising avenue for rural women to achieve both labor and economic independence, ultimately fostering confidence in their abilities. The support of the government, society, and family members is crucial for the advancement of women.

4. CONCLUSIONS

The role of women in BSFL activities has been under estimated and valued. In determining smallholder farmers' awareness on BSF farming, the results revealed that few farmers in two provinces are aware of the potential use of BSFL as feed. Farmers participated, shared their experiences in small scale production and expressing their enthusiasm for adopting this technology, not only for their work on BSFL production in the fields but also in the management of organic waste at home. The main determinants of farmers' awareness were years of education and participates in agricultural groups. Women have little access to formal extension services, and awareness about important agricultural innovations. Innovations the BSF production are often not shared through these groups. Some are members of youth organizations active in organic waste management and the production of BSFL, and others are business man or graduate students interested in BSFL farming.

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DUCK-RICE REARING SYSTEM IN THE DIRECTION OF ORGANIC CIRCULATION IN BINH THANH COMMUNE - THANH BINH DISTRICT - DONGTHAP PROVINCE

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ABSTRACT

The experiment took place at a farm household located in Binh Thanh commune, Thanh Binh district, Dong Thap province. A total of 240 Dutch ducks, aged 3-13 weeks, were raised in a 2,000m² rice field, and the experiment consisted of 5 treatments: T4, T8, T12, T16, and T20 (corresponding to 4, 8, 12, 16, and 20 ducks for each 100m² rice field, respectively). The experiment was replicated 4 times, resulting in a total of 20 experimental units. All treatments were subjected to identical conditions and received the same basic diet throughout the 10-week trial period, involving ducks aged 3 to 13 weeks. The study's findings indicated that Ducks in the T16 and T20 treatments exhibited lower daily weight gain (DWG) compared to those in the T4, T8, and T12 treatments. The T4 treatment showed the most favorable feed conversion ratio (FCR), attributed to the fact that ducks found more feed in the field when the duck population was only 4. As the number of ducks in the treatment increased, the FCR also rose. The highest profits were observed in the T20, T16, and T12 treatments, with profits declining gradually, while the lowest profits were recorded in the T4 treatment. Raising Hoa Lan ducks, with a quantity of 12 ducks/100m² (or 120 ducks/1,000m²) on a rice field, resulted in high profits, along with favorable daily weight gain, FCR, and carcass quality.

Keywords: *Hoa Lan ducks, Duck-Rice system, organic.*

1. INTRODUCTION

In recent years, the model of livestock combined with crop cultivation has been positively responded to by many localities, especially in the Red River Delta and the Mekong Delta, in which the duck - rice model brings the most benefits and people also most applicable. This is a highly feasible model, suitable for the actual conditions in each locality, creating conditions for people to produce, increase income, improve life.

This model is considered an advantage in the Mekong Delta because it is where the largest duck population in the country is located and also the largest area of wet rice cultivation. According to statistical data, by 2022 the Mekong Delta region produced 852 million eggs. Currently, one of the 13 Mekong Delta provinces and cities has a total duck population growth. The large fields in these places are a favorable environment for the duck farming industry. This is an ecological resource utilization that our farmers have had a lot of experience with for a long time (Gau and Nha, 2013).

Recently, agricultural production has focused on productivity and output according to linear thinking, not paying enough attention to sustainable development, environmental friendliness, the surplus of the production process (organic fertilizer) to nourish and improve soil structure, and protecting biodiversity... As a result, it causes waste of agricultural by-products and livestock waste...

Therefore, it is necessary to encourage renewable energy, return organic matter to the soil, prevent burning of straw and slash-and-burn fields, promote the use of environmentally friendly products, and perfect and develop eco-friendly models. Circular economy models such as the Duck-Rice model in Vietnam's Mekong Delta. The goal of the research is to find the most appropriate combination method and farming density to increase income for farmers.

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2. MATERIALS AND METHODS

2.1. Experimental birds and design

The experiment was conducted at a farm household, Binh Thanh commune, Thanh Binh district, Dong Thap province. A total of 240 Dutch ducks aged 3-13 weeks were raised in a rice field with an area of 2.000m². Experiment with 5 treatments: Tr4, Tr8, Tr12, Tr16 and Tr20 corresponding 4, 8, 12, 16 and 20 ducks for each 100m² rice field. The experiment was repeated 4 times, for a total of 20 experimental units.

All treatments were raised under similar conditions and with the same basic diet. The trial lasted 10 weeks with ducks from 3 to 13 weeks old. The food composition of the basic diet (BD) is presented in Table 1.

Table 1. Ingredients, chemical composition of BD

Variables		3-13 weeks age
Ingredients, %	Maize meal	35.3
	Broken rice	22.3
	Rice bran	12.0
	Fish meal	6.5
	Soya meal	19.5
	Lysine	0.05
	Methionine	0.15
	Bone meal	2.0
	Seashell meal	1.5
	Premix	0.8
Chemical composition and Metabolisable energy, %	ME, kcal/kg feed	2950
	ME, MJ/kg DM	12.11
	EE	3.95
	CP	20.0
	CF	4.11
	NFE	67.1
	Ca	2.90
	P	1.08

The basal diet was formulated to contain 12.3 MJ ME/kgDM and 19% CP.

2.2. Treatments and feeds

There were 5 treatments:

Tr4: BD + 4 ducks on 100m² of rice field

Tr8: BD + 8 ducks on 100m² of rice field

Tr12: BD + 12 ducks on 100m² of rice field

Tr16: BD + 16 ducks on 100m² of rice field

Tr20: BD + 20 ducks on 100m² of rice field

2.3. Breeding method combines the Duck-Rice model

Once rice is planted, it is time to raise ducks. When the ducks are 2 weeks old, the rice plants have grown well. Then the ducks were released into the rice fields. When the rice blooms, we lock up the ducks. When the rice is ripe and harvested, the ducks will be released again for about 2 more weeks to end the experiment.

2.4. Model correlation

Limit rice-damaging insects, weeds, snails, and rice diseases, because ducks take advantage of available food sources in the fields. Reduce the use of pesticides and fertilizers that are toxic to humans and the environment. Save on seeds, fertilizers, etc. Take advantage of farmers' free time during the rice crop and when flood water rise.

2.5. Housing and management

Ducks are raised in spacious enclosures, comprising 20 separate sections measuring 10x10x1m each, and these sections are divided by nets. Each of these sections is furnished with both feeding and drinking troughs. The ducks receive three daily feedings at 7:00, 13:00, and 17:00. The amount of food provided is adjusted weekly in accordance with the ducks' actual growth, incrementally increasing from 2% to 5% per week. Additionally, the ducks have unrestricted access to water and are permitted to roam freely within the rice fields. Before commencing the experiment, the ducks underwent vaccination to safeguard against common diseases such as duck cholera, H5N1, and pasteurellosis.

The daily consumption of food and nutrients was assessed by gathering and weighing both the provided food and any remaining portions every morning. Weekly, the body weight of the ducks was documented. To calculate feed conversion efficiency (FCR), the amount of feed consumed was divided by the weekly weight gain.

FCR=Daily feed consumed/Daily weight gain

2.6. Chemical analyses

The feeds given were subjected to a chemical composition analysis, which covered parameters such as dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE), crude fiber (CF), and ash. These analyses adhered to the protocols specified in AOAC (1990). For NDF (neutral detergent fiber) analysis, the procedure outlined by Van Soest *et al.* (1991) was followed, and ME was computed utilizing the formula devised by Janssen (1989).

2.7. Statistical analysis

The data collected were subjected to analysis utilizing the GLM within the Minitab program, version 18.1.0 (Minitab, 2018). To identify any noteworthy distinctions between the two treatments, the Tukey method available in Minitab (2018) was utilized.

3. RESULTS AND DISCUSSION

3.1. Effects of different rearing densities on growth performance of Hoa Lan ducks

According to table 2, the daily weight gain (DWG) of ducks was found to be lower in the Tr16 and Tr20 treatment compared to the Tr4, Tr8 and Tr12 treatments.

Table 2. Weight gain, and FCR of Dutch duck

Item	Treatments					SEM	P
	Tr4	Tr8	Tr12	Tr16	Tr20		
IW (g)	95	97	95	93	94	4.50	0.876
FW (g	2,145^{a}	2,105^{a}	2,040^{ab}	1,905^{b}	1,840^{c}	25.18	0.001
DWG (g)	29.3^{a}	28.7^{a}	27.8^{ab}	25.9^{c}	24.9^{d}	1.05	0.003
DM intake	71.8^{e}	72.6^{de}	72.5^{cd}	73.5^{b}	75.1^{a}	1.09	0.05
FCR	2.45^{e}	2.53^{de}	2.61^{cd}	2.84^{b}	3.01^{a}	0.03	0.04

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

The DWG of ducks decreased when the number of ducks in the treatment increased because the ducks had to compete to find feed, leading to a decrease in DWG.

The best feed conversion ratio (FCR) was observed in the Tr4 treatments, when only raising 4 ducks during the treatment period. The decrease in FCR is because ducks find more feed in the field when the number of ducks is only 4. As the number of ducks in the treatment increases, the FCR also increases. These FCR values were lower than about 3.05-3.86 reported by Pham Tan Nha (2020), showing that when ducks were raised in the rice field FCR has decreased.

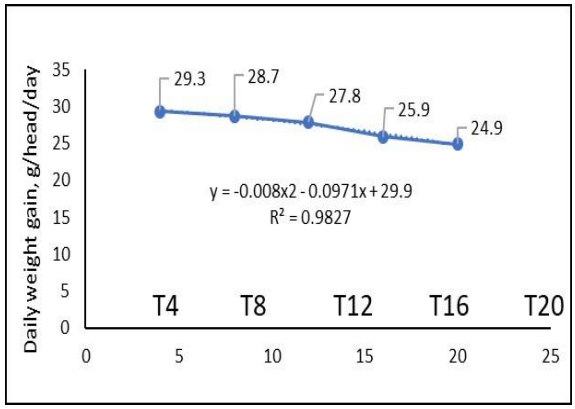

Figure 1. Effect of treatments on DWG

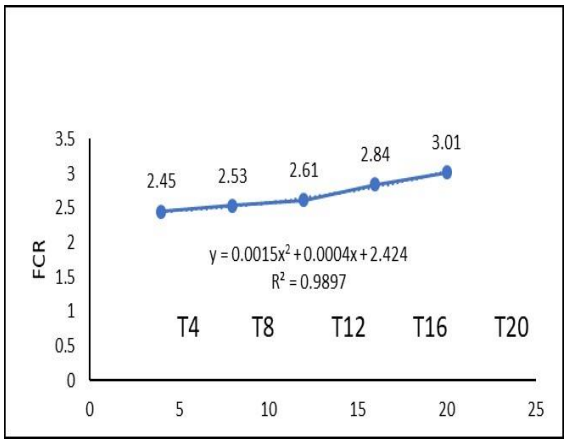

Figure 2. The effect of treatments on FCR

The Tr20 was the highest FCR (3.01) compared to the remaining Trs (Tr4, Tr8, T1r2 and Tr16), with FCR values of 2.45, 2.53, 2.61 and 2.84, respectively. The final LW of the duck in this trial ranged from 1,840g to 2,145g, which were lower than the weights reported in the previous trial conducted by Phuong and Nha (2017).

Table 3. Carcass and weights of internal organs in Dutch ducks raised on rice fields

Item	Tr4	Tr8	Tr12	Tr16	Tr20	SE	P
Slaughter LW (g)	2,145^{a}	2,105^{a}	2,040^{ab}	1,905^{b}	1,840^{c}	18.6	0.03
Carcass weight (g)	1,562^{a}	1,518^{b}	1,467^{bc}	1,358^{c}	1,303^{d}	15.8	0.05
% Carcass	72.8	72.1	71.9	71.3	70.8	0.84	0.25
Thigh meat weight (g)	322^{a}	308^{a}	293^{ab}	266^{b}	249^{c}	5.75	0.03
% Thigh meat	20.6	20.3	20.0	19.6	19.1	1.18	0.80
Breast meat weight (g)	251^{a}	240^{a}	227^{ab}	205^{bc}	198^{c}	4.08	0.03
% Breast meat	16.1	15.8	15.5	15.1	15.2	0.36	0.85
Heart weight (g)	10.1	10.2	10.5	11.0	11.1	0.85	0.76
Liver weight (g)	19.0	19.5	20.0	20.1	20.0	5.11	0.46
Cecal length (cm)	21.5	21.0	19.8	19.2	19.6	1.34	0.77

The number of ducks in the treatment had a clear effect on the value of carcasses and internal organs. Regarding carcass weight, the Tr4, Tr8 and Tr12 showed significantly higher weights compared to the other treatments (P<0.05), as indicated in table 3.

Nevertheless, the carcass percentage did not display any notable variances among the treatments (P>0.05), with values aligning with the range of 70.8-72.8% as reported by Nguyen Thi My Linh (2011). Notably, the weights of breast meat and thigh meat exhibited significant increases in the Tr4, Tr8 and Tr12. However, the percentages of breast meat and thigh meat did not demonstrate any significant fluctuations across the treatments (P>0.05). Furthermore, no significant distinctions were observed in the weights of internal organs among the treatments.

3.2. Economic efficiency

Analysis of economic efficiency between treatments presented in table 4 shows that the lowest investment capital in Tr4 being 12.2 USD/Tr, investment capital tends to increase gradually as the number of ducks raised increases and investment capital increases. The highest in the Tr20 was 63.4 USD/Tr. However, the profit/Tr has the opposite trend. The highest profits/Tr were in Tr20. Profits tend to decrease and the lowest profits/Tr were in Tr4.

Table 4. Economic efficiency of ducks-rice (USD)

Item/Trs	Tr4	Tr8	Tr12	Tr16	Tr20
Duck, 0.5USD/head	2.0	4.0	6.0	8.0	10.0
Feed, kg/Trs	21.0	42.6	63.9	86.6	110.8
Feed, USD/Trs	9.5	19.2	28.8	39.0	49.9
Veterinary medicine	0.7	1.4	2.1	2.8	3.5
Investment	12.2	24.6	36.9	49.8	63.4
Income from ducks	27.5	53.9	78.3	97.5	117.8
Profit/Tr	15.3	29.3	41.4	47.7	54.4
Profit/Investment (%)	125	119	112	96	86

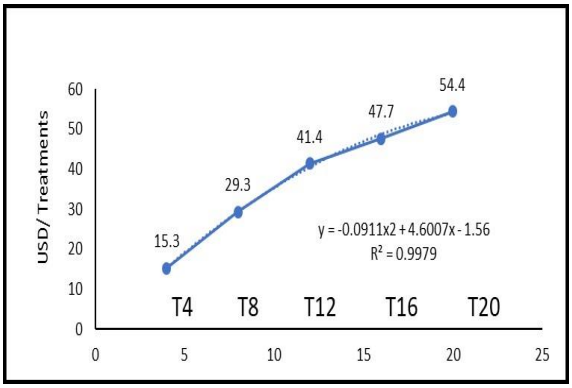

Figure 3. Effect of different treatments on profit

This shows that the higher the density of ducks raised, the higher the profits/Tr. However, Tr12 gave quite high profits/Tr (36.9 USD/Tr) and Duck WG/day is equivalent to the Tr4. This result is also consistent with Nguyen Tan Loi (2022). Raising ducks in the field brings higher

profits than raising ducks in captivity in the same duck breed and in the same season.

3.3. The effect of duck production on the yield of rice

Raising ducks on rice has increased rice productivity. The treatments with the highest rice yield were Tr12, Tr16 and Tr20 (63.9, 54.1 and 64.7kg, respectively), which were higher than the remaining treatments.

Table 5. Effect of duck farming on rice yield, kg/100m²

Item	Tr0	Tr4	Tr8	Tr12	Tr16	Tr20	SE	P
Rice-duck system	54.5^{d}	57.3^{c}	60.8^{b}	63.9^{a}	64.1^{a}	64.7^{a}	2.34	0.04
Rice-duck system, %	100	105.1	111.6	117.2	117.6	118.7	-	-

4. CONCLUSIONS

Raising Dutch ducks with a quantity of 12 ducks/100m² (120 ducks/1,000m²) on a rice field resulted in high profits, while also achieving good results in daily weight gain, feed conversion ratio, and carcass quality.

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BLOOD PHYSIOLOGICAL INDICATORS OF GOAT CROSSBREDS F_1(BOER x BACH THAO) AND F_1(SAANEN x BACH THAO)

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ABSTRACT

This article presents the results of research on blood physiological indicators of F_1(Boer x Bach Thao) and F_1(Saanen x Bach Thao) goat hybrids raised at the Binh Duong Large Cattle Research and Development Center. Blood samples from 160 clinically healthy goats were collected according to age and sex. Blood physiological indicators: white blood cell count (WBC), types of white blood cells: Lymphocytes, Mono, Neutral; red blood cell count (RBC), hemoglobin (HGB), hematocrit (HCT), mean HC volume (MCV), mean amount of HGB in a red blood cell (MCH), mean hematocrit (MCHC), red blood cell distribution width (RDW) was measured using a fully automatic hematology analyzer Hemascreen 18. The lymphocyte, monocyte and neutrophil indexes of each goat breed vary with age and gender ($P<0.05$). The number of white blood cells, red blood cells, and red blood cell distribution did not differ between ages and genders ($P<0.05$). The indexes of lymphocyte, mono, neut, MCH, MCHC in males tend to be higher than in females of the same age ($P<0.05$), but do not differ significantly in the same sex according to age. This study provides data on blood physiological indices of hybrid goats, contributes to knowledge of reference ranges for hybrid goat breeds and evaluates management, nutrition, and health monitoring, diagnose diseases to increase efficiency in goat farming.

Keywords: *Blood physiological index, goat hybrid, Bach Thao, Boer, Saanen.*

1. INTRODUCTION

The population is increasing while the quality and quantity of natural resources used for livestock are decreasing. This requires improving and developing innovative and sustainable livestock farming methods to create higher quality products to meet increasing human needs. Cattle farming is the foundation of the livestock system, in which goat meat and milk are the main products in the consumer diet. As the global population grows and developing countries strive to consume more meat, the need for animal protein and dairy to maintain good nutrition and diet for consumers is also increasing. Increasing livestock production requires an understanding of biological characteristics to increase efficiency and improve animal characteristics such as fertility, growth and development, meat quality and yield, milk.

Researching blood physiological indicators to understand the nutritional status and health of animals is extremely necessary. Blood physiological indicators have a role and significance in genetic characteristics, growth and development process, breed quality, fertility, and adaptability of animals in different environmental conditions. (Campora *et al.*, 2011; David *et al.*, 2013). Blood physiological indicators in goats are important data to help goat farmers easily diagnose goat diseases and thereby find the causative agent and appropriate treatment methods. Blood physiological indices can also be used to monitor or evaluate the health, nutritional and physiological status of ruminants (Al-Eissa *et al*., 2012). It reflects the health status of livestock that we cannot observe from the outside. Factors such as nutrition, stress, reproductive status, age, sex, genetics, management, housing and environmental factors all have a profound influence on hematological and biochemical indicators of animals. small ruminants (Samira *et al.*, 2016).

According to the approval to adjust the master plan for socio-economic development of Binh Duong province to 2020, supplementing the planning to 2025 by the Prime Minister

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(2014), Agriculture sector will shift in the direction of reducing the proportion of the crop industry, increasing the proportion of livestock and agricultural services. Among them, goat farming has an important position in the economy. Recently, goat farming in Binh Duong has developed strongly, many new imported goat breeds, and large-scale goat farms have been formed and developed.

Large livestock research and development center with the task of importing, domesticating and cross-breeding livestock breeds, including goats. Here, the indigenous Bach Thao (BT) goat breed is cross-bred with the imported Saanen (Sa) and Boer (Bo) goat breeds to create hybrids that improve meat and milk productivity. However, studies on their physiological and blood biochemical indicators are still quite modest. Meanwhile, biochemical or hematological analysis of blood is important to evaluate the general physiological condition of the animal. Determining reference values can assist in evaluating management practices, nutrition and diagnosing animal health status. On the other hand, variables such as nutrition, stress, temperature, climatic conditions, disease, age, gender and breed can affect blood parameters. Therefore, studying the physiological and biochemical indicators of goat breeds and their hybrids is very necessary. The purpose of this study is to evaluate blood physiological indicators of Boer x Bach Thao and Saanen x Bach Thao hybrids. The values determined in this study contribute to the knowledge of reference ranges for crossbred goat breeds and assessments of management, nutrition, health status monitoring, and disease diagnosis to increase efficiency fruit in goat farming.

2. Materials and Methods

2.1. Materials

Farm: Goats were raised at the Large Livestock Research and Development Center, Lai Hung commune, Ben Cat district, Binh Duong province.

Food, water, barn cleaning

Goats were raised intensively at a density of 2 m^2/head. The barn was designed in the style of a stilt house, the wooden floor was 1m away from the cement floor, and the roof was made of cement corrugated iron. Goats were fed 2 meals/day at 7.00AM and 5.00PM, including green elephant grass, synthetic bran (De Heus), leaves (bottle, or jackfruit leaves, mother of pearl). On average, each animal receives 2-4kg of grass, 0.5-0.8kg of bran, and 0.5kg of leaves/day. Roll dry straw and put it on troughs to feed the goats at night. Drinking water was clean water, put into a clean trough placed in the barn for the goats to drink freely. The water trough was cleaned every day and the water was changed once a day.

The barn was cleaned with water spray every day. Cleaning and disinfection were carried out every 2 weeks. The antiseptic solution used is BESTAQUAM-SR with the ingredient: didecyl dimethyl ammonium bromide, mix at a ratio of 1/400.

2.2. Collect samples and analyze blood biochemical indicators

2.2.1. Sample selection

Randomly select 20 goat individuals 3-6 months old (sexually immature age) and 20 individuals 12-20 months old (adult age) for F_1(BoxBT) and F_1(SaxBT) goat crosses, according to each group: male, female. All individuals were in good health based on clinical monitoring (eating, walking, and activities are normal; there were no signs of abnormalities in eating, living, or excretion activities). Pregnant or sick animals were excluded. An information sheet was used to collect information on age, sex, breed, type of feed used and sample collection date.

Table 1. Exp. blood physiology sample collection

Hybrid	Male		Female	
	3-6month	>12month	3-6month	>12month
F_1(BoxBT)	20	20	20	20
F_1(SaxBT)	20	20	20	20

2.2.2. Survey criteria

Blood physiological indicators: red blood cell count (RBC-Red blood cells), hemoglobin (HGB), red blood cell capacity (HCT-Hematocrit), mean corpuscular volume (MCV), mean hemoglobin amount Mean corpuscular hemoglobin in a red blood cell (MCH-Mean corpuscular hemoglobin), mean red cell concentration (MCHC-Mean corpuscular hemoglobin concentration), red cell distribution width (RDW-Red cell distribution width), platelets (PTL-Platelet) , mean platelet volume (MPV-Mean platelet volume), platelet distribution width (PDW-Platelet distribution width), platelet capacity (PCT-Plateletcrit).

All indicators are monitored in normal health condition. The goats are considered normal based on clinical monitoring, with no pathological signs or abnormalities in eating and activity.

2.2.3. Blood sample collection and analysis

Blood samples were taken through the jugular vein at 7-8am, without feeding, using a 3ml syringe (25 Gx1 size needle) to take 1.5-2ml of blood/individual. After taking blood, the sample was quickly put into an anticoagulant (EDTA) tube, gently shaken, and the name and symbol of the symbol of the sampled anima was recorded. Blood physiological parameters were performed on a fully automatic hematology analyzer Hemascreen 18.

2.3. Statistical analysis

ANOVA and Post hoc test analysis with Tukey Kramer test to evaluate differences between groups ($P<0.05$). Statistical parameters were processed using MS Excel 2020 software. Results was expressed as Mean and Standard Deviations values (Mean±SD).

3. RESULTS AND DISCUSSIONS

The results of monitoring blood physiological indicators of F_1(BOxBT) AND F_1(SAxBT) goats hybrids were studied and presented in Tables 2 and 3.

The results in table 2 showed that the WBC of female and male goats of Bo x BT hybrids at the same age are not significantly different, but there was a statistical difference in this same index between the two ages. In male goats 3-6 months old, WBC was at $18.52x10^9$/l, higher than that of male goats 12-36 months old ($13.61x10^9$/l) ($P<0.05$). This result is also consistent with previous publications, WBC of young goats were higher than that of adult goats. WBC of BT female goats reached $18.07x10^9$/l, higher than that of male goats ($16.78x10^9$/l). WBC of Bo goats has the reference value in male goats ($12.52x10^9$/l) and goats. Female board ($10.6x10^9$/l); The WBC count of young goats is $19.32x10^9$/l, significantly larger than that of adult goats ($11.56x10^9$/l) (Nguyen Thi Thu Hien *et al*., 2022). This result is equivalent to the study of Washaya (2019), male goats were $13.63x10^9$/l and female goats ($11.98x10^9$/l) and higher than goats in Salta, Argentina in the study of Analía *et al*. (2021). The total white blood cell (WBC) count in this study was higher than the value obtained in Sokoto Red goats (Tambuwal *et al*., 2002).

The composition of lymphocytes in males ($69.08x10^9$/l) was higher than in female goats ($61.03x10^9$/l) and this difference was statistically significant, while, Mono, Neut in females was higher at both ages monitored (Table 2). The results were similar in F_1(SAxBT) goat hybrids (Table 3), all indicators related to Leukocytes in both groups of hybrids were within the normal values published by Merck (2016). Gender was observed to have a significant influence on the lymphocyte and neutrophil values of the goats examined. Male crossbreeds had increased lymphocyte values compared to female goats, while female goats had increased neutrophil values compared to male goats. This finding is similar to the observation reported for Sokoto red goats by Tambuwal *et al*. (2002). This may show that the composition of WBC fluctuates with the age of the goat. WBC is an important

component participating in the immune system to help the body fight against harmful agents. The increase or decrease in WBC is related to the immune system. physiological states, diseases and when infected with bacteria, the number of WBCs will increase (Al-Seaf and Al-Harbi, 2012). Monocytes often increase in the following cases: viral infections, parasitic infections, bacterial infections, diseases related to cancer, enteritis, mononucleosis, lymphoma, myeloma... (Samira, 2016). Neut index represents the level of neutrophils in peripheral blood cells. Neut is a type of mature cell found in blood cells and they play an essential role in blood formation and immunity to attack or destroy foreign viruses and bacteria that enter the body. Neut index increases when animals are infected, infected, stressed, lose a lot of blood and decrease when exposed to poisoning, heavy metal contamination, exhaustion, labor, treatment with chemicals and immunosuppressants. According to tables 2 and 3, the Neut index of young male goats and adult goats has no significant difference (P<0.05) and is similar to previous research reports on BT, Bo, and Sa goats (Nguyen Thi Thu Hien *et al*., 2022). Other parameters did not have significant differences with other reports on goats (Merck, 2016; Analía *et al*., 2021). Thus, in crossbred animals, the immune system has developed with such a number of immune cells to bring good health and the ratio of lymphocytes, monocytes, neutrophils (%) is similar to the reports. foxes previously on goats.

Males and females had no statistically significant difference (P>0.05). Table 2 shows that the RBC of F_1(BoxBT) were 2.63; 2.87; 2.19; 2.65 x10^{12}/l (Table 2). The results in F_1(SaxBT) were 2.17; 2.21; 2.18; 2.19 x10^{12}/l (Table 3). All of the above figures are within the RBC reference value published by Merck (2016) from 1.3-3.7 x10^{12}/l. According to Nguyen Thi Thu Hien *et al.* (2022), RBC indices also did not have a significant difference between the studied goat breeds (P>0.05), however, higher levels were found in the goat breeds. foreign varieties (Bo, Sa). In each breed of goat, these indicators do not differ between males and females and between age groups. RBC was within reported normal limits in male Sa goats. RBC in adults and juveniles had no difference but was significantly lower than the study by Dhuha *et al.* (2021) with the red blood cell index in goats Sa 3.24x10^{12}/l. WBC in adult goats (17.36x10^9/l) is higher than in young goats (13.66x10^9/l). This result is similar to Elitok (2012) study with the lymphocyte index of young goats 14.64x10^9/l and adult goats 6.44x10^9/l.

Table 2. Blood physiological indices of age, sex (n=20)

Parameter	3-6 Months		>12 Months	
	Male	Female	Male	Female
WBC, 10^9/l	18.52^a±5.72	17.37^a±4.35	13.61^b±5.16	11.31^b±4.28
Lympho, 10^9/l	12.57^a±4.35	11.71^b±3.62	9.22^c±3.41	8.76^c±2.76
Mono, 10^9/l	2.92^a±0.56	3.63^b±0.71	2.09^c±0.68	2.03^c±0.73
Neut, 10^9/l	2.86^a±1.53	3.49^b±1.21	3.33^c±1.43	3.97^d±1.25
Lympho, %	69.48^a±5.58	62.53^b±6.53	66.27^a±5.82	63.36^b±4.46
Mono, %	16.61^a±2.73	18.36^b±3.62	17.86^b±4.56	20.62^c±4.41
Neut, %	15.47±4.47	18.64±4.62	16.62±5.38	17.62±3.45
RBC, 10^{12}/l	2.63±1.08	2.87±0.89	2.19±1.01	2.65±0.85
HGB, g/dl	7.87±1.22	7.31±1.62	6.91±1.33	5.79±1.25
HCT, %	8.17±1.74	7.46±2.65	4.71±1.39	5.83±2.2
MCV, fl	34.52±2.56	33.36±1.94	32.35±1.56	34.27±1.32
MCH, pg	34.82^a±3.35	30.65^b±3.91	35.50^a±4.72	31.85^b±5.37
MCHC, g/dl	108.72^a±13.21	101.55^b±12.43	107.26^a±15.13	102.63^b±21.16
RDW-SD, fl	24.69±3.25	23.78±3.22	23.56±3.35	22.26±2.87
RDW-CV, %	18.35±2.14	17.97±2.33	17.27±2.75	15.83±1.76

Note: Mean values with different letters in the same row are statistically significant differences (P<0.05).

HGB - the amount of hemoglobin in a volume of blood, is a protein molecule found in red blood cells that carries oxygen and gives red blood cells their red color. The amount of HBG increases when animals are dehydrated or have heart or lung disease and decreases when animals are injured with bleeding, blood loss, and hemolytic reactions (Maria *et al.*, 2018). The amount of HGB in the two stages of young goats and adult goats is

similar, however this data is different from the statistical data of Dhuha (2021) with the amount of HGB in males (10.34 g/l) and in female (10.30 g/l) (Elitok *et al*., 2012). Hemoglobin (Hb) concentrations in this study were within the range of high values obtained in Sokoto Red goats (Tambuwal *et al*., 2002). Hb in adult WAD goats was higher than the value obtained in young WAD goats. West African Dwarf goats appear to have relatively high Hb values and this is an advantage in terms of their ability to transport oxygen in the blood. The HGB content of Bo x BT hybrids ranged from 6.79-7.87 g/dl, juveniles were higher than adults; and that were 7.69-7.89 g/dl in SaxBT hybrid goats. These values in Bach Thao, Boer, and Saanen goats were 7.01-7.68 g/dl respectively; 4.91-8.17 g/dl; 7.68 g/dL (Nguyen Thi Thu Hien *et al*., 2022). All of these values are within the normal range studied in many other goat breeds of 9-15 g/dl (Merc, 2016).

Table 3. Blood physiological indices of age
(n=20/lot)

Parameter	3-6 Months		>12 Months	
	Male	Female	Male	Female
WBC, 10^9/l	15.13±2.23	16.9±3.57	17.66±5.32	16.56±4.12
Lympho,10^9/l	7.84^a±0.89	13.79^b±2.01	10.85^c±2.25	10.82±3.05
Mono, 10^9/l	2.48±0.79	2.29±0.21	3.04±0.97	2.60±0.80
Neut, 10^9/l	3.31^a±0.72	1.68^b±0.41	2.8^c±1.01	2.59^c±1.02
Lympho, %	56.95^a±4.47	78.24^b±2.50	64.13^c±8.63	56.44^c±10.58
Mono, %	20.42^a±5.42	13.9^b±2.74	17.04^c±2.57	17.12^c±4.67
Neut, %	23.42^a±2.95	18.67^b±1.50	18.9^b±7.34	16.99^b±7.73
RBC, 10^{12}/l	2.17±0.49	2.21±0.52	2.18±0.17	2.19±0.44
HGB, g/dl	7.69±1.29	7.77±1.32	7.89±0.65	7.78±1.16
HCT, %	7.09±1.74	7.22±1.83	7.42±1.17	7.24±1.64
MCV, fl	32.42±0.83	32.98±0.51	32.96±0.47	32.78±0.68
MCH, pg	38.53^a±5.05	33.29^b±1.22	37.03^b±2.30	34.61±3.94
MCHC,g/dl	109.22^a±11.38	101.51^b±4.89	108.17^a±8.73	102.29^b±9.23
RDW-SD, fl	24.1±2.93	25.83±1.24	25.89±1.09	25.27±2.12
RDW-CV,%	17.99±1.44	18.81±0.63	19.08±0.80	18.62±1.12

The average molecular weight of red blood cells in the blood (MCV) of F_1(BoxBT) and F_1(SaxBT) crossbred goats in male adulthood is 32.35 and 32.96 fl, respectively, not significantly different from that of female goats, which is 34.27fl and 32.78 fl, these values are within the normal range of 28-40fl determined in goats (Merck, 2016). MCV is used to evaluate the size of red blood cells. If MCV is low compared to normal, it can be diagnosed that goats are suffering from microcytosis, often caused by iron deficiency. On the contrary, if MCV is high, goats will suffer from macrocytosis due to weak liver, vitamin B12 deficiency, and folic acid deficiency. If the goat shows some unusual signs such as: bruises on the body, bleeding, pale skin, etc., it may be due to unstable MVC index (Richard, 2016). This result is similar to that published on Bach Thao, Boer, and Saanen goats (Nguyen Thi Thu Hien *et al*., 2022). This observation parallels the values obtained for Sokoto red goats in Nigeria (Tambuwal *et al*., 2002).

The average hemoglobin count in a red blood cell (MCH) and mean red blood cell concentration (MCHC) in both tables 2 and 3 showed that males tend to be higher than females of the same age ($P<0.05$), however there was no statistically significant difference in the same gender in 2 age groups. According to El Nasri *et al*. (2016), in each breed of goat, these indicators also differ between males and females and between age groups. The parameters RDW-SD and RDW-CV of male crossbred goats were not different from those of females and between ages ($P>0.05$). This result is similar to study of Elitok (2012); Dhuha (2021) with an MCH level of 32.63pg.

4. CONCLUSIONS

The results obtained from this study show that the blood biochemical indices of F_1(Boer x Bach Thao) and F_1(Saanen x Bach Thao) goat hybrids are within the normal limits published in goats. This study contributes to supplementing the data source on blood biochemical indicators of goats raised in Vietnam, and also serves as a reference when assessing the health of hybrid

goats based on physiological and blood biochemical indicators. It is necessary to continue to research the effects of nutritional factors, climate change... on these indicators on goat breeds raised in Vietnam.

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SEASONAL TESTOSTERONE CHANGES IN SAANEN GOATS

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ABSTRACT

The aim of this study was to monitor changes in serum testosterone concentrations of Saanen goats by season and by age. The study was conducted on 30 adult male Saanen goats (15 2-year-olds and 15 3-year-olds) raised semi-intensively at the Binh Duong Large Livestock Research and Development Center. Blood was taken from the jugular vein on the 10th day of every month, serum was collected and testosterone content was determined by electrochemiluminescence testing, with the Cobas E601 immunoassay system. The results determined the overall average level of testosterone content in male Saanen goats over 12 months. The influence of age factor on testosterone production in Saanen goats (2 and 3 years old) is statistically significant ($P<0.05$). The testosterone hormone concentration of male Saanen goats changed significantly ($P<0.05$) with the seasons, decreasing significantly during the non-breeding season. The lowest plasma testosterone concentration was 1.95±0.16ng/ml (in 2-year-old goats) and 1.61±0.85 ng/ml (in 3-year-old goats) in April (dry season) and the highest in 11 months 11.17±2.46 ng/ml (in 2-year-old goat) and 11.85±2.14ng/ml (in 3-year-old goat) in September (rainy season). This result is used as a reliable reference in research, when using reproductive support tools and applying breeding strategies to improve reproductive performance in Saanen goats.

Keywords: *Goat, reproduction, Saanen, testosterone.*

1. INTRODUCTION

Reproduction is a major factor contributing to increased goat production efficiency. Determination of reproductive hormone content indicates the physiological reproductive status of livestock and is meaningful in selecting, mating, and supporting effective reproduction. On the other hand, many variables such as nutrition, stress, temperature, climatic conditions, illness, and age can affect reproductive hormone levels. Therefore, determining the reference values of these parameters for goat breeds being raised is very important. Due to this fact, many studies have investigated reproductive hormone parameters for many goat breeds raised in various locations around the world (Charallah *et al*., 2000; Chentouf *et al*., 2011). In Vietnam, research on these parameters for pure domestic and imported goat breeds being raised today is still quite modest. Recently, the Estradiol and Progesterone content of Bach Thao goats and Boer goats during pregnancy have been studied (Thi Thu Hien Nguyen and Thanh Binh Nguyen, 2022), seasonal changes in testosterone of Bach Thao goats have been studied (Thi Thu Hien Nguyen, 2023). The results of this study show that Bach Thao goats, a native goat breed of Vietnam, have plasma testosterone levels that change over the months of the year, with clear seasonality, with high testosterone levels in the summer. rainy season and lower in the dry season. However, there have been no studies on testosterone in male Saanen goats. On the other hand, climate change is a challenge for developing countries like Vietnam. Therefore, a strategy in the agricultural sector is required to mitigate these challenges, including improving and promoting imported genetic material for future use or developing adapted varieties for the destination for meat. Conservation programs aimed at maintaining genetic diversity of native breeds using reproductive biotechnology require a basic understanding of reproductive biology and the influence of environmental factors (Delgadillo *et al*., 2002; Delgadillo *et al.,* 2004).

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Many studies showed that season seems to be the basic sign that affects semen quality and testosterone content in goats (Barkawi *et al*., 2006). Goat fertility varies seasonally mainly due to changes in day length during the year (Delgadillo *et al*., 2002; Delgadillo *et al*., 2004). Short days stimulate the secretion of luteinizing hormone (LH), which in turn stimulates testicular development, testosterone release, sperm production, mating activity, and fertility (Delgadillo *et al*., 2004). Testosterone is the hormone responsible for spermatogenesis and sexual behavior, so seasonal patterns of testosterone secretion may limit male reproductive performance during certain times of the year (Eitedal *et al*., 2007).

In Vietnam, the Saanen goat breed was imported to crossbreed with native goats, creating hybrid goats to improve meat and milk productivity. However, based on our knowledge, to date there have been no studies evaluating testosterone levels in Saanen goats during different seasons of the year. Therefore, this study aimed to evaluate plasma testosterone content using electrochemiluminescence assay (ECLIA) to provide this data in Saanen goat breed, as a reference in reproductive management and breeding more effective.

2. MATERIALS AND METHODS

2.1. Experimental animals

Thirty adult male Saanen goats (15: 2-year-olds and 15: 3-year-olds), selected on the basis of known history, were healthy based on clinical monitoring, and successfully bred. The goat herd is raised in semi-intensive conditions at the Large Livestock Research and Development Center, Lai Hung commune, Bau Bang district, Binh Duong province. All goats are identified by their ear tag number. Vaccination and deworming of goats are carried out according to the center's schedule.

2.2. Food, water, barn cleaning

Goats were raised semi-intensively, with a density of 2 m^2/head. The barn was designed in the style of a stilt house, the wooden floor is 1m away from the cement floor, and the roof is made of cement corrugated iron. The goats were grazed during the day and fed in the barn when the herd returns to the barn. Goats were fed 2 meals/day and night, including 1 breakfast (about 7-8am) and 1 afternoon meal (4am-5pm), including green elephant grass, synthetic bran (De Heus), leaves (bottle, or jackfruit leaves, mother of pearl). On average, each animal received 2-4kg of grass, 0.5-0.8kg of bran, and 0.5kg of leaves/day. Dry straw was rolled up into troughs ready for the goats to eat at night. Drinking water was clean water, put into a clean trough placed in the barn for the goats to drink freely. The water trough was cleaned every day and the water is changed once a day. The barn was cleaned with water spray every day. Cleaning and disinfection are carried out every 2 weeks. The antiseptic solution was used BESTAQUAM-SR with the following ingredients: didecyl dimethyl ammonium bromide, mixed at a ratio of 1/400.

2.3. Sample collection and processing procedures

Goat blood samples were taken at 6-8am, 10^{th} day of each month, for 12 consecutive months.

Take 2ml of jugular vein blood properly, the sample was quickly put into an anticoagulant tube (EDTA-K2), barcode was pasted on the sample tube, gently shaken, preserved and transferred to the laboratory, the blood sample was centrifuged. Centrifuge the sample for 15min at 1,000xg at 4^0C within 60min of collection. After centrifugation, collect 1ml of extracted plasma into a 1.5ml Eppendorf bottle and store the sample at -20^0C. Do not repeat freeze/thaw cycles.

Testing: Thaw once, leave the serum sample at room temperature (20-25^0C) and shake well before testing. To avoid any

influence on the results, serum samples were analyzed within 2hrs.

Hormone testing: Testosterone amount was determined by electrochemiluminescence testing method, with Cobas E601 immunoassay system (Roche Diagnostics, Switzerland). The testing procedure followed the instructions of the testosterone KIT (Roche, Germany).

2.4. Data processing

Peak hormone concentrations are defined as values greater than the average of all remaining values from the study group. ANOVA analysis and Post hoc test with Tukey-Kramer test to evaluate differences between groups (P<0.05). Statistical parameters were processed using MS-Excel 2020 software. Data were expressed as Mean±SD.

3. RESULTS

3.1. Changes in the hormone testosterone by month of the year

Table 1. Testosterone content (ng/ml, Mean±SD)

Month	2 years (n=15)	3 years (n=15)	Average (n=30)	MIN	MAX
1	3.23^{a}±0.63	2.83^{a}±0.38	3.04±0.47	0.78	3.46
2	2.72^{a}±0.56	2.46^{a}±0.36	2.59±0.51	0.75	4.74
3	2.51^{a}±0.84	1.83^{b}±0.82	2.17±0.72	1.03	3.96
4	1.95^{b}±0.16	1.61^{b}±0.85	1.78^{b}±0.58	0.91	4.86
5	3.34^{a}±0.87	2.66^{a}±0.72	3.00^{a}±0.75	1.16	5.81
6	5.38^{c}±1.21	2.83^{a}±0.46	4.11^{c}±0.91	1.45	3.85
7	4.51^{c}±1.71	3.97^{c}±1.35	4.24^{c}±1.24	2.22	6.37
8	8.66^{d}±2.74	8.35^{d}±1.27	8.53^{d}±1.96	4.69	12.08
9	11.17^{e}±2.46	11.85^{e}±2.14	11.51^{e}±2.35	6.27	13.35
10	8.82^{d}±2.28	8.57^{d}±2.13	8.70^{d}±1.82	4.23	11.72
11	7.76^{d}±1.73	7.91^{d}±1.22	7.84^{d}±1.53	3.26	11.78
12	4.85^{c}±1.32	5.48^{c}±0.87	5.17^{c}±1.23	2.25	7.63
Average	5.41^{c}±1.29	5.03^{c}±0.92	5.22^{c}±0.98		

Note: Mean values with different letters in the same row or column have statistically significant differences (P<0.05).

The results of changes in testosterone hormone in Saanen goats aged 2 and 3 over a period of one year are presented in Table 1 and Figure 1. Average level of testosterone hormone in Saanen goats aged 2 and 3 over the periods of a year starting from January are 3.23±0.63, 2.72±0.56, 2.51±0.84, 1.95±0.16, 3.34±0.87, 5.38±1.21, 4.51±1.71, 8.66±2.74, 11.17±2.46, 8.82±2.28, 7.76±1.73, 4.85±1.32 ng/ml and 2.83±0.38, 2.46±0.36, 1.83b±0.82, 1.61±0.85, 2.66±0.72, 2.83±0.46, 3.97±1.35, 8.35±1.27, 11.85±2.14, 8.57±2.13, 7.91±1.22, and 5.48±0.87 ng/ml, respectively. In terms of these values, September has the highest level, April has the lowest level in both age groups and the difference compared to other months is statistically significant (P<0.05).

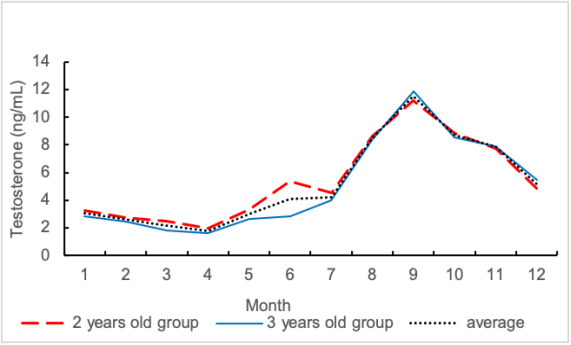

Figure 1. Testosterone content of Saanen goats during the year

3.2. Seasonal changes in the hormone testosterone

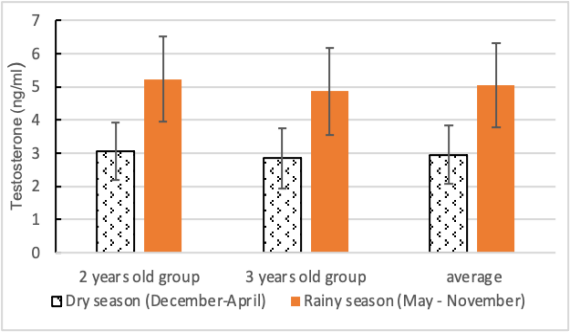

Figure 2. Testosterone content of Saanen goats by season

Figure 2 shows the findings of seasonal changes in testosterone hormone in Saanen goats aged 2 and 3. Average testosterone hormone concentrations of Saanen goats aged 2 and 3 in the dry season (December of the previous year to April of the following year) and rainy season (May to November)

are (3.05±0.86), (2.84±0.91), (5.23±1.28), (4.86±1.32) ng/mL. For Saanen goats in both age groups the difference between average testosterone levels in the wet and dry seasons was statistically significant ($P<0.05$). It can also be seen from Table 2 that for both Saanen goats at 2 and 4 years of age, testosterone hormone concentrations increased during the wet season compared to the dry season.

4. DISCUSSIONS

Testosterone is an anabolic steroid and the primary sex hormone in males. It plays important roles, including testicle, penis and prostate development, muscle and bone growth and sperm production. Photoperiod is the main factor affecting reproduction in goats. Levels of hormones (hypothalamic-pituitary-gonadal axis) undergo changes depending on photoperiod. In goats, as in other farm animals, the production of the hormone testosterone is under the control of gonadotropin-releasing hormone (GnRH) and luteinizing hormone (LH). The level of secretion of the hormone testosterone, shows a dependent increase in the level of LH secretion as the male goats approach the reproductive period (Delgadillo *et al*., 2002; Delgadillo *et al*., 2004; Şogorescu *et al*., 2011).

A fundamental link between environment and reproduction has been established that causes species to reproduce during the most optimal times of the year (Qun *et al*., 2012). Although the exact mechanism of this seasonal reproductive process is not yet fully understood, it is clear that "rhythms" are detected by nerve cells in the brain, which then control reproductive function. Changes in the photoperiod provide a representation of circadian time, and these changes in day length are sensed by the pineal gland and translated into physiological signals of prompting hypothalamic neurons to release gonadotropin (GnRH) in the brain, stimulates the pituitary gonadotropins to synthesize and secrete luteinizing hormone (LH) and LH stimulates interstitial cells in the testicles to produce testosterone (Abecia *et al*., 2012). During the non-breeding season, gonadotropin-releasing hormone (GnRH) and gonadotropin secretion are reduced, thereby affecting testosterone production.

Binh Duong province has two distinct seasons: rainy season from May to November, Dry season from December of the previous year to April of the following year, no winter. The average annual air temperature is 27.96°C, the highest is April (30.5°C), the lowest is December (26°C). In the dry season, the average annual humidity ranges from 76%–80%, the highest being 86% (in September) and the lowest being 66% (in February) (People's Committee of Binh Duong Province, 2017).

This study showed that testosterone values in male Saanen goats vary seasonally, confirming the basic arguments stated above that for male goats, testosterone production and excretion are controlled by photoperiod. Although seasonal patterns of hormone production and secretion are generally similar for all goat breeds, changes in hormone levels essentially occur due to different latitudes and subsequently other factors. Other factors such as genotype, diet and age (Zarazaga *et al*., 2009). In this study, the average level of testosterone hormone in Saanen Goats (1.61-5.58 ng/ml) remained approximately the same from December to July and reached high levels (7.76-11.65 ng/ml) in August-November, which are the months with low temperatures of the year. In previous studies, plasma testosterone levels were low (2 ng/ml) from January to August, increased sharply in August to the highest level (20 ng/ml) and gradually decreased after August to August. December (Delgadillo *et al*., 2002; Delgadillo *et al*., 2004). Research on male goats of two different breeds (*Capra hircus ibex*) (*Capra ibex nubiana*), has stated that the highest level of plasma testosterone occurs in August and the largest

size of the testes occurs around September and October (Zarazaga *et al*., 2009).

Although the effect of age on testosterone production in Saanen goats was generally significant (P<0.05) for an age group across months, differences between age groups within the same month Is negligible. This finding corresponds to the findings of Chentouf and co-authors (2011) for indigenous Northern Moroccan goat breeds showing that differences between age groups with regard to the production of the hormone testosterone are significant, the reproductive function of male goats is less dependent on the season than that of female goats. Therefore, the most important characteristics of male fertility (testosterone production, reproductive stimulation and spermatogenesis) do not stop completely but continue at low levels through seasons other than the breeding season. However, there is an increase in the frequency and severity of these characteristics as the breeding season approaches (Delgadillo *et al*., 2002). The results of our study indicate that in terms of the overall average of serum testosterone hormone concentrations observed in male Saanen goats, both inter-month (January to December) and inter-month variation The difference between seasons (dry season and rainy season) within a period of one year is statistically significant (P<0.05). The effect of age on testosterone production in Saanen goats (2 and 4 years old) was generally significant (P<0.05) for differences between months in each age group, but not significant differences between age groups in each month.

Seasonal changes in goat reproductive function are related to environmental factors such as changes in photoperiod and changes in air temperature. These results are consistent with the findings of Barkawi and co-authors (2006) with Zaraibi goats. As with other goat breeds, the highest values for most parameters were found in the fall and the lowest values were recorded for many parameters in the early summer (Charallah *et al*., 2000). In this study, seasonal plasma testosterone patterns were similar to those reported by Eitedal and co-authors (2010), who found that mean plasma testosterone concentrations from goat breeds were affected. according to the sampling season, higher in fall than in winter. Northern Moroccan male goats exhibit reproductive seasonality related to photoperiod. Testicular measurements, sperm characteristics, and plasma testosterone concentrations were low during winter, increasing during spring and summer (Chentouf *et al*., 2011). In Zaraibi goats in Egypt, Barkawi *et al*. (2006) concluded that this breed has a distinct seasonal sexual activity. In this goat breed, libido, semen characteristics and plasma testosterone concentrations are highest in summer and lowest in spring. In this breed, the histological structure of the testicles shows clear differences between seasons. The gonads are more active in summer and fall than in spring and winter. Şogorescu and co-authors (2011) showed that male Carpathian goats exhibit seasonal changes in plasma testosterone concentrations. The highest values are recorded in autumn and the lowest in spring. Studying the effects of season and feeding level on reproductive performance and semen quality in Payoya male goats concluded that this breed exhibits significant reproductive seasonality with strong sexual activity from May August (mid-summer) to November (mid-late fall) (Zarazaga *et al*., 2009). Male Creole goats in subtropical northern Mexico show marked seasonal variations in testosterone secretion. Low concentrations are observed from November to mid-June, after which plasma testosterone concentrations increase and remain high from July to October (Delgadillo *et al*., 2002; Delgadillo *et al*., 2004). Thus, the seasonal changes in testosterone content in male Saanen goats in this study are also consistent with studies in other goat breeds around the world.

When compared with published research results on Bach Thao goats aged 2 and 4. The average testosterone hormone concentration of Bach Thao goats aged 2 and 4 in the dry season (December of the previous year to April of the following year) and summer rain (May to November) were 2.68±0.83, 2.33±0.92, 4.78±1.22, 4.41±1.31 ng/ml, respectively (Thi Thu Hien Nguyen, 2023). Testosterone levels vary between small ruminants, which can be explained by their different genetic potential, while also noting that genetic traits for different responses to light and temperature have been prove (Abecia *et al*., 2012). However, in terms of seasonal changes, the results of these studies are similar. Research on testosterone in Bach Thao goats under farming conditions in Binh Duong also showed that there were seasonal changes, the rainy season has a higher concentration than the dry season (Thi Thu Hien Nguyen, 2023). This is the first report of testosterone in Saanen goats using a seasonal pattern study, the results showed that testosterone increases during the rainy season, which is important as this is also the breeding season of Saanen goats. Therefore, seasonal changes manifested by testosterone feedback are quite clear indicators of seasonal reproduction.

5. CONCLUSIONS

In summary, the results of this study showed that in Saanen goats, plasma testosterone levels change over the months of the year, with clear seasonality, with high testosterone levels in the rainy season and lower levels in the dry season. There needs to be continued research on reproductive hormones on other goat breeds in Vietnam, especially assessing the effects of climate change and feeding regimes on these indicators.

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IDENTIFICATION OF CO-INFECTION OF INFECTIOUS LARYNGOTRACHEITIS VIRUS AND GYROVIRUS GALGA 1 IN DOMESTIC CHICKENS IN HA NOI AND BAC GIANG

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ABSTRACT

This research was conducted to evaluate the coinfection of Infectious laryngotracheitis virus (ILTV) and Gyrovirus galga 1 (GyVg1) in diseased chicken flocks and utilize by polymerase chain reaction (PCR) in diagnosis. A total of 42 pooled tissue samples were collected from clinically suspected flocks and diseased chickens farmed in Ha Noi and Bac Giang from October 2023 to February 2024. The results indicated that the positive rates for the ILTV genome according to individual and farm levels were 11.90 and 22.22%, respectively; for the GyVg1 genome were 28.57 and 55.56%, respectively; and for coinfection of the two viral genomes were 7.14 and 22.22%. The finding of this study gains a better understanding of ILTV and/or GyVg1, leading to the development of disease prevention strategies in the chicken industry.

Keywords: *Coinfection, ILTV, GyVg1, PCR, chicken.*

1. INTRODUCTION

Infectious Laryngotracheitis (ILT) is an acute and highly contagious respiratory diseases in the poultry industry. ILTV is caused by infectious laryngotacheitis virus, which is a herpes virus *Gallid alphaherpesvirus 1* of the family *Alphaherpesvirinae* (Maekawa *et al*., 2019). In addition, Gyrovirus galga 1 (GyVg1), a member of the genus Gyrovirus, was first detected in diseased chicken exhibiting clinical such as apathy and loss of weight in 2011 in Brazil (Rijsewijk *et al*., 2011). ILTV has been recognized in broiler chickens as young as three weeks of age (Dormitorio *et al*., 2013). Chicken infected ILT exhibits diverse clinical symptoms. Acute infections show with dyspnea, tracheal muscosal hemorrhage, and even hemotypsis, while moderate infections showed conjunctivitis, tracheitis, and rhinitis (Hughes *et al*., 1991).

To date, the understanding of GyVg1 epidemiology and pathogenesis remain poorly. Furthermore, the pathogenicity of GyVg1 in chicken is still unknown. The only report of the pathogenicity of this virus is a case presented in South Africa (Abolnik *et al*., 2014). The report stated that GyVg1 infections in chickens can result in brain damage, mental decline, and weight loss (Abolnik *et al*., 2014).

In the study, we reported cases of identification ILTV and GyVg1 in domestic chickens in Ha Noi and Bac Giang by PCR technique.

2. MATERIALS AND METHODS

2.1. Sample collection

A total of 42 pooled samples including larynx, lung, and brain were collected from diseased chickens in Ha Noi (n=23) and Bac Giang (n=19). In the study, all chickens were collected from non ILTV-vaccinated chicken farms. To date, the chicken farms are not applied the GyVg1 vaccine. They were transferred to and processed at the laboratory of Microbiology and Infectious Disease, faculty of Veterinary Medicine, Vietnam National University of Agriculture to be examined for ILTV and/or GyVg1 from October 2023 to February 2024.

The Medivac ILT vaccine (Medion-Bandung-West Jave, Indonesia) and the

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positive control with GyVg1 detected in the previous study were used as positive control for ILTV and GyVg1.

2.2. DNA extraction

DNA were extracted from homogenous samples using Viral Gene-spin™ Viral DNA/RNA Extraction Kit (iNtRON Biotechnology, Seoul, South Korea) according to the manufacturer's instructions.

2.3. Polymerase chain reaction (PCR) assay

The positive and negative samples for ILTV and/or GyVg1 by conventional PCR using two set of primers as previous reported (Table 1) (Pang *et al*., 2002; Ye *et al*., 2015).

The mPCR technique was demonstrated using Gotaq® Green Master Mix (Promega, USA). It was performed in a total of 25µl volumes, in which the reaction mixture included 12.5µl of Gotaq® Green Master Mix 2X, 1µl of forward and reverse primers with final concentration 0.25 pmol/µl of each virus (Table 1), 1µl of cDNA, and 1µl of DNA, and 6.5µl of distilled water. The amplification condition was conducted as follows: 95°C for 5min; followed by 40 cycles of 95°C for 10sec, 55°C (for ILTV) or 60°C (for GyVg1) for 30sec, and 72°C for 1min; and finally 72°C for 5min. After that, 10 µl of PCR product was separated on 1% agarose gel electrophoresis.

Table 1. Primers were used in the study

Name	Sequence (5'–3')	PCR Product (bp)	References
GyVg1-F	CGTGTCCGCCAGCAGAAACGAC	346	Ye *et al*. (2015)
GyVg1-R	GGTAGAAGCCAAAGCGTCCACGA		
ILTV-F1	ACGATGACTCCGACTTTC	647	Pang *et al*. (2002)
ILTV-R1	CGTTGGAGGTAGGTGGTA		

2.4. Data analysis

Data were analyzed by Excel software.

3. RESULTS

3.1. Detection ILTV and GyVg1 genomes in the field samples

PCR reactions conducted with field samples showed that the amplified PCR product had only a single band for both the GyVg1-F/R and ILTV-F1/R1 primer pairs used to determine the presence of GyVg1 (346bp) and ILTV (647bp), with no nonspecific PCR product bands (Figure 1). Ye *et al*. (2015) and Pang *et al*. (2002) used these primer sets to identify ILTV and GyVg1 in the field samples. The primer pair has been shown to have high specificity for diagnosing the presence of this virus.

The ILTV and/or GyVg1 genomes detected in the diseased chickens by PCR were showed in Table 2. In total of 42 samples, 5 (11.90%) samples were determined positive with the ILTV genome, while 12 (28.57%) samples were positive with the GyVg1 genome. In detail, the positive rate of ILTV genome were 13.04 and 10.52% in Ha Noi and Bac Giang, respectively. Regarding GyVg1 detection, the positive rate is 30.43 and 26.32% in Ha Noi and Bac Giang, respectively.

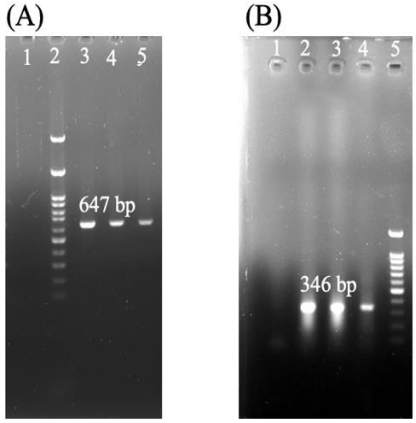

Fig 1. The PCR products of ILTV (647bp) **genome (A) and GyVg1** (346bp) **genome (B) by PCR**
(A): 1: negative control, 2: Marker (100bp), 3: positive control, 4 and 5: field samples;
(B): 1: negative control, 2: postive control, 3 and 4: field samples, 5: Marker (100bp)

Table 2. Detection of ILTV and GyVg1 genome in domestic chickens in Ha Noi and Bac Giang

Location	No. of Samples	ILTV		GyVg1	
		No. of positive samples	Positive rate (%)	No. of positive samples	Positive rate (%)
Ha Noi	23	3	13.04	7	30.43
Bac Giang	19	2	10.52	5	26.32
Total	42	5	11.90	12	28.57

Of the 9 farms with suspected infections, 2 (22.22%) and 5 (55.56%) flocks were positive for ILTV and GyVg1 genomes, respectively (Table 3). In detail, ILTV showed a positive rate of 16.67 and 33.33% in Ha Noi and Bac Giang; while the positive rate of GyVg1 genome is 50 and 66.67% in Ha Noi and Bac Giang, respectively.

Table 3. Detection of ILTV and GyVg1 genome in domestic chicken flocks in Ha Noi and Bac Giang

Location	No. of Flocks	ILTV		GyVg1	
		No. of positive flocks	Positive rate (%)	No. of positive flocks	Positive rate (%)
Ha Noi	6	1	16.67	3	50
Bac Giang	3	1	33.33	2	66.67
Total	9	2	22.22	5	55.56

It is interesting to note that the coinfection-positive rate for ILTV and GyVg1 was detected in the study. In detail, the positive rate of ILVT and GyVg1 was 7.14% (3/42) at the individual level and 22.22% at the flock level (Table 4). At the individual level, 2 (8.69%) and 1 (5.26%) samples were considered positive with ILTV and GyVg1 in Ha Noi and Bac Giang, respectively.

Table 4. Coinfection of ILTV and GyVg1 genome in domestic chicken flocks in Ha Noi and Bac Giang

Location	Individual level			Flocks level		
	No. of samples	No. of positive samples	Positive rate (%)	No. of Flocks	No. of positive flocks	Positive rate (%)
Ha Noi	23	2	8.69	6	1	16.67
Bac Giang	19	1	5.26	3	1	33.33
Total	42	3	7.14	9	2	22.22

4. DISCUSSION

To date, ILT, a contagious disease of poultry, is spread all over the world, especially it is present in regions with developed poultry industry. ILTV outbreaks were presented in commercial layer flocks in some Asian countries (Yan *et al*., 2016; Yang *et al*., 2020). In Southeast Asian countries, ILT was reported in chickens from major Myanmar poultry farms with some clinical signs of respiratory pathogen observed (Yang *et al*, 2000). In term of GyVg1, the virus has also been detected in some European, South American, African, and Asian countries, suggesting its global distribution (Yao *et al*., 2016; dos Santos *et al*., 2012; Smuts E.M., 2014; Ye *et al*., 2015).

Although ILTV has been reported in commercial flocks in some countries, the information on ILTV still limits in Vietnam (Dormitorio *et al*., 2013; Maekawa *et al*., 2019). Recently, the genome of ILTV was reported in chickens farmed in Hanoi (Vietnam) in 2022 (Hieu *et al*., 2023; Tran *et al*., 2023). In the present study, we detected the ILTV and GyVg1 genome in suspected disease chickens in Ha Noi and Bac Giang.

The previous reported revealed that low pathogenic NDV and GyVg1 were detected in the case in South Africa (Abolnik *et al*., 2014).

The report suggested the possibility of a synergistic pathogenic effect between avirulent NDV and GyVg1. In the study, we found the coninfection of ILTV and GyVg1 genomes in diseased chickens in Ha Noi and Bac Giang proviences. The data is the first report of the coinfection of theses virus in chicken farms in VietNam. Therefore, increasing the number and geography of sample collection is necessary for understanding well the coinfection of ILTV and GyVg1.

5. CONCLUSION

The positive rate for ILTV (11.90%), GyVg1 (28.57%), and coinfection of ILTV and GyVg1 (7.14%) in domestic chickens were reported in the present study. Regarding the chicken flocks, the positive rate for ILTV (22.22%), GyVg1 (55.56%), and coinfection of ILTV and GyVg1 (22.22%).

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ANTIMICROBIAL SUSCEPTIBILITY OF *ESCHERICHIA COLI* ISOLATED FROM MILK IN DAIRY HOUSEHOLD FARMS IN GIALAM, HANOI

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ABSTRACT

Milk is well-known as a nutritious food source and is widely used in many countries. However, milk also provides a favorable environment for the growth of bacteria, causing spoilage of milk and milk products, even can cause foodborne diseases. This study was conducted to determine the prevalence and antimicrobial susceptibility of *Escherichia coli* isolated from milk in dairy household farms in Gialam, Hanoi. Milk samples were collected in 82 household farms from May to September 2023. *E. coli* was isolated and test for antimicrobial susceptibility using disk diffusion method. The results revealed that *E. coli* was detected in 42 samples, accounting for 51.22%. The highest resistance rate was observed in ampicillin (16.67%), followed by cefotaxime (9.52%) and sulfamethoxazole/trimethoprim (7.14%). 26.19% of isolates showed resistance to at least one antimicrobial with 10 resistant patterns and 2 multidrug resistant strains were observed.

Keywords: *Antimicrobial susceptibility, dairy household farm, E. coli, milk.*

1. INTRODUCTION

In Vietnam, people's income has steadily improved, thus the demand for fresh milk and milk products also increases. Therefore, the number of dairy cows as well as milk production has increased rapidly, especially after Decision No. 167/2001/QĐ-TTg of the Prime Minister on a number of measures and policies to develop milch cow farming was issued. The General Statistics Office (GSO) reported that the milk production of the whole country was estimated at 1165.7 thousand tons in 2023 (GSO, 2023).

According to the Food and Agriculture Organization of the United Nations (FAO), milk is a widely used food source in many countries, because milk consists of high-quality protein, fat, minerals and vitamins, which meet the required nutrient intakes of human diets (https://www.fao.org/dairy-production-products/products/milk-composition/en). However, milk also provides favorable environment for the growth of bacteria, which leads to spoilage of milk and milk products, and even can cause foodborne diseases for consumers (Oliver *et al*., 2005).

Escherichia coli (*E. coli*) is one of the bacteria commonly found in milk (Malik and Fatima, 2021). *E. coli* is a Gram-negative bacterium, belongs to *Enterobacteriaceae* family, and can be found in the digestive systems of animals and human. *E. coli* is used as a hygiene indicator, the presence of *E. coli* in milk indicates direct or indirect fecal contamination (Desmarchelier and Fegan, 2016). In addition, *E. coli* is one of the common causes of mastitis in dairy cows (Cobirka *et al*., 2020; Dalanezi *et al*., 2020). Besides, antimicrobial resistance of *E. coli* in cow's milk has been reported in many countries, which demonstrates a great concern for public health (Liu *et al*., 2021; Mwasinga *et al*., 2023).

Gialam is one of the main dairy farming districts in Hanoi. Up-to-date, there is limited information about antimicrobial resistance of

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bacteria in milk, therefore, the study was performed to determine the prevalence and antimicrobial susceptibility of *E. coli* isolated from milk in dairy household farms in GiaLam, HaNoi.

2. MATERIALS AND METHODS

2.1. Materials

Media used in the study includes: Buffer Peptone Water (APW) (Merck, Germany), MacConkey agar (MAC) (Merck, Germany), Nutrient agar (NA) (Merck, Germany), Mueller - Hinton agar (MHA) (Merck, Germany), Eosin-Methylene Blue Agar (EMB) (Merck, Germany), and biochemical reagents.

Antimicrobial disks: ampicillin (AMP, 10µg/disk), chloramphenicol (C, 30µg/disk), ceftazidime (CAZ, 30µg/disk), ciprofloxacin (CIP, 5µg/disk), gentamicin (CN, 10µg/disk), cefotaxime (CTX, 30µg/disk), doxycycline (DO, 30µg/disk), cefoxitin (FOX, 30µg/disk), imipenem (IPM, 10µg/disk), streptomycin (S, 10µg/disk), sulfamethoxazole/trimethoprim (SXT, 23,75µg/1,25µg/disk), and tetracycline (TE, 30µg/disk).

2.2. Methods

2.2.1. Sampling

Milk samples were collected in 82 dairy household farms in Gialam, Hanoi from May to September 2023. At each household, one samples was taken from milk container, kept in sterile tubes and stored at 4°C in a cool box, then immediately transferred to the laboratory for analysis.

2.2.2. Isolation and identification of E. coli

Milk samples were enriched in BPW medium and incubated at 37℃/24h. After incubation, a loop of enrichment was streaked onto MAC agar, then incubated at 37℃/24h. Colonies of *E. coli* on MAC agar are round, smooth and dark pink. Typical colonies were selected and streaked onto NA agar and incubated at 37℃/24h. Colonies on NA agar were streaked onto EMB agar and used for biochemical tests, including Gram staining, indole test, methyl red test, voges proskauer test, growth on Simmons citrate and TSI agar.

2.2.3. Antimicrobial susceptibility test

All *E. coli* isolates were tested for antimicrobial susceptibility using disk diffusion method based on the standard procedure of the Clinical and laboratory standards institute (CLSI).

2.3. Data analysis

Obtained data was entered and analyzed using Microsoft Excel.

3. RESULTS AND DISCUSSION

3.1. Prevalence of *E. coli* in milk

A total of 82 milk samples were collected, the results revealed that *E. coli* were detected in 42 samples, accounting for 51.22%. All isolates were Gram-negative, indole positive, methyl red positive, voges proskauer negative, fermentation of glucose and lactose/sucrose. In addition, all *E. coli* isolates did not grow on Simmons citrate agar, but grew on EMB agar with green metallic sheen colonies (Figure 1).

Of the 82 selected households, 79.27% of the households used milking machines, while 20.73% of the households applied hand milking. All households shared that they cleaned cow udders before milking, however only 39.02% of the households used teat disinfectants, whereas majority of the households (61.98%) only used towels for teat cleaning. If milking hygiene is not carried out appropriately, improper hand washing, or milking equipment such as milking machines and containers are not properly cleaned, milk will be contaminated with bacteria. Currently, there have not been many publications on the status of *E. coli* contamination in milk in Vietnam. A study of Cam Thi Thu Ha *et al.* (2019) showed that the amount of *E. coli* in fresh cow milk collected from households and milk collection stations

in Phudong, Gialam ranged from 0 - 16 MPN/ml, of which 5/40 samples exceeded the standard. The prevalence of *E. coli* in milk collected from dairy households in this study was lower than that in a previous study in Ghana (68.0%) (Adzitey *et al.*, 2022), while higher than that reported in Ethiopia (27.91%) (Tadesse *et al.*, 2018) and China (34.4%) (Liu *et al.*, 2021).

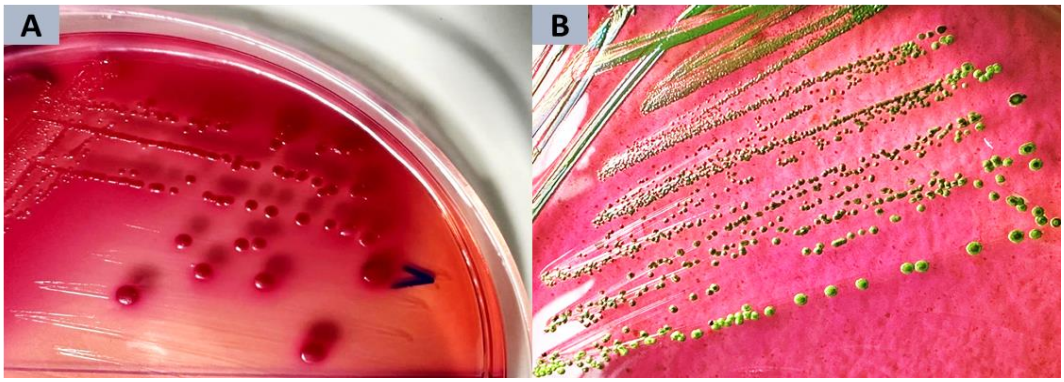

Fig 1. *E. coli* colonies on different agars. *A: MAC agar, B: EMB agar*

3.2. Antimicrobial susceptibility of *E. coli* isolates

Table 1. Antimicrobial susceptibility of *E. coli* isolated from milk (n = 42)

Antimicrobials	Resistant		Intermediate susceptible		Susceptible	
	No.	%	No.	%	No.	%
AMP	7	16.67	3	7.14	32	76.19
C	2	4.76	0	0	40	95.24
CAZ	2	4.76	2	4.76	38	90.48
CIP	1	2.38	9	21.43	32	76.19
CN	1	2.38	0	00	41	97.62
CTX	4	9.52	1	2.38	37	88.10
DO	0	0	0	0	42	100
FOX	1	2.38	0	0	41	97.62
IPM	0	0	0	0	42	100
S	1	2.38	11	26.19	30	71.43
SXT	3	7.14	0	0	39	92.86
TE	2	4.76	3	7.14	37	88.10

E. coli isolates from milk were tested for antimicrobial susceptibility against 12 antimicrobials. The analysis demonstrated that all *E. coli* isolates were completely sensitive to imipenem and doxycycline, followed by gentamicin (97.62%), cefoxitin (97.62%) and chloramphenicol (95.24%). Whereas, *E. coli* isolates showed the highest resistance rate to ampicillin (16.67%), followed by cefotaxime (9.52%) and sulfamethoxazole/trimethoprim (7.14%). Low resistance rates were also observed for chloramphenicol, ceftazidime, tetracycline, ciprofloxacin, gentamicin, cefoxitin and streptomycin (Table 1).

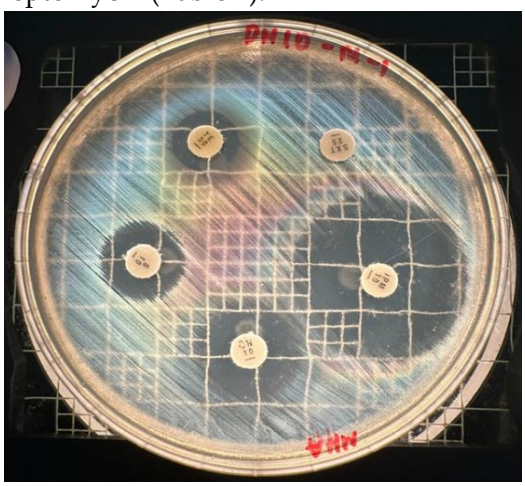

Figure 2. Inhibition zones on MHA agar

Tab 2. Antimicrobial resistant patterns of *E. coli* isolates

No.	Patterns	No. of isolates
1	AMP	2
2	CTX	1
3	FOX	1
4	SXT	1
5	AMP+C	1
6	AMP+CTX	1
7	AMP+CAZ+CTX	1
8	CAZ+CTX+TE	1
9	AMP+S+SXT+TE	1
10	AMP+C+CIP+CN+SXT	1

Among 42 *E. coli* isolates, 11 isolates were resistant to at least one antimicrobial tested, accounting for 26.19%. The analysis of antimicrobial resistant phenotypes showed that there were 10 resistant patterns and 2 multidrug resistant (MDR) isolates (resistant to at least one agent in three or more antimicrobial classes) (Table 2).

Recent studies also reported high resistance rates against ampicillin in *E. coli* isolated from milk samples. A study of Yoon and Lee (2022) in Korea reported that 64/183 *E. coli* isolates from bulk tank milk in 290 dairy farms were resistant to at least one antimicrobial and 15/64 isolates were MDR. The highest resistance rate was observed against tetracycline (37.5%), followed by cephalothin (35.9%), ampicillin (34.4%), gentamicin (26.6%), colistin (21.9%), chloramphenicol (14.1%), and cefazoline (10.9%); none of isolates was resistant to nalidixic acid, imipenem and ciprofloxacin (Yoon and Lee, 2022). Another study of Liu *et al.* (2021) in China showed that *E. coli* isolates from milk in 195 dairy farms were resistant to ampicillin (46.3%), amoxicillin-clavulanic acid (16.4%), tetracycline (13.4%), trimethoprim/sulfamethoxazole (13.4%), cefoxitin (11.9%), chloramphenicol (7.5%), kanamycin (7.5%), streptomycin (6.0%), tobramycin (4.5%), azithromycin (4.5%), and ciprofloxacin (1.5%). The presence of antimicrobial resistant *E. coli* in milk reflects the improper use of antimicrobials in dairy farms and households. Antimicrobial resistant bacteria in milk might enter the food chain and cause serious health problems for consumers.

4. CONCLUSION

The study showed a high rate of *E. coli* contamination in milk collected from dairy households in Gialam, Hanoi. In addition, the antimicrobial resistance rate of *E. coli* isolates was also relatively high, with the presence of MDR isolates. Therefore, it is necessary to improve milking hygiene conditions as well as management of antimicrobial use in dairy farming to minimize microbial contamination and control antimicrobial resistance.

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SURVEY OF COMMON SKIN TUMORS IN DOGS AND CATS AND ASSESSING THE EFFECTIVENESS OF TREATMENT

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ABSTRACT

The study aimed to investigate skin tumors in dogs and cats in Ho Chi Minh City by collecting data from Thuan Pet Veterinary Clinic and DC Vet Veterinary Clinic. The results recorded 1541 cases of dogs and cats brought to the clinics for treatment, among which 304 cases were related to skin diseases and 14 cases involved skin tumors. Nine cases of skin tumors underwent surgical treatment, accounting for 64.2% of tumor cases. Skin tumors were easily identified, with the highest occurrences being epidermal cysts (35.7%) and papillomas (35.7%), followed by benign lipomas (14.2%), fibroids (7.1%), and malignant lymphoma (7.1%). The proportion of animals with skin tumors differed significantly by sex ($P<0.01$), with 78.5% affecting dogs and cats of foreign breeds, and 21.5% affecting those of domestic breeds. Skin tumors were statistically significant in dogs and cats aged 5 to 10 years and over 10 years (42.9%). The incidence of skin tumors did not significantly differ by breed, with females accounting for 64.2% of cases compared to 35.8% in males. The treatment success rate was nearly 100%, with a complication rate of 44.4% after surgical removal of skin tumors in pets. Healing time ranged from 4 to 16 days.

Keywords: *Tumors, dogs, cats, skin*

1. INTRODUCTION

Dogs and cats have been long-standing companions of humans in daily life and are increasingly regarded as family members, rather than merely animals for guarding homes or catching mice as in the past. Owners are not only concerned about their pets' health but also their beloved fur. Consequently, skin diseases significantly impact the appearance of dogs and cats, particularly skin tumors.

According to Lawhead (2016), mitosis is a vital process essential for the growth and maintenance of animals. This cellular division is regulated by various factors present in both the cell and extracellular fluid. When uncontrolled, cells may undergo frequent mitosis, resulting in the excessive production of new cells, leading to the accumulation of cells in a specific area. This uncontrolled growth of cells is referred to as a tumor. Nguyen Van Khanh (2018) explains that all tumors, whether benign or malignant, consist of two fundamental components: parenchyma and stroma. The parenchyma is the primary component that determines the nature and developmental characteristics of a tumor. Based on the parenchyma, it is possible to classify tumors as epithelial, connective tissue, or a combination of both. In benign tumors, cells are mature and well-differentiated, closely resembling the parent cells but arranged irregularly without a specific pattern. Conversely, malignant tumors typically contain immature cells that are poorly differentiated, often resembling embryonic cells with little to no resemblance to the parent cell, and arranged irregularly. Stroma refers to the supportive framework of connective tissue that binds and provides nutrients necessary for tumors to survive and grow. It plays a crucial role in facilitating tumor growth and progression.

Skin tumor diseases in dogs and cats in Vietnam, particularly in Ho Chi Minh City, have received limited research attention. Tumors in dogs and cats share similarities with those in humans, encompassing both benign and malignant forms. Therefore, accurate diagnosis is crucial for effective treatment. This study aims to gather additional data and fundamental information

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about existing skin tumors in dogs and cats across selected districts of Ho Chi Minh City.

2. MATERIAL AND METHODS

2.1. Subject, time and place

All dogs and cats underwent examination and treatment at the HCM City Veterinary Clinic from Dec 2023 to Jun 2024.

2.2. Methods

Observe the general condition of the animal, location, shape, size, and color of the tumor. Use your hands to touch and observe the animal's reaction. Palpate the surrounding tissues and adjacent lymph nodes to determine the hardness, firmness, mobility, and location of the tumor.

Animals fast for 12 hours and do not drink for about 4 hours before surgery if using medical anesthesia. Conversely, if gas anesthesia is used, the animal does not need to fast or restrict water intake. Shave the surrounding skin to facilitate the surgical process and post-operative care. Wash the shaved area with water to remove loose hair. Apply povidone iodine to the surgical site. Use a surgical marker to outline the tumor for precise surgical planning.

Determine skin tension lines. Depending on the type of tumor, location and size, the length of the incision will vary. Use an electric scalpel to make cuts through the skin. Dissect the subcutaneous connective tissue with metzenbaum scissors and tweezers. The dissection should be wide enough to reduce skin tension. Use forceps and threads to clamp and tie off all the blood vessels that feed the tumor. Use your hands to palpate to find the base of the tumor and remove it. In case the tumor has roots embedded in muscle or bone tissue, it must be very gently removed. Dredge remaining tumor parts and surrounding tissues. Recheck for vascular leakage, examine and scrape away any remaining traces of the tumor. Disinfect the incision with povidine solution, adjust the wound edges and determine the skin tension line before sewing the wound closed.

Sample immobilization aims to preserve tissue and cellular structures while maintaining their morphology similar to when they were alive. Process fresh specimens (within 30 minutes of removal from the body). Cut specimens into small, 5mm thick pieces that fit into a plastic specimen transfer mold. Immediately place specimens in a vial containing 10% neutral buffered formalin solution, ensuring specimens do not stick to the vial wall. The volume of fixing solution should be 20 - 30 times greater than the specimen volume.

Provide a clean, dry environment for the animal and nutritional support to aid quick wound healing, enhance strength, and reduce pain. Apply nano silver spray to the wound as needed. Administer antibiotics and anti-inflammatories based on wound condition and for the prescribed duration. Address fluid accumulation by reopening and cleaning the wound, inserting a drain for fluid drainage, and re-suturing if necessary. Survey criteria, monitoring and calculation formulas:

- % dogs and cats total examined
- % dogs and cats by breed
- % dogs and cats by age group
- % dogs and cats by gender

2.3. Data processing

Data were processed by Microsoft Excel 2016 and Minitab 17 statistical software.

3. RESULTS AND DISCUSSION

3.1. Dogs and cats with skin tumors treated

Out of 1,541 cases of dogs and cats brought to the clinic for treatment, 304 cases were related to skin diseases (19.7%), and 14 cases were identified as skin tumors (0.9%). Among these tumor cases, 9 were treated surgically, accounting for 64.2% of all tumor cases and 2.96% of the total skin disease cases. The remaining 5 tumor cases were recorded but did not undergo surgical intervention.

Table 1. Tumors on the skin of dogs and cats

Tumor type	Surgical treat		Quantity		Total	%
	Dog	Cat	Dog	Cat		
Epidermoid cyst	3	0	2	0	5	35.7
Papilloma	5	0	0	0	5	35.7
Lipoma	0	0	2	0	2	14.4
Malignant fibroids	0	0	0	1	1	7.1
Malignant lymphoma	1	0	0	0	1	7.1
Total	9	0	4	1	14	100

Out of 14 cases of skin tumors, Epidermal cyst and papilloma 5 cases (35.7%), lipoma 2 cases (14.3%), malignant fibroid and malignant lymphoma 1 case each.

3.2. Pets with skin tumors by breed

During the survey, we divided into two groups of dog and cat breeds: domestic dog and cat breed group and foreign dog and cat breed group.

The results showed that in 14 cases of skin tumors in dogs and cats, the domestic dog and cat breed group accounted for 79% (11 cases), while the foreign dog breed group accounted for 21% (3 cases). This difference is statistically significant (P<0.01). The disparity may be attributed to the lower level of attention and care typically received by dogs and cats in the domestic breed group. Conversely, animals in the foreign dog and cat breed group are well cared for, closely managed, and less likely to roam freely. Consequently, the incidence of skin tumors is higher in the domestic dog and cat breed group compared to the foreign dog and cat breed group.

3.3. Dogs and cats with skin tumors by age

The risk of tumors increases with age in most cases of tumors on the skin of dogs and cats. To clarify, we conducted a survey according to four age groups with the results.

Table 2. Dogs and cats with skin tumors by age

Age	Dogs and cats with skin tumors	P
<1	0	<0.05
1–5	2	
5–10	6	
>10	6	

Based on the above results, we observe that the incidence of skin tumors increases progressively from one year old to over ten years old. The lowest incidence is observed in animals under one year old (0%), with a gradual increase observed in the 1 to 5 years old age group (14.2%), peaking in dogs and cats aged 5 to 10 years old and over 10 years old (42.9%). The difference in skin tumor incidence between age groups is statistically significant (P<0.05).

3.4. Pets with skin tumors by gender

Survey results showed that in 14 cases of dogs and cats with tumors on the skin brought for examination, there were 9 cases of female dogs and cats with tumors on the skin (64.2%), higher than 5 cases of male dogs and cats (35.8%). However, the difference in the rate of skin tumors in dogs and cats according to gender is not significant (P>0.05).

Table 3. Dogs and cats with skin tumors by gender

Gender	Pets with skin tumors	P
Male	5	>0.05
Female	9	

3.5. Treatment effectiveness

Investigating the treatment effectiveness of 9 cases of skin tumors treated with surgical excision. Wound healing time is calculated from the day of surgery to the day the wound heals and sutures are removed.

Table 4. Treatment effectiveness

Tumor type	Times of recovery (days)			Recover from illness
	<8	8–14	>14	
Epidermoid cyst, n=3	7	8	16	10.3
Papilloma, n=5	4, 4, 5	8, 9		6
Malignant lymphoma, n=1		-		-*

We observed that wounds resulting from epidermal cyst removal had the longest healing time (16 days), followed by wounds from papilloma removal (9 days). One case each of epidermal cyst and papilloma removal required longer healing times compared to others. This could be attributed to the aggressive nature of epidermal cysts, which are difficult to access and manage, and papillomas causing hyperactivity in animals, leading to increased movement and licking of

the incision. In contrast, wounds healed faster in other cases, possibly due to smaller incision sizes and surgical locations away from high-mobility areas like the abdomen or near joints, thus reducing the risk of infection. In cases of malignant lymphoma, wound healing, while not complete, showed significant progress

4. CONCLUSION

There were 304 cases related to skin diseases and 14 cases of skin tumors (0.9%). Among these, 9 tumor cases underwent surgical treatment, accounting for 64.2% of skin tumor cases. Skin tumors manifest in various locations, with the most prevalent being epidermal cysts (5 cases, 35.7%) and papilloma tumors (5 cases, 35.7%). Other tumor types include benign lipomas (14.2%), malignant fibromas (7.1%), and malignant lymphomas (7.1%). The disparity in skin tumor rates between dog and cat breeds is highly significant, with 78.5% of cases involving foreign breeds and 21.5% involving domestic breeds. Skin tumors predominantly occur in dogs and cats aged 5-10 years (42.9%) and over 10 years (42.9%). There is no significant difference in skin tumor rates between genders, with females accounting for 64.2% of cases and males for 35.8%. The success rate of treatment is nearly 100%, except for cases of malignant lymphoma that cannot be monitored. The rate of complications after surgery for skin tumors in dogs and cats is 44.4%. Wound healing time varies from 4 to 16 days and depends significantly on tumor location, animal age, wound size, and post-operative care

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EVALUATION OF THE TREATMENT REGIMEN FOR INTESTINAL PARASITIC INFECTION IN DOGS AT VETERINARY CLINICS

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ABSTRACT

The study was conducted from Feb 2024 to Jun 2024, surveying 235 cases brought in by owners for examination and treatment. There were 82 cases with symptoms of gastrointestinal disease infected with roundworms (32.34%), infected with melon seed fluke (*Diphylidium caninum*) (2.13%), infected with coccidia (*Isospora canis*) (0.43%). Of which, the infection rate of *Toxocara canis* was 20.00%, *Ancylostoma* spp was 12.34%. *Toxocara canis* infection was high in dogs <3 months old (30.10%) and decreased with age. *Ancylostoma* spp infected all ages. No cases of whipworm infection were found. Domestic dogs had a higher infection rate (38.85%) than foreign dogs (22.92%). The infection rate of males was 40.66% and females was 31.25%. The rate of parasitic infection in dogs with typical clinical symptoms: vomiting symptoms of roundworm infection 26.47%, hookworm infection 11.76%; diarrhea symptoms with or without blood roundworm infection 21.57%, hookworm infection 25.49%, malnutrition with roundworm infection 12.00%, hookworm infection 4.00% and no obvious signs of roundworm infection 22.73%, hookworm infection 9.09%. The treatment results of dogs infected with parasitic worms were 85.37%; infectious diseases associated with *parasitic worms*: Parvovirus infection with *parasitic worms* had a cure rate of 60.87%, Carre infection with *parasitic worms* had of 40%.

Keywords: *Parasites, intestines, dogs.*

1. INTRODUCTION

In recent years, the cross-breeding and importation of many dog breeds into Vietnam have quickly introduced numerous new pathogens that infect local dog breeds. In addition to infectious, internal, surgical, and obstetric diseases, parasitic diseases in dogs caused by helminths also inflict significant harm. Diseases caused by parasites often occur silently, weakening the animals' bodies and creating conditions for other diseases to spread and develop, especially infectious diseases. They cause great harm to dogs' health, primarily leading to anemia, enteritis, and potential mortality. More dangerously, many parasitic species in dogs can infect and seriously threaten human health, particularly children, including certain species of *Ancylostoma* spp., *Toxocara canis*, and *Dipylidium caninum*. Several studies on gastrointestinal parasites in dogs in Vietnam have indicated prevalence rates of hookworms ranging from 43.85% to 69.49%, and roundworms from 10% to 38.3% (Bui Khanh Linh *et al*., 2018; Vo Thi Hai Le *et al*., 2011; Nguyen Phi Bang *et al*., 2016; Anh *et al*., 2016). Additionally, dogs are also infected with whipworms (3.28-5%), tapeworms *Dipylidium* sp., *Spirometra* sp. (Nguyen Phi Bang *et al*., 2016), and the protozoan Giardia duodenalis (Sam *et al*., 2018). Le Van Huan and Le Huu Khuong (1995), Bui Ngoc Thuy Linh (1998), and Tran Thien Xuan (2002) similarly concluded that the prevalence of parasitic infections in dogs is alarmingly high. Therefore, the diagnosis, treatment, and prevention of parasitic infections in dogs are critical concerns within the veterinary industry.

2. MATERIALS AND METHODS

2.1. Materials

All dogs were brought for examination and treatment at K9 Veterinary Clinic in Ho Chi Minh City from Feb 2024 to Jun 2024.

2.2. Research Methods

2.2.1. Clinical diagnostic methods

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Medical history: Record owner information such as name and address. Record information about the animal's disease, including name, breed, coat color, sex, weight, care regimen, method of raising, vaccination and deworming schedule, duration of illness, normal food intake, appetite (whether reduced or normal), frequency of bowel movements per day, stool appearance (consistency, color, odor), presence or absence of vomiting, frequency of vomiting episodes, medications administered, duration of medication use, etc.

Clinical examination: Assess the nutritional status (whether the animal is overweight or underweight), neurological status (level of alertness, responsiveness, presence of lethargy), condition of the nasal skin, condition of mucous membranes (moist or dry), color of mucous membranes (pale, pink, cyanotic), stool color and consistency, evaluate skin elasticity, palpate the chest and abdomen to identify abnormalities and assess pain responses in the digestive organs. Perform physical examinations such as auscultation (listening) of heart and lung sounds, measurement of heart rate, respiratory rate, and body temperature, among others.

2.2.2. Testing method is by flotation method

Flotation method: Prepare the sample: Place 1-2g of feces in a test tube and add a small amount of saturated salt water to dissolve the feces.

Filter sample: Filter the mixture through a mesh with 81 holes/cm² into a narrow-mouthed bottle.

Add saturated salt water: Fill the bottle's mouth with saturated salt water.

Allow sedimentation: Cover the bottle with a lid and let it settle for 15-20 minutes.

Observation and determination: Quickly remove a sample with a pipette, place it on a slide, and observe under a microscope at 40x magnification.

Sedimentation method:

Prepare sample: Place 5-10g of feces in a cup and add clean water until it reaches 2/3 of the cup's volume.

Filter sample: Stir thoroughly and filter the mixture through a sieve to remove impurities and large particles.

Decantation: Transfer the filtered liquid into a narrow-bottomed jar and let it settle for 3-5 minutes.

Replace water: Gently pour out the water from the top (approximately 2/3 of the jar) and refill with fresh water.

Repeat decantation: Allow it to settle for another 3-5 minutes after each water change (repeat this process 3-5 times until the water runs clear, then stop).

Observation and processing of samples: After completing the decantation steps, remove the excess water and place the sediment on a slide or petri dish for observation of helminth eggs.

Survey criteria, monitoring and calculation formulas

Infection rate (%)=(Infected/surveyed)x100

Cure rate (%)=(cured dogs/treated dogs)x100

2.3. Statistical analysis

Use the Chi-square test with Minitab 17 software to compare ratios and process data on Microsoft Excel 2010.

3. RESULTS AND DISCUSSIONS

3.1. Overall infection rate

Stool tests were conducted on 235 dogs displaying signs of gastrointestinal disease, among which 82 dogs were found to be infected with helminths, accounting for 34.89%. These dogs were subsequently treated at the K9 Veterinary Clinic using the flotation method with saturated NaCl solution and the decantation method.

Table 1. Overall infection rate

Survey	Manifestations of gastrointestinal diseases	Helminthine infection
Nº of dogs	235	82
%	100	34.89

Out of the 235 dogs examined and treated for gastrointestinal disease, 172 dogs received full deworming and vaccination. Our survey findings exceed those reported by Nguyen Anh Chung (2011) and Tran Ngoc Nhan (2011) at

Nong Lam University Veterinary Hospital in Ho Chi Minh City by 29.68% and 8%, respectively, but are lower than the results reported by Nguyen Thi Diem Phuong (2010) at Can Tho University Veterinary Clinic (51.67%) and Bui Ngoc Lanh (2010) at Nong Lam University Veterinary Hospital in Ho Chi Minh City (75.91%). This disparity may be attributed to differences in location, time, and sample size of the surveys

3.2. Prevalence of intestinal parasite infection by parasite species

Through stool tests on 235 dogs, we identified four species of parasitic worms in the dogs' intestinal tracts: roundworms, hookworms, tapeworms, and coccidia. Among them, the group of hookworms parasitizing dogs includes three species (Ancylostoma caninum, Ancylostoma braziliense, and Uncinaria stenocephala). According to Table 2, the infection rate of roundworms (Toxocara canis) is the highest at 20.00%. This is followed by hookworms (*Ancylostoma* spp.) and tapeworms (*Diphylidium caninum*) with infection rates of 12.34 and 2.13%. Coccidiosis (Isospora canis) exhibits the lowest infection rate at 0.43%.

Table 2. Intestinal parasite infection by parasite

Species	Survey dogs	Infected dogs	%
Roundworms	235	47	20.00
Hookworms	235	29	12.34
Tapeworms	235	5	2.13
Bridge Worm	235	1	0.43

Our roundworm infection rate of 20.00% is lower than reported rates by Nguyen Anh Chung (2011) and Bui Ngoc Lanh (2010) at Nong Lam University Veterinary Hospital, which are 40.29 and 35.06%, respectively, showing statistically significant differences ($p < 0.001$). However, our results exceed those of Le Huu Khuong (2005), who reported a roundworm infection rate of 6.57% in dogs from several southern provinces of Vietnam. The dogs we surveyed at the clinic were mostly indoor pets, with owners who prioritize parasite prevention and dog care practices, which likely contributes to the lower roundworm infection rate. Our recorded hookworm infection rate of 12.34% is lower than findings by previous authors such as Nguyen Thi Diem Phuong (2010) at Can Tho University Veterinary Clinic (62.32%), Nguyen Huu Trung (2013) at Go Vap Veterinary Station (64.44%), and Bui Thi Bich Phuong (2010) at K-9 Veterinary Clinic (60%). The tapeworm infection rate of 2.13% is also lower than Nguyen Anh Chung's (2011) finding of 3.88% at Binh Thanh District Veterinary Clinic. Our coccidiosis rate is 0.43%, which is lower than the 0.71% reported by Tran Ngoc Nhan (2011); overall, coccidiosis rates remain low.

3.3. Prevalence of intestinal roundworm infection by age

Age is a crucial factor influencing disease rates in animals, with varying infection levels observed across different age groups. Among the 235 dogs surveyed for gastrointestinal disease, we categorized them into four age groups, detailed in table 3.

Table 3. Intestinal roundworm infection by age

Age	Survey dogs	Infected dogs	%	Roundworms		Hookworms	
				Infected	%	Infected	%
<3	103	47	45.63	31	30.10	20	19.42
3-6	44	15	34.09	8	18.18	3	6.82
6-12	59	11	18.64	7	11.86	1	1.69
>12	29	3	10.34	1	3.45	5	17.24
∑	235	76	32.34	47	20.00	29	12.34

Roundworm infection rates are observed across all age groups and tend to decrease with age. Dogs under 3 months old exhibit the highest infection rate (30.10%), followed by dogs aged 3-6 months (18.18%), 6-12 months (11.86%), and the lowest rate is among dogs over 12 months old (3.45%). Statistical analysis indicates these differences are highly significant ($P<0.01$), highlighting puppies' greater susceptibility to roundworm infections compared to adult dogs.

According to Urquhart (1996), puppies can acquire roundworm infections transplacentally and via colostrum during the first three weeks after birth. Additionally,

puppies generally have weaker immune systems than adult dogs when exposed to pathogen-rich environments. Consequently, it is crucial for mother dogs to undergo regular deworming during pregnancy and while nursing puppies to prevent transmission of roundworms.

3.4. Rate of roundworm infection according to dog breed origin

The 235 dogs surveyed were categorized into two groups: foreign dog breeds (such as Chihuahua, Fox Terrier, Pekingese, Poodle, and some hybrid breeds of foreign origin) and domestic dog breeds (native breeds like village dogs and Phu Quoc Ridgebacks). The prevalence of worm infection among dogs exhibiting signs of gastrointestinal disease is detailed in table 4.

Table 4. Rate of roundworm infection by dog breed origin

Breeds	Number of dogs surveyed	Infected dogs	%	Roundworms		Hookworms	
				Infected dogs	%	Infected dogs	%
Domestic	139	54	38.85	32	23.02	19	13.67
Foreign	96	22	22.92	15	15.63	10	10.42
Total	235	76	32.34	47	20.00	29	12.34

According to the survey findings, the proportion of domestic dog breeds infected with worms is 38.85%, whereas the proportion among foreign dog breeds is 22.92%. Statistical analysis indicates a highly significant difference in the prevalence of worm infections among dogs based on breed ($p < 0.01$). This suggests that the likelihood of worm infection in dogs varies with breed. Domestic dog breeds often have greater exposure to environments where they can freely roam, thus increasing their risk of worm infections. Conversely, foreign dog breeds are typically valued and well-cared-for breeds that receive meticulous care.

3.5. Rate of roundworm infection according to symptoms

To contribute to the clinical diagnosis of diseases caused by helminths in dogs, we have recorded common symptoms, and the results are presented in Table 5.

Table 5. Rate of roundworm infection by symptoms

Symptom	Surveyed dogs	Infected dogs	%	Roundworms		Hookworms	
				Infected dogs	%	Infected dogs	%
Vomiting	68	27	39.71	18	26.47	8	11.76
Skip eating	34	11	32.35	4	11.76	3	8.82
Diarrhea, bloody diarrhea	51	16	31.37	11	21.57	13	25.49
Emaciated	25	7	28.00	3	12.00	1	4.00
Anal itching	13	3	23.08	1	7.69	0	0.00
No manifestation	44	18	40.91	10	22.73	4	9.09
Total	235	76	32.34	47	20,00	29	12.34

We observed that dogs infected with intestinal roundworms commonly exhibit symptoms such as vomiting, loss of appetite, diarrhea, and emaciation. Additionally, less frequent symptoms include edema, anal itching, or scooting. Generally, dogs without clear symptoms represent the highest proportion (40.91%), followed by vomiting (39.71%), loss of appetite (32.35%), bloody diarrhea (31.387%), and emaciation (28.00%). Anal itching is the least common symptom reported (23.08%).

Specifically, in cases of roundworm infection, vomiting is the most prevalent symptom, occurring at a rate of 26.47%. Conversely, dogs infected with hookworms tend to experience diarrhea and bloody diarrhea more frequently than other symptoms, with a rate of 25.49%.

3.6. Rate of infection with melon seed tapeworm according to age

During the survey period, we recorded 5 cases of melon seed tapeworm infection. Among these cases, there was 1 instance of co-infection with Parvovirus in dogs, but no dual infections with other intestinal parasites were observed.

According to Table 6, melon seed tapeworm primarily infects dogs under 6 months old, with the highest prevalence among dogs under 3 months old. Additionally, there was a case of melon seed tapeworm infection combined with Parvovirus disease in a dog under 3 months old. The infection rate of melon seed tapeworm with Parvovirus disease is 0.97%. Main symptoms observed include malnutrition, emaciation, presence of tapeworm segments in feces, and some infected dogs may be asymptomatic.

Table 6. Melon seed tapeworm infection by age

Age, months	Surveyed dogs	Infected dogs	%	With Parvovirus	
				Infected	%
<3	103	3	2.91	1	0.97
3-6	44	2	4.55	0	0.00
6-12	59	0	0.00	0	0.00
>12	29	0	0.00	0	0.00
Total	235	5	2.13	1	0.43

3.7. Rate of infection with melon seed tapeworm (*Diphylidium caninum*) by breed

To investigate the influence of breed origin on the likelihood of intestinal parasitic infection in dogs, we surveyed 235 dogs exhibiting symptoms of gastrointestinal diseases brought to the K9 Veterinary Clinic for treatment. Among them, 139 were domestic dogs and 96 were foreign dogs.

According to Table 7, the rate of tapeworm infection in domestic dog breeds is 2.16%, while in foreign dog breeds it is 2.08%. There was one case of concurrent infection with Parvovirus disease, with an infection rate of 0.72%. The survey results indicate that tapeworms can infect both domestic and foreign dog breeds when dogs are not regularly dewormed. Statistical analysis shows that the difference is not significant (P>0.05).

Table 7. Melon seed tapeworm infection by breed

Breed	Surveyed dogs	Infected dogs	%	With Parvovirus	
				Infected	%
Domestic	139	3	2.16	1	0.72
Exotic	96	2	2.08	0	0.00
Total	235	5	2.13	1	0.43

3.8. Effective treatment and prevention measures for intestinal helminths

According to table 8, we observed a recovery rate of 85.37% among dogs showing symptoms of gastrointestinal disease infected with worms. Our results surpass those reported by Nguyen Thi Diem Phuong (2010), who recorded a recovery rate of 83.06%. During the treatment process, 12 cases (14.63%) could not be cured due to severe worm infestations, resulting in significant debilitation and dehydration.

Table 8. Treatment results for intestinal helminths

Tracking Indicators	Dogs suspected of being infected with helminths	%
Number of dogs off	70	85.37
Dead dogs	12	14.63
Total	82	100

With a treatment duration of 5-7 days, out of 82 suspected cases of helminthiasis, 70 dogs successfully recovered (85.37%). This high treatment success is attributed to owners promptly bringing their sick dogs to us. In cases of severe vomiting and diarrhea, owners sought intravenous fluids and regular injections for their pets.

3.9. Effective treatment of dogs infected with infectious diseases and helminths

According to table 9, we documented the treatment outcomes for dogs suspected of infectious diseases with helminth co-infection: 14 out of 23 dogs recovered, accounting for 60.87% of those suspected of having Parvovirus disease with helminth co-infection, and 2 out of 5 dogs recovered, accounting for 40% of those suspected of Carre's disease with helminth co-infection.

Symptoms observed in suspected cases of Carre's disease with helminth co-infection include anorexia, high fever (40-41°C), vomiting, diarrhea with mucus and blood, conjunctivitis, excessive ocular and nasal discharge, and dry nasal mucosa. Additionally, some dogs exhibited hyperkeratosis of paw pads, abdominal pustules, and neurological symptoms. Suspected cases of Parvovirus with helminth co-infection presented with lethargy, anorexia, mild to absent fever, profuse vomiting, loose diarrhea with a foul odor, and bright red blood mixed with sloughed intestinal mucosa, emitting a characteristic fishy odor. Severe dehydration severely weakened the dogs' bodies.

Table 9. Treatment with helminth co-infection

Tracking Indicators	Suspected disease caused by helminthic Parvovirus		Suspected disease caused by Carre grafting helminths	
	Number of dogs	%	Number of dogs	%
Number of dogs off	14	60.87	2	40
Number of dead dogs	9	39.13	3	60
Total	23	100	5	100

Helminth infections, while not as lethal as other diseases, compromise a dog's immunity and exacerbate the severity of concurrent infectious diseases. Infectious diseases in dogs, notably Parvovirus, lack specific treatment and progress rapidly, leading to severe debilitation and dehydration, particularly when compounded by helminth co-infection. Failure to adhere to prescribed treatment protocols by owners contributes to lower recovery rates.

4. CONCLUSION

The prevalence of intestinal roundworm infection among surveyed dogs is 32.34%. Two species of intestinal roundworms were identified, with roundworms (20.00%) showing a higher infection rate than hookworms. No cases of whipworm infection were detected. Roundworm infection predominates among dogs under 3 months of age, with infection rates decreasing with age. In contrast, hookworm infection shows high prevalence across all age groups. The rate of tapeworm infection is 2.13%. Fluke infections are predominantly found in dogs under 6 months old, with instances of concurrent infection with Parvovirus observed. The domestic dog breed group shows a higher infection rate compared to the foreign dog breed group. Following treatment of 82 dogs infected with intestinal parasites, 70 dogs recovered, resulting in a recovery rate of 85.37%.

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INFORMATION OF THE AAAP AAAP20 IN MELBOURNE – AUSTRALIA, JULY 9-12, 2024 AND AAAP21 IN HANOI – VIETNAM, OCTOBER 28-31, 2026

Assoc. Prof. Dr. Nguyen Van Duc,
Vice-President of AAAP20 Council
Vice-President of Scientific committee of JAHST

The Australian Association of Animal Sciences (AAAS) and the Asian-Australasian Association of Animal Production Societies (AAAP) Congress has been organised on July 9-12, 2024 in the Melbourne Convention & Exhibition Center, Melbourne, Australia.

On behalf of the Australian Association of Animal Sciences (AAAS), Dr Dianne Mayberry AAAS Federal President and the Asian-Australasian Association of Animal Production Societies (AAAP), Professor Michael Friend President WELCOME TO THE AUSTRALIAN ASSOCIATION OF ANIMAL SCIENCES 2024 CONFERENCE in Melbourne, Australia.

The 35th AAAS Biennial Conference is being held as a joint event with the 20th AAAP Congress, providing an opportunity for members of our Associations to connect with old friends and new colleagues, build collaborative networks, support our early career researchers, and share our science.

Livestock producers in the Asia-Pacific region are being challenged by a changing climate, consumer expectations, government regulations, biosecurity threats and geopolitical tensions, so the conference theme of Embracing disruption as an opportunity for animal science seems especially appropriate as we strive to create more resilient, ethical, and sustainable animal enterprises.

The AAAS was established in 1954 as the Australian Society of Animal Production. The Association has evolved over time, but we are particularly proud of our recent efforts to foster a diverse and inclusive network of animal scientists. Our Federal Council for the past two years has been compromised of both established and early-career scientists from across the animal industries, representing a variety of roles within research organisations, government agencies, and industry associations. In addition to our existing membership categories, the Association now offers discounts for retired professionals and parental leave, and our Branch committees have been exploring shared executive positions, with these initiatives facilitating engagement from a broader range of animal scientists. We would like to thank the members of the AAAS Branch Committees, National Committees and Federal Council who have volunteered their time to create value for our members. Recent activities have included seminars, symposia, BBQs, and a special session at the British Association of Animal Science conference in Belfast. The achievements of members have been recognised and supported through student awards, domestic and international travel grants, and the induction of new AAAS Fellows. It has also been wonderful to see AAAS members seeking each other out and connecting at other conferences and industry events, both within Australia and overseas.

The AAAP was established 1980 and the 1st AAAP Animal Science Congress was held that year in Kuala Lumpur, Malaysia, some 44 years ago. AAAP exists to support collaborative activities between its 19 association members. The Association's journal was established in 1988 as the Asian-Australasian Journal of Animal Sciences and relaunched in 2021 as Animal Bioscience. The citations per paper have increased substantially, from 0.4 in 1999, to 3 in 2023, resulting in it being a Q1 ranked journal in Animal Science and Zoology for the past three years. This is testament to the strength of our Association, the quality of the work of our researchers, and the hard work of the editorial team. The other major collaborative activity of AAAP is the Congress, which represents the pinnacle of supporting collaboration between the 19 member organisations. This meeting is only the second hosted in Australia, the last being in Sydney in 2000. I fondly remember that conference, where as an early career researcher I established career networks that remain to this day. It is my hope that this conference will also foster the strengthening of existing and development of new networks. The diversity of both Associations and broader animal science community is reflected in the breadth of science offered at this week's conference, with submitted papers covering wildlife, feral, sporting and companion animals, in addition to production animals. Presentations on the people and communities involved in the business of animal production, and indigenous approaches to sustainability, are also offered alongside those focused on the latest advances in animal nutrition, health, reproduction, genetics, and digital technologies. iv We trust you will enjoy this week's conference, along with all that the wonderful city of Melbourne offers, and invite you to join us in Adelaide, South Australia in 2026 for the next AAAS conference, before we return to Melbourne again in 2028 for the World Association of Animal Production Conference.

1. Scientific programs

Scientific programs offered eight plenary speeches, four oral presentation sessions, one poster session and a large area for exhibitors.

A total of 354 scientific papers and about 200 posters presented in the 20th AAAP Animal Science Congress.

Total numbers of 573 participants coming from 21 countries and regions.

Vietnamese participants in the AAAP20 Congress at Melbourne Australia

The Animal Bioscience (AB) is the new name of the Official Journal of AAAP has been changed from year 2021 of the AJAS as informed by Editor-in-Chief Prof. Jong Kyu Ha.

Discussion bettween the Journal of Animal Husbandry Sciences and Technics of Vietnam and the Animal Bioscience Journal of AAAP at the AAAP20 Congress at Melbourne Australia

The AAAP20 Council Meeting at Melbourne, Australia

Australian Association Animal Science and Animal Husbandry Association of Vietnam Meeting in the AAAP20 at Melbourne - Australia

AAAP20 Conference dinner at Melbourne Australia

At the AAAP20 Conference dinner at Melbourne Convention Center Melbourne, Australia, Prof. Frank Dunshea informed that the 35th Biennial Conference of the Australian Association of Animal Sciences and the 20th Asian-Australasian Association of Animal Production Societies has been organised successfully. Prof. Frank Dunshea also informed that the Conference papers in different subjects avalable in the AAAP20 Proceedings:

Subject	Number of paper
Key-notes/Plenary speeches	08
Adoption, Education and Extension	10
Animal Genetics	24
Animal Health and Disease	30
Animal Welfare	14
Beef and Buffalo Science	18
Dairy Science	11
Environmental Sustainability	38
Food Efficiency and Global Security	08

Global Farm Platform	07
Heat and/or Environmental Stress	24
Meat Science, Carcass Science and Cellular Agriculture	27
Nutrition	78
Pig Science	31
Poultry Science	14
Precision Agriculture	18
Reproduction	34
Small Ruminants (Goat and Sheep)	15
Wildlife/Human Disruption	05

2. AAAP21 Conference in Vietnam

At the same times of AAAP20 Conference dinner, after the final report by Prof. Frank Dunshea President of the 35th Biennial Conference of the Australian Association of Animal Sciences and the 20th Asian-Australasian Association of Animal Production Societies that the AAAP20 Conference has been organised successfully, on behalf of AAAP council, haned the AAAP flag to Dr. Nguyen Xuan Duong, Chairman of Animal Husbandry Association of Vietnam hosted AAAP21 and Assoc. Prof. Dr. Nguyen Van Duc, Vice-President of Scientific committee of JAHST, Vice-President of AAAP20 Council.

On behalf of Animal Husbandry Association of Vietnam the host of AAAP21, after receving the AAAP flag, Dr. Nguyen Xuan Duong express a lot ofthanks to Prof. Frank Dunshea, President of AAAP20 and all members of AAAP council and promises to Secretary General Professor Sang Jip Ohh and the AAAP Council members to open the official homepage of the congress as early as possible.

The host of AAAP21 Vietnam receving the AAAP flag from the AAA20 hosted by Australia

Dr Duong also invites all AAAP council members, as well as Scientists and Students related to the 11 themes to attend the AAAP21 in HaNoi, Vietnam. Dr Duong also mentioned the 11 categories will be available for AAAP21 Congress in Vietnam on October 28-31, 2026 at the National Cenvention Center (NCC) in HaNoi, Vietnam:

1. Animal breeding and genetics
2. Animal production systems
3. Animal nutrition
4. Sustainable livestock systems
5. Digital management & smart farming
6. Animal welfare
7. Carbon emission management
8. Food safety & security
9. Companion animals
10. Smallholder livestock farming
11. Agricultural biomass utilization

The 21st AAAP ASC will be help on October 28-31, 2026 at the National Cenvention Center (NCC), Ha Noi, Vietnam. Welcome!!!