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ANIMAL GENETICS AND BREEDING

ANIMAL FEEDS AND NUTRITION

ANIMAL PRODUCTION

SCIENTIFIC NEWS

ASSOCIATION OF HEAT SHOCK PROTEIN 70 GENE (HSP70) POLYMORPHISM WITH mRNA EXPRESSION IN CHICKEN UNDER HEAT STRESS CONDITIONS

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ABSTRACT

The present study was designed to ivestigate the relationship between Hsp70 genotypes and the Hsp70 mRNA expression in embyos and chicken under heat stress conditions. One hundred and eighty eggs from Ri chicken breed (20 eggs/genotype: H1H1, H1H2, H1H3, H1H4, H2H2, H2H3, H2H4, H3H3 and H3H4) were divided in to two groups: control group (10 eggs/genotype) was maintained at 37.8°C and 56-60% relative humidity throughout the incubation period; the experiment group was subjected to heat stress at 39.5°C for 6 h/day and 56-60% relative humidity from 12th to18th day during embryogenesis. Ninety chickens at the age of 40 days (10 chicks/each Hsp70 genotype) were divided into two groups: control group was still maintained at the same environment conditions; experiment group was subjected to heat stress at 41-42°C for 6h/day and the experiment was repeated for 3 days. The liver and pectoral muscle tissues of embryos and chicken were collected in both control and experiment groups for total RNA extraction. The expression of mRNA of Hsp70 genes in liver and muscle tissue was evaluated by RT-qPCR. The results showed that the expression of Hsp70 among different genotypes was different under heat stress conditions. The highest expression of Hsp70 was found in the H3H3 genotype and followed by the genotype contain H3 haplotype, it means that chicken with H3H3 genotype was the most tolerant to heat stress and could be used as a marker for selecting heat tolerant breeds of chickens.

Keywords: *Ri chicken, Hsp70 genotype, mRNA expression, RT-qPCR.*

1. INTRODUCTION

Heat stress is considered one of the most challenging environmental conditions in poultry industry, (Yu and Bao, 2008). Poultry is warm-blooded animal (40.5-41.5°C). Poultry does not have sweat glands, and almost all of the poultry body is covered by feathers. Therefore, releasing of body heat to the environment is difficult, and poultry is quite susceptible to heat stress (Tamzil *et al*., 2014; Aryani *et al*., 2019). Stressed poultry can be identified by panting frequency (Gaviol *et al*., 2008), a decrease in feed consumption, an increase in water consumption and manure water content (Garriga *et al*., 2006), an increase in serum corticosterone concentration and the expression of Hsp70 protein (Zhen *et al*., 2006; Yu and Bao, 2008). Heat shock proteins are a group of highly conserved proteins that are rapidly synthesized in tissues subjected to thermal stressors. On the molecular level, Hsps play an important role in maintaining the integrity of structural proteins, prevention of protein aggregation, and folding-refolding of damaged proteins (Al-Zhgoul *et al*., 2013). In addition, Hsps facilitate the degradation of unstable proteins and translocation of proteins across mem branes (Morimoto, 1998). Of the many expressed Hsps, those with a molecular weight of approximately 70 kDa appear to be most closely associated with heat tolerance. Many studies reported that mRNA Hsp70 expression in some organs of chickens increased significantly after being exposed to heat stress (Mahmoud *et al*., 2004, Al-Zhgoul *et al*., 2013; Zhen *et al*., 2006; Yu and Bao, 2008).

The Hsp70 gene in chicken is located in the fifth chromosome and consists of only one exon with a coding-region length of 1,905bp (Morimoto *et al*., 1986). In our previous study

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(Lan *et al*., 2019), four haplotypes of Hsp70 gene (H1, H2, H3 and H4) were detected in four chicken breeds raised in Viet Nam, LV, HA, TP and Ri chickens based on the polymorphisms at positions A258G and C276G in the protein coding region of the Hsp70 genes, and Ri chicken breed showed a highest genetic diversity of Hsp70 gene. This study was conducted to investigate the relationship between Hsp70 genotypes and the Hsp70 mRNA expression in Ri chicken under heat stress conditions.

2. MATERIAL AND METHODS

2.1. Experiment design and sampling

2.1.1. Incubation and heat stress during embryogenesis

We used 180 eggs from Ri chicken breed, twenty eggs per each Hsp70 genotype: H1H1, H1H2, H1H3, H1H4, H2H2, H2H3, H2H4, H3H3 and H3H4. The eggs were divided into two incubation treatment groups: control group (ten eggs per each genotype) was maintained at 37.8°C and 56-60% relative humidity throughout the incubation period; the experiment group (10 eggs/genotype) was subjected to heat sock at 39.5°C for 6 h/day and 56-60% relative humidity from day 12^{th} to 18^{th} during embryogenesis. At day 18 of the incubation, samples from the pectoral muscle and liver of embryos were collected in both control and experiment group and put in liquid nitrogen within a 10 to 20 mins and stored at -80°C for total RNA isolation and real time RT-PCR.

2.1.2. Chickens at the age of 40 days exposed to heat sock

A total of 90 Ri chickens, being 10 chicks per each Hsp70 genotype (H1H1, H1H2, H1H3, H1H4, H2H2, H2H3, H2H4, H3H3 and H3H4) were raised under the same environment conditions. At the age of 40 days, chicks were divided into two groups and each group had total 45 chicks, five chicks per each genotype: control group was still maintained at the same environment conditions; experiment group was subjected to heat sock by adjusting room temperature at 40-41°C for 6h and the experiment was repeated for 3 days. At the end of the exposure period, chickens were killed and liver and pectoral muscle samples were collected and stored according to the method described above.

2.2. Total RNA extraction and reverse transcription

Total RNA was extracted from pectoral muscle and liver of embryos and chicks using a GeneJet RNA Purification Kit (ThermoFisher Scientific-TFS). DNA was removed using the DNA-FreeTM DNA Removal Kit (TFS) and the RNA samples were checked for concentration and purity by QubitTM3.0 (Invitorgen). RNA (1µg) was reverse transcribed to a cDNA in a reaction mixture using RevertAid Fist Stand cDNA Synthesis Kit (TFS).

2.3. Relative-quantitative Real-time RT-PCR

The relative-quantitative real-time RT-PCR was performed using a Maxima SYBR Green/ROX qPCR Master Mix (2x) Kit (TFS) on 7500 Fast Real-Time PCR system (ABI, USA). The RT-qPCR primers were used as described by Gan *et. al.* (2013): cHsp70 (F: 5′-GCGCCAGGCC ACCAAAGATG-3′; R: 5′-GCCCCCTCCCAAGTCAAAGATG-3′) and housekeeping gene β-actin (F: 5′-CTCCCCCATGCCATCCTCCGTCTG-3′; R: 5′-GCTGTGGCCATCTCCTGCTC-3′). The reaction mix of RT-qPCR was performed in total 25µL including: 12.5µL of master mix; 2µL forward primer (10 pmol); 2µL reverse primer (10 pmol); 2µL cDNA of the sample; and 9 µL of nuclease-free water. Cycling parameters were 95^0C for 10 min, 40 cycles of 95^0C for 15s, followed by 30s at 58^0C (or each primer's annealing temperature), and 72^0C for 30s with final melting at 95^0C for 30s. A negative control (ultra-pure water) also was added in each assay. The C_T values were exported to Microsoft Excel to calculate the C_T mean, standard deviation. The specificity of the target amplification was confirmed

by melting curves and the gene expression was normalized with β-actin which is a housekeeping gene. The related gene expression was calculated by $2^{-\Delta\Delta CT}$ method (Livak *et al*., 2001), where $\Delta\Delta C_T = (C_{T,\ Hsp70}\text{-}C_{T,\ \beta\text{-}actin})_{Treatments}\text{-}(C_{T,Hsp70\text{-}CT,\ \beta\text{-}actin})_{Control}$.

2.4. Statistical analysis

The program normalizes the data using the ΔΔCT method, thus generating Fold Change, which is the value of the relative expression between the control and the treatment (Livak *et al*., 2001). Statistical analysis of the mRNA expression differences between each genotype was carried out using a one-way ANOVA using the Statistical Package for Social Sciences (SPSS version 25) followed by all-pairs Tukey test. All data were expressed as means ± standard deviation, and the significance level was set at P<0.05.

3. RESULTS AND DISCUSSION

The primers specificity for β-actin and Hsp70 genes were evaluated through the melting curve, which showed only one peak indicating no primer dimers were detected and presenting excellent performance (Figure 1).

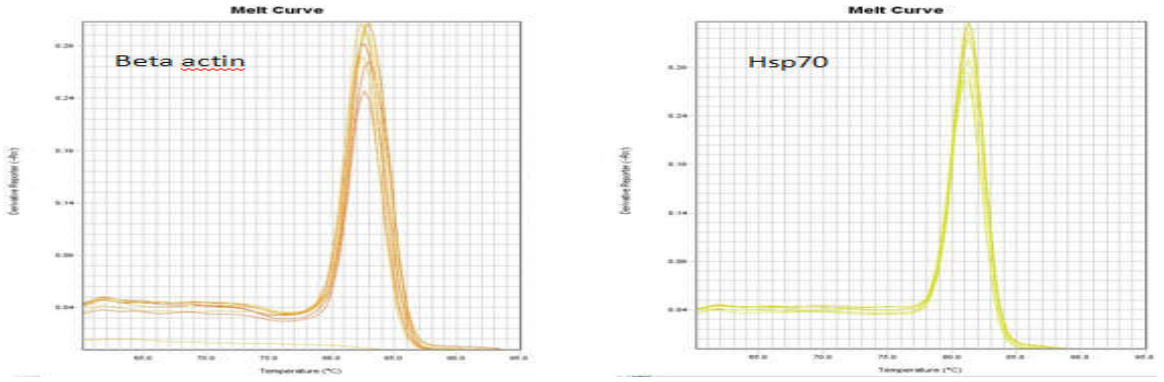

Figure 1. The melt curve of efficiency test for Beta actin and Hsp70 genes derived from RT-qPCR reactions

The data of Hsp70 mRNA expression levels in liver and muscle tissues under heat stress condition during embryogenesis are presented in table 1. Using the $2^{-\Delta\Delta CT}$ method, the data are presented as the fold change in Hsp70 gene expression normalized to a housekeeping gene (β-actin) and relative to the untreated control for each of genotype. For the untreated control sample, $\Delta\Delta C_T$ equals zero and 2^0 equals one, so that the fold change in gene expression relative to the untreated control equals one, by definition. For the treated samples, evaluation of $2^{-\Delta\Delta CT}$ indicates the fold change in gene expression relative to the untreated control. The results in Table1 revealed that the mRNA expression of all Hsp70 genotypes significantly increased in response to heat stress. The relative levels of mRNA expression among different Hsp70 genotypes ranged from 2.67±0.09 to 4.96±0.24 in liver and from 2.48±0.08 to 4.51±0.1 in muscle tissues. Comparing between the mRNA expression levels among different genotypes in liver and muscle tissues, we found that there was a significant difference. The level expression of homozygous H3H3 genotype was highest and significantly higher compared with the other genotypes (P<0.05).

The results of Hsp70 mRNA expression level in liver and muscle tissues of chicken at the age od 40 days old are shown in Table 2. The data in Table 2 showed that, during the exposure to heat stress, liver and muscle tissues of Ri chickens exhibited increased amounts of Hsp70 mRNA. The levels of mRNA expression among different Hsp70 genotypes

ranged from 2.69±0.13 to 4.92±0.08 in liver and from 2.41±0.09 to 4.36±0.1 in muscle tissues. There was a significant difference between the mRNA expression levels among different genotypes in liver and muscle tissues. The level expression of homozygous H3H3 genotype was highest in both tissues type (P<0.05).

Table 1. mRNA expression levels of Hsp70 in different genotypes under heat stress conditions during embryogenesis

Genotype	mRNA expression in liver ($2^{-\Delta\Delta CT}$)			mRNA expression in muscle ($2^{-\Delta\Delta CT}$)		
	Ne	Nc	Mean±SD	Ne	Nc	Mean±SD
H1H1	4	4	2.78±0.07^{de}	4	4	2.48±0.08^{e}
H1H2	4	4	2.86±0.14^{de}	4	4	2.60±0.16^{de}
H1H3	4	4	3.82±0.07^{b}	5	5	3.26±0.05^{b}
H1H4	4	4	2.98±0.05^{d}	5	5	2.81 ± 0.08^{cd}
H2H2	4	4	3.32±0.07^{c}	5	5	2.87±0.19^{c}
H2H3	5	5	3.76±0.12^{c}	5	5	3.43±0.07^{b}
H2H4	4	4	2.67±0.09^{e}	4	4	2.61±0.16^{de}
H3H3	4	4	4.96±0.24^{a}	5	5	4.51±0.12^{a}
H3H4	5	5	3.60±0.08^{b}	5	5	3.37±0.10^{b}

Within the same column, Means with different superscripts differ significantly (P<0.05). Ne: number of embryoes analyzed for each genotype in experiment group; Nc number of embryoes for each genotype in control group.

Table 2. mRNA expression levels of Hsp70 in different genotypes during the exposure to heat stress of chicken at the age 40 days

Genotype	mRNA expression in liver ($2^{-\Delta\Delta CT}$)			mRNA expression in muscle ($2^{-\Delta\Delta CT}$)		
	Ne	Nc	Mean±SD	Ne	Nc	Mean±SD
H1H1	4	4	2.80±0.15^{de}	4	4	2.42±0.13^{d}
H1H2	4	4	2.69±0.13^{e}	4	4	2.41±0.09^{d}
H1H3	4	4	3.51±0.08^{b}	5	5	3.17±0.13^{bc}
H1H4	4	4	2.80±0.08^{de}	5	5	2.76±0.09^{cd}
H2H2	4	4	2.96±0.12^{c}	4	4	2.78±0.17^{cd}
H2H3	5	5	3.68±0.20^{b}	4	4	3.32±0.12^{bc}
H2H4	5	5	2.73±0.15^{e}	5	5	2.42±0.12^{d}
H3H3	4	4	4.92±0.08^{a}	4	4	4.36±0.24^{a}
H3H4	5	5	3.58±0.06^{b}	4	4	3.10±0.31^{bc}

Ne: number of chickens analyzed for genotype in Exp group; Nc number of chickens for genotype in Con group

High temperatures can cause several damages to livestock production, especially in poultry farming causing financial losses. De Nada *et al*. (2011) indicated that exposure to thermal stress can promote expression of heat shock factors and heat shock protein (Hsps). Heat shock proteins produce responses to temperature rise and are driven by some factors besides heat, such as microbial infection, tissue trauma and genetic injury. According to their molecular size, there are six main families of Hsps namely Hsp100, Hsp90, Hsp70, Hsp60, Hsp40, and the small Hsps (Morimoto, 1998). In heat environments, Hsp70 gene expression plays a better role in cellular functions than other Hsps (Ali and Banu, 1991). Enhanced Hsp70 expression may be a response to stressful environments, and may improve cell survival by protecting proteins from degradation and facilitating their refolding. The Hsp70 gene is considered as an ideal biological marker for heat stress in livestock (Archana *et al*., 2017). The induction of Hsp70 gene expression occurs when stress induces and activates transcription factors, such HSF, to bind to HSE. The binding of HSF to HSE allows the transcription of the Hsp70 gene by RNA polymerase II (Akerfelt *et al*., 2010). The results of this study and many other scientific researches have confirmed that the Hsp70 expression significantly increased in several tissuse when exposed to heat stress. (Mahmoud *et al*., 2004., Al-Zhgoul *et al*., 2013; Zhen *et al*., 2006; Yu and Bao., 2008).

Research on the polymorphisms of Hsp70 gene between breeds of chickens has progressed based on the sequence of Hsp70 gene of chickens (Mazzi *et al*., 2003; Zhen *et al*., 2006; Aryani *et al*., 2019, Lan *et al*., 2019). The association between polymorphisms of the HSP70 gene and heat tolerance has been regarded as a marker for selecting heat resistant breeds of chickens (Mazzi *et al*., 2003; Gaviol *et al*., 2008; Tamzil *et al*., 2013; Duangjinda *et al*., 2017). In this study, the heat stress was

implemented during embryogenesis and chicken at age of 40 days and the expression level of mRNA among different Hsp70 genotypes in liver, muscle tissues were assayed. The results showed that Hsp70 was expressed in both liver and muscle tissues for all Hsp70 genotypes but that expression levels varied in a tissue dependent manner under heat stress conditions. This study demonstrated that the liver, and pectoral muscle of embryos and chicken exposed to high temperature exhibited increased amounts of Hsp70 protein and mRNA. Under normal growth conditions, Hsp70 is synthesized constitutively; however, its expression increases following thermal challenge or stimulation from a variety of other environmental stressors. Our results are consistent with the finding by Zhen *et al*. (2006) that the haplotype combinations, which contain H3, H1H3, H2H3, H2H3, H3H4 and H3H3, were associated with much higher expression of hsp70 than that of any other haplotype combinations. The level expression of homozygous H3H3 genotype was highest and significantly higher compared with the other genotypes ($P<0.05$). These results further confirm the deduction that the haplotype H3 was probably advantageous to the heat resistance of chickens.

4. CONCLUSION

This study demonstrated that heat stress induces an increase in the levels of Hsp70 mRNA in the liver and pectoral muscle tissues during embryogenesis and chicken at age of 40 days old. There is an association between the polymorphism of Hsp70 gene and its Hsp70 mRNA expression. The genotype H3H3 was highest expression of Hsp70, it means that chicken with H3H3 genotype was the most tolerant to heat stress.

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LIVE WEIGHT AND BODY DIMENSIONS OF TAM HOANG CHICKENS SUPPLEMENTED WITH PROBIOTIC *LACTOBACILLUS* SP.

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ABSTRACT

This study was carried out to investigate the influences of *Lactobacillus* sp. as probiotic supplementation on body weight and dimensions in Tam Hoang chickens from 28 to 56 days old. A total of 135 Tam Hoang chickens were randomly divided into 15 cages subjected to five treatments, three replications per treatment, including Control (basal diet without additives), AMOX (basal diet with 100ppm (w/w) of the antibiotic amoxicillin trihydrate), PRO-1 (basal diet with 0.5% probiotic *L. acidophilus* K1), PRO-2 (basal diet with 0.5% probiotic *L. plantarum* L6) and PRO-3 (basal diet with 0.5% probiotic *L. acidophilus* K1 and *L. plantarum* L6). The number of bacteria in both single-strain probiotic and two-strain mixture (1:1 ratio) were at 1.0×10^8 cfu/g. As results, the addition of probiotic, particularly the mixture of two *Lactobacillus* strain significantly improved neck length, chest girth and drumstick circumference of broilers compared to the Control and AMOX treatments. The findings suggested the beneficial implication of probiotic in poultry production. In addition, the high, positive and significant correlations between body weight and linear body measurements observed in this study can be used as criteria to predict the body weight and the desired traits of chickens.

Keywords: *Body dimensions, Lactobacillus sp., live weight, probiotic, Tam Hoang chickens.*

1. INTRODUCTION

Poultry meat and eggs are important food sources to fulfill the ever-growing dietary needs of human population. Over the few decades, broiler chickens have gone through tremendous growth; however, they are raised under various kinds of stresses due to production pressure, which adversely affects their growth performance (Mund *et al.*, 2017). In such circumstances, antibiotics and synthetic antimicrobial agents have been favorably used in animal production as it effectively and economically improved poultry performance (Dhama *et al.*, 2011). At the same time, the development of antibiotic-resistant bacterial population into the environment and their further transmission to humans via the food chain could lead to serious consequences on public health (Hao *et al.*, 2014; Agyare *et al.*, 2018). Some strategies, therefore, have been applied to reduce the use of antibiotics in chicken farms with similar beneficial effects of growth promoters. Among these, the most popular are probiotics, prebiotics, enzymes, organic acids, phytogenic feed additives, phytoncides, nanoparticles and essential oils (Mehdi *et al.*, 2018).

Probiotic was defined as a live microorganism which beneficially affected the host animals by improving their intestinal balance (Fuller, 1989). Thus, probiotic is one of the approaches that has a potential to enhance intestinal health and subsequently results in the improvement of performance traits such as body weight (BW), body dimensions (BD) and carcass quality. Numerous authors confirmed the positive effects of probiotic supplementation on body conformation which regard in broiler meat production, especially in the breasts, thighs and drumsticks. It has been stated

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that supplementation of that probiotic *Bacillus subtilis* (>10^{11} spores/g diluted with 5l of water) significantly improved BW, back length (BL), and breast diameter of Noi chickens (Khoa *et al*., 2019). The inclusion of the probiotic in the feed (*L. fermentum*, *L. plantenrun* and *Weissallaciberia*) for 6 weeks also promoted carcass quality and proximate analysis of chicken meat, as reported by Ebu *et al*. (2019). However, there are few studies taken into account the implications of probiotic on BD of Tam Hoang (TH), a dual -purpose chicken breed in Vietnam. Therefore, the objective of this study was to evaluate the effects of administering *L. acidophilus*, *L. plantarum* and their combination on body weight and conformation traits of TH chickens.

2. MATERIALS AND METHODS

The experiment was conducted from Feb to May 2020, at the Animal Experimental farm of An Giang University. A total of 135 day-old TH chicks obtained from a private hatchery were kept in a brooder for 21 days. After that, all birds were randomly splited into 15 dependent barns (9 broilers per each: 4 males and 5 females) and fed until reaching 56 days old by five diets corresponding to five experimental treatments. The treatments included: Control (basal diet without additives), AMOX (basal diet with 100 ppm (w/w) of the antibiotic amoxicillin trihydrate), PRO-1 (basal diet with 0.5% probiotic *L. acidophilus* K1), PRO-2 (basal diet with 0.5% probiotic *L. plantarum* L6) and PRO-3 (basal diet with 0.5% probiotic *L. acidophilus* K1 and *L. plantarum* L6). The basal diet was formulated to meet the nutrient requirements of broiler chickens according to NRC (1994), as shown in Table 1.

The *Lactobacillus* strains were isolated from fermented foods and stored in the laboratory of An Giang University. The probiotic preparations were processed into freeze-dried cultures and subsequently mixed into the diet. The number of bacteria in both single-strain probiotic and two-strain mixture (1:1 ratio) were at 1.0×10^8 cfu/g.

Table 1. The basal diet for experiment

Ingredients (%)	Grower-finisher diet
Broken rice	27.0
Rice bran	37.5
Maize	16.0
Fish meal	10.0
Soybean meal	9.0
Premix*	0.5
Dry matter	89.5
Ash	4.99
Crude protein	18.4

Note: Supplied per kg of premix: vitamin B1 1,500mg, vitamin B2 500mg, vitamin A 1,000,000IU, vitamin D3 500,000IU, vitamin E 1,000mg, copper 2,250-2,500mg, iron 9,000-10,000mg, zinc 9,000-10,000mg and Manganese 9,000-10,000mg. The chemical composition was determined according to standard procedures of AOAC (2005).

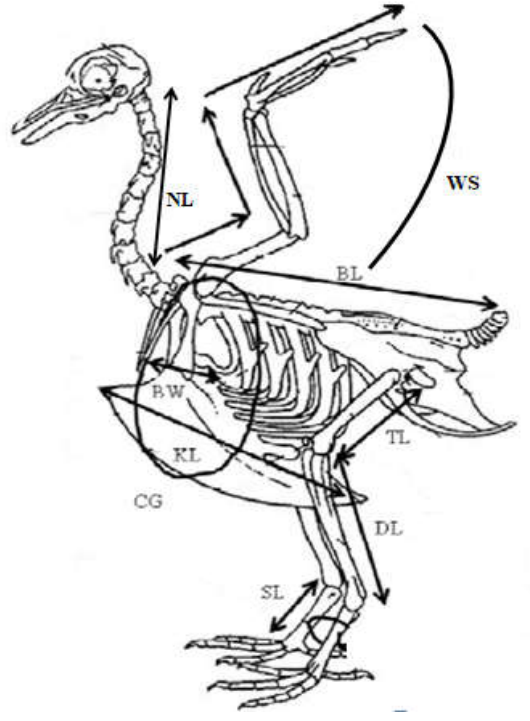

Fig 1. Body dimensions of TH chickens (Rachma *et al.*, 2013)

The chickens were weighed and randomly divided into five dietary treatments with three replicates of 9 birds each, providing a space of $0.2m^2$/bird. The birds were vaccinated against Newcastle, Gumboro, and avian influenza

disease prior to the experiment. Feed and water were provided *ad libitum*.

At 28, 42 and 56 days of age, 6 birds (3 males and 3 females) at an average treatment weight were selected to measure for BW (g) and BD (cm) according to previous studies (Rachma *et al.*, 2013; Kokoszyński *et al.*, 2017; Khoa *et al.*, 2019). The parameters included neck length (NL), back length (BL), wingspan (WS), chest girth (CG), breast width (BW), keel length (KL), thigh length (TL), drumstick length (DL), drumstick circumference (DC), and shank length (SL).

The numerical data were statistically analyzed using the GLM procedure of the Minitab 16.0 software. A probability value of less than 0.05 was considered to be significant. Pearson's coefficients of correlation were calculated to determine the relationships between body weight and other measurements.

3. RESULTS AND DISCUSSION

3.1. Live weight and dimensions of Tam Hoang chickens

Table 2. Live weight and dimensions of Tam Hoang chickens

Age	Treatments	LW	NL	BL	WS	CG	BW	KL	TL	DL	DC	SL
28 days	Control	482.50	6.62^{a}	14.13	29.75	16.78	7.15	6.60	6.78	7.58	7.38^{a}	5.78
	AMOX	491.17	7.17^{ab}	14.00	30.33	17.00	7.28	6.87	7.33	7.58	7.92^{ab}	5.83
	PRO-1	494.33	7.42^{ab}	14.42	30.33	16.65	7.12	6.53	6.75	7.33	7.95^{ab}	5.50
	PRO-2	470.33	7.70^{b}	13.85	30.75	16.18	7.50	6.73	7.12	7.17	8.22^{b}	6.00
	PRO-3	466.33	6.77^{ab}	13.50	30.83	17.25	7.47	6.70	6.65	7.42	8.22^{b}	5.67
	SEM	19.42	0.23	0.24	0.63	0.30	0.34	0.20	0.22	0.29	0.20	0.23
	P	0.802	0.013	0.127	0.758	0.153	0.895	0.785	0.192	0.830	0.038	0.612
42 days	Control	895.17	9.83	18.17	35.83	20.42	9.67	8.05	8.48	9.50	9.33^{a}	7.35
	AMOX	856.50	9.53	18.08	36.33	21.08	9.85	8.50	9.00	9.75	9.42^{a}	7.17
	PRO-1	833.17	9.83	17.92	35.08	19.75	9.38	8.02	8.23	9.25	10.08^{ab}	6.92
	PRO-2	773.33	9.33	17.00	34.50	19.58	10.02	8.47	8.75	9.75	10.53^{ab}	7.27
	PRO-3	868.33	9.22	17.75	35.50	20.92	9.65	8.70	8.58	10.25	10.65^{b}	6.97
	SEM	44.64	0.31	0.52	0.73	0.41	0.31	0.25	0.25	0.35	0.29	0.23
	P	0.396	0.520	0.529	0.469	0.057	0.686	0.260	0.297	0.358	0.008	0.620
56 days	Control	1,304.00	11.33	21.87	43.67	22.23^{ab}	10.45	9.67	9.88	11.67	10.67^{a}	8.25
	AMOX	1,334.33	10.92	22.25	44.83	22.75^{ab}	10.62	9.97	9.97	12.30	11.30^{a}	8.03
	PRO-1	1,278.17	11.17	22.25	44.17	23.00^{ab}	10.60	9.55	9.58	11.92	11.55^{ab}	7.92
	PRO-2	1,242.83	11.42	21.17	45.00	21.88^{a}	10.90	9.83	10.33	11.78	11.82^{ab}	8.25
	PRO-3	1,345.83	11.00	21.83	44.17	23.37^{b}	11.22	10.10	9.92	12.33	12.65^{b}	7.92
	SEM	64.15	0.15	0.33	0.73	0.35	0.27	0.26	0.29	0.29	0.28	0.21
	P	0.787	0.144	0.152	0.695	0.042	0.305	0.565	0.494	0.382	0.001	0.656

Note: Means on the same column having different superscripts are significantly different (P<0.05).

The BW of the evaluated treatments, which ranged from 466.33 to 1345.83g from 28 to 56 days of age, may be indicative of their normal development resulting mainly from appropriate nutrition and housing conditions during the rearing period. The similar LW of the chickens among the treatments at all observation points may contribute to the lack of statistically significant differences (P>0.05) of other BD, such as BL, WS, BW, KL, TL, DL and SL with some exceptions. The addition of probiotics (particularly PRO-3, the mixture of

two *Lactobacillus* strains) positively increased NL and DC at 28 days old, CG at 42 days old and CG, DC of chickens at 56 days old. It also showed that probiotic supplementation (PRO-1, PRO2 and PRO-3) had similar or higher results compared to the antibiotic inclusion (AMOX), suggesting the beneficial implication of probiotic in poultry production.

These findings are consistent with those found by Softić *et al*. (2011) and Khoa *et al.* (2019). Considering the fattening period of Cobb chickens, birds consumed the commercial probiotic had higher values of body measurements on breast circumference, keel length, breast depth, drumsticks, circumference, shank length and breast angle (Softić *et al*., 2011). Khoa *et al.* (2019) found positive effects on body weight, back length, breast diameter by application of *B. subtilis* agents in Noi chickens. Breast and thigh muscles are the most preferred parts of chicken carcasses (Wideman *et al*., 2016) and higher relative values of chest girth (CG) and drumstick circumference (DC) may express higher meat yield. By this criterion, a more favorable conformation achieved by the groups of chickens supplemented with *Lactobacillus* probiotics.

3.2. Coefficients of correlation between live weight and dimensions of Tam Hoang chickens

Table 3. Coefficients of correlation between live weight and dimensions of Tam Hoang chickens

Traits	LW	NL	BL	WS	CG	BW	KL	TL	DL	DC	SL
LW	1.00										
NL	0.90***	**1.00**									
BL	0.95***	0.90***	**1.00**								
WS	0.95***	0.88***	0.93***	**1.00**							
CG	0.91***	0.85***	0.92***	0.88***	**1.00**						
BW	0.80***	0.79***	0.83***	0.80***	0.84***	**1.00**					
KL	0.90***	0.83***	0.88***	0.89***	0.88***	0.81***	**1.00**				
TL	0.87***	0.84***	0.90***	0.88***	0.83***	0.81***	0.90***	**1.00**			
DL	0.91***	0.86***	0.90***	0.91***	0.91***	0.80***	0.88***	0.85***	**1.00**		
DC	0.85***	0.79***	0.85***	0.85***	0.86***	0.85***	0.86***	0.81***	0.85***	**1.00**	
SL	0.89***	0.86***	0.86***	0.86***	0.83***	0.77***	0.87***	0.86***	0.87***	0.77***	1.00

Table 3 presents the correlations between live weight and conformation traits pooled from all the experimental chickens. The correlation ranged from r=0.77 to r=0.95. The relationships between live weight and all the body measurements were positive and highly significant (P<0.001). The highest correlation was obtained between body weight and back length and wingspan while correlations between shank length and breast width or drumstick circumference were observed to be the least. Correlation coefficient was consistently high (r>0.77) among the other traits. Similar results were observed that body weights were highly related to all morphometric traits of the chickens (Egena *et al*., 2014; Fayeye *et al*., 2014; Rasheed, 2019). The high, positive and significant correlations between body weight and linear body measurements observed in this study can be used as criteria to predict the body weight and the desired traits of chickens.

5. CONCLUSION

This study provides evidence that the supplementation of *Lactobacillus* sp. to feed positively increased neck length, drumstick circumference and chest girth of Tam Hoang chickens from 28 to 56 days old. Furthermore, the high, positive and significant correlations

between body weight and linear body measurements can be used as criteria to predict the body weight and the desired traits of chickens.

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APPLICATION DNA BARCODE TECHNIQUES ON SPECIES IDENTIFICATION OF EARTHWORM IN VIETNAM

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ABSTRACTS

The development of raising of earthworms (EW) in Vietnam for the purpose of animal feeds was an increasing. Studies on indentification of EW species, which were reared in Vietnam, have been still limited in the methods, especially the molecular methods. This study aimed to make the practical approach using DNA barcoding as a tool to determine the EW species. The study was carried out on 10 collected worm samples in Hanoi, Thanh Hoa, HCM city. The EW DNAs were extracted and stored to carry out the PCR reaction. The COI gene was amplified as 640bp fragment length and was sequenced and analyzed alignment to the Nucleotide Basic Local Alignment Search Tool in NCBI Genbank. The results showed four EW species have been identified from 10 samples of collected EW. These were *Eisenia andrei* (HN02, TH01, HN03), *Eudrilus eugeniae* (HT01, HT03, HN04), *Peryonix sansibaricus* (HN01, HT02, SG01), *Eisenia fetida* (TH02). The genetic relationships between identified species showed their correllation. This first study provided the knowledge of EW species in Vietnam as well as information services for agricultral management and production.

Keywords: *COI, sequencing, DNA barcode, earthworms.*

1. INTRODUCTION

Earthworms have long been recognized as soil benefactors and their presence is commonly associated with good quality soils (Chan KY, 2001). By tunneling through the soil, the earthworms (EW) help to aerate the soil, improve the water and nutrient holding capacity of loose soil, sandy soil in the same way it lightens heavier soils. Besides, EW are a potential source of protein, high content and good quality of EW protein, the high content of vitamin B and other bioactive substances, it is very likely that EW could become an important source of animal protein. Therefore, it has been considered a high potential value as livestock feed (Truong Thi Bich Hong, 2014).

Recently, raising EW is of interest to farmers in Vietnam as an additional source protein for animal, etc. Hence, many large EW farms have been established in different provinces such as Thanh Hoa, Hanoi, Thai Binh... The species of EW as well as the productivity is yet unknown by farmers.

There are more than 5,000 different EW species (Drilo BASE, 2014). They vary in size, from some centimeters to several meters long of body, and in behaviour determining a particular depth of residence in the soil, and levels of incidence on surface. It is difficult to determine the EW species based on their morphological and anatomical characteristics. Currently, species classification can be complemented by molecular techniques. In particular, the use of "DNA barcoding" based on a standardized region of the mitochindrial cytochrome oxidase I gene (COI) has been widely used as a genetic marker to discriminate animal species (Hebert *et al*., 2003). Thus, COI gene, defined as the DNA barcode has been used to identify species of birds (Hebert *et al*., 2003), spiders (Barrett and Hebert, 2005), insect pests (Ball and Armstrong, 2006).

Understanding about the EW species is very important and necessary because in Vietnam, there are only few studies on identification of EW species. Tran Thi Thanh Binh and Nguyen Thi Ha (2014) studied on classification and density of EW species in

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the North of Vietnam. Nguyen Thanh Tung and Tran Thi Anh Thu (2008) studied on classification and distributed characteristics of EW species along the delta of Tien River. However, no previous study has applied molecular methods for EW identification. Hence, this study aimed to introduce the use of molecular techniques for identification of EW species in Vietnam based on DNA barcoding, in order to achieve variablility and obtain a comprehensive range of species for COI sequencing as well as agricultural exploits of EWs.

2. MATERIALS AND METHODS

2.1. Materials

There was 10 samples were collected from three provinces: Hanoi (HN01, HN02, HN03, HN04, HT01, HT02 and HT03), Thanh Hoa (TH01 and TH02), HCM city (SG01).

2.2. Sample storage and DNA extraction

For taxonomic identification, each collected EW was kept in 100% ethanol and stored in 4^0C. DNA was extracted by phenol-chloroform isolation method followed by ethanol precipitation (Palumbi and Cipriano, 1998) at Vietnam National University of Agriculture.

2.3. PCR protocol

PCR amplications were perfomed in an Axygen™ Maxygene™ Gradient Thermal Cycler (Axygen Scientific THERM1001, USA). A typical PCR protocol consisted of template DNA, 1µl; AmpliTag polymerase, 1.25U; primers concentration, 25pM with dNTP concentration of 200µM, $MgCl_2$ concentration of 2mM, and 5µl 10xPCR buffer. Sterile water was added for a total volume of 50µl. For most taxa, a 640bp fragment of the COI gene was amplified using the forward primers LCO1490 (5′-GGTCAACAAATCATAAAGA TATTGG-3′) and reverse primers HCO2198 (5′-TAAACTTCAGGGTGACCAAAAAAT CA-3′) (Folmer *et al*., 1994).

The reaction mixtures were denaturated to 94^0C for 3min, followed by 38 cycles at 94^0C for 30s, annealing at 52^0C for 45s and 1min at 72^0C, followed by a final elongation step of 10min at 72^0C and hold at 4^0C.

2.4. DNA sequence processing

The PCR products were sent to 1st Base Company (Singapore) for purification and sequencing. The DNA sequence were edited manually with Chromas Lite Software and subjected to Nucleotide Basic Local Alignment Search Tool (BLAST-N) in NCBI Genbank. Sample sequences and the sequence of same species (Table 1) was checked for similar. A neighbor-joining (NJ) tree was constructed using MEGA version 7.0 with Kimura 2-parameter model and the bootstrap values of the phylogenic tree were estimated with 10,000 repetitions (Kumar *et al*., 2016).

Table 1. COI gene reference in Genebank database

Species	Sequence accession reference
Perionyx sp3	HM219174.2
Perionyx sansibaricus	JX535190.1
Amynthas corticis	KF205966.1
Metaphire posthuma	KF205454.1
Amynthas	KF205398.1
Amynthas sp1	KT429010.1
Rhinodrilus alatus	HM535997.1
Eudrilus eugeniae	KX832073.1
Glossoscolecidae	GU014208.1
Amynthas amis	JX290397.1
Amythas phaselus	AB542519.1
Pheretima apoensis	LC127228.1
Pheretima sp1	LC268872.1
Pheretima sp2	LC268886.1
Pithemera	LC268861.1
Acanthodrilidae	GU014208.1
Pontodrilus litoralis	LC018724.1
Aporrectodea nocturna	JN850546.1

3. RESULTS AND DISCUSSION

3.1. DNA isolation and DNA qualification

DNA isolation of EW was extracted using protocol of Palumbi and Cipriano (1998). The DNA qualification was checked on the agarose gel 1.5%. The result showed that the production of DNA was quality, very clear and clean and it can be using for PCR reaction.

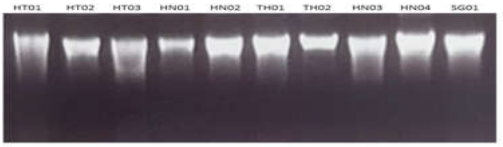

Figure 1. DNA electrophoresis on agarose gel

3.2. COI gene amplifcation

DNA products were used to amplify COI gene by using forward primers LCO1490 (5′-GGTCAACAAATCATAAAGATATTGG-3′) and reverse primers HCO2198 (5′-TAAACTTCAGGGTGACCAAAAAATCA-3′) measured by the ladder SiZer™-100bp. Examination of the COI gene on agarose 1.5% showed that the DNA length was 640bp.

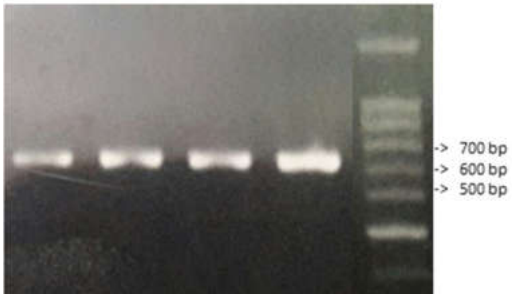

Figure 2. COI gene fragment length on agarose gel

3.3. DNA sequencing and genetic relationship between identified earthworm species

This was the result of DNA sequencing of COI gene which was 640bp in length. 5′-GGATCTCCACCCCCAGCTGGGTCGAAGAATGAAGTATTGAGATTACGATCTGTGAGAAGTATTGTGATTGCCCCCGCTAAGACTGGAAGTGATAGAAGAAGTAGCACTACAGTGATTACTACTGCTCATACAAATAGTGGAATTCGTTCTAATCGAAGACCGGATCATCGTATATTAATTACTGTTGTAATAAAGTTAATTGCCCCTAAAATAGATGATGCCCCTGCAAGATGAAGAGAGAAAATGGCTAGGTCTACTGAGGGCCCCGCATGAGCAAGATTTCTTGCAAGTGGTGGGTAAACAGTTCATCCTGTACCTGCACCCTTTTCAACTGCAGCTGACGAAACTAAGAGAATTAGTGAAGGAGGTAATAATCAAAATCTTAAATTATTTAGTCGGGGGAATGCTATGTCGGGCGCTCCCAGTATTAGTGGGAGTAATCAATTTCCAAATCCGCCAATAAAAACTGGCATTACAAGGAAAAAGATTATTAGAAAAGCATGAGCTGTAACGATAGTGTTATAGAGTTGGTCTCTTCCTAGAAAAGCACCCGGCTGTCTTAGTTCAATTCGAATAAGAAGCCTTATACCGGCACCGATTATACCAGCTCACACACCTAGAATAAAGTACAGTGTTCCAATGTCTTTATGNTTGGGTTGAACAAA-3′.

Submitting the DNA sequence to the Nucleotide Basic Local Alignment Search Tool in NCBI Genbank, the results suggested a strong correlation between the collected EW and the EW species published in the Database. The results was presented in the table 2.

The results of the Table 2 showed that four EW species have been identified from 10 samples of collected EW. These were *Eisenia andrei* (HN02, TH01, HN03), *Eudrilus eugeniae* (HT01, HT03, HN04), *Peryonix sansibaricus* (HN01, HT02, SG01), *Eisenia fetida* (TH02). The correllation of COI gene between COI sequenced gene and reference species was highest in *Eudrilus eugeniae* (97.34-100%) and lowest in *Eisenia andrei* (78.39-99.11%). The correllation of COI gene was high in *Peryonix sansibaricus* (93.42-94.46%) and *Eisenia fetida* (99.56%). There was no species of *Peryonix excavatus* that has been identified in those EW farms.

Table 2. Results of correlated analysis of COI gene with EW species in Genebank database

Samples	Species identification	Sequence accession reference	Correllation
HT01	African nightcrawler (*Eudrilus eugeniae*)	KX832073.1	100%
HT02	Earthworm (*Peryonix sansibaricus*))	JX535190.1	94,96%
HT03	African nightcrawler (*Eudrilus eugeniae*)	KX832073.1	99,52%
HN01	Earthworm (*Peryonix sansibaricus*)	JX535190.1	94,75%
HN02	Earthworm (*Eisenia andrei*)	LC006116.1	99,11%
TH01	Earthworm (*Eisenia andrei*)	LC006116.1	98,52%
TH02	Red worm (*Eisenia fetida*)	HQ224516.1	99,56%
HN03	Earthworm (*Eisenia andrei*)	LC006116.1	78,39%
HN04	African nightcrawler (*Eudrilus eugeniae*)	KX832073.1	97.89%
SG01	Earthworm (*Peryonix sansibaricus*)	JX535190.1	93,42%

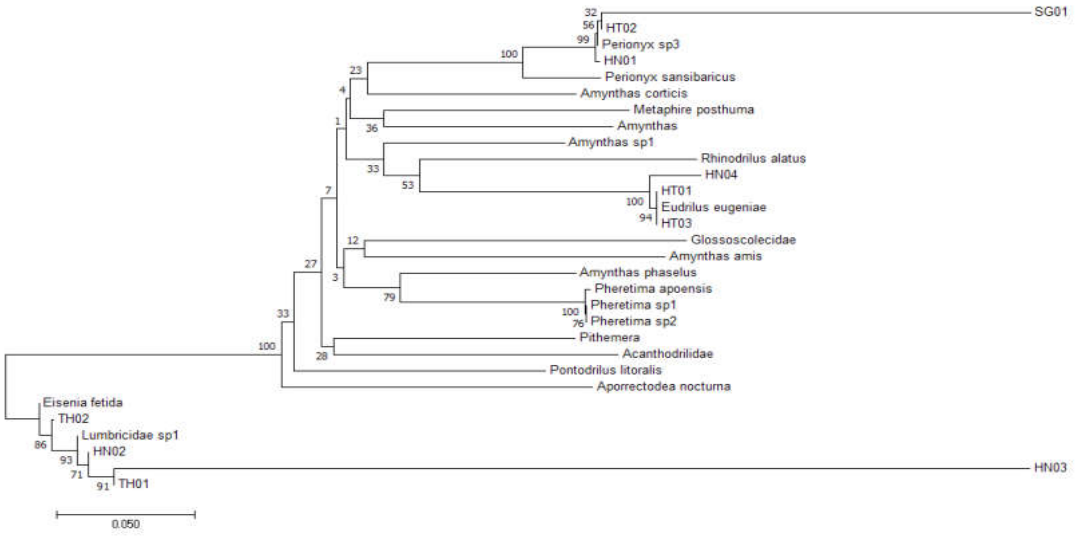

Figure 3. Phylogenic relationships of identified EW species

The result of genetic relationship between identified EW species was shown in the Figure 3. Using the Clustal X 2.1 Software and MEGA 7 with the bootstrap values from 1000 replicates, this study indicated that there was a correlation within one species.

Besides, the results also showed the genetic distinction of identified EW species. These findings will be helpful in analyzing genetic variation in EW.

4. CONCLUSION

Based on results of this study, we suggested that COI gene barcoding provided a powerful tool for species discrimination in EW. We were able successfully identify 10 individuals representing 4 species. This work could represent the first step toward a DNA barcode system for EW and an effective method. Future studies should examine larger sample sizes, increased taxonomic diversity. Therefore, this practical method allowed for identification of EW species with access to a basic DNA sequencing laboratory.

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FERTILITY AND MILK YIELD OF SAANEN, BACH THAO AND F_1(SAANEN X BACH THAO) GOATS

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ABSTRACT

An experimental evaluation on the growth and fertility of F_1 crossbred goats who were crossbred from purebred goats Bach Thao (BT) and Saanen (Sa), thereby conducting comparision on the milk yield of F_1(SaxBT). The experiment was arranged in a completely randomized design with 3 treatments on 3 breeds of BT, F_1(SaxBT) and Sa, six replications for each treatment. Results showed that the weight of F_1(SaxBT) goats at birth, 3, 6 and 9 months of age were 2.2, 13.1, 18.58 and 23.34kg, respectively. There was no difference in first farrowing age of 3 breeds (P>0.05). Farrowing interval of F_1(SaxBT) (330.9 days) was higher than BT and lower than Sa. The litter size of F_1(SaxBT) with 1.35 for litter one and 1.39 for litter two was lower than that of BT but higher than Sa. F_1(SaxBT) had a better improvement in milk yield with 2 kg/head/day, lactation period with 189 days for parity 1 and 209 days for parity 2, and total milk yield with 382kg for litter one and 430kg for litter two compared to BT at both parities. In addition, F_1(SaxBT) also showed better adaptation to hot and humid climates in Vietnam than the pureblood Sa.

Keywords: *Saanen×Bach Thao goat, litter size, growth performance, milk yield, lactation period.*

1. INTRODUCTION

Goats provide an important source of protein by converting various lower quality natural resources (Dubeuf *et al*., 2004). Goat farming is a major source of milk and meat products for people in rural areas. Goat milk plays an important role in nutrition and socio-economic wellbeing of developing and underdeveloping countries (Park and Haenlein, 2007).

The number of goats and their production in the world has increased sharply in recent years because of realizing the practical opportunities of goat production. While in Vietnam, raising goats has not attracted much attention leading to low milk production but in contrast to high demand for goat milk. Being aware of the above advantages, recently there has been a research project on breeding to improve performance of goat such as goat research of Son Tay Goat and Rabbit Centers in period 2000-2005. This research has identified that two high milk yielding hybrid goats are F_1(SA*BT) and SA*BT-Co (www.sanvatbavi.com.vn/detail.asp? id=523 and cate_id=18 & parent_id=16). Although this research has been achived advanced genetic in heterosis testing, there have been no assessments of fertility as well as the affected factors such as weather and lactation period of F_1(SA*BT) goat.

2. MATERIAL AND METHODOLOGY

2.1. Subbject, location and time

Pure Bach Thao (BT) and Saanen (Sa) goat were purchased in Ninh Thuan province and Son Tay Goat and Rabbit Center. The experiment was conducted at the Research and Technology Transfer Center, Nong Lam University of Ho Chi Minh City from Oct 2017 to Apr 2020.

2.2. Experimental design

2.2.1. Crossbreeding of pure Bach Thao and Saanen goat to create F_1(Sa×BT)

Selection of 30 female BT goats: body weight from 20-25 kg/head. A well-proportioned appearance such as a long neck, well-muscled in head-neck, the straight back, the straight forelegs and well proportioned, white marking on black coat, the downed ears. The breasts are attached to the abdomen firmly and neatly forward, the veins are prominent on the breasts, the nipples are big and long

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(4-6cm). The genitals are not misshapen and there are no reproductive diseases.

Selection of 2 male Sa goats based on the records of weight, short broad head, big ears, big neck, broad chest, and strong limbs. The testicular is big and well-proportioned, the circumference of the scrotum is about 25cm, body weight reaches 45kg.

F_1(Sa×BT) goats aged 8-10 months and the ratio of male and female of the herd is 1:15. Cross-formular: ♂pure Saanen x ♀pure Bach Thao –> F_1(SaxBT).

Table 1. Breeding calendar

Month	
1	Selcetion of breeding stock
2	Mating starts: Putting buck to females for 42 days
3	End of mating
4	- Pregnant
5	- Pregnant
6	Separate pregnant and nonpregnant
7	Vaccination
8	Kidding start
9	End of kidding
10	Care of kids
11	Vaccination for kids Weaning
12	Flushing of females

Sikosana and Senda (2011).

2.2.2. Comparison of milk yield of F_1(Sa×BT) with pure BT and Sa goats

The experiment was designed completely randomize with 3 treatments (T) and 6 replicates. In which, T1 is pureblood female Sa goat, T2 is pureblood female BT goat, T3 is F_1 crossbred goat that selected from crossbreeding experiment in Section 2.2.2. Goats in each treatment were equipvalent in terms of age, litter, weight and were fed in the same diet.

2.3. Rearing sytem, housing and feeding

Rearing system: Goats were reared under semi-intensive system in first 10 days, grazing 4-6 hours per day. After adaptation, goats were confined exclusively in sheds.

Housing: The housing of goat is designed in an open barn style. The height of floor is 0.8-1m from the ground. Space for raising goats with kids 0.2 m^2/head; weaned kids 0.3 m^2/head; gilts 0.6 m^2/head; aldult females 0.8 m^2/head.

Feeding: Kids from 1-3 months of age were crept feeding according to Table 2.

Table 2. Feeding kid goats (1-3 months of age)

Age (day)	Milk V,ml	Milk time/day	Concentrate (g/day)	Roughage
1-3	350	3	-	-
4-14	350	3	-	-
15-30	350	3	50	Creeping
31-60	400	2	100-150	*Ad libitum*
61-90	200	2	200-250	*Ad libitum*

Aldult, pregnant and lactating goat in the treatments were fed total mixed ratio diet (TMR), DM offer is in range 3-5% of body weight, based on reproductive performance and milk yield. The TMR diet was mixed according to Table 3.

Table 3. Chemical composition and ME in diet

Ingredient	%	DM (%)	CP (% in DM)	CF (%)	ME (kcal)
Concentrate	30	89	15.8	14.9	2,874
Brewery	34.8	27	25	16.0	2,080
Cassava pulp	11	89	1.98	13.6	2,763
Elephant grass	20	19	11.2	34.3	2,078
Urea	0.2	100	280	-	-
Molasses	1	70	4	-	11.7
Biochar	1	90	-	-	-
Premix vitamine	1	-	-	-	-
Premix minerals	1	-	-	-	-
Total, %	100				
Crude protein, g	15.9				
Crude fiber, g	21.8				
ME, kcal	2,288				
ME, MJ	9.6				

Note: ME= metabolism energy (National Institue of Animal Science, 2001)

Goats are vaccinated against pox disease, pasteurellosis and FMD twice a year.

2.4. Data collection

Weight of birth and Survival rates of birth at 3, 6 and 9 months of age.

Fertility: first farrowing age, farrowing intervals, number of kid per parities.

Average milk yield, average milk yield, lactation period.

Making a personal tracking sheet to record changes in weight gain, fertility, disease situation. Weight of newborn goat was recorded immediately after drying. For the weight of goats 3, 6 and 9 months of age, the goats were weighed in the morning before feeding.

Survival rate is percentage of surviving kid in each growth period from birth to 3 months of age, from 3 to 6 months of age and from 6 to 9 months of age.

The farrowing intervals (days) was calculated from the average number of days that pass between a farrowing and the next one. Number of newborn babies per parities is the number of live births in a parity within 24hrs.

Milk production was recorded during lactation period. Milk yield in the first 12 days was measured by weighing baby goats before and after breastfeeding. On day 13, the kid goat will be separated from the mother. Milk production would be collected twice daily at 8 and 15 hours. After milking, the young goats were breastfed to exploit all the mother's milk, weigh the kid before and after sucking to record this milk. Total milk production/cycle (kg) = number of days for milk/cycle (days) × milk yield/day.

2.5. Data analysis

The data was analyzed by using the General Linear Model option of the ANOVA program in Minitab 16.2 software. The standard deviation (SD) corrected for the experimental factors that would be used to assess the effect of factors on the parameters.

3. RESULTS AND DISCUSSION

3.1. Appearance, weight and survival rate of F_1(Sa×BT) goats

Assessment of appearance characteristics can show the health status of animal, it additionally shows the level of genetics that the animal was received from the parent generation, thereby showing productivity and growth. of animals. The male Saanen goat used in the experiment was characterized by short white coat, standing ears and horn, while the BT female goat had black coat, a white striped face, horns, and downed ears toward cheek. The result of F_1(Sa×BT) appearance was 61.1% the white color trait inheritance from the father, the remaining 38.9% had a white coat and some brown stripes, and most had horns.

Table 4. Live weight and survival rates in growth periods of F_1(SaxBT) goats

Age	Newborn (n=23)	3 months (n=20)	6 months (n=19)	9 months (n=19)
Live weight	2.20±0.4	13.11±1.34	18.58±1.61	23.34±1.66
Survival rate, %	-	87	95	100

The survival rate of F_1(SA*BT) goats was lowest in the period of birth to 3 months of age with 87%. Over each period, the survival rate increased gradually and was highest at 9 months of age with 100%. Most of the goats' deaths at this period are due to coccidiosis, leading to depression and further complication of pneumonia, mainly occurring on goat between 2 and 4 months of age.

3.2. The fertility of F_1(Sa×BT) and pure BT and Sa

There was no difference in age at first estrus among goat breeds, however, the farrowing interval of pureblood BT was shorter than that of pure Sa, whereeas F_1(Sa×BT) goats had a farrowing interval fall between BT and Sa. The gestation length of the goat breeds is relatively similar from 145 to 150 days, therefore, gestation interval between parities depends on the length of time after birth is long or short. One of the factors affecting parity intervals is the lactation period in each individual. The days of lactation period for BT was much shorter than Sa, while F_1 crossbred goats probably inherited the trait from their father (pure male Sa), hence their lactation period was also last longer than pure BT.

In our comparative experiment, pure BT goats gave a higher number of kid/parity with 1.65 compared to pure Sa and F_1(Sa×BT) goats. This value is higher than that recorded by Dau Van Hai (2006) on Bach Thao goat with 1.58, but lower than that recorded by Nguyen *et al*. (2012) was 1.7. F_1(Sa×BT) goats had the number of kid/parities tended to improve more than pureblood Sa, this improvement was significant in parities 1 and 2.

Table 5. Fertility parameters of pure Saanen, Bach Thao and F_1(Sa×BT) goats

Parameters		**Saanen** (n=6)	**Bach Thao** (n=6)	**F_1** (n=6)	P
Traits	Age at first farrowing, day	432±13.7	422±7.91	423±11.4	0.121
	Farrowing intervals, day	352^a±11.2	289^b±14.4	331^a±14.2	0.001
Parity	P1	1.26^a± 0.03	1.65^b± 0.07	1.35^a±0.09	0.002
	P2	1.29^a±0.22	1.63^b±0.05	1.39^a±0.08	0.001

Mean values in rows without common letter are different at P<0.05

3.3. Milk production of pure BT, Sa and F_1(Sa×BT) goats

Milk production is mostly influenced by a combination of factors such as genetic make-up in terms of the use of improved breeds selected for milk production, a favorable nutritional environment, and improved management. Therefore, the genetic make-up of dairy animals plays a major role in changes in milk yield and composition (Sammy *et al*., 2012). F_1(BT×Sa) goats in this study showed more genetic improvement compared to pureblood BT in terms of milk, days of lactation period and total milk yield/cycle. Milk yield of F_1(Sa×BT) goats achived averaged 2 kg/head/day, higher than that of pure BT at 1.19 kg/head/day but lower than that of pure Sa (2.2 kg/head/day). However, milk yield of Sa goats tended to decrease from 2.2 in pariry (P) 1 to 1.98 in P2, equivalent to that of F_1(Sa×BT) goats. The days of lactation period of the F_1(Sa×BT) goat in both parities was 189 and 209 days, respectively (P=0.79), longer than that of BT, only 149 days. Meanwhile, the lactation period of Sa was 209 days at P1 but tended to reduce to 191 days at P2, even lower than lactation period of F_1(Sa×BT) goats at P2. This led to total milk production of Sa net tended to reduce in parity 2 compared with parity 1, while total milk production of F_1(Sa×BT) goat had a significant improvement in P2 compared to P1.

Table 6. Milk yield and lactation period by parity

Parity	**Saanen** (n=6)	**BT** (n=6)	**F_1** (n=6)	**P** Breed	**P** BxP
Milk yield, kg/head/day					
P1	2.2^a±0.09	1.19^b±0.13	2.00^c±0.05	0.001	0.001
P2	1.98^a±0.09	1.21^b±0.13	2.1^a±0.11		
Lactation period, day					
P1	209^a±5.23	149^b±5.78	189^c±3.30	0.001	0.001
P2	191^a±2.92	149^b±5.75	209^c±2.00		
Total milk yield/cycle, kg					
P1	453^a±24.8	177^b±20.7	382^c±12.0	0.001	0.001
P2	378^a±21.2	179^b±19.5	430^a±20.5		

The experiment recorded the effect of weather on milk yield and lactation period among breeds. Milk yield in the dry season is higher than the rainy season in all breeds, however, the effect of the season on milk yield is quite evident on the Sa goats with a reduction in milk yield/head/day from 2.26kg in the dry season to 2.06kg in the rainy season. The second parity was in the rainy season, the hot and humid weather was not suitable for goat production and it affected the reduction of milk production. According to our observations, Sa purebred goat got more health problems such as cough, runny nose, eye pain ... than pure BT and F_1(Sa×BT) goat. As originally predicted, this suggests that F_1(Sa×BT) goats may have received genetic advantages from their mother (pure BT) to adapt to the experimental weather conditions.

Table 7. Seasonal effects on the lactation (n=20)

Traits	Season	Sa	BT	F_1	P_{breed}	P_{BxP}
Milk yield, kg	Dry	2.26	1.24	2.02	0.001	0.167
	Rainy	2.06	1.15	2.00		
	P			0.001		
Lactation period, kg/head	Dry	213	152	190	0.001	0.012
	Rainy	202	147	186		
	P			0.001		

Results showed the advantages of F_1(Sa×BT) on milk production and the ability to adapt to hot and humid weather in Vietnam. Milk production is a factor of genetic-environmental interaction. It is important to selectively balance both characteristics of production, e.g. milk yield and composition. Therefore, a comprehensive evaluation of the genetic improvement of F_1(Sa×BT) goats on milk production requires longer-term assessments, greater numbers of goats, and further analysis of milk composition.

4. CONCLUSIONS

The appearance of F_1(Sa×BT) goat has almost white color trait inheritance from the father, the remaining had a white coat and some brown stripes, and most had horns. The weight of F_1(Sa×BT) goats on growth periods has improved weigh gain compared to pureblood goats for newborns; 3, 6 and 9 months. F_1 crossbred goats had a better improvement in milk yield and lactation period compared to pureblood goats. Besides that, F_1(Sa×BT) goats showed the ability to adapt to hot and humid climates in Vietnam than the pure Sa goat.

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EFFECTS OF CURCUMIN AND CINNAMON POWDERS ON GROWTH PERFORMANCE, LAYING REPRODUCTION AND EGG QUALITY OF JAPANESE QUAIL

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ABSTRACT

Two experiments were conducted with the purpose of evaluating the effect of dietary supplementation of curcumin and cinnamon powders on growth performance, laying reproduction and egg quality of Japanese quails. The experiment 1: 240 fourteen days old quail chicks were assigned to four dietary treatments, each treatment consisted of six replications of 10 chicks per replicate. The experiment 2: a total of 36 laying Japanese quail hens at 36 days old which had been in production for 10 weeks was randomly divided into 4 dietary treatments with nine replicates (one female per each). The treatment diets were: (1) Control (Con) contained a basal diet without additive; (2) N0.1 contained the basal diet plus 0.1% curcumin powder; (3) Q250 contained the basal diet plus 250mg cinnamon powder/kg of feed; and (4) N0.1Q250 contained the basal diet plus 0.1% curcumin and 250mg cinnamon powder/kg of feed. Results showed that there were no effects of dietary supplementation with curcumin and cinnamon powders on FI, BW and FCR during the growing and laying periods. The supplementation of 0.1% curcumin and 250mg cinnamon powder/kg of feed resulted in significant effect on the improvement of ADG as compared to other supplemented diets. Egg laying rate, egg shell weight and yellowness (b*) score were significantly different among treatments, highest on control (89.21%, 1.26g and 48.61) and lowest on N0.1Q250 (80.93%, 1.11g and 43.56). It is possible suggested that supplementation of 1% turmeric or 0.025% cinnamon powders to improve the growth and reproductive performances of quails.

Keywords: *Japanese quail, curcumin, cinnamon, weight gain, laying rate, egg yolk color.*

1. INTRODUCTION

Using herbs and spices in animal nutrition as feed additives for improving the animal performance and health (Windisch *et al.*, 2006) as well as an alternative approach to replace the use of antibiotics, especially after the ban of antibiotic feed additives within EU (Greathead, 2003). The biological activities of herbs and spices have examined their beneficial impact as dietary supplements in nutrition (Christaki *et al.*, 2012). In poultry nutrition, regarding the use of aromatic plants as performance enhancers was reported (Botsoglou *et al.*, 2005).

Turmeric (*Curcuma longa* L.) is used as a food spice, colorant and medicine in the tropical regions. Besides, turmeric is a potent antioxidant (Osawa *et al.*, 1995), antifungal (Wuthi-udomler *et al.*, 2000), anti-inflammatory (Pal *et al.*, 2001), imunomodulatory (Antony *et al.*, 1999) and antimutagenic (Soni *et al.*, 1997) activities. According to Kumar *et al.* (2005), supplementation of turmeric at 1g/kg of diet increased in broiler chickens' WG. On quail, Rondonuwu *et al.* (2014) reported that, turmeric rhizome at 2% in quail diet can increase egg yolk color scores from 7-9.

Cinnamon (*Cinnamonum verum*) is a common spice in human nutrition which can be used as medicinal purposes and as feed additive in poultry. Cinnamaldehyde is the major bioactive compound of cinnamon, producing 65% of the extracted essential oil (Mountzouris *et al.*, 2009), which contribute to its different biological activities such as stitmulate immune system (Nofrarias *et al.*, 2006), antibacterial (Chang *et al.*, 2001),

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antioxidant (Singh *et al.*, 2007) and antifungal properties (Soliman and Badea, 2002).

In the study of Hasan *et al.* (2004), dietary cinnamon supplementation did not affect feed intake but affected effectively feed conversion ratio, health status (Al-Kassie, 2009). Park (2008) shown that broilers fed diet containing 3% cinnamon extract had higher BW than control, while another authors found that BW and FCR of broilers were improved on dietary inclusion of 2 g/kg cinnamon (Toghyani *et al.*, 2011). The feed intake were increased when diet combined cinnamon with other herbs (Al-Kassie, 2009), improved broilers growth performance and meat quality (Sang-Oh *et al.*, 2013), improved performance and feed efficiency (Al-Kassie, 2009).

Based on our previous researches indicated that the optimum concentration of cinnamon powder supplementation at 0.025% (Nguyen Thi Kim Khang *et al.*, 2015) or curcumin at 0.1% (Nguyen Thi Kim Khang *et al.*, 2016) to broiler diets increased feed intake, improved performance, feed efficiency and health status, increased breast meat yield as well as economic efficiency compared to other levels.

On the background above, therefore, the objective of this study was to further confirm the effect of dietary supplementation of curcumin and cinnamon powders on growth performance, laying reproduction and egg quality of Japanese quails.

2. MATERIALS AND METHODS

2.1. Animals and management

The experiment was conducted at the experimental farm located on Thuan An, Binh Minh, Vinh Long province. The one-day-old Japanese quail chicks originated from Tien Giang and Ben Tre provinces were used in this experiment, all birds were raised in the opened house system. Chicks were fed *ad-libitum* during the growing period, and restricted regime was applied at the laying period. Water was available for free access.

Curcumin product, a dried fine powder, yellow color and flavor, was purchased from Baongoc Limited Company, Thao Dien, Dist 2, HCMC. Cinnamon product, a dried fine powder, dark brown color with a strong aroma and slightly spicy and acrid taste, was obtained from Viet Pepper Ltd. Company, Binh Trung Tay, Dist 2, HCMC. The commercial feeds contained mainly corn, soybean meal, fish meal were formulated into three continuous phase feedings of 0-14, 15-35, 36-106 days of age to match the bird nutrient requirements. The CP diets contained 23, 20, 20% corresponding to ME 2,900; 2,850; 2,750 kcal/kg of each stage, respectively.

2.2. Experimental design and data collection

In the experiment 1: 240 one-day-old quails were assigned to four dietary treatments*, each treatment consisted of six replications of 10 chicks per replicate.

In the experiment 2: a total of 36 laying Japanese quail hens at 36 days old which had been in production for 10 weeks was randomly divided into 4 dietary treatments* with nine replicates, Each replicate of one hen was kept in a cage.

The treatment diets* were as followed: (1) Control (Con) contained a basal diet without additive; (2) C0.1 contained the basal diet plus 0.1% curcumin powder; (3) Q250 contained the basal diet plus 250mg cinnamon powder/kg of feed; and (4) C0.1Q250 contained the basal diet plus 0.1% curcumin and 250mg cinnamon powder/kg of feed.

Fresh feed was mixed weekly and not stored for more than one week. Feed and water were provided *ad libitum* consumption during the starter and grower phases. At 14 days old, 240 birds were housed in cages (10 birds per cage). The body weight were recorded at 21, 28 and 35 days old. Feed offered and feed refused were weighed daily for calculation

of feed intake and feed conversion ratio. The growth performance parameters such as weight gain, feed intake and feed conversion were determined according to the procedures of McDonald *et al.* (2011). The birds' health status, culling and mortality were also recorded daily.

At the 35 days of age, 36 female quail were randomly selected and housed in individual cage under similar environmental conditions. All birds had full access to feed and water. The photoperiod was 16 hours of light per day throughout the experimental period, which lasted for two and a half months. Records were kept for egg production, feed consumption, egg weight, average body weight change, and deadth of hen.

Daily feed consumption was recorded and calculated as g/day/hen, and FCR was calculated as the ratio of g of feed consumption per g of egg weight production. Measurements of egg quality were taken on average of 14 eggs from each treatment at 70 days of age. The egg internal and shell quality characteristics were determined. Each egg was weighed and their shape index and shell thickness were measured. The yolk height, albumen height, yolk width, albumen width and albumen length were determined to calculate the yolk index, albumen index according to Anderson *et al.* (2004) and Haugh unit (Haugh, 1937).

Using a Konica Minolta Chroma Meter CR-400 (aperture 8mm) (Konica Minolta, Tokyo, Japan) with Spectra Magic NX software to determine the color in the yolk. The instrument was calibrated using a Minolta calibration plate CR-A43 (Konica Minolta, Tokyo, Japan). The CIELAB coordinates (L*, a* and b*) were measured. The parameter a* takes positive values for reddish colors and negative values for greenish ones, whereas b* takes positive values for yellowish colors and negative values for bluish ones. L* is an approximate measurement of luminosity, which is the property according to which each color can be considered as equivalent to a member of the grey scale, between black and white (Pathare *et al*., 2013).

2.3. Statistical analysis

All recorded data were analyzed by using the GLM procedure of Minitab 16.0 and the Tukey test was used to compare mean differeneces among treatments at P<0.05.

3. RESULTS

3.1. Growth performance of Japanese quail chicks

Results showed that dietary treatments did not significantly affect feed intake, feed conversion ration and body weight of birds (P>0.05). However, the average daily weight gain was significant difference among treatments, highest on control and lowest on N0.1 (P<0.05).

Table 1. Growth performance of Japanese quail

Parameters	Treatments				SEM	P
	Con	N0.1	Q250	N0.1Q250		
BW_{14d}	54.27	54.14	53.80	54.59	0.10	0.95
BW_{21d}	90.65	91.21	88.85	89.03	1.25	0.47
BW_{28d}	117.05	124.88	117.59	118.74	3.35	0.35
BW_{35d}	159.8	156.2	149.7	155.2	3.10	0.17
ADG_{14-21}	6.06	6.18	5.84	5.74	0.20	0.40
ADG_{22-28}	3.77	4.81	4.11	4.24	0.39	0.33
ADG_{29-35}	4.75^{a}	3.48^{b}	3.56^{ab}	4.06^{ab}	0.33	0.04
FI_{14-21}	11.66	11.45	10.13	11.88	0.68	0.30
FI_{22-28}	16.27	15.10	15.28	14.88	0.90	0.71
FI_{29-35}	16.68	17.10	15.85	14.19	1.05	0.25
FCR_{14-21}	1.91	1.96	1.77	1.96	0.12	0.65
FCR_{22-28}	3.45	3.76	3.67	4.07	0.25	0.40
FCR_{29-35}	3.64	5.11	4.52	3.72	0.47	0.12

^{a,b} mean on the same row with different superscripts are significantly difference at P<0.05

3.2. Reproduction characteristics and egg paremeters of Japanese laying quail hens

Results on initial (Ini) and final (Fin) body weights did not differ among treatments (P>0.05). The weight gain of the quail hens in all treatments were no significant differences (P>0.05). Moreover, EW and FI at 36-70 and 36-106 days of age were not affected by dietary supplementation of turmeric and cinnamon

powders (P>0.05). At 71-106 and 36-106 days of age, addition of 0.1% turmeric or 250mg cinnamon powders/kg feed had significantly higher laying ER and lower FCR in comparison with the combination of turmeric and cinnamon powder treatments (P<0.05). Feed intake of the addition of cinnamon powder to the basal diet at 71-106 days of age led to a significantly highest compared the lowest on the addition of turmeric powder (P<0.05).

Table 2. Reproduction of Japanese laying quail

Parameters	Treatments				SEM	P
	Cont	N0.1	Q250	N0.1Q250		
IniBW, g	174.9	187.2	178.4	179.9	4.91	0.36
FinBW, g	199.2	209.7	203.7	201.9	4.04	0.32
WG, g	24.32	22.52	25.32	22.00	4.23	0.94
$ER_{36\text{-}70,\%}$	74.60	74.29	72.38	74.38	2.69	0.92
$ER_{71\text{-}106,\%}$	110.79^{a}	105.08^{a}	105.08^{a}	93.33^{b}	2.94	0.01
$ER_{36\text{-}106,\%}$	92.70^{a}	89.68^{ab}	88.73^{ab}	83.97^{b}	2.08	0.04
$EW_{36\text{-}70,g}$	10.08	9.93	9.73	9.83	0.15	0.41
$EW_{71\text{-}106,g}$	11.26	11.09	11.08	10.88	0.15	0.62
$EW_{36\text{-}106,g}$	10.79	10.62	10.53	10.42	0.16	0.37
$FCR_{36\text{-}70,kg}$	2.87	2.94	3.13	3.09	0.15	0.55
$FCR_{70\text{-}106,kg}$	2.12^{b}	2.21^{b}	2.38^{ab}	2.69^{a}	0.15	0.01
$FCR_{36\text{-}106,kg}$	2.36^{b}	2.47^{b}	2.64^{ab}	2.85^{a}	0.08	0.01
$FI_{36\text{-}70,g/day}$	21.21	21.32	21.80	22.47	0.62	0.48
$FI_{70\text{-}106,g/day}$	25.99^{ab}	25.59^{b}	27.64^{a}	27.05^{ab}	0.62	0.03
$FI_{36\text{-}106,g/day}$	23.60	23.46	24.72	24.76	0.43	0.07

Table 3. Egg quality of Japanese laying quail

Parameter		Treatments				SEM	P
		Cont	N0.1	Q250	N0.1Q250		
EW, g		10.87	10.59	10.47	10.46	0.23	0.56
AW, g		6.13	5.89	6.02	5.97	0.60	0.74
YW, g		3.48	3.50	3.30	3.38	0.10	0.31
ShW, g		1.26^{a}	1.21^{ab}	1.19^{ab}	1.11^{b}	0.03	0.03
ShT,mm		0.20	0.19	0.19	0.19	0.00	0.56
YR, %		31.96	33.08	31.25	32.22	0.60	0.21
AR, %		56.42	55.41	57.39	57.15	0.69	0.19
ShR, %		11.62	11.51	11.36	10.63	0.32	0.13
SI		0.80	0.78	0.79	0.79	0.01	0.29
AI		0.12	0.09	0.10	0.10	0.02	0.75
YI		0.35	0.33	0.35	0.34	0.60	0.46
Yolk color	L*	53.23	53.47	52.93	53.10	1.01	0.99
	a*	-1.84	-2.02	-1.51	-1.70	0.23	0.44
	b*	48.61^{a}	46.81^{ab}	46.47^{ab}	43.56^{b}	1.16	0.03
HU		86.55	84.95	86.84	88.36	2.69	0.85

The addition of turmeric or cinnamon powders alone or mixture in the diet of laying hens had no significant effect (P>0.05) on the values of internal and external egg quality characteristics (Table 3), except the egg shell weight (SW) and yellowness (b*) score (P<0.05) with highest values on control (1.26g and 48.61), N0.1 (1.21g and 46.81), Q250 (1.19g and 46.47) and lowest on N0.1Q250 (1.11g and 43.56).

3. DISCUSSION

The addition of turmeric or cinnamon or their mixture to diets was actually hoped to be able to enhance the metabolism (Al-Sultan and Gameel, 2004) that later on could improve feed efficiency of broilers (Nguyen Thi Kim Khang *et al.*, 2015; 2016). However, the present finding showed that feed intake, body weight and egg weight of quails did not affect significantly. In agreement with the present results, Cabuk *et al.* (2006) reported that no differences between control and an essential oil mixtures of oregano oil, laurel leaf oil, sage leaf oil, myrtle leaf oil, fennel seed oil and citrus peel oil. Similar findings reported by Demir *et al.* (2003) with oregano and thyme powder, and Lee *et al.* (2004) with carvacrol and/or cinnamaldehyde. Conversely, Toghyani *et al.* (2011) and Nguyen Thi Kim Khang *et al.* (2015, 2016) who reported that turmeric improved feed efficiency of broilers at starter and finisher phases. Baghban *et al.* (2016) indicated that Ross 308 broiler birds treated with heat stress (32^{o}C) and fed 0.5% turmeric, 0.5% cinnamon, and a blend of cinnamon and turmeric (0.25% each) increased feed intake and body weight gain of broiler chickens due to reduce lipid peroxidation.

In both experiments of this study, supplementing the basal diet with turmeric, cinnamon and mixture did not significantly increased laying egg rate and improved FCR in comparison with the control. However, laying egg rate (ER) at 71-106 and 36-106 days old of quails were similarly higher among N0.1, Q250 and control treatment groups compared N0.1Q250 whereas their FCRs were lower. These findings are in agreement with

those of Bozkurt *et al.* (2012)who indicated that a diet supplemented with essential oil mixture had no beneficial effects on egg production or FCR in laying hens. Bolukbasi *et al.* (2007) who reported that dietary supplementation with mixed essential oils and thyme oil to a layer diet had no significant effects on FCR. Egg weight did not differ between the different treatment groups. The present findings are in agreement with those of Cabuk *et al.* (2006), Bozkurt *et al.* (2012) and Cabuk *et al.* (2014) who indicated that a diet supplemented with essential oil mixture had no beneficial effect on egg weight. In contrast to these results, Bolukbasi *et al.* (2008) observed that the addition of essential oils to a layer diet increased egg weight. Egg quality characteristics, including HU, shell thickness, albumen weight, yolk weight, albumen index, yolk index, L* and a* yolk color were not significantly affected by supplementing the diet with turmeric, cinnamon and their mixtures in laying quails. These results are in agreement with those of Bozkurt *et al.* (2012) and Cabuk *et al.* (2014) who examined the effects of EOM on shell thickness (ShT), and albumen index (AI), eventhough HU, yolk weight (YW), AI, and yolk index (YI) (Cabuk *et al.*, 2014). The lowest ShW and b* yolk were observed with N0.1Q250 supplementation. These results were in disagreement with Cabuk *et al.* (2014).

4. CONCLUSION

The study revealed that N0.1 or Q250 dietary supplementation may improve the growth and reproductive traits of quails.

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EFFECTS OF SUPPLEMENTAL DIETARY DRIED ORANGE PEEL POWDER ON PERFORMANCE OF CROSSBRED NOI PULLETS

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ABSTRACT

The effects of supplemental dietary of dried orange peel (DOP) powder on reproductive performance and egg quality traits of crossbred Noi pullets were evaluated, the trial was conducted on a total of 84 crossbred Noi pullets to randomly allocate to 4 dietary treatments in 7 replicates of 3 pulllets each. The formulated basal diet was supplemented with four levels of DOP (0, 0.5, 1.0 and 1.5 g/kg) namedly DC, C0.5, C1.0 and C1.5, respectively. Results showed that the age at point egg production of DC, C0.5, C1.0 and C1.5 was 144, 149, 150 and 154 days, respectively. The age of 50% egg production was earlier in C0.5 (157 days) than in C1.5 and DC (167 days) or C1.0 (181 days). Besides, hen-day egg production and egg yield in C0.5 (46.94% and 41.29 eggs/hen) had a higher tendency (P>0.05) than in DC (42.86% and 40.43 eggs/hen), C1.5 (38.44% and 37.86 eggs/hen) and C1.0 (34.69% and 23.14 eggs/hen). No significant differences were detected among treatments on egg quality parameters except for yolk color a* (P<0.05) with the highest in C1 (10.3) among other treatments. It can be concluded that additional DOP did not have significant effects on egg yield and egg quality of the crossbred laying Noi pullets in the experiment, dietary supplementation of 0.5g DOP/kg feed may improve the laying performance of hens.

Keywords: *Dried orange peel, laying Noi pullet, yolk color, egg production.*

1. INTRODUCTION

Feed additives as herbal plants and plant derived products are used as alternative antibiotics in animal nutrition for improving the animal performance and health (Toghyani *et al*., 2010). Based on their biological and medicinal properties are illustrated, some researchers have studied to find out the positive effects of the herbs and their essential oils as their additional forms, or their combinations in poultry diets with expected effects on improvement the performance and health of poultry.

Sweet orange (*Citrus sinensis*) is one of the most important and oldest horticulture products in many tropical and sub-tropical areas. Orange peels are a primary by-product produced by the fruit processing industry, so many researches were studied to utilize dried SOP as natural feed additive, especially as medicinal supplement for animals (Callaway *et al*., 2008). Chemically, sweet oranges are valuable source of vitamin C as well as energy (Hasin *et al*., 2006; Yang *et al*., 2011). Besides, sweet orange peel contains 2,440-2,890 kcal/kg, 7.44-10% crude protein and high concentrations of carotene (Oluremi *et al*., 2006) as well as phenol (Manthey, 2004).

Some studies have found that sweet orange rind can be used in broiler up to 15% level without any adverse effect on growth performance (Oluremi *et al*., 2006). Mourao *et al*. (2008) reported that adding citrus pulp reduced daily gain when birds fed 10% citrus pulp compared with the control diet; conversely, feed intake increased in broilers fed with 5 or 10% of citrus pulp, which resulted in higher feed efficiency in birds fed diets contained 10% of citrus pulp. It was previously revealed that BWG, FI were enhanced in broiler chicks fed diets contained 1or 2% of SOP (Abbasi *et al*., 2015), who found that sweat orange peel with different levels from 0.5 to 2% as feed additives of broiler chickens had no effects on glucose, cholesterol, and liver enzymes. Abbas *et al*. (2013) reported

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that dried SOP in the proportion of 1.5% of feed seems to promote feed intake and weight gain in the period between the 1-21 days of age, indicating that DSOP can constitute a useful additive in the feeding of broilers. Recently study of our group shown that supplementation of 150-250mg *Pericrpium citri* Reticlatae/kg feed improved growth performance of Cobb500 broilers (Nguyen Thi Kim Khang *et al*., 2019).

Therefore, the objective of this study was to evaluate the effect of different dietary levels of dried orange peel powder supplementation on reproduction performance and egg yolk quality of crossbred Noi pullets.

2. MATERIALS AND METHODS

2.1. Animals and management

The experiment was conducted at an Experimental Poultry Farm of Department of Animal Sciences located on Thuan An commune, Binh Minh district, Vinh Long province, Vietnam.

The crossbred Noi pullets started at 16 weeks old and ended at 28 weeks old were used in this experiment, all pullets were similar body weight and raised in the opened house system and were vaccinated all common diseases followed the vaccination procedure guide. The poultry house was oriented east to north and contained conventional battery cages arranged in a three-tier design, namely bottom, middle and top. The dimensions of each cage were 60x40x40cm. At 15 weeks of age, the pullets were randomly allocated to the treatments, the three tiers, with seven replicates of three hens per treatment. During the experimental period, birds were freely accessed for drinking water. The lighting was constantly provided to 16h until the end of the experiments

Sweet orange powders were purchased from Mai Tran Gia Ltd. Company located in 2A/1 Nguyen Thi Minh Khai, DaKao str., District 1, HCM city. The commercial feeds contained mainly corn, soybean meal, fish meal were formulated to match the bird nutrient requirements. The crude protein diet contained 17 corresponding to ME 2,700 kcal/kg.

2.2. Experimental design and data collection

A total of 84 crossbred Noi pullets to randomly allocate into 4 dietary treatments and seven replicates with 3 pullet chickens per each. The control fed only a basic diet and other different levels of DOP (0.5; 1.0 and 1.5 g/kg feed) named C0.5, C1.0 and C1.5, respectively.

The body weight of birds were recorded at 16, 20, 24 and 26 weeks old. Feed offered and feed refused were weighed daily for calculation of feed intake and feed conversion ratio. The growth performance parameters such as weight gain, feed intake and feed conversion were determined according to the procedures of McDonald *et al.* (2011). The birds' health status, culling and mortality were also recorded daily. Eggs from each replicate were collected twice a day, at 09:00 and 15:00, and weighed with an electronic balance at the same time every day to calculate hen-day egg production (HDEP, egg/hen), percentage hen-day egg production (HDP, %), and egg weight (EW, g) from 16 to 28 weeks of age (North, 1984).

The eggs were analysed for interior and exterior quality traits, weight, shape index, shell weight, thickness, albumen index, yolk index and Haugh unit (HU) score (Bui Huu Doan *et al*., 2011). Yolk color was measured by using a Konica Minolta Chroma Meter CR-410 which allows determination of L* (lightness), a* (redness) and b* (yellowness). These parameters were performed two times and the final values were calculated as the averages of the two corresponding values measured.

2.3. Statistical analysis

All recorded data were analyzed by using the GLM procedure of Minitab version 16.0, the Tukey test was used to compare mean differences among treatments at $P<0.05$.

3. RESULTS AND DISCUSSION

3.1. Growth paremeters of the crossbred Noi pullet chickens

The effect of DOP on body weight of the crossbred Noi pullets at 16, 20, 24 and 26 weeks old were not significant differences among treatments (Table 1). Similarly, there were no significant differently among treatments on daily feed intake (DFI), ADG and FCR (P>0.05). No culled birds were found over the experimental period, however the overall mortality of the experimental birds was less than 5%, except C1.0 had higher mortality (9.5%). The age of 5% egg production of the crossbred Noi pullets varied between 144-154 days, and 157-181 days at 50% egg production with C0.5 was being 157 days earlier than C1.5 and control (167 days) or C1.0 (181 days). The reports of Nguyen Van Quyen and Vo Van Son (2008) shown that the first age at 5% laying yield for Noi hens was 219.1 days, while Chau Thanh Vu (2018) reported that it was at 164-177.9 days. The difference between this research result with the above reports may be due to nutrient diet, body weight,… or may be due to the addition of DOP considered as phyto-estrogen (Mazur *et al.*, 1998), however the present study did not explain the reason why C1.5 and C1.0 had delayed the laying age at 5 and 50% egg production compared to control. The results of this study indicated that DOP supplementations had no effect on body weight, daily feed intake, average weight gain and feed conversion ratio.

Table 1. Body weight, age of yield and mortality of crossbred Noi pullets

Parameters		Treatments				SEM	P
		Control	C0.5	C1.0	C1.5		
Body weight (g)	16 wks old, g	1,042.9	1,042.9	1,032.9	1,041.9	11.4	0.91
	20 wks old, g	1,369.5	1,328.6	1,359.1	1,321.0	28.8	0.58
	24 wks old, g	1,468.6	1,423.8	1,364.8	1,309.5	66.5	0.37
	26 wks old, g	1,491.4	1,414.3	1,451.0	1,501.9	77.1	0.85
	DFI, g/bird/day	57.7	58.5	58.2	58.2	2.11	1.00
	ADG, g/bird/day	12.1	10.6	12.1	10.3	1.11	0.54
	FCR	5.3	5.6	4.9	6.1	0.60	0.60
Age of yield (days)	5%	144	149	150	154	-	-
	50%	167	157	181	167	-	-
	Mortality, %	4.8	4.8	9.5	4.8	-	-

The performance, yield and egg quality traits of crossbred Noi pullets are shown in Table 2. C1.0 and C1.5 had lower FCR, HHP (%) and HEP (egg/hen) than C0.5 and control, however there were no significantly different among treatments (P>0.05). The addition of DOP treatments were tendency higher egg weights (EW) than control (P>0.05). Previous studies have reported the egg production of Noi pullets after 12 months old was 94.5 eggs/hen (Chau Thanh Vu, 2018) or the laying rate of Noi laying hens after 28 weeks old was 0.35-5.34% (Nguyen Van Quyen and Vo Van Son, 2008).

In overall, pullets in C1.0 and C1.5 performed worse for most of the relevant reproduction performance traits, indicating that the addition of dried orange peels in a diet could due to in a dose-depended way. The results of this study indicated that DOP supplementations had no effect on feed conversion ratio, hen house egg production, total hen egg production and egg weight. The use of different citrus wastes as feed ingredients have reported by Oluremi *et al.* (2006) and Agu *et al.* (2010) replaced up to 15-20% of the corn content in broiler diets with dried *C. sinensis* peel and found no

negative effects on growth performances of broiler chickens. The results of Mourao *et al.* (2008) shown that adding of citrus pulp in chicken diet resulted in higher feed efficiency compared to birds fed diet including up to 10% of citrus pulp. The finding of this study partly agreed with that of Ly Huu Phuong (2016) who stated that the effect of different levels of tangerine peel powders on the body weight, feed intake, egg reproduction and egg weight of Hisex Brown laying hens at 22-28 weeks oldwas not significantly different from the control. In contrast, Ebrahimi *et al.* (2013) and Ani *et al.* (2015) reported that the sweet orange peel levels of 3-5% as a feed additive in broiler chickens had negative effects on growth performances because of attributing to high crude fiber and pectin presented in the sweet orange peel which may have a deteriorating effect on gastrointestinal tract through increasing viscosity within intestinal tract which lower the digestive enzymes (Serena *et al.*, 2007).

No significant effects of DOP supplementations on shape index, albumen and yolk index, albumen, yolk and shell percentages, eggshell thickness and Haugh unit score were found (P>0.05), except for yolk color *a** varied significantly highest on C1.0 (10.3) and lowest on C0.5 (3.96) (P<0.05). The color score indicates that the use of sweet orange peel in the diet improved yolk coloration. Hasin *et al.* (2006) explained that this was because total xanthophyll consumption in the orange skin group was higher than that of control diet. However, the yolk color a* was significantly decreased in the C0.5 supplementation compared to control, the reason why the supplemented 0.5g orange peel/kg feed prevented the pigmenting ability of egg yolk is unknown.

Table 2. Performance, yield and egg quality traits of crossbred Noi pullets

Traits	Parameters	Treatments				SEM	P
		Control	C0.5	C1.0	C1.5		
Egg production	FCR20-24wks	2.18	2.17	2.07	1.97	0.12	0.56
	FCR24-26wks	2.28	2.23	1.94	1.98	0.09	0.053
	HHP20-24wks, %	24.15	25.00	12.33	26.19	5.59	0.29
	HHP24-26wks, %	42.86	46.94	34.69	38.44	4.55	0.28
	Total HEP, egg/hen	40.43	41.29	23.14	37.86	6.22	0.16
	EW20-24, g	32.32	34.14	33.14	33.20	1.40	0.84
	EW24-26, g	35.93	37.00	37.52	36.81	0.88	0.64
Egg quality	EW, g	33.06	31.84	35.86	32.59	1.43	0.29
	Shape index	1.45	1.31	1.39	1.35	0.10	0.80
	Albumen index	0.09	0.08	0.06	0.08	0.01	0.23
	Yolk index	0.39	0.46	0.38	0.39	0.03	0.39
	L*	51.0	54.8	51.6	53.2	1.29	0.23
	a*	6.82^{ab}	3.96^{b}	10.30^{a}	5.74^{ab}	1.18	0.03
	b*	46.01	41.37	34.78	48.18	4.05	0.17
	Shell thickness, mm	0.33	0.35	0.33	0.32	0.01	0.60
	Albumen, %	46.00	41.37	34.78	48.18	4.05	0.26
	Yolk, %	28.20	27.93	32.72	29.51	1.71	0.26
	Albumen:Yolk ratio	2.12	2.13	1.71	2.00	0.17	0.34
	Eggshell, %	12.16	12.78	13.10	11.71	0.65	0.48
	Haugh unit	85.12	82.03	71.73	80.20	3.16	0.08

3. CONCLUSION

The study revealed that dietary supplementation of 0.5g DOP/kg feed may improve the laying performance of the crossbred laying Noi pullets.

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EFFECTS OF SUPPLEMENTAL DIETARY LEMON GRASS AND CINNAMON POWDERS ON GROWTH PERFORMANCE AND CARCASS TRAITS OF COBB500 BROILER CHICKENS

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ABSTRACT

This study was done to evaluate the effect of dietary supplementation of lemon grass and cinnamon powder on growth performance and carcass traits of Cobb500 broilers. A total of 360 Cobb500 broiler chicks at 14 days old was completely randomized design into 4 dietary treatments and three replicates with 30 chicks per each. The experimental diets were as followed as: control fed a basic diet without any herbal supplementation; S10 fed the basal diet plus 10g LGP/kg of feed; S10Q150 and S10Q250 fed the basal diet plus 10g LGP plus 150 or 250mg CP/kg of feed. Results showed that S10Q150 tendency reduced the infectious coccidiosis disease of broiler chicks compared to control and others. Moreover, S10Q150 was significantly highest BW and ADG whereas lowest values were on control. Slaughtered weight, carcass weight, dressing percentage, breast and thigh weights, breast and thigh meat weight as well as drip loss of breast meat recorded at 36 and 48h after slaughtered of dietary supplementations were improved significantly compared to control. It is possible suggested that supplementation of 1% LGP plus together with 0.015 or 0.025% CP to improve the growth performance and carcass traits of broiler chickens.

Keywords: *Broiler, carcass, cinnamon, lemon grass, performance*

1. INTRODUCTION

Herbal plants and plant derrived products are used as feed additives in animal nutrition for improving the animal performance and health (Toghyani *et al*., 2010). They are suggested potentially as an alternative approach to replace antibiotic growth promoters in animal production. With respect of their biological and medicinal properties are illustrated, some researchers have studied to find out the positive effects of the herbs and their essential oils as their additional forms, or their combinations in poultry diets with expected effects on improvement the performance and health of poultry. However, these findings have given different results in the literatures and therefore more studies need to be clearly proven.

It has been reported that Lemon grass (*Cymbopogon citratus*) acts as anti-inflammation, antioxidant, antibacterial, antiobesity, antihypertensive, anxiolytic, anti-nociceptive, anti-ungiantimicrobial (Shah *et al*., 2011). Recent studies have revealed that broiler chickens fed diets supplemented Lemon grass (LG) leaf meal or oil improved significantly broilers body weight by increasing in feed taste, palatability lead to enhance faster passage and digestion of nutrients (Mukhtar *et al*., 2012).

Cinnamon (*Cinnamon verum*), a common spice in human nutrition which can be used as medicinal purposes and as feed additive in poultry. Cinnamaldehyde is the major bioactive compound of cinnamon, producing 65% of the extracted essential oil (Mountzouris *et al*., 2009), which contribute to its different biological activities such as stitmulate immune system (Nofrarias *et al*., 2006), antibacterial (Chang *et al*., 2001), and antioxidant (Singh *et al*., 2007) properties.

The added cinnamon powder (CP), cinnamaldehyde alone or mixture of other

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herbs or their essential oils have showed the beneficial effects in poultry. Park (2008) reported that broilers fed diet containing 3% CP extract had higher BW than control, while another author found that BW and FCR of broilers were improved on dietary inclusion of 2 g/kg CP (Toghyani *et al*., 2011). The feed intake were increased when diet combined cinnamon with other herbs (Al-Kassie, 2009), improved broilers growth performance and meat quality (Sang-Oh *et al*., 2013), improved performance and feed efficiency (Al-Kassie, 2009); increased pancreatic and intestinal lipase activity (Kim *et al*., 2010), improved in health status (Al-Kassie, 2009).

This study was aimed to evaluate the effect of dietary supplementation of lemon grass and cinnamon powder on growth performance and carcass traits of broiler chicks.

2. MATERIALS AND METHODS

2.1. Animals and management

The experiment was conducted at the broiler farm located on Thuy Thuan hamlet, An Phuoc commune, Mang Thit district, Vinh Long province, Vietnam.

The 14 days old Cobb500 broiler chicks were used in this experiment, all birds were raised in the closed house system and were vaccinated all common diseases followed the vaccination procedure guide by Cobb-Vantress (2013). During the experimental period, birds were fed ad-libitum and freely accessed for drinking water.

Lemon grass and cinnamon powder were purchased from Vietanco Vietnam Spice, Vianco Joint Venture, Ho Chi Minh city, Vietnam. The commercial feeds contained mainly corn, soybean meal, fish meal were formulated into four continuous phase feedings of 0-7, 8-21, 22-35 and 36-42 days of age to match the bird nutrient requirements. The CP diets contained 22, 20, 19, 18% corresponding to ME 2,900; 3,000; 3,100; 3,100 kcal/kg of each stage, respectively.

2.2. Experimental design and data collection

A total of 360 Cobb 500 broiler chicks at 14 days old were randomly allocated into 4 dietary treatments with 3 replications of 30 chicks per each replicate. The experimental diets were followed as control fed a basal diet without any herbal supplement; S10 fed the control diet plus 10g lemon grass powder (LGP)/kg of feed; S10Q150 fed the control diet plus 10g LGP and 150mg cinnamon powder (CP)/kg of feed; and S10Q250 fed the control diet plus 10g LGP and 250mg CP/kg of feed.

The body weight of birds were recorded at 14, 21, 28, 35 and 40 days old. Feed offered and feed refused were weighed daily for calculation of feed intake and feed conversion ratio. The growth performance parameters such as weight gain, feed intake and feed conversion were determined according to the procedures of McDonald *et al.* (2011). The birds' health status, culling and mortality were also recorded daily. At the 40 days of age, four female and male chickens per treatment were randomly selected and slaughtered to evaluate their carcass parameters. These chickens were only supllied water within 24hrs prior slaughter. The body parts and internal organs were calculated as percentage of carcass weight, and only breast meat were taken into plastic bags and stored by hanging in refrigurator at 4^0C to measure the driploss at 36 and 48hrs after slaughter.

The growth performance parameters such as weight gain, feed intake and feed conversion were determined according to the procedures of McDonald *et al.* (2011). The health and mortality of the experimental birds were recorded throughout the experimental period.

2.3. Statistical analysis

All recorded data were analyzed by using the GLM procedure of Minitab version 16.0, the Tukey test was used to compare mean differeneces among treatments at $P<0.05$.

3. RESULTS AND DISCUSSION

3.1. Health status and mortality of the experimental birds

Broiler chicks fed diets containing LGP with CP had tendency lower coccidiosis infection and chronic respiratory disease compared to control, especially on S10Q150. Recording time of 50% birds exposed the outward signs of both coccidiosis and CRD on control have found on day 17th and 26th of bird age while the supplemented diets were recorded lately at days of 19-21 and 32-34, respectively. Besides, chicks fed LGP with or without CP supplemental diets were low mortality and culling rates compared to control. The results indicate that supplementation of LGP and CP to diet have great impact on the health status of birds. This may be because of the bioactive compounds identified in these herbs as flavonoids, lococha cone A and B (Abd-El *et al*., 2010), contributed to the antioxidant (Singh *et al*., 2007; Abd-El *et al*., 2010; Abeysekera *et al*., 2013), antimicrobial and antifungal properties (Chang *et al*., 2001; Sang-Oh *et al*., 2013; Singh *et al*., 2014). Recently, cinnamaldehyde has been found to be effectively in reducing oocyst output from *E.acervulina* infection and increasing *E. tenella* stimulated parasite antibody response when supplemented in the chicken diets at 14.4 mg/kg (*E. maxima* or *E. tenella*) or 125 mg/kg (*E. acervulina*) (Lee *et al*., 2011).

Table 1. Health status and death of broiler chicks

Parameters	Con	S10	S10Q150	S10Q250
Coccidiosis inf,%	46.7	50	22.2	36.7
Bird age, day	17	19	21	19
CRD, %	35.5	26.7	20	21.1
Bird age, day	26	32	34	32
Mortality rate, %	2.2	1.1	1.1	1.1
Culling rate, %	2.2	1.1	0	0

3.2. Growth performance and carcass paremeters of the experimental birds

The effect of LGP and CP on growth performance of broiler chicks (Table 2) showed that supplemented LGP with 0.015% CP significantly improved the final body weight and weight gain of broilers by 8.7-10.8% compared to control. No significant differences among treatments were found on DFI and FCR. The improvement of FBW and ADG on S10Q150 may be due to the bioactive compounds in both herbs by digestion stimulating, leading greater efficiency in the utilization of feed, resulting in growth improvement. The present results are in agreement with of Al-Kassie (2009), who found that chicks fed with 200ppm essential oil derived from thyme and cinnamon had the positive effect on the live weight gain and improvement of the health of broiler chickens. Contrary, Hernandez *et al.* (2004), who reported that broilers fed the mixture of 200ppm of oregano, cinnamon and pepper essential oils in broilers diets had no effect on their performance at 21 and 42 days of age.

Table 2. Growth performance of Cobb 500 broiler chicks

Parameters	Treatments				SEM	P
	Control	S10	S10Q150	S10Q250		
Initial weight, g	448.8	445.5	447.7	448.8	4.94	0.96
Final weight, g	2,354^{b}	2,373.3^{b}	2,559^{a}	2,482.3^{ab}	36.96	0.01
ADG, g/bird/day	70.56^{b}	71.40^{b}	78.20^{a}	75.31^{ab}	1.28	0.01
DFI, g/bird/day	111.6	111.5	111.0	102.5	3.95	0.34
FCR	1.58	1.56	1.42	1.36	0.07	0.13

Note: Mean on the same row with different superscripts are significantly difference at P<0.05;

The effects of dietary supplementation of lemon grass and cinnamon powder on carcass traits and breast pH of Cobb500 broilers are shown in Table 3. There were significantly different among treatments on slaughtered weight, carcass weight, dressing percentage, breast and thigh weights, breast and thigh meat weight, highest values on S10Q150 and lowest on control. Moreover, drip loss of breast meat at 36 and 48 hours after slaughtered were improved significantly on S10Q150 compared to control. In contrast to these results, Sadeghi *et al*. (2012) reported that the mixture of cinnamon, thyme and turmeric significantly reduced carcass weight. Consistent with present results, broiler chicks fed different levels of oil extract derived from thyme and cinnamon had significant effects on dressing percentage, abdominal fat, and internal organs percentages (Al-Kassie, 2009).

Table 3. Carcass characteristics and breast pH of Cobb500 broiler chicks

Parameters	Treatments				SEM	P
	Control	S10	S10Q150	S10Q250		
BW, g	2280^{c}	2360^{c}	2597.5^{a}	24800^{b}	23.40	0.00
BW after 24h, g	2190^{d}	2300^{c}	2525^{a}	2425^{b}	23.14	0.00
BW loss, %	3.94^{a}	2.53^{b}	2.82^{ab}	2.20^{b}	0.34	0.01
Carcass yield, %	70.30^{b}	73.28^{a}	73.60^{a}	72.70^{a}	0.36	0.00
Liver, %	3.69^{a}	3.46^{ab}	3.15^{b}	3.24^{b}	0.09	0.01
Abdominal fat, %	3.77	3.23	3.13	3.13	0.19	0.08
Breast weight, g	511.3^{c}	575.0^{b}	677.5^{a}	606.3^{b}	9.30	0.00
Breast weight, %	33.17^{b}	34.08^{b}	36.33^{a}	34.22^{b}	0.29	0.00
Breast meat, g	410.0^{c}	475.0^{b}	573.8^{a}	502.5^{c}	8.20	0.00
Thigh weight, g	392.5^{c}	457.5^{b}	522.5^{a}	475.0^{b}	9.57	0.00
Thigh weight, %	25.46^{b}	27.07^{a}	27.82^{a}	26.81^{ab}	0.36	0.00
Thigh meat, g	326.3^{c}	390.0^{b}	455.0^{a}	405.0^{b}	9.44	0.00
DL36hrs	1.93^{a}	1.62^{b}	0.97^{d}	1.24^{c}	0.03	<0.05
DL48hrs	4.10^{a}	3.39^{b}	2.16^{d}	2.70^{c}	0.05	<0.05

4. CONCLUSION

The study revealed that dietary supplementation of 1% LGP plus together with 0.015% or 0.025% CP may improve the growth performance and carcass traits of Cobb500 broiler chickens and has a potential growth promoters.

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EFFECTS OF TRIBUTYRIN AND PROBIOTIC IN DIET ON EGG PERFORMANCE AND *E. COLI* IN FECES OF HENS IN THE EARLY STAGE OF LAYING CYCLE

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ABSTRACT

An experiment was conducted to determine the effect of diet supplementing with tributyrin (TBU) or probiotic (PRO) in diet on egg performance and quality of laying hens from 20-30 weeks age. A total of 2160 Hisex Brown laying hens at the 20 weeks of age were randomly distributed in a completely randomized design experiment, with 3 treatments and 3 replicates, each replicate consisted of a line with 60 pens (4 birds/pen). The experimental data was collected during 10 weeks. Treatments used: (1) Control (Cont): Basal diet (B) without any supplementation; (2) TBU: B + Tributyrin; (3) PRO: B + Probiotic, all supplements were supplied at 0.5 g/kg feed in the diets. The results showed that, average daily feed intake was not affected by tributyrin or probiotic supplementation in the diets (P>0.05). But a little improvement hen day production, egg weight and feed conversion ratio in TBU and PRO compared to Cont group. Tributyrin supplementation significantly increased eggshell thickness (P<0.01), the higher yellow color of egg York was found in chicken eggs fed TBU and PRO treatments. Howerver, the *E. coli* content in feces were decreased in TBU and PRO supplementation diets compared with control group. The conclusion of this experiment is adding tributyrin or probiotic at 0.5 g/kg feed could lightly improve hen day production, feed conversion ratio, increased eggshell thickness and reduce *E. coli* in feces.

Keywords: *Hen- day production, Hisex Brown, laying hen, Tributyrin, Probiotic.*

1. INTRODUCTION

Commercial laying hens is now developing, because egg production play an important product beside poultry meat production in the Mekong Delta of Viet Nam. Hisex Brown laying hen is a popular breed, which have earlier age at first lay (19-21 weeks age), then slowly reach the peak at around 28-32 weeks age, this laying hens are normally culled after 72-76 weeks of age due to low egg production (Haider and Nath, 2014). Therefore, improving the egg production in the early laying cycle is importance to reach the peak of laying performance and gain the benefit for farmers. In order to achieve these productions, there are not only considering the quantity of feed but also the supplement factors. The tendency of chicken producers in the world now should minimize and completely stop using antibiotics in the animal diets (Sheikh *et al*., 2011). Thus, the study found the solution for replace antibiotics in the laying hen diets are really becoming an urgent need. Probiotic and organic acid have an additional dimension, which can control the pathogen microorganisms such as salmonella and coliforms, and also enhance the digestive microflora with beneficial microorganisms, inhibits the desease cause of harmful bacteria, and enhance immunity in poultry (Mountzouris *et al*., 2010). There are many kinds of organic acids with diferent effects, there are some studies focus on mixture of organic acid, but many research on using probiotic in laying hen. Howerver, study on using butyric acid in the form of tributyrin in laying hens have very few research up to now. Therefore, this study was conducted to evaluate the effect of tributyrin or probiotic on egg production, egg quality parameters and *E. coli* in feces during the early production stage of laying hens.

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2. MATERIALS AND METHODS

2.1. Materials

Tributyrin product was provided by Menon Animal Nutrition Technology Co., Ltd, mixture of white powder, with the main ingredients of butyric acid. Probiotic (ProbiP) was a product of Vemedim Company. The experiment was conducted in a laying farm in Can Tho city in the Mekong Delta of Viet Nam from October to December, 2019. The hen house was a tunnel ventilated house with 3 floor cages in a line inside. A total of 2,160 Hisex Brown laying hens were housed in cages (pen), the hens were 20 weeks age, lasted at 30 weeks age. Water was supplied *ad-libitum*. Feed formulation and composition are showed in Table 1, and was supplied twice a day. The study was arranged as a completely randomized design with 3 treatments and 3 replicates, each replicate consisted of a line with 60 pens (4 birds/pen).

Table 1. Feed composition of basal diet

Composition		Basal diet
Ingredients (%)	Rice bran	11.0
	Maize	51.0
	Broken rice	8.0
	Soya bean meal	20.0
	Fish meal	6.0
	Premix-vitamin[1]	1.0
	Limestone	3.0
Chemical composition (%)	DM	88.0
	Crude protein	17.0
	Ether extract	5.05
	Ash	10.4
	Crude fiber	5.0
	Ca	3.0
	P	0.7
	NaCl	0.2
	NFE	63.0
	ME (MJ/kg feed)	11.4

Treatments were:

Cont: Basal diet (B) without any supplemented products in the diet

TBU: B + Tributyrin 0.5 g/kg feed

PRO: B + Probiotic 0.5 g/kg feed

Chemical composition of basal dietary is showed in Table 1. The basal dietary was formulated following laying hen requirement with amount of metabolizable energy (11.4 MJ/kg) and crude protein (17.0%) content. Feed ingredients in basal diet included: Maize, broken rice, rice bran, fish meal, soya bean meal, amino acids and premix vitamin. The supplementation products were supplied in the diets every day at level of 0.5 g/kg feed.

2.2. Measurement methods

Egg performance: During the 10-weeks experimental period, the averages of FI, hen day production, and egg weight were recorded daily for computing the average daily egg production, egg mass and feed conversion ratio. Egg mass was determined by calculating hen day production x egg weight. FCR was determined by calculating FI (g)/egg mass (g). Egg classification (broken, unnormal, double york egg) was separated for computing each class percent from the total egg production.

Egg quality: At the 25 weeks old, 180 eggs/treatment (1 egg/pen) were randomly collected for egg quality analysis. Egg shape index was determined by calculating (egg width/egg length) x 100 (Sandi *et al.*, 2013), the same eggs were broken to weight albumin, york and shell individually (Englmaierová *et al.*, 2014). Shell thickness was determined by calculating the mean of triplicate measurement from different sides of shell (Güçlü *et al.*, 2008). Haugh Unit was measured using formula $HU=100xlog(H-1.7W^{0.37}+7.57)$ (Saleh, 2013). York color was recorded using a colorimeter, which indicated degrees of lightness of a york sample (L), redness (a) and yellow-ness (b).

E. coli in feces: At the weeks 25-30th, the feces in the caecum were collected immediately from ten hens of each replication after exsanguination, placed into sterile centrifuge tubes, put on ice and transported (within one hour after collection) to the laboratory for bacterial enumeration. The infestation of *E. coli* (CFU/g) in feces was determined by

colony counting. Homogenous samples were implanted in appropriate agar environment containing lactose, and then incubated at 44^0C for 24h. The number of characteristic colonies having the shape of coliforms was counted and confirmed as *E. coli* by IMViC (Indol, Methyl Red,Voges Proskauer and Citrate) (Tran Linh Thuoc, 2006). The quantity of *E. coli* (CFU/g) was calculated as: (CFU/g) = N/(n1vf1+ … + nivfi) * R. *Where, N: The total number of colonies counted; f1: Dilution at each plate; ni: The number of plates in each dilution; R: The positive rate, and v: The volume of dilution to grow in each plate*

Chemical analysis: The chemical composition of basal feed was determined following Association of Official Analytical Chemists methods (AOAC, 1990). York color was recorded using a colorimeter (Chromameter Minolta, CR-400 Head, DP-400/Japan), which indicated degrees of lightness of a york sample (L), red-ness (a) and yellow-ness (b).

2.3. Statistical analysis

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

3. RESULTS AND DISCUSSIONS

3.1. Egg performance and feed efficiency

Hen day production (HDP) and FCR of laying hens during the period between 20-30 weeks of age is presented in Table 2. Results showed that the supplement of tributyrin or probiotic trend to lightly increase egg production but not with daily feed intake of hens. This may explain that tributyrin in the ferm of acid butyric could reduce intestinal pH, increased enzyme activity, so it should improve the digestion and absorption of protein, then improve egg production (Sakdee *et al.*, 2018). Also Sheikh *et al.* (2011) reported that, supplementation of an organic acid in diet increased intestinal villi height in all segments of the small intestine, especially in the ileum, thereby improving the absorption and increased feed efficiency. Because, tributyrin is a triglyceride which not only supplies energy, but also provides butyric acid for the development and maintenance of the epithelium of the intestinal tract (Sakdee *et al.*, 2018), and tributyrin contain three molecules of butyrate that can be broken down by lipase and then release a large amount of butyrate into the intestine (Miyoshi *et al.*, 2011; Vinolo *et al.*, 2012). In addition, tributyrin has no offensive odor and can bypass the stomach to reach the hind gut (Augustin *et al.*, 2011), so butyrin has been developed to overcome the drawbacks of butyrate in application.

Table 2. Hen day production and feed intake

Variables	Treatments			SEM	P
	Cont	TBU	PRO		
FI, g/bird/day	106.1	105.2	107.2	0.32	0.21
HDP, %	76.7	78.3	78.5	1.16	0.18
EW, g/egg	52.1	53.2	54.1	0.32	0.36
EM, g/bird	40.0	41.5	41.9	0.57	0.18
FCR, g/g	2.65	2.53	2.56	0.07	0.07

Probiotic also have many advantages when supplying in laying hen diet. Zhang *et al.* (2012) reported that probiotics as alternatives to antibiotic growth promoters to counteract problems derived from endotoxin exposure. The resutls of this study showed that probiotic supplementation can also improve slightly egg production and egg weight, this is in agreement with research from Mohan *et al.* (1995), in the 100mg probiotic in diet, egg production improved by 5%, and shell thickness improved slightly, with fewer thin-shelled eggs than in the control 8.6% compared to 18.6%. Also Kurtoglu *et al.* (2004) showed that, feed conversion ratios were positively affected by supplementation of 250 and 500

mg/kg probiotic compared with controls, but there was no difference between the control and probiotic treatment groups on feed consumption, egg weight and egg yolk weight. Similarly with research from Quanhang *et al.* (2019), dietary supplementation with probiotic may be beneficial with respect to hen performance, egg quality, and gut health (Moataz *et al.*, 2018).

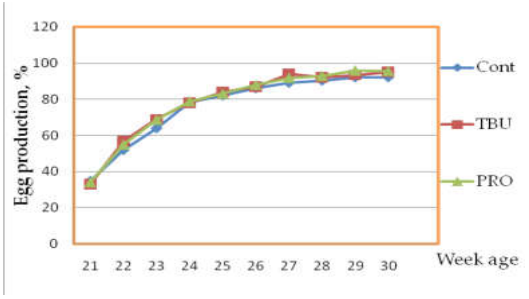

Figure 1. Hen day production 21-30 weeks age

Egg weight and egg mass were higher in TBU and PRO diets, this is in agreement with research from Langhout and Sus (2005), who found the egg weight increased in higher organic acids supplementation diet. This result also was consisted with research by Rahman *et al.* (2008), who found that the FCR improved in the layer when their diet was supplemented with organic acids. Nguyen Thi Thuy *et al.* (2018) showed similar results when supply with mixture of organic acids showed higher egg production and weight.

3.2. Egg quality

Egg quality parameters are important factor in the economic profitability of egg production. Normally, the internal egg quality and egg shell quality decline with the increasing age of hen, due to the decrease in nutrient absorption for egg shell formation and the increased in egg size and shell surface area (Roberts *et al.*, 2013). The results in Table 3 showed that egg shape (EShaI), York (YI) and Albumin index (AI) did not differ as a result of supplemented products. But a little increased egg shell thickness (EShel) of hens egg supplied with TBU to compare with control diet, and the yellowness of the yolk was increased by TBU and PRO diet supplementations. It may be because butyric acid in tributyrin is quickly absorbed and metabolized by mucosa cells, the absorption and metabolism of butyric acid begins in the mucosa of the crop and this process continues throughout the gastro intestinal tract, and also is active in the small intestine and encapsulated butyrate is active the ceca and colon (Moquet *et al.*, 2017). This result also similar with report of Kaya *et al.* (2015) who have been reported the supplementation of organic acids or short chain fatty acids positively improved the eggshell quality, and tributyrin supplementation significantly increased the eggshell thickness and tended to increase the eggshell percentage (Dani-el Ruth Hanna, 2019). The resuts of this experiment was inconsistent with the study of Soltan (2008), who found that the eggshell thickness was improved in laying hens fed organic acids 0.078% supplemented compared with control treatment. Hence, tributyrin may be valuable and useful for egg producers in terms of the improvement in egg shell quality and gut health.

Table 3. Egg quality of laying hens 20-30 weeks

Variables		Treatments			SEM	P
		Cont	TBU	PRO		
EW, g		52.4	53.3	52.7	0.27	0.07
EShaI		79.3	79.8	79.1	0.21	0.35
EShеT,mm		0.37^{b}	0.38^{a}	0.37^{b}	0.03	0.04
YI		0.46	0.46	0.47	0.01	0.18
AI		0.09	0.10	0.11	0.01	0.06
HU		88.2	88.4	87.2	0.75	0.35
Yolk color	L*	46.7	47.3	47.5	0.51	0.32
	a*	6.35	6.19	6.59	0.27	0.22
	b*	43.3^{b}	44.8^{a}	44.30^{a}	0.03	0.02

Means within rows with different letters differ at P<0.05

3.3. Egg classification

Effect of supplements on broken egg, unnormal egg and double york egg proportion of laying hens during the period between 20-20 weeks age are presented in Table 4. The

proportion of unnormal and broken eggs was lower in TBU diet than control and PRO diets, in contract with double york eggs.

Table 4. Egg classification and liveweigh gain

Variables	Treatments			SEM	P
	Cont	TBU	PRO		
Egg proportion,%	3.15	1.55	1.95	0.35	0.07
Unnormal egg, %	0.51	0.42	0.50	0.10	0.21
Double york, %	1.52	1.97	1.89	0.27	0.06
Hen ADG, g/day	3.71	3.65	3.77	0.18	0.07

3.4. *E. coli* in feces

The addition of TBU and probiotic into laying hen diets has significantly diferent *E. coli* density in fesces compared to control. In theory, intestinal bacteria, especially *E. coli* causes diarrhea, enteritis and high mortality in laying hens, so that the probiotic prevented the growth of harmful microorganisms in the intestinal tract of laying hen led to increased digestibility, then reduce the incidence of disease and death (Fatufe and Matanmi, 2011). when supplying TBU in the diet causes lower pH to below 3.5, it will limit the activity of harmful bacteria, and enhance the activity of beneficial bacteria (Adil *et al.*, 2011). Butyric acid can improve epithelial cell development (Levy *et al.*, 2015), and provides carbons which acts as an energy source for gut epithelial cells and promotes their proliferation, differentiation and multiplication (Qaisrani *et al.*, 2015). In the present study, the supplementation of tributyrin as a source of butyric acid may improve gut physiology and ecology, and consequently promote egg production and eggshell quality of young laying hens.

Table 5. *E. coli* in feces by age (10^5 CFU/g)

Ages	Treatments			SEM	P
	Cont	TBU	PRO		
25 weeks	5.16^a	4.14^b	4.02^b	0.32	0.03
30 weeks	6.19^a	5.16^b	4.62^c	0.22	0.04

4. CONCLUSIONS

Supplementation of tributyrin and probiotic in diets at 0.5 g/kg feed could lightly improve hen day production, egg shell thickness, and egg york color compared to control group, and both tributyrin or probiotic supplementation reduced *E. coli* in feces of laying hen in the early of laying cycle.

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EFFECTS OF ADDING MULTIVITAMIN SEPARATE OR MIX WITH ACID AMIN IN DRINKING WATER ON GROWTH AND FEATHER PECKING OF HISEX BROWN CHICKS

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ABSTRACT

An experiment was conducted to evaluate the effect of multivitamins (Vi) or vitamins mix with acid amin (ViA) in drinking water on growth performance and feather pecking rate of Hisex Brown chicks during brooding stage until 9 weeks age. A total of 2,070 chicks at one day of age was allocated in 3 treatments and 3 replications. The experiment was arranged in completely randomly design into 9 pens (230 chicks/pen). The treatments were: 1) Control treatment (Cont): Basal feed (B) without any supplemented product in drinking water; 2) MV: B+Vi at 1ml/l of drinking water; 3) MVA: B+ViA at 1ml/l of drinking water. The results showed that the final live weight of chicks in treatments supplemented with Vi (912.3 g/bird) and ViA (924.5 g/bird) were higher than the chicks in control group (852.1 g/bird). The ADG of chicks in supplemented treatments was higher than that in control group. FI did not difference between the treatments. Lead to FCR was lowest in MVA (2.30kg), next to the chicks fed MV (2.36kg), and highest in Cont (2.44kg). The morbidity and death rate of chicks in the MV treatment was lowest, and highest in control group. Feather pecking rate was lower in chicks fed supplemented treatments to compare with control chicks, in which chicks in MVA was lowest. In general, supplementation with multivitamins or vitamins mix with amino acid products at the level of 1ml/l of drinking water improved ADG, FCR and feather pecking rate of Hisex Brown chicks during brooding stage until 9 weeks age.

Keywords: *Amino acid, brooding stage, growth performance, multivitamins, Hisex Brown, feather pecking rate.*

1. INTRODUCTION

Poultry production has an important role in our country's agricultural economy, in which Hisex Brown is a commercial laying breed that is very popular for commercial laying farm. In order to have a high productivity, there is not only providing adequate nutrition for laying stage, but also should consider nutrition and supplements for prelaying period as young chick stage. In commercial scale, Hisex Brown young chicks often raised in a flooring pen where have some hundred chicks in a pen, this condition lead to feather pecking problem. Feather pecking is generally considered to be caused by a complex of genetical and environmental factors. Among the environmental factors, nutrition and feeding behaviour play a prominent role. Nutritionists assume that feather pecking is caused by nutrient deficiencies or imbalances, and the primary causes of feather pecking related to feed intake (Bessei *et al.*, 1999). Because feather pecking problem has often resulted in reducing the growth performances and further egg production of the chickens. So, in order to improve the young chick performance, additive nutrients should be considered. One of the most important nutrients for young chicks is vitamins and amino acids (AA) in the early stages after hatching. According to Hoseein *et al.* (2013) when vitamin A,E or D deficiency, poultry will affect the growth and efficiency of using feed, and also affect the bone development and reproduction in the laying age. In addition, essential AA play an extremely important role that need for normal development of chicks, so vitamins or AA should be more important for keeping good health for chickens. Therefore, the objective of the experiment is to evaluate the effects of multivitamin separate or mix with AA supplementation into drinking water to growth and feed efficiency of Hisex Brown chicks from 1 day old to 9 weeks age.

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2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Location and experimental chicks

The experiment was conducted during a period of 9 weeks in a experimental farm, O Mon district, Cantho City, located in the Mekong Delta of Vietnam. The experiment was conducted from January-March 2019. Hisex Brown chicks at one day old were raised in an open-sided house, in 9 pens (4mx7m) separated by netting, with 230 chicks/pen. Feed and water were provided continuously from feeders and automatic drinkers, the chicks were vaccinated against common diseases (Gumboro, H_5N_1 and fowl pox) during the experimental period.

2.1.2. Experimental treatments and feed

Table 1. Ingredient, chemical composition of diet

Ingredients and Chemical composition		1-4 weeks	5-9 weeks
Ingredients (%)	Maize	46.35	51.44
	Rice bran	14.0	15.0
	Fish meal	10.0	6.0
	Soya bean	24.5	22.53
	Methionin	0.15	0.13
	Bone meal	2.0	2.0
	Oster meal	2.27	2.27
	Nacl	0.06	0.06
	Embavit 2	0.47	0.47
	Lactozym	0.2	0.2
Chemical composition (%)	ME,kcal/kg feed	3,000	3,100
	EE	7.41	7.15
	CP	21.0	18.2
	CF	5.0	3.12
	NFE	60.3	64.08
	Ca	0.8-1.2	1.59
	P	0.6-1.0	0.58

Treatments were: (1) Cont: Basal diet (B); (2) MV: B + Multivitamin at 1ml/l of water; and (3) MVA: B + Multivitamin mixed AA at 1ml/l.

Feed ingredients included maize meal, rice bran, broken rice, fish meal, soya meal, bone and shellfish meal and premix vitamin. The experimental diets (Basal feed) were formulated to satisfy nutritional requirements of chicks 1-4 and 5-9 weeks of age (Tables 1). Multivitamin product (Vi) and Vitamin mix with acid amin product (ViA) were produced by Vemedim Company, they are both in water form. The main ingredients of 2 products, they were added into drinking water everyday and continuously for 9 weeks.

Table 2. Composition of supplemental products

Compositions	MV	MVA
Vitamin A, IU	10,000	12,000
Vitamin D3, IU	2,000	3,000
Vitamin E, mg	1,500	1,500
Thiamin, mg	2,000	2,000
Riboflavin, mg	2,000	2,000
Pyridoxine, mg	1,250	1,250
Cyanocobalamin, mg	-	10,000
Nicotinamide, mg	-	5,000
L-Methionine	-	700
L-Cysteine	-	20
L-Lysine	-	880
L-Arginine	-	960
L-Threonine	-	160
L-Glycine	-	5,200
L-Leucine	-	600

2.2. Measurement methods

The chicks were weighed weekly in the early morning before feeding and watering, the parameters included ADG (g/head/day), daily FI (g/head/day), FCR (kg/kg), feed and water were offered *ad libitum*. FI was calculated by measuring the amount of feed offered and residue left over after 24h. FCR was calculated by dividing the FI by weight gain. Morbility and morbidity were mearsured by calculating the number of desease chicks and death chicks everyday. The feather pecking rate was measured by observation the number of chicks were pecking everyday. The chemical composition of basal feed was determined following Association of Official Analytical Chemists methods (AOAC, 1990).

2.3. Statistical analysis

Data was analyzed by ANOVA using GLM of Minitab 16. Tukey pair-wise comparisons

were used to determine differences between treatment means at P<0.05. The statistical model used is as follows: $Y_{ij} = \mu + \alpha_i + e_{ij}$, *Where, Y_{ij} is growth performances and FCR; μ is overall mean averaged over all treatments; α_i is effect of treatment; e_{ij} is random error associated with treatment and replicate within treatments.*

3. RESULTS AND DISCUSSIONS

3.1. Growth performance and feed efficiency

Growth performance of chicks during the period between 9 weeks of age is presented in Table 3. The results of the experiment showed that supplementing multivitamin separate or mix with AA tended to have better growth results than the control. In fact, according to Rahman *et al*. (2012) who has suggested that in order to improve the growth of chickens, it is necessary to provide enough food with necessary additive such as vitamin, AA or other additives. And study of Islam *et al.* (2004) showed that adding vitamins to chicken diets showed the improvement of weight better than the control.

Table 3. Body liveweight 1-9 weeks age (g/bird)

Age	Treatments			SEM	P
	Cont	MV	MVA		
Week 1	99.67	105.1	106.8	3.33	0.07
Week 2	158.3	159.4	162.0	4.10	0.03
Week 3	205.3^{b}	216.6^{a}	218.8^{a}	3.44	0.02
Week 4	277.0	282.4	289.8	7.11	0.06
Week 5	358.0^{b}	371.3^{ab}	384.0^{a}	9.46	0.01
Week 6	450.0^{b}	475.0^{a}	489.0^{a}	11.1	0.04
Week 7	560.6^{b}	590.5^{a}	600.4^{a}	5.11	0.03
Week 8	695.6^{b}	740.3^{a}	760.5^{a}	8.15	0.04
Week 9	852.1^{b}	912.3^{a}	924.5^{a}	9.23	0.04

The differences between treatment means at P<0.05 if the Mean with diferent leter.

Thus, in order to achieve high productivity and efficiency in poultry production, it is not only setting up perfect and balanced feeding rations but also supplying enough vitamin or AA in the diets for chickens. It can be explained that these additive help to increase the absorption of intestinal parenchyma, stimulate the activity of coenzymes to promote the process of breakdown and metabolism of substances in the body (Hoseein *et al.*, 2013). Moreover, almost vitamins are needed in the diet of chickens, because they have a positive effect on feed metabolism, stimulating the immune system and reducing stress for chickens (Sahin *et al.*, 2003). Chickens are susceptible to vitamin deficiencies because the intestinal bacteria system can synthesize very little vitamin, and therefore if there is not absolutely vitamin in the diet or deficiency, chickens are very susceptible to stress (Jang *et al.*, 2014).

Table 4. ADG, FI, FCR of chicks during 9 weeks

Age	Treatments			SEM	P
	Cont	MV	MVA		
IW,g/bird	51.67	52.78	52.67	1.13	0.75
FW, g/bird	852.1^{b}	912.3^{a}	924.5^{a}	9.23	0.04
ADG, g/day	12.71^{b}	13.65^{a}	13.84^{a}	0.25	0.02
AFI, g/day	33.16	33.94	33.54	0.48	0.07
FCR, g/g	2.44^{a}	2.36^{ab}	2.30^{b}	0.05	0.04

The results in table 4 also showed the daily gain tended to lightly improve in MV and MVA compared to control group. In fact, according to Moravej *et al.* (2012) who showed that vitamins take part in many enzyme structure in the system to catalyze biological reactions to maintain all normal life activities such as growth, reproduction, body protection, meat production, etc. Vitamin deficiency leads to metabolic disorders, damaging poultry body, because these vitamins help chickens stimulate appetite, help them grow faster, and have an important role in the oxidation of substances, hydrocarbon metabolism and energy. Moreover,vitamins are also important components of coenzyme-A function and important function in the metabolism of fat synthesis (Duong Thanh Liem, 2008). So supplementation of multivitamin in drinking water help chicks to consume more enough vitamins than supplementation in the diet, because in the hot weather the consumption of

high volum of water is nessesary of the chicken requirement. The results of this experiment are similar to those of Jang *et al.* (2014), who showed the addition of vitamins C and E to the diet on broilers increased the weight to compare with control. Because in this research feed intake of chicks in all treatments were almost similar, but the daily gain of chicks in MV and MVA tended higher than control, the reason is that chickens in MV and MVA have high levels of essential vitamins, which stimulate the absorption and metabolism of nutrients more effectively. This led to a feed conversion ratio of chickens in MV and MVA tended to be better than chickens in control group. According to research by Khajali *et al*. (2006); Maiorka *et al.* (2002), vitamins play a part in protecting the body while the substances that are absorbed from food will be used for weight gain.

3.2. Feather pecking rate and health status of chicks

The morbidity rate and death rate of chicks in the MV treatment was lowest, and feather pecking rate was lower in chicks fed supplemented treatments to compare with control chicks. It can be explain that the chicks in MV and MVA consume enough vitamins, which take part in enzyme structure to catalyze biological reactions of body protection and improve chicken health (Moravej *et al.*, 2012). In theory,when living conditions with relatively high stocking densities during hot weather, chickens under stress produce biting each other (Adebiyi *et al.*, 2011). And if food does not guarantee necessary nutrition, especially protein, AA, molting stage, lack of vitamins such as A, E, H, D3, K ... also lead to chicken bites (Khan *et al.*, 2011). From the results of this experiment, the addition of multivitamins or mix with AA have partly prevent missing of vitamins and essential AA such as lysine or methionine, which helps chickens to avoid biting each other. So the results showed that the addition of vitamins or mixed AA to drinking water showed a reduction in the incidence feather pecking in chickens compared to no supplementation.

Table 5. Feather pecking rate and health status of chicks during 9 weeks (%)

Parameters	Treatments		
	Cont	MV	MVA
Initial chicken number	690	690	690
Final chicken number	662	678	670
Number of morbidity	45	38	41
Morbidity rate (%)	6.52	5.51	5.94
Number of mortality	28	12	20
Death rate (%)	4.06	1.74	2.90
Feather pecking rate (%)	22.2	16.7	11.1

Many research has been showned that nutrition influences feather pecking in different poultry species, and inadequate supply of protein and AA, mainly methionine and lysine, stimulate feather pecking. The effect of low protein levels in the diet was obviously compensated by the increased feed and protein intake in the low energy diet. However, feather pecking also occurs when the feed contains adequate levels of all nutrients and a surplus of certain specific nutrients does not reliably prevent it (Kjaer and Bessei, 2013). In order to solve the problem it will be necessary to identify birds, which show this particular preference for feathers under favourable nutritional and environmental conditions. So in this research, the chicks were supplied more AA and vitamins showed reducing feather pecking rate than control. Because, there is a consistent interrelationship of nutrition and feather pecking as deficiencies in protein, AA increase the risk of feather pecking in all poultry species (Danner and Bessei, 2000). Supply of nutrients beyond the known requirement for production, or high vitamin and AA adequate diets reduce the risk of feather pecking, so this particular affinity to feathers is considered the cause of feather pecking under optimum feeding systems.

4. CONCLUSIONS

Supplementation of multivitamin separate or mix with AA both improved growth performance and reduced feather pecking rate in Hisex Brown young chicks in brooding stage until 9 weeks age.

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EFFECTS OF REPLACEMENT OF FISH MEAL AND SOYBEAN MEAL BY BREWER'S YEAST EXTRACT ON CARCASS PERFORMANCE AND MEAT QUALITY OF LXY PIGS

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ABSTRACT

In Vietnam, beer production output is estimated from 2.5 to 3.0 billion liters annually, that means about 3,500 to 4,000 tons of brewer yeast extract powder is produced per year. The using this by-product for livestock production as a protein feed resource is a way to reduce waste and pollution. Therefore, our research was focused on effect of this by-product on performance of fattening pigs. The study was conducted to investigate the effects of replacement of fish meal and soybean meal by brewer yeast extract (BYE) in the diets on carcass traits and meat quality of L×Y fattening pigs. Pigs were fed diets in which fish and soybean meals were replaced by 0, 2, 4 and 6% high-protein (73% CP) BYE on equal protein basis. In the end of fattening period, 24 pigs (12 gilts and 12 barrows) including one male and one female per replication and 6 pigs per treatment for investigating carcass traits (slaughter weight, hot carcass weight, dressed carcass weight and carcass length, killing-out percentage, carcass yield) and organoleptic meat quality (pH at 45 minutes and 24 hours post mortem, meat colour, drip loss percentage, cooking-loss percentage, shear force at 24 hours post mortem) and meat chemical compositions (dry matter, crude protein, lipids and ash). The results showed that replacement of BYE in the diets did not affect carcass traits (P>0.533), organoleptic meat quality (P>0.206) and meat chemical compositions (P>0.084). This suggests that BYE could be used as a protein resource in the diets for fattening pigs without effect on carcass traits and meat quality.

Keywords: *Brewer yeast extract powder, fattening pigs, pork quality, carcass traits.*

1. INTRODUCTION

The pig production plays an important role in livestock sector in Vietnam, which provide the food sources for domestic consumption and exportation. The improvement of carcass characteristics and meat quality are the most important objectives of commercial pig production. The increasement of carcass characteristics leads to improved carcass yield per head while aims of meat quality improvements are creation of products that have high nutritive values, good flavour and taste, attractively organoleptic quality.

Beside genetic selection and breeding, feeds and feeding are very important factors that may influence the carcass traits and meat quality in pigs. Many researches have been conducted to find out the alternative sources for protein ingredients in feed that may improve the growth performance and meat quality in pigs but possibly reduce the production costs (Sivilai *et al.*, 2018, Nguyen Cong Oanh *et al.*, 2019). The idea is the production of feed should not compete directly with human food production due to both economical and ethical reasons, as the food shortages in the world is critical (Gabriel *et al.*, 2014). BYE is a by-product from beer industry that contain high levels of crude protein, vitamin B and mineral exceeding the nutrition requirement of almost livestock (Shurson, 2018). The use of BYE as an animal feed was reported by many authors (Nguyen Thi Hoang Anh *et al.*, 2008; Trinh Vinh Hien, 2010; Pham Quynh Trang, 2012; Ha Xuan Bo *et al.*, 2020). The effect of BYE on growth performance of L×Y fattening pigs was reported in the study of Ha Xuan Bo *et al.* (2020). The authors confirmed that replacement of fish meal and soybean meal by BYE in the diet increased growth rate and improved feed conversion. However, further study should be conducted to examine the effects of BYE on carcass traits and meat quality in crossbred fattening pigs.

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The aim of present study was to investigate the effects of replacement of fish meal and soybean meal by BYE supplementation on carcass performance, organoleptic quality and chemical compositions of meat from L×Y fattening pigs.

2. MATERIALS AND METHODS

The experiment was conducted on 184 L×Y fattening pigs (92 gilts and 92 barrows) of 2 months of age at Phu Dong pig farm in in Gia Lam district, Hanoi from Jun to Dec 2018. There were 3 replicates for each of the 4 diets with 46 pigs (23 gilts and 23 barrows) in each diet. The experiment was divided into two phases: growing (bodyweight from 20 to 45kg) and finishing phase (bodyweight from 45 to 100kg). The pigs were fed diets in which fish and soybean meals were replaced by 0, 2, 4 and 6% high-protein (73% CP) BYE on equal protein basis. The pigs were kept in closed pens, with free access to feed and water. The pigs were vaccinated according to the standard procedures of the farm. In the end of fattening period, 24 pigs (12 gilts and 12 barrows) including one male and one female per replication and 6 pigs per treatment were randomly selected for investigating carcass traits and meat quality. The pigs were slaughtered according to the standard procedures QCVN 01-150:2017/BNNPTNT for pig slaughtering in Vietnam.

Source of BYE, experimental diets and the chemical composition of these diets were described in the study of Ha Xuan Bo *et al.* (2020).

Twenty-four pigs were slaughtered at age of 182.76±7.05 days with body weight of 98.01±8.48kg on Dec 2018. Before slaughter, live weight was individually recorded by digital scale (Mettler Toledo). The hot carcass weight with head, feet, and leaf fat was weighed. The killing-out percentage was calculated as a ratio of hot carcass to live weight. Dressed carcass weight (kg) were measured as weight of hot carcass without head and legs. Carcass yield was calculated basing on dressed carcass weight and live weight. Carcass length (cm) was measured as the distance between Atlas bone and symphysis Pubis by tape measure. The samples of longissimus dorsi muscle were collected from the left half-carcass from the first to fourth last ribs immediately after slaughter. Each sample was divided into two slices of 3cm thickness (one of those were stored at 4°C for analyses of organoleptic quality at 24hrs post mortem, the another was stored at -50°C for analyses of meat chemical compositions).

The organoleptic quality were pH values, meat colour (L*, a, and b*), DL, CL and shear force (SF). The pH, meat colour, and SF were measured using a portable pH-meter (Testo 230 with an electrode, type 03 pH; Lenzkirch, Germany), a colorimeter (Chroma Meter CR-410; Minolta, Tokyo) with settings of illuminant D65 and 2° standard observers, and a SF (SF Warner Bratzler 2000D, G-R Manufacturing, Manhattan, KS), respectively. Drip loss (%) were calculated basing on the weights of samples before and after 24hrs of preservation. Cooking loss (%) were defined basing on weight loss during cooking process at 75°C in 50 minutes by Waterbach Memmert. The chemical compositions of meat samples including percentages of dry matter (DM), crude protein (CP), lipids and ash were analyzed following methods by AOAC (1990). The meat quality and chemical compositions were analyzed in the laboratory of the Department of Animal Genetic-Breeding and Central laboratory, Faculty of Animal Science, Vietnam National University of Agriculture respectively.

A linear model including the fixed effects of diet, sex and interaction between these factors is presented in the statistical model below: $Y_{ijk} = \mu + DIET_i + Sex_j + DIET_i*Sex_j + e_{ijk.}$ *Where, $Y_{ijk:}$ Parameters of carcass performance, organoleptic quality and chemical compositions of meat from LxY fattening pigs; μ: overall mean; $DIET_i$: fixed effect of diet i (0, 2, 4 and 6); Sex_j: fixed effect of sex j (gilt and barrow); $DIET_i*Sex_j$: interaction between diet and sex; e_{ijk}: residual errors*. The pairwise comparison of least square means was made using Tukey's test. Statistical parameters are least square mean (LSM) and

standard error (SE). A significant difference was considered when P-value <0.05. The data were analyzed using the general linear model (GLM) procedure of SAS software (SAS, 1989) to identify significant sources of variation.

3. RESULTS

The carcass traits from L×Y fattening pigs according to the diets are shown in Table 1. The carcass traits were not significantly different between diets (P>0.533). However, slaughter weights in the diets with BYE (97.32, 97.82 and 98.38kg for 2, 4 and 6% BYE respectively) trended higher than that without BYE (95.67kg).

Table 1. Effects of brewer yeast extract replacement in the diet on carcass performance of L×Y pigs

Variable	**0** (n=6)	**2** (n=6)	**4** (n=6)	**6** (n=6)	**SEM**	**P**
Slaughter weight (kg)	95.67	97.32	97.82	98.38	1.45	0.59
Hot carcass weight (kg)	79.21	79.54	80.37	80.42	1.32	0.89
Dressed carcass weight (kg)	72.16	72.34	73.08	72.54	1.16	0.95
Killing-out percentage (%)	82.79	81.77	82.16	81.81	0.96	0.87
Carcass yield (%)	75.42	74.36	74.71	73.78	0.79	0.53
Carcass length (cm)	98.33	99.58	98.00	99.17	1.80	0.92

No effect of BYE on organoleptic meat quality (Table 2) and chemical compositions of meat (Table 3) was observed.

Table 2. Effects of brewer yeast extract replacement in the diets on organoleptic meat quality of L×Y pigs

Variable	**0** (n=6)	**2** (n=6)	**4** (n=6)	**6** (n=6)	**SEM**	**P**
pH45	6.74	6.88	6.66	6.68	0.08	0.24
pH24	5.95	5.93	5.82	5.87	0.07	0.52
L*	57.24	56.14	55.59	56.41	0.79	0.54
a*	13.66	14.18	14.23	14.11	0.24	0.35
b*	6.97	7.77	7.16	7.30	0.37	0.48
DL (%)	1.16	1.11	1.06	1.30	0.16	0.74
CL (%)	30.28	30.94	31.14	31.66	0.82	0.70
SF (N)	45.56	53.92	53.76	45.45	3.68	0.21

Table 3. Effects of brewer yeast extract replacement in the diets on chemical compositions of L×Y meat

Variable	**0** (n=6)	**2** (n=6)	**4** (n=6)	**6** (n=6)	**SEM**	**P**
DM, %	25.88	25.70	25.89	25.56	0.18	0.51
CP, %	22.59	22.41	22.39	22.29	0.14	0.52
Ash, %	1.45	1.47	1.43	1.35	0.06	0.50
Lipid, %	1.39	1.62	1.76	1.51	0.10	0.08

We remarked that a*, b*, lipids content in the pork from diets with BYE supplementation were higher than those without BYE but this difference was not significant (P>0.084). Additionally, effects of sex and interaction between sex and diet were not observed (P>0.05). Therefore, these results were not presented.

4. DISCUSSION

The effect of BYE on growth performance of LxY fattening pigs has been shown before (Ha Xuan Bo *et al.*, 2020). It was found that increasing replacement of fish and soybean meal by BYE in the diet leaded to improve ADG and decrease FCR. However, in the current study, this replacement did not affect carcass traits and meat quality. This finding is in agreement with Zhang *et al.* (2018a); Zhang *et al.* (2018b); Suzuki *et al.* (2019); Zhang *et al.* (2019). The study of Zhang *et al.* (2019) on D(LY) pigs revealed that increasing supplementation with brewer's yeast in diet was to improve the growth performance with body weight and feed efficiencies, but no effect on carcass weight and meat quality. Similarly, carcass traits of D(LY) pigs were not significantly different between the control diet with 0% brewer's yeast and treatment group with 9.75% liquid brewer's yeast (Suzuki *et al.*, 2019). It is in agreement with a study of Sreeparvathy *et al.* (2012) and Zhang *et al.* (2018a). Inversely, dressing out percentage and carcass length were significant difference between supplementation levels of dried brewer grains (Murashi *et al.*, 2015). Suzuki *et al.*, 2019 confirmed that BYE did not affect

meat colour (L*, a* and b*), drip loss (DL) and cooking loss (CL). The meat colour (L*, a* and b*) in our study was higher than that in study of Salmi *et al.* (2010). On the other hand, the DL percentage of meat was lower than that in mentioned studies, indicating that the water-holding capacity of muscle may be enhanced. This results are in agreement with study of Vo Trong Thanh *et al.* (2017). To our knowledge, there is no publication of effect of BYE on chemical compositions of pork meat allowing comparison with current study. However, these results are consistent with study of Vo Trong Thanh *et al.* (2017).

5. CONCLUSION

Replacement of fish meal and soybean meal by BYE in the diets at 2, 4 and 6% did not affect carcass, organoleptic meat quality traits, and chemical compositions of meat from L×Ypigs. This suggests that BYE could be used as a protein resource in the diets for fattening pigs without effect on carcass performance and meat quality.

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UNDERSTANDING BAN PIG VALUE CHAIN IN HOA BINH PROVINCE, NORTHWEST VIETNAM

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ABSTRACT

The study provides the understanding of Ban pig value chain in Hoa Binh province regarding marketing channels and linkages between chain actors to comprehend potentials and challenges for the market improvement. This study was conducted in Da Bac district and Hoa Binh city in Hoa Binh province, using a participatory approach. Three focus group discussions with farmers at communes of different remoteness, 20 in-depth interviews with collectors, slaughterers, owners of restaurants were conducted to understand distribution channels of Ban pigs and trading relationships. A stakeholder meeting with various chain actors was carried out for the validation of findings. Results indicate that marketing channels of Ban pigs in Hoa Binh were categorized by weights, and trading patterns of Ban pigs vary by remoteness. While farmers in remote villages mainly sold heavy pigs to a group of consumers and local slaughterers, selling pigs at small weights to collectors was a predominant pattern in villages near the district center. Relationships between pig farmers and with other actors were weak; while restaurants, slaughterers and district collectors had complex trading relationships with commune collectors.

Keywords: *Ban pig, value chain, market improvement, Vietnam.*

1. INTRODUCTION

Pig production plays an important role in Vietnam due to the significant contribution to human nutrition and agricultural production development. It provides the livelihood for more than four billion smallholders in the country and supplies 80% of total pork for domestic consumption (Nga *et al.*, 2015). Local pig production is a part of traditional culture and a means of improving the livelihoods of ethnic minorities in remote and limited-resource access areas. Ban pig is a local breed prevalent in marginal regions in Northwest of Vietnam with good adaptation abilities to harsh conditions. Consumers favor Ban pork as it is tastier, more tender, and healthy than pork from exotic breeds (Huong *et al*., 2009). Huyen *et al*. (2016) showed the difference in the preference of chain actors in pig value chain in Son La province, and the potential of high prices in urban markets. Understanding on marketing channels of Ban pig products and linkages between actors will help to enhance the development of Ban pig production in the region. The current study looks inside the Ban pig value chain in Hoa Binh province, especially in marketing channels and linkages between actors in order to understand potentials and challenges for the improvement of Ban pig value chain.

2. MATERIALS AND METHODS

The study was conducted in Da Bac district and Hoa Binh city, belonging Hoa Binh province in the framework of project *LPS/2016/143 "Safe Pork": Marketbased approaches to improve the safety of pork in Vietnam.* Da Bac district is a mountainous area in the West of Hoa Binh city, about 15 km away from the city center. The total pig population in Da Bac was 28.98 heads, of which, the number of Ban pigs accounted for 40% of the pig herds (JICA, 2015). Three communes in Da Bac district were selected, with different geographical features: Muong Chieng is a remote commune, Doan Ket is an intermediate commune, and Cao Son is a commune near the district center

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Ban pig farmers, collectors in Da Bac district, slaughterers, owners of restaurants in Da Bac and Hoa Binh city were interviewed at the time of the study.

Data were collected based on a participatory approach, through focus group discussions, semi-structured interviews, and a stakeholder meeting.

Focus group discussions (FGDs) with Ban pig keepers were held in Cao Son, Doan Ket and Muong Chieng communes. Each discussion involved 12 participants of both males and females. They were separated into male and female groups to map and discuss marketing channels of Ban pig products and problems in production and market access. This classification also aimed to encourage the participation of females in discussions. In total, 36 participants were involved.

In-depth interviews were conducted with collectors, slaughterers, restaurants in Hoa Binh province, using a set of semi-structured questionnaires including both closed and open-ended questions. These stakeholders were asked about general information, trading and consumption activities, preferences, and constraints. In total, 20 stakeholders were interviewed (six collectors, nine slaughterers, five restaurants).

A stakeholder meeting was organized in Da Bac district center, with the participation of nine different stakeholders. They were representatives of various stakeholders in the Ban pig value chain. Major results of the survey were presented, and then participants discussed to approve, make revisions on findings and gave explanations for changes.

Qualitative data from FGDs and interviews were analyzed using synopsis analysis and descriptive statistics.

3. RESULTS AND DISCUSSIONS

3.1. Marketing channels of Ban pigs

In general, live Ban pigs at different weights of <10kg, 10-20kg and >40kg were mainly sold in Hoa Binh province (Figure 1). Piglets were given to other farmers for breeding while fatterners 10-20kg and >40kg were marketed to different chain actors.

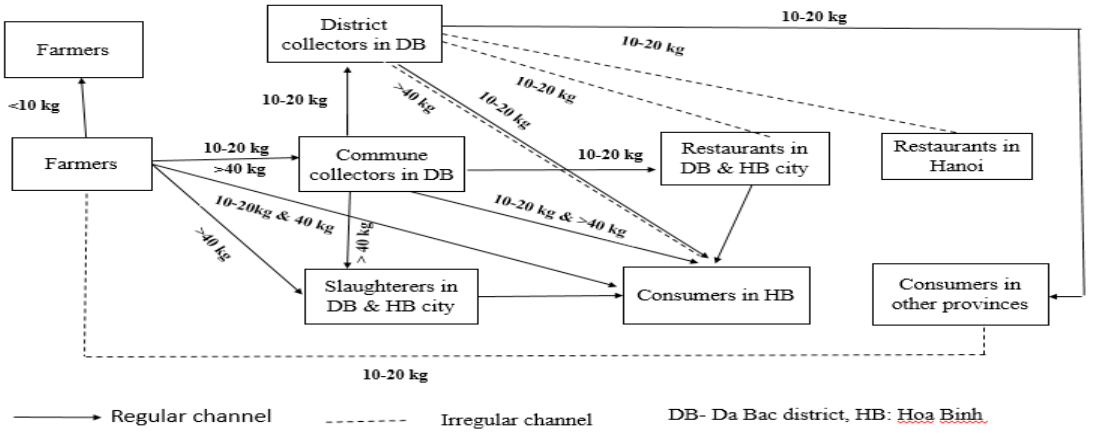

Figure 1. General marketing channels of Ban pigs in Hoa Binh province (Stakeholder meeting, 2019)

For pigs 10-20kg, farmers mainly sold pigs to collectors at commune levels, who then distributed pigs to collectors at district levels in Da Bac, restaurants in Da Bac and Hoa Binh city, and consumers within the province. From district collectors, most of pigs were sold to consumers in the province and in other provinces, including Hanoi and Ho Chi Minh cities, Ha Nam, Nam Dinh, Phu Tho provinces. A small share of their pigs were transferred to restaurants in Da Bac district and Hoa Binh city because either restaurants

in these areas did not purchase pigs frequently, or, district collectors were afraid of bad debts. Few district collectors could connect with restaurants in Hanoi to sell pigs. The channel of small Ban pigs to local restaurants is consistent with finding of Huyen and Sautier (2017) who studied Black pig value chain in Mai Son, Son La. The marketing channel of pigs to restaurants in Hanoi is similar with results by Huyen *et al.* (2016) on Ban pig value chains in Thuan Chau district and Son La city. Huyen *et al.* (2016) state that restaurant owners in Hanoi had strict requirements on quality and a certificate of veterinary inspection. That might be a reason for an irregular channel of Ban pigs in Hoa Binh to restaurants in Hanoi.

With regards to pigs above 40kg, farmers sold heavy pigs to both commune collectors in Da Bac district and slaughterers in Da Bac district and Hoa Binh city, who sold pork cuts at local markets. According to Huyen *et al.* (2016), this type of pigs was mainly distributed to local slaughterers due to high fat content and the preference of local consumers to fat pork to super-lean pork. In addition, the current study found that commune collectors also collected pigs above 40kg for slaughterers, and sold pigs to a group of consumers who shared pigs for cheaper purchasing costs. District collectors sometimes sold heavy pigs to consumers in the province when they had orders. A variation in trading patterns of Ban pigs have been found in different regions in Da Bac district.

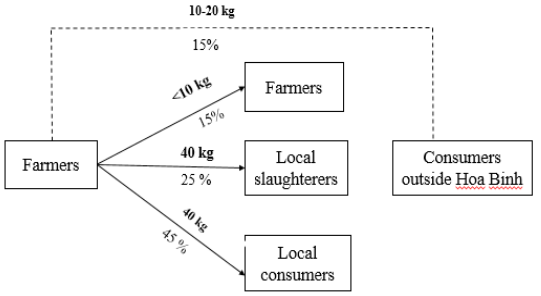

Figure 2. Trading pattern in a remote commune

In remote villages, farmers sold a great share of pigs above 40kg to a group of consumers (45%) and local slaughterers (25%). No local collectors were available, and collectors in other areas found difficult to access this commune due to far distances. According to interviewed slaughterers, Ban pigs were high prices, so they only slaughtered pigs once a week. Only businesses and teachers in the village could afford the high prices of pork. Other consumers often purchased heavy pigs in groups of five to six people to reduce costs. Pigs from 10 to 20kg sometimes were transported to acquainted people of farmers outside the province (15%). Piglets for breeding purposes at 5-6kg were given to other farmers after Lunar New Year.

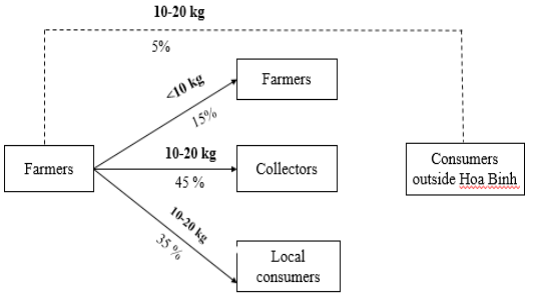

Figure 3. Trading pattern in intermediate commune

Farmers in the intermediate region sold most of pigs 10-20kg to a collector (45%) in Tan Minh, a neighboring commune and local consumers (35%). Farmers did not want to pay for feeds and other production costs at latter growth periods. Heavy pigs were only available for slaughterers in case farmers could not sell pigs at small weights. Pigs were randomly marketed outside the commune (5%).

It can be seen that farmers in near town and city mainly sold pigs from 10 to 20kg to collectors (60%) and consumers within the province (30%). The finding is incomparable with Huong *et al.* (2007) that Ban pig keepers near towns in Son La province mainly traded pigs with slaughterers. The reason for this tendency was that farmers preferred to sell

pigs at low weights for high prices. Piglets less than 10kg were given to other farmers (10%).

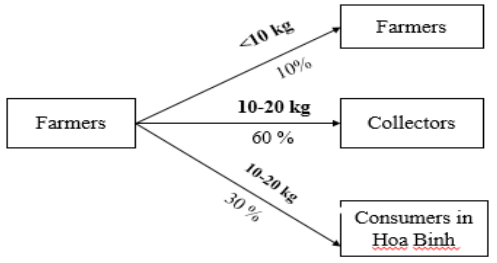

Figure 4. Trading pattern in near the center

3.2. Linkages between actors in marketing channels of Ban pigs

3.2.1. Linkages between farmers and with other chain actors

Relationships between farmers and with other actors were weak at the time of the study. Results from FGDs show that farmers normally sold pigs individually and at the time they were in demand for cash or when collectors asked to buy pigs. This is similar to the situation in Son La province described by Huyen and Sautier, (2017). Among the interviewees in the current study, four interviewed slaughterers visited farmers when farmers called them, but they had not had any agreements in advance. It is different to some villages in Mai Son district, Son La province in which slaughterers had made oral agreements to buy pigs as pigs were ready for sale (Huyen and Sautier, 2017). The other slaughterers sought pigs themselves or contacted with acquainted people. All investigated collectors came to pig farms without announcement. It was because either they lacked information about pig sources, or, they wanted to check for farming conditions to ensure that pigs have been kept without commercial feeds. Besides, unplanned visits helped them to avoid the situation of feeding pigs before selling to gain weight.

3.2.2. Linkages between collectors at commune and district levels

All 3 out of 3 interviewed district collectors connected with commune collectors to receive information about pig sources. There was no trading ties or regulations between them. For those who knew about pig sources, would get 50,000VND per pig, and up to 200,000VND if they could find many pigs. Those commune collectors continued shared 20,000 to 30,000VND to other commune collectors or their acquainted people at different areas to get information in case they could not find pigs in their areas. This finding is comparable with results obtained by Nga *et al.* (2014) that pig brokers received commissions of 20,000VND per pig.

Two interviewed district collectors also had agreements with communal collectors for purchasing pigs. The requirements regulated kinds of pigs, weight ranges, regular selection activities and commissions. Each of them had three to four commune partners to ensure the trading capacity. Commune ones were responsible for managing and seeking for pigs in certain areas in Da Bac district. They could receive commissions at 100,000VND per pig, or 300,000VND per tour (about 100kg of pigs/ tour). Apart from that, they also agreed on ways of contacts for transactions. Accordingly, commune collectors had to take pictures and make videos of farm conditions and send them to district ones for quality approval. Based on their experiences, district collectors would decide to purchase or refuse pigs.

Especially, one district collector had a trading relationship with a restaurant in Hanoi, in which pigs have been purchased on a stable and scheduled plan, twice a month. Prices were agreed in advance and stable within the year. This situation is similar in Son La province (Huyen *et al.*, 2016). Phuong *et al.* (2014) suggest long-term agreements between producers and restaurants as an option to sustain the growth of Ban pig production. However, selling pigs to restaurants has been reported by an interviewed collector to be risky due to bad debt.

3.2.3. Linkages between slaughterers and commune collectors

Slaughterers collaborated with commune collectors in two main patterns. In the first pattern, commune collectors provided the information and sought pigs in their areas for slaughterers. All interviewed slaughterers contacted with commune collectors when they wanted to find pigs or collectors had information on sources of pigs in their areas. Like the relationship with district collectors, commune collectors did not have any trading ties and also got 50,000VND for the information provision.

In another pattern, slaughterers had stable trading relationships with commune collectors and made verbal agreements with them for seeking pigs. Two of nine interviewed slaughterers had this type of trading relationship. One slaughterer in Da Bac district made a verbal agreement with commune collectors for seeking pigs. Agreements included a common understanding of breeds, weight ranges, and slaughtering schedules. For instance, they only got Ban pigs above 40kg, with pure black and long hair; and they needed pigs on every Thursdays and Sundays. Commune collectors received 150,000VND per pig in this relationship. One slaughterer in Hoa Binh city had a long-term relationship with commune collectors. Commune collectors had to meet some requirements on the quality, including breeds, weight ranges. To specify, pigs should have at least 50% of blood of parent Ban pigs. Those pigs were called as "hai bề lui" or "crossbreds with more genes of local breeds". The slaughterer would not accept pigs with more than 75% of exotic or crossbreeds. Commune collectors had to call the slaughterer and described the characteristics of pigs and farming conditions. The slaughterer raised prices for commune collectors to negotiate prices with farmers. The prices would be agreed on transactions. If collectors would negotiate with farmers for lower prices, they would get more commissions.

3.2.4. Linkages between restaurants and commune collectors

Four of five interviewed restaurants collaborated with commune collectors in Da Bac district seasonally. One of them had a verbal supply contract with a collector in Lao Cai province as he did not believe in the quality of Ban pigs in the province. In general, all investigated restaurants did not have a planned schedule for purchasing pigs. They only called collectors to select a good Ban pigs from 10 to 20kg when they had orders from consumers. Collectors should understand the characteristics of a qualified pig. They could negotiate prices on calls, or collectors would raise prices depending on the quality of pigs. For example, Ban pigs with small ears and feet, flat belly would be expected to be lean and get good prices. According to investigated restaurant owners, prices of Ban pigs were stable within the year. It usually increased from 10,000 to 20,000VND per kg of live pigs in two months before Lunar New Year.

4. CONCLUSION

Current marketing channels of Ban pigs in Hoa Binh is categorized by weight levels and varied by remoteness. Small Ban pigs are mainly traded among different stakeholders to consumers, both inside and outside province. This channel is mainly found in near town regions while heavy pigs are mainly traded at commune level in local markets.

Linkages among farmers and with other actors were weak. District collectors, slaughterers and restaurants had relationships with commune collectors for the information in pig sources, pig selection and collection.

A cooperative of pig farmers and formal agreements between farmers and chain actors are essential to improve linkages between actors in the Ban pig value chain in Hoa Binh province. Furthermore, diversifying marketing

channels by weights and developing short channels based on the community demand will become a potential to reduce transaction costs in marketing channels.

ACKNOWLEDGEMENT

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STUDY ON THE PREFERENCE OF URBAN CONSUMERS FOR QUALITY ATTRIBUTES OF BAN PORK

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ABSTRACT

The study analyzes characteristics and the preference of Ban pork consumers in Hanoi city for pork quality attributes in order to help Ban pig keepers meet consumer expectations and obtain a competitive advantage in the urban market. This study was conducted in Hanoi city, using a participatory approach. Thirty in-depth interviews with Ban pork consumers were conducted, using semi-structured questionnaires and a 5-point Likert Scale to evaluate the consumer preference for quality attributes. Results indicated that Ban pork consumers in Hanoi belong to a group of high family income and literacy. They purchased slaughtered pigs more frequently than live Ban pigs. Green food stores, traditional markets, and pig keepers were the most popular places where consumers buy Ban pork. Leanness, freshness, colour had a high preference of consumers at the point of purchase. Taste was the most significant trait after the purchase. Flavour, tenderness and juiciness were less important than other traits at purchase, but useful to distinguish Ban pork from other kinds of pork after the purchase.

Keywords: *Ban pork, consumer preference, quality traits, urban, Hanoi, Vietnam.*

1. INTRODUCTION

Local pig production is important for rural farmers in Vietnam due to its contribution to socio-culture value and household livelihoods. Ban pig is a local breed prevalent in marginal regions in Northwest of Vietnam with good adaptation abilities to harsh conditions, and mainly kept by ethnic minorities. Ban pigs have a marketing potential in terms of price premiums and high evaluation of consumers for the quality in urban markets (Huong *et al*., 2009; Huyen *et al*., 2016). However, they are mainly sold in local markets, with low prices (Huong *et al*. 2009) due to weak linkages between smallholder pig keepers with other chain actors and insufficient information on consumer preferences (Lapar *et al*., 2012).

Several studies have been done in Vietnam concerning on the market improvement for Ban pigs, for instance, about market marketing channels of Ban pigs (Huong *et al*., 2009), influencing factors to prices of Ban pigs (Phuong *et al*., 2014), and the discrimination of Ban pork from crossbred pork (Muth *et al*., 2017). Huyen *et al*. (2016) and Huyen and Sautier (2017) show the potential of a niche market for Ban pigs in the urban market and the preference of different stakeholders for Ban pigs. However, these researches only studied attributes of live Ban pigs and understood consumer preferences under the viewpoint of suppliers. Consumer characteristics regarding consumption patterns, frequencies and consumption places have not been detected. The current study aims to analyze characteristics and the preference of urban consumers in order to improve the market competitiveness of Ban pigs. These will help smallholder pig keepers to meet consumer expectations and gain a competitive advantage in urban markets.

2. MATERIALS AND METHODS

The study was conducted in Hanoi city in 2019, in the framework of project LPS/2016/143 "Safe Pork": Marketbased approaches to improve the safety of pork in Vietnam. Hanoi city was selected because of high income of residents and their willingness to pay for pork from local breeds (Nga *et al*., 2015).

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Ban pork consumers were interviewed at the time of the study. The sampling selection was carried out via two steps: (i) Consumers in Hanoi who have consumed Ban pork products within the last two years were stratified and collected; (ii) Samples of Ban pork consumers were then selected randomly from the above list. In total, 30 consumers of Ban pork were selected. These people were located in different areas, including Dan Phuong, Cau Giay, Gia Lam districts of Hanoi.

Data were collected based on a participatory approach, through in-depth interviews. In-depth interviews were conducted with consumers in Hanoi, using semi-structured questionnaires. They were asked about socio-economic characteristics, consumption activities of Ban pork, preferences for Ban pork attributes. A 5-point Likert scale (1: least important and 5: most important) adapted from Huong (2007) was utilized to evaluate the consumer preference for quality attributes. A variety of quality attributes were assessed, including leanness, taste, tenderness, color, flavor, juiciness, freshness (Huyen *et al*., 2016; Muth *et al*., 2017).

Data from interviews were analyzed using synopsis analysis and descriptive statistics. Mean, percentages, standard deviations and ranges were used to indicate consumer characteristics, consumption frequencies and consumer preferences.

3. RESULTS AND DISCUSSIONS

3.1. Characteristics of Ban pork consumers

The characteristics of Ban pork consumers is shown in Table 1. Most respondents were female at the age of about 35 years. They illustrated high level of literacy, with 93.3% having tertiary education and 6.7% completing high schools. More than two-third of respondents had a total household income of above 180 million VND per year, while a very small number (6.7%) fell into low income groups of 30-60 million VND and 60 to 90 million VND per year. They spent about 1 million, on average, up to 2 million on food weekly. According to GSO (2018), the per capita income of urban residents was 67.2 million VND per year, which is equal with a family income of 134.4 million VND per year. It means that most Ban pork consumers in this study was higher than an average level of family income and belonged to a high-income group.

Table 1. Characteristics of investigated consumers

Indicators		Percentage
Age (years)		34.8
Sex (%)	Male	10.0
	Female	90.0
Education (%)	High school	6.7
	Tertiary education	93.3
Range of family income per year	30-60 million VND	6.7
	60-90 million VND	6.7
	90-180 million VND	16.7
	>180 million VND	70
Weekly food expenditure (thousand VND)	Mean	1006.7
	Min	500
	Max	2000

The consumption frequency and categories of Ban pork products is given in Table 2.

Table 2. Consumption frequency and Ban pork product categories

Indicators		Percentage
Consumption frequency (%)	Less than 2 weeks	25.0
	2 weeks to 2 months	57.1
	3 to 4 month	17.9
Categories of Ban pork products (%)	Live pigs	16.7
	Pig carcass	26.7
	Pork cuts	56.6

Interviewed consumers in the study area bought Ban pork fairly frequent. 57.1% of them bought pork every couple of weeks up to two months for household consumption. One fourth of them bought Ban pork weekly. These people often bought pork cuts of Ban pork two to three times a week or the whole carcass every two weeks, and used Ban pork instead of pork from exotic pigs sold in traditional markets. About 17.9% of interviewed consumers

consumed Ban pork three to four times per year at restaurants or only bought Ban pork on special days of the year.

The results show that purchasing pork cuts was the dominant consumption pattern by interviewed consumers in Hanoi. 56.6% of respondents often bought Ban pork cuts, followed by the consumption of pig carcass by 26.7% of respondents. These consumers bought the whole carcass of Ban pigs for weekly consumption. That finding was comparable with results by Huyen and Sautier, (2017) that consumers in Hanoi preferred a half to the whole carcasss of Black pigs from 20 to 25kg for consumption. Less than one-fifth of respondents in the current study purchased live pigs and slaughterred pigs themselves for ceremonies or on holidays of the year.

Figure 1 describes places of Ban pork consumption by interviewed consumers in Hanoi.

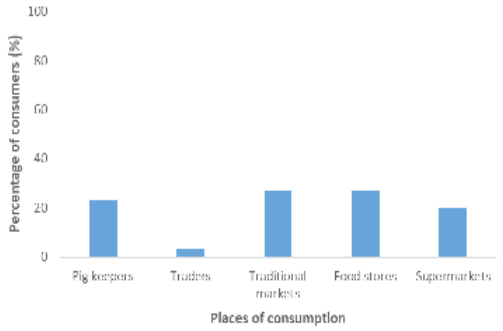

Figure 1. Places of Ban pork consumption

It is clear that green food stores and traditional markets were the most common places to buy Ban pork in Hanoi. 26.7% of respondents bought Ban pork at green food stores and traditional markets each. These consumers decided to buy Ban pork at green food stores because they trusted in the pork safety and sellers' reputation. On the other hand, the decision to purchasing Ban pork at traditional markets was due to short distances to their places. The consumption of Ban pork in green food stores is correlated with the results of Huyen *et al.* (2016) about the demand of food stores in Hanoi on Ban pigs. The authors also detected the difference of Ban pork products sold in food stores from traditional markets. Food stores often consumed pork from small Ban pigs at an average weight of 16.5kg rather than pork from heavy pigs selling by retailers in traditional markets. The authors also state that food store owners required rigorously for the quality of Ban pigs that Ban pigs should have a veterinary certificate and be kept in traditional conditions. This evidence partly explains why the interviewed consumers in the current study decided to buy Ban pork at food stores in spite of high prices.

23.3% of respondents purchased Ban pork from pig keepers, followed by the consumption of Ban pork at supermarkets by 20% of respondents. Only very small proportion bought Ban pork from traders owning to lack of the information about traders from production regions. In comparison to the study of Ha (2018), almost consumers in apartment buildings in Hanoi bought pork at supermarkets or acquainted people more frequently than in traditional markets. It was explained that they felt secured to buy pork from known people, or, they believed in the quality and safety of branded pork provided by large companies. This implies the necessity of a quality brand to boost consumers' beliefs in the quality of pork.

3.2. The preference of consumers for quality attributes of Ban pork

The preference of consumers for quality attributes of Ban pork is described in Table 3.

Table 3. The preference of consumers for quality attributes of Ban pork

Attributes	Consumer preference	
	Percentage	Mean±SD
Taste	76.67	3.9±1.6
Freshness	73.3	3.7±1.7
Leanness	70.0	3.6±1.3
Colour	60.0	2.8±1.7
Flavor	46.67	2.8±1.9
Tenderness	43.3	2.6±1.8
Juiciness	30.0	2.1±1.7

It can be seen that taste, leanness, freshness were most preferred traits of Ban pork by a majority of respondents. A total of 76.7% of interviewed consumers liked taste of Ban pork during cooking and eating as they supposed that Ban pork were tastier than other kinds of pork. More than 70% of respondents considered leanness and freshness at the point of purchase. The preference of urban consumers for lean pork is comparable with the findings found by other authors (Nga *et al.*, 2015; Huyen and Sautier, 2017; Ha, 2018). Huyen and Sautier (2017) found that consumers in Hanoi preferred lean parts of Black pork like buttock pork or shoulder lean meat, with more than 40% of lean meat. Ha (2018) indicated that Vietnamese pork eaters did not like fat as purchasing.

Other traits including colour, flavour, tenderness, juciness were less significant traits to almost interviewed consumers as purchasing Ban pork. Pork colour were considered by 60% of interviewees at the point of purchase, but its importance varied (Mean=2.8; SD=1.7). Some of them believed that pork with nice colour would be fresher. On the other hand, some of respondents were afraid of red pork colour as they thought that pig producers might use chemical substances to colour meat. According to Lapar *et al*. (2010), Vietnamese consumers supposed fresh pork with nice colour as newly slaughtered pork. Nevertheless, Muth *et al.* (2017) found the relevance between pork colour and pig breeds as well as the variance of colour by position of pork cuts. The authors indicate that the colours of pork from pure Ban pigs had been lighter and redder compared to that of hybrid pigs, and the colour also varied by positions of pork cuts (Muth *et al*., 2017).

Flavour and tenderness of Ban pork were responded by 40% of interviewed consumers. They said that Ban pork had better flavour and tenderness compared to other kinds of pork as cooking and eating. That finding was similar to results by Phuong *et al.* (2013); Huyen *et al.* (2016); Muth *et al.* (2017). These attributes are only recognized after purchasing, or during cooking (Muth *et al*., 2017). Juiciness was used as a cue to evaluate the quality of Ban pork by only 30% of respondents (Mean=2.1; SD=1.7). For these people, they distinguished Ban pork from other kind of pork based on the water amount pork produced. They thought that Ban pork were drier and produced little water during cooking rather than pork from exotic pigs.

4. CONCLUSION AND RECOMMENDATIONS

Ban pork consumers in Hanoi belonged to a group of high family income and literacy. They mainly purchased slaughterred pigs more regular than live Ban pigs. Green food stores, traditional markets, and pig keepers were the most popular places of Ban pork consumption in Hanoi.

Leanness, freshness, colour were most significant quality traits of Ban pork preferred by interviewed consumers at the point of purchase. Taste was the most significant attribute after the purchase. Flavour, tenderness and juiciness had less significance than other traits as purchasing Ban pork, but useful to distinguish Ban pork from other kinds of pork after the purchase. Marketing for Ban pork via a tasting program and branding for Ban pork will help consumers to trust on the products at purchase as many of quality attributes can only be recognized after being bought or cooked.

5. ACKNOWLEDGEMENTS

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EFFICACY OF TMR FEEDING METHOD IN DAIRY HUSBANDRY ON MILK YIELD AND QUALITY ON HOUSEHOLD FARMS

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ABSTRACT

The aim of this study was to evaluate the efficacy of TMR feeding method in dairy husbandry on milk yield and quality under natural condition of household dairy farms in Ho Chi Minh City from Feb to Jun 2019. The experiment was arranged into a completely randomized block design with 2 feeding methods (Control treatment with cows fed with the traditional feeding method and TMR treatment with cows fed with the TMR feeding method), and lasted 3 months. Twenty four HF crossbred cows from the 2nd to 3rd parities were divided into 2 treatments. The results showed that the average milk yield/day of cows fed with the traditional feeding method was 17.88 kg/cow/day and lower than that of cows fed with the TMR feeding method of 21.32 kg/cow/day ($P \leq 0.01$). There were significant improvements in milk quality in TMR feeding method ($P \leq 0.05$). The dry matter intake/kg milk of cows fed in the control treatment (0.86kg DMI/kg milk) was higher than that of cows in the TMR treatment (0.77kg DMI/kg milk) ($P \leq 0.05$). The feed cost for producing 1kg milk decreased about 863.7 VND/kg milk in the TMR treatment. The rate of digestive diseases of cows in the control treatment (25.00%) was higher than that in the TMR treatment (8.33%) ($P \leq 0.05$). In brief, TMR feeding method improved milk yield, milk quality and DMI/kg milk, as well as reduced feed cost and prevalence of digestive diseases in dairy cows.

Keywords: *Dry matter intake/kg milk, milk quality, milk yield and TMR feeding method.*

1. INTRODUCTION

The popular feeding method in household dairy farms is the traditional method of 97.5% and much higher than the TMR method of only 2.5% (Nguyen Thanh Hai, 2017), which is not the suitable supplying way of nutrients for physiological characteristics of dairy cows. This is one of the main causes for lameness, post-partum anestrus, hard conception, post-partum infertility, low lactation peak and reduction of milk quality (Lieu Kieu, 2017). Therefore, the average milk yield in household dairy farms with the traditional feeding method in Ho Chi Minh City is very low with 11.98 kg/cow/day (Nguyen Vo Thu Truc *et al*., 2017).

Total mixed ration (TMR) is a method of feeding cows that combines feeds formulated with specific nutrients into a complete feed mixture. It contains roughage, concentrate and feed additives which are the most suitable for dairy cows with the balanced and adequate diet of nutrients (energy, protein, minerals, vitamins and etc). TMR helps stabilize rumen pH and the ecosystem, improving better feed efficiency and reducing digestive diseases. It also improves fertility, body condition score, milk productivity and quality, and lactation durability. Therefore, farmers will effectively control amount of feed used through fluctuation in daily milk yield and adjust the suitable ration to cow demand (Wongnen *et al*., 2009; Mai Thi Ha *et al*., 2011; Lammers *et al*., 2016). The first study of TMR feeding method on the intensive and large-scale dairy farms in Ho Chi Minh City showed that it increased milk yield from 2.9-6.9 kg/cow/day, reached peak milk soon at the end of the 2nd month of lactation circle, and maintained the lactation peak period over 60 days (Lieu Kieu, 2017).

In order to find the suitable feeding methods for household condition to improve production capacity, production cost and economic efficiency is essential. It requires specific calculations in both economic and technical terms in order to find the optimal feeding method that meets dairy cows'

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requirements of nutrients to achieve the highest economic efficiency in the present household farm conditions. It is necessary to have some field studies on a household scale to assess accurately the efficacy of TMR feeding method.

2. MATERIALS AND METHODS

2.1. Location

The experiment was conducted at the household dairy farm of Phan Thanh Thuan in Dong Thanh Commune, Hoc Mon District, Ho Chi Minh City from Feb to Jun, 2019.

2.2. Experimental design, animals and housing

The study was arranged into a completely randomized block design with 2 treatments of feeding methods. The treatments included (1) control with 12 cows fed with the traditional feeding method and (2) TMR with 12 cows fed with the total mixed ration feeding method. The cows were housed in the same cubical barn with rubber mats on the concrete floor. Twenty four HF crossbred cows with parities from the 2nd to 3rd were divided into 2 treatments with 12 cows/treatment and lasted 3 months (90 days). Cows in 2 treatments prior to the experiment period were almost equal at parity, live weight and milk yield (P>0.05, Table 1).

Table 1. Experimental design

Treatment	Control	TMR
Replication	12	12
1st-3rd lactation month	4	4
4th-6th lactation month	4	4
7th-10th lactation month	4	4
Parity (*P=0.54*)	2.36±0.63	2.51±0.61
Live wt (kg), *P=0.91*	498.4±30.82	501.8±29.64
MY (kg/day), *P=0.86*	16.38±2.67	16.25±2.95

2.3. Daily ration of cows

All cows were fed twice a day with 2 different feeding methods (7:00 and 14:30 every day) and *ad libitum*. All cows received the same basal diet, which was calculated to supply roughly 15.36kg dry matter (DM)/cow/day with the same variety of ingredients available in the local area (3% DM requirement according to NRC, 2001), namely king grass, rice straw, corn silage, complete feed, cassava pulp, brewers grain and molasses (Table 2). All cows were washed and cleaned twice a day, and the house was cleaned twice a day before milking. Drinking water for cows came from well, and each treatments had 2 drinking troughs having free access to water and was cleaned every day.

Table 2. Ingredients of daily diets

Ingredients	Feed intake (35% DM, kg)	Dry matter intake (kg)	Rate (%)
King grass	20.5	3.67	23.89
Rice straw	1.0	0.91	5.92
Corn silage	6.0	1.88	12.26
Complete feed	6.5	5.72	37.25
Cassava pulp	4.0	0.52	3.41
Brewers grain	6.5	1.37	8.88
Coconut meal	1.0	0.91	5.93
Molasses	0.5	0.38	2.44
Roughage feed	27.5	6.46	42.08
Concentrate feed	18.5	8.89	57.91
Total	46.0	15.36	100

2.4. Sample collection and measurements

Milk yield: All cows were milked into a specialized bucket twice a day (7:30 and 15:00), using scale 30kg for weighing individually, and then merging two times into average milk yield/cow/day.

Milk quality: Take one milk sample per cow at the end of month to analyze milk quality (fat, protein and solid non fat) each treatment. The sampling method of fresh milk was applied according to TCVN 6400:2010 with 200ml/sample. Milk samples must be stored in cool condition at 2-6°C and transported quickly to the laboratory. The milk quality indicators were quickly analyzed by Ekomilk Total machine about 60 seconds/sample in the testing and analyzing laboratory of breeds, Center for Plant, Livestock and Aquaculture Breeds.

Dry matter intake/kg of milk (DMI/kg milk): The total amount of feed for each treatment

was recorded before feeding and refusal feed was collected in the early morning for calculation the DMI/day. Then, apply the equation to calculate DMI/kg milk as the total dry matter intake per day each treatment divided by the total milk yield each treatment.

Feed cost/kg milk (VND/kg milk): Feed cost for producing 1kg milk was calculated as total cost in a comsumption ration of each treatment divided by cow's milk production/day (kg).

Digestive diseases: All cows were observed and recorded about all issues related to digestive diseases in the experimental period to calculate the percentage of digestive diseases. In particular, the percentage of digestive diseases were calculated as the amount of digestive disease cows in the experiment period divided by the total cows each treatment x 100.

2.5. Statistical analysis

Data were analyzed as a completely randomized block factorial design by ANOVA using the GLM procedure of Minitab 16.2. The individual cow was considered the experimental unit for all the parameters. The average values were compared by the Tukey test and the % were compared with the χ^2 test, the differences were considered significant at P≤0.05.

3. RESULTS AND DISCUSSION

3.1. Milk yield

The average milk yield (MY)/day of cows fed with the traditional feeding method was 17.88 kg/cow/day, lower than that of cows fed with the TMR of 21.32 kg/cow/day (P=0.01). This result is appropriate with the result of TMR feeding trial program with Israel Technology of the Center for Plant, Animal and Aquatic Breeds in Ho Chi Minh City, MY increased 2.9-6.9 kg/cow/day with TMR feeding method compared to the traditional feeding method (Lieu Kieu, 2017). Thus, the feeding method had a great influence on digestion and improved milk productivity of dairy cows (Mai Thi Ha *et al*., 2011; Duong Nguyen Khang and Tran Xuan Lam, 2019). The reason for this may be that TMR method had benefits on rumen microorganisms, palatability, dry matter intake, and increased milk yield (David, 2017; Jim, 2019; Truong La, 2019).

Table 3. Milk yield during experimental period

Treatment	n	Mean±SD	CV (%)	SEM	P
Control	12	17.88^{a}±3.21	6.85	0.02	0.01
TMR	12	21.32^{b}±2.54	5.2		

Means within a column with different superscript letters differ (P≤0.05)

3.2. Milk quality

The percentage of milk fat of cows fed with the traditional feeding method was 3.46%, lower than that of cows fed with the TMR of 3.72% (P=0.05), a growth of 7.5%. Milk protein of cows fed with the traditional feeding method was 3.98%, lower than that of cows fed TMR method of 4.39% (P=0.04). Solid non fat (SNF) in milk of cows in the control treatment was 8.37% and lower than that of cows in the TMR treatment of 8.98% (P=0.04). These results showed that there were significant improvements in milk quality of three indicators (P≤0.05). According to research by Schroeder *et al*. (2003), the standard milk yield and milk protein with TMR method were higher than those of traditional feeding method of 3.70 and 3.45%, respectively. Therefore, TMR method improved milk quality compared to traditional method (Mai Thi Ha *et al*., 2011; Duong Nguyen Khang and Tran Xuan Lam, 2019).

Table 4. Effect of 2 feeding methods on the milk compositions during experimental period (n=12)

Treatment	Control	TMR	SEM	P
Fat	3.46^{a}±1.45	3.72^{b}±1.21	0.12	0.05
Protein	3.98^{a}±0.49	4.39^{b}±0.37	0.06	0.04
SNF	8.37^{a}±1.09	8.98^{b}±1.23	0.08	0.04

3.3. Dry matter intake

The DMI/day/cow fed with the traditional feeding method (Control) was 15.69kg and lower than that of cows fed with the TMR of 16.43% (P=0.06), a growth of 0.74kg DMI/day. Thus, our studied results were suitable with previous surveyed results, DMI was increased about 0.5-3.6kg DMI/day compared to cows fed only fresh grass (Kolver and Muller, 1998) and can be increased 4% (Le Dang Danh, 2015). However, the DMI/kg milk of cows in the control treatment was 0.86kg, higher than that of cows in the TMR treatment of 0.77kg DMI/kg milk (P=0.04), a decline of 0.09kg DMI/kg milk. This may be because TMR feeding method lowers the risk of digestive upset, stabilizes rumen pH and optimizes rumen feed digestion, resulting in better fermentation activities of rumen microorganisms as well as higher feed efficiency (Lammers *et al*., 2016).

Table 5. The dry matter intake (n=12, cows)

Treatment	Control	TMR	SEM	P
DMI/day	15.69±3.01	16.43±2.78	0.57	0.06
DMI/kg milk	0.86^{a}±0.29	0.77^{b}±0.31	0.01	0.04

3.4. Feed cost per kg of milk

Feed cost for producing 1kg milk of cows fed with traditional feeding method was 7,094.5 VND, higher than that of cows fed with TMR method of 6,230.8 VND, a decrease of 863.7 VND/kg milk. This showed that using TMR feeding method would bring higher economic efficiency than the traditional feeding method because it helped reduce about 12.17% of feed cost/kg milk, and it was suitable with previous experiments of Mai Thi Ha *et al*. (2011) and Lammers *et al*. (2016).

Table 6. Effect of 2 feeding methods on feed cost/kg milk (VND/kg milk)

Ingredients	Price/kg (VNĐ)	Control		TMR	
		Quantity (kg/day)	Total amount (VND)	Quantity (kg/day)	Total amount (VND)
King grass	500	20.9	10,472.8	21.9	10,967.5
Rice straw	1,100	1.0	1,123.9	1.1	1,177.0
Corn silage	2,200	6.1	13,487.0	6.4	14,124.0
Complete feed	7,800	6.6	51,802.2	7.0	54,249.0
Cassava pulp	2,300	4.1	9,400.0	4.3	9,844.0
Brewers grain	4,900	6.6	32,542.4	7.0	34,079.5
Coconut meal	5,000	1.0	5,108.7	1.1	5,350.0
Molasses	5,700	0.5	2,912.0	0.5	3,049.5
Feed cost/cow/day			126,848.9		132,840.5
Feed cost/kg milk			7,094.5		6,230.8

3.5. Digestive diseases

This is an important indicator to assess the health status of cows during the experiment in specific and the practice in general. There were three cases of digestive disorder diseases (diarrhea with bubbles in liquid feces) in the control treatment which accounted for 25.00%, higher than that of TMR treatment, which had one case of digestive disorder disease of 8.33% (P=0.05). This showed that TMR feeding method reduced the disorder risk in the gastrointestinal tract and gastrointestinal diseases for milking cows and it was appropriate with the experiments of Duong Nguyen Khang and Tran Xuan Lam (2019) and the judgment of Le Dang Danh (2015).

Table 7. Cows with digestive disease (%)

Treatment	n cows	Cows with digestive disease	%	P
Control	12	3	25.00^{a}	0.05
TMR	12	1	8.33^{b}	

4. CONCLUSIONS

TMR feeding method improved the average milk yield/day, milk quality, DMI and feed efficiency. Besides, it reduced DMI/kg milk, gastrointestinal diseases and feed cost for producing 1kg milk, bringing higher economic efficiency.

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EVALUATION OF THE TOTAL TRACT APPARENT DIGESTIBILITY AND N RETENTION IN GROWING PIGS GIVEN DIETS WITH SUPPLEMENT OF COCONUT OIL AND CATFISH BY-PRODUCTS

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ABSTRACT

The coefficients of total tract apparent digestibility of nutrient and nitrogen retention of diets with four different coconut oil levels were determined in 4 LY growing pigs with average body weight of 17±0.5kg at start. The four diets were based on maize and soybean meal (SBM); maize and head, bone and skin catfish by-products and supplementation of coconut oil at level 3% (HBSCO3), 6% (HBSCO6) and 9% (HBSCO9) in dry matter. The four diets were fed to four growing pigs in a 4x4 Latin Square design and was conducted in the experimental station of An Giang University. The average daily dry matter and crude protein intake of SBM diet were higher than in catfish by-product diets (P<0.05), while among the head, bone and skin catfish by-product diets which supplementation at 3% of coconut oil were lower than in at 6 and 9% (P<0.05). In contrast, average daily ether extracted was lower in SBM diet than in HBSCO diets (P<0.05). There were differences in the total tract apparent digestibility of nutrients among the diets (P<0.05). In which, dry matter and crude protein was higher in the diet SBM than in the HBSCO3, HBSCO6 and HBSCO9 diets, but ether extracted in the diet HBSCO9 and HBSCO6 were higher HBSCO3 and SBM diets (P<0.05). There were no significantly different in feces and urine nitrogen, percentage of total nitrogen intake and percentage of nitrogen digested between diets. There was significant differences in nitrogen intake and retained. Nitrogen intake of MSB diet was higher than other diets (P<0.05), while both HBSCO6 and HBSCO9 diets was similar, but was higher than HBSCO3 diet (P<0.05). There was no significant in nitrogen retained among HBSCO3, HBSCO6 and HBSCO9 treatment, but was lower than MSB treatment (P<0.05).

Key words: *Catfish by-product, coconut oil.*

1. INTRODUCTION

Pig production in Vietnam and Mekong delta in 2018 was 28,151,900 and 3,456,400 head/year (General Statistics office of Viet Nam, 2018). Smallholder farms in Mekong delta utilized nutrient sources from crop production and vegetables for pig production as sustainable (Kamakawa *et al*., 2003). In 2018, the Tra catfish production had increased dramatically estimated 5.400 ha in Viet Nam and exported of fish fillet about 1.42 million tons (Viet Nam News Agency, 2019), resulting catfish by-product was abundant. The by-products of catfish processing, include heads, skin and viscera, which are the main products, and account for almost 60% of the volume that enters catfish processing factories (Richard and Lovell, 1980). According to Bui Xuan Men (2005), the CP content in the residue meal is not influenced by the processing method of the catfish by-product. This means that catfish by-product is a potential source of nutrients of high value for livestock feed. The present experiment was carried out to determine the total tract apparent digestibility of nutrients and nitrogen retention in diets based on maize, SBM and HBSCO at 3, 6 and 9% on growing pigs.

2. MATERIALS AND METHODS

2.1. Animals and management

The pigs used to conduct in the experiment were bought from a private farm in An Giang Province. Four LY castrated pigs with average body weight at 60 days of age of 17±0.5kg

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were used. All pigs were vaccinated against hog cholera and foot and mouth disease and were treated against round worms before starting the experiment. The pigs were kept in metabolism cages (0.8x0.8m) made of wood and bamboo and designed to allow recording of feed intake and separate collection of feces, urine and to access feed and water freely. The experiment was conducted in the Experimental Station of An Giang University.

2.2. Experimental design

The apparent digestibility experiment was designed as a 4x4 Latin Square with 14 days for each period and 3 days of inteval periods, all pigs were fed only maize. The first seven days of each periods were for adaptation to the experimental diets, which were fed *ad libitum*. For the last seven days the amount of offered feed was reduced to 90% of the previous FI, and the last 5 days were for feces and urine collection.

The treatments were Control treatment (SBM): Maize meal was mixed with soybean meal and half of percent of a premix of minerals and vitamins; Treatment HBSCO3: Maize meal was mixed with head, bone and skin catfish by-product meal and a 0.5 premix of minerals and vitamins; and plus 3% coconut oil; Treatment HBSCO6: Maize meal was mixed with head, bone and skin catfish by-product meal and a half of percent of a premix of minerals and vitamins; and plus 6% coconut oil; and treatment HBSCO9: Maize meal was mixed with head, bone and skin catfish by-product meal and 0.5% of a premix of minerals and vitamins; and plus 9% of coconut oil.

During the last five days of each period, the amount of offered and refused feed was recorded to calculate feed intake (FI). Samples of feed offered, feces and urine of individual pigs were collected in the morning and stored at -4^{o}C. At the end of the experiment, total samples were pooled and sub-samples taken for analysis.

Samples of feed and feces were analyzed for DM, ether extracted (EE) and ash according to the standard methods of AOAC (1990), and CP was determined by the Kjeldahl procedure.

2.3. Statistical analysis

The data for apparent digestibility of dry matter, crude protein, ether extracted and ash were analyzed as a Latin Square design by using the GLM of the ANOVA procedure of the Minitab 16. The Tukey test for pair-wise comparisons was used to separate means when the differences were significant at 0.05.

3. RESULTS AND DISCUSSION

3.1. Ingredient and chemical composition of the diets

The diets were based on maize meal, soybean meal (SBM), head, bone and skin catfish by-products and supplementation of coconut oil (HBSCO) at level 3, 6 and 9% in DM; and plus at 0.5% of premix vitamins and minerals. The DM content among the diets were different, ranging from HBSCO6, HBSCO9, HBSCO3 and SBM ($P<0.05$). Moreover, there were differences among the diets in CP content, that was lowest in SBM diet and highest in HBSCO3 and HBSCO9. In comparison with ether extracted and ash content among diets, the SBM diet were lower than catfish by-product diets, however between diets which supplemented higher level of coconut oil was higher ether extracted and ash content.

The DM content in the diets of present experiment was slightly higher than found in the previous study carried out in An Giang province by Tuan and Brian (2018). The probable explanation for difference is catfish by-products input used in this trial being different from processing procedures of factory in each catfish by-product meal. However, the CP content in these diets were quite lower in HBSCO3 and HBSCO6 (14.5 and 14.3%) than compared with head and

bone diet (18.3%) by Tuan and Brian (2018), it can be explained that head, bone and skin catfish by-product containing high ash, therefore, to reduce ash level in diets by used less catfish by-product meal ratio resulting to low CP level in diets and lower ash content in HBSCO3 and HBSCO9 (10.1 and 13.3%) compared with 20.3% of ash content of head and bone diet by Tuan and Brian (2018). Moreover, ether extracted content among treatments was different by adding coconut oil with different levels in diets.

Table 1. Ingredient and chemical composition (%) of the experimental diets

Theme	Item	SBM	HBSCO3	HBSCO6	HBSCO9	P	SE
Ingredient	Maize meal	82	76	72	68.5		
	Soybean meal	17.5	0	0	0		
	Head, bone and skin	0	20.5	21.5	22		
	Coconut oil (%in DM)	0	3	6	9		
	Premix vitamin+mineral	0.5	0.5	0.5	0.5		
	Total	100	100	100	100		
Chemical composition of diets (%, dry matter basis)	Dry matter	88.8^{c}	89.8^{b}	90.1^{a}	89.8^{b}	0.001	0.11
	Crude protein	14.1^{c}	14.5^{a}	14.3^{b}	14.5^{a}	0.001	0.04
	Ether extracted	3.69^{d}	6.85^{c}	9.17^{b}	11.9^{a}	0.001	0.17
	Ash	3.49^{c}	10.1^{b}	10.2^{b}	13.1^{a}	0.001	0.62

Means within row with different letters differ significantly (P<0.05).

3.2. Coefficients of total tract apparent digestibility

The average daily DM and CP intake of SBM diet were higher than in diets of catfish by-product (P<0.05), while among the HBSCO at 3% of coconut oil were lower than in at 6 and 9% (P<0.05). In contrast, average daily ether extracted and ash intake of SBM diet were lower than in HBSCO diets (P<0.05), there was higher in comparison with between the HBSCO diets when increasing supplementation of coconut oil at 3, 6 and 9% were increased (P<0.05).

There were differences in the coefficients of total tract apparent digestibility (CTTAD) of dry matter, crude protein, ether extracted and ash among the diets (P<0.05). In which, there was no significant in the dry matter and crude protein among HBSCO3, HBSCO6 and HBSCO9 diets, but were significantly lower than SBM diet (P<0.05). Moreover, there was no significantly different in ether extracted between HBSCO6 and HBSCO9 diets, but apparent digestibility of ether extracted in these diets was higher than HBSCO3 diet, and the SBM diet was significantly lowest (P<0.05).

Table 2. Nutrients intake (g) and coefficients of total tract apparent digestibility (%) of diets in pigs

Theme	Item	SBM	HBSCO3	HBSCO6	HBSCO9	P	SE
Daily Intake (g)	DM	577^{a}	436^{c}	505^{b}	495^{b}	<0.001	9.23
	CP	81.2^{a}	63.0^{c}	72.2^{b}	71.7^{b}	<0.001	1.30
	EE	21.1^{d}	29.8^{c}	46.0^{b}	58.6^{a}	<0.001	0.86
	Ash	21.3^{c}	44.8^{b}	51.7^{b}	66.7^{a}	<0.001	2.67
Coefficients of total tract apparent Digestibility (%)	DM	85.7^{a}	80.5^{b}	82.1^{b}	81.2^{b}	<0.001	0.55
	CP	80.8^{a}	76.4^{b}	78.3^{b}	77.3^{b}	<0.001	0.34
	EE	76.7^{c}	83.5^{b}	87.3^{a}	90.0^{a}	<0.001	0.76
	Ash	70.9^{a}	65.9^{b}	68.3^{ab}	67.3^{ab}	0.066	1.34

Average daily dry mater intake in SBM diet (577 g/d) was higher than catfish by-product diets (436, 505 and 495 g/d), this could be explained that SBM diet was contained less CP and ether extracted than the other diets, 14.1 and 3.39% compared to 14.5 and 6.85%, 14.3 and 9.17%, 14.5 and 11.9%, respectively, therefore pigs tried to adjust their FI. Furthermore, there were positive relationships between apparent digestibility of DM and CP and the DM intake Tuan and Brian (2018). Pomar *et al.* (2003) and Speedy (1997) confirmed that voluntary FI is limited when the feed is unbalanced. In the case of DM intake in HBSCO3 was lower than HBSCO6 and HBSCO9 with 436 g/d in comparison to 505 and 495 g/d. The probable explanation for the difference is coconut oil supplementation when increasing coconut oil level in diets (3 to 6%) dry matter intake was increased, but up to 9% resulting high in ash intake from 44.8, 51.7 and 66.7 g/d, therefore DM intake in HBSCO9 diet was slightly reduce by adjusting FI of pigs.

The total tract apparent digestibility of DM (85.7, 80.5, 82.1 and 81.2% of SBM; HBSCO3, HBSCO6 and HBSCO9, respectively) in present study was slightly higher than in comparison with previous study (77,5%) by Tuan and Brian (2018) on head and bone catfish by-products meal. This could be explaned for such as ash content in diets differs. Noblet and Perez (1993) reported that high ash content in diets, nutrients was lower fecal apparent digestibility (65-95%). The total tract apparent digestibility of crude protein in this experiment was equivalent to the previous trial (75.9%) by Tuan and Brian (2018) and (77.8 and 78.8% of catfish by-product meal and ensiled catfish by-product) was reported by Thuy *et al.* (2011). These results were also agreement with previous studies by Jørgensen *et al.* (1984) and Knabe *et al.* (1989) on diets containing fish meal and meat and bone meal. The total tract apparent digestibility of ether extracted was increased when dietary fat content had higher, which can be explained that a low fat content in diet was increased secretion of fecal endogenous fat, resulting lower digestion. The results of HBSCO3, HBSCO6 and HBSCO9 diets (83.5, 87.3 and 90.0%) were higher than head and bone catfish by-product diet (79.5%) reported by Tuan and Brian (2018) and catfish by-product, ensiled catfish by-product and processing waste water (71.9, 72.0 and 73.2) found by Thuy *et al.* (2011).

3.3. Nitrogen retention

There were no significantly different in nitrogen feces and urine, percentage of total nitrogen intake and digested between diets. However, there was significant differences in nitrogen intake and retained. In which, nitrogen intake of MSB diets was higher than other diets (P<0.05), while both HBSCO6 and HBSCO9 diets was similar, but was higher than HBSCO3 diet. Moreover, there was no significant in nitrogen retained among HBSCO3, HBSCO6 and HBSCO9 treatment, but was lower than MSB treatment (P<0.05) (Table 3).

Table 3. Nitrogen balance in pigs fed head, bone and skin catfish by-product supplemented coconut oil

Item	SBM	HBSCO3	HBSCO6	HBSCO9	P	SE
N Intake	13.0[a]	10.1[c]	11.6[b]	11.5[b]	<0.001	0.21
N feces	2.47	2.34	2.49	2.59	0.180	0.08
N Urine	4.50	3.68	4.25	4.46	0.090	0.25
N Retained	6.01[a]	4.06[b]	4.80[b]	4.44[b]	<0.001	0.25
% of total N intake	45.8	39.0	41.5	38.9	0.081	2.12
% of N digested	56.7	50.8	53.0	50.2	0.309	2.64

The data showed that there was not affected on nitrogen retention when diets were supplemented coconut oil. This study was lower than the reported from Hieu and Terdsak (2014) (ranging from 6.8 to 7.9 g/ day) that diets based on soybean, fish meal, yellow maize and supplemented palm oil from 3 to 12% and Chhay *et al.* (2003) found that there was not effect on nitrogen retention when supplemented palm oil at 5, 10 and 15% into growing pig diets. This can be explaned that low nitrogen retention in this result probable due to nutritive value of protein that connected composition of amino acids. Thuy *et al*. (2007) found that amino acid composition in head and bone catfish by-product is less well balanced.

4. CONCLUSIONS

It can be, therefore, concluded that there were different in nutrient intake among diets. In which, DM and CP intake of SBM were highest and ether extracted was lowest, in contrast dry matter and crude protein of HBSCO3 diet were lowest. The total tract apparent digestibility of dry matter and crude protein in SBM diet were higher than others, while ether extracted of HBSCO6 and HBSCO9 diets were highest. Nitrogen retention among catfish by-product diets was non-significant, but lower than SBM diet.

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PREVALENCE OF REPRODUCTIVE DISEASES CATTLE UNDER HOUSEHOLD CONDITION IN CHO GAO, TIEN GIANG

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ABSTRACT

The study was conducted on beef cattle at households in Cho Gao district, Tien Giang province by investigating 396 cows of 111 households to determine the reproductive disease situation using clinical diagnosis and ultrasound technic to identify some common ovarian diseases that cause delayed reproduction. It was shown that the conception rate in cows was 74.0% with the average mating coefficient of 1.35. The percentage of reproductive diseases in cows was 35.6% and the percentage of cows with delayed reproduction was 27.3%. Ovarian diseases of cows were identified by three forms including underdeveloped ovaries (31.8%), cysts (31.8%), and corpus luteum (36.4%). Differences in frequencies in the breed group and disorders were also identified with the highest proportion being Brahman breed (37.4%) and the predominant disorder being calving difficulty (37.4%).

Keywords: *Beef cattle, ovarian disorders, reproductive diseases.*

1. INTRODUCTION

In recent years, the cattle industry in Tien Giang province has developed rapidly to become a high-income source for farmers. As part of restructuring the province's cattle, efforts have been made to raise the cows' meat production and reproduction. However, diseases related to reproductive disorders in cows responsible for remarkable economic losses to farmers still exist (Talukder *et al*., 2005; Rabbani *et al*., 2010; Fareed *et al*., 2016). In this context, major problems that have a direct impact on the reproductive performance of cows are abortion, dystocia, retained placenta (retention of the fetal membrane), metritis, prolapse (uterine and/or vagina), anoestrus and repeat breeder. These result in considerable economic loss to the cattle industry due to slower uterine involution, reduced reproductive rate, prolonged inter-conception and calving interval, negative effect on fertility, increased cost of medication, reduced calf crop and early depreciation of potentially used cows (Lobago *et al*., 2006). Although there have been attempts to improve the reproductive efficacy of cattle, the incidences of reproductive disorders in bovine have still been increasing over the years in Tien Giang province. The exact cause of this may not be evident and is often complicated by multiple causative agents. Thus, there is a need for a multi-faceted approach via investigation to understand the effects of various factors related to reproductive disorders in cattle. From the results of this study, it is possible to propose prevention methods related to epidemiological factors to limit the rate of reproductive disorders in cattle.

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2. MATERIALS AND METHODS

2.1. Materials

A total of 396 beef-type cows showing a decided tendency syndrome toward slow breeding were investigated from 111 households at Cho Gao, Tien Giang province.

2.2. Methods

A questionnaire was prepared according to the objectives of the investigation and was designed in a simple way so that the farmers could understand easily. It included questions to collect information on breed and reproductive diseases (metritis, mastitis, vaginitis, miscarriage, difficult calving), other diseases, etc. The data were collected

directly from the owner by interviewing and examination of records. Cows seemed to suffer from delayed puberty, repeated breeding, post-calving anoestrus, and metritis were examined by per rectal palpation of genital tracts and ultrasound (using a real-time B-mode ultrasound scanner come in 5.0 and 7.5 MHz transducers) to confirm the diagnosis. Ultrasound is the most common approach for scanning bovine reproductive organs per rectum, using a transrectal transducer and a linear-array scanner. Accordingly, a 5.0 MHz is a general-purpose one, providing reasonably detailed images of ovaries and uterus, whereas a 7.5 MHz transducer will give minimum tissue penetration yet maximum resolution.

2.3. Statistical analysis

Chi-square test (c^2) with statistical significance set at 95% confidence level was used for statistical comparisons of prevalence. Chi-square was also used for comparison of proportions.

3. RESULTS AND DISCUSSIONS

3.1. Conception rate and fertility coefficient on cows by breed group

The surveying results on 396 beef cows at 111 households were recorded with 393 mating times and 291 pregnant cows as shown in Tab 1. The conception rate in the local cow breed was the highest (85.2%), followed by Charolais (76.4%), Red Angus (73.6%), Brahman (68.6%) and Lai Sind breed (62.4%), the lowest proportion was BBB breed (45.5%).

The cause of the uneven pregnancy rate was due to different breeds and even individuals of the same breed have different reproductive abilities. However, the genetic coefficient and fertility were very low, and therefore the differences in reproduction were mainly caused by externalities through interaction with the genetic basis of each breed and individual cows. Breeds or individuals that were highly adaptable to the climate and well-tolerated in a specific environment will have a higher reproductive capacity (Nguyen Xuan Trach, 2003).

Table 1. Conception rate of cows by breed

Breed	Pregnant cows	Mating time	Mating coefficient	Conception rate (%)
BBB	10	22	2.20	45.5
Brahman	24	35	1.46	68.6
Charolais	68	89	1.30	76.4
Local	69	81	1.17	85.2
Lai Sind	53	85	1.60	62.4
Red Angus	67	91	1.36	73.6
Total	291	393	1.35	74.0

The investigation of this study also showed that the mating coefficient was not uniform among breed groups. The average mating coefficient was 1.35 times, in which the highest mating coefficient was the BBB breed (2.2 times), the lowest was the local breed (1.17 times). In general, the results of this research were consistent with the studies of Dinh Van Cai and Nguyen Ngoc Tan (2007). In fact, it takes an average of 1.5 to 2.0 times mating for a conception per cow. When this number was higher, the fertility situation was not normal. Mating ratio and uneven pregnancy rate were influenced by many factors such as nutritional rations, raising methods, less movement of cow, hormonal imbalance and other diseases could have effects on fertility and production of cows. In addition, weak nutrition for cattle diet will be susceptible to diseases, thus reducing fertility. On the contrary, if the nutrition, especially glucid, was high, it will cause an increase in fat in the body and accumulated fat in the ovaries, thus reducing the reproductive function (Nguyen Xuan Trach, 2003).

3.2. Reproductive disease situation in the beef-rearing herd

During the study period, a total of 396 beef cattle were surveyed for reproductive diseases, of which the Lai Sind breed had the highest rate of reproductive diseases. Specificially, the rates were 40 cases with 47.1%, followed by Brahman breed with 15 cows with a rate of 42.9%. Charolais breed had 33 cows with the rate of 36.3%, Red Angus breed had 33 cows with the rate of 35.9% and the local breed had 18 cows infected with the rate of 22.2%, BBB breed had 2 cases with the lowest rate at 16.7%. The mentioned results

showed that the rate of reproductive diseases of cow breeds was quite high. The differences in the prevalence of reproductive disease were due to the technical stage, environment, age of different cow breeds, leading to disparities. Besides, due to crossbred generation and a high proportion of crossbred blood breeds in cows that were raised in hot weather in Vietnam, it was easy to get reproductive diseases (Dinh Van Cai, 2007).

Table 2. Prevalence of reproductive diseases by breed

Breed	No. of household	Reproductive cows		Reproductive disorder cows	
		n	Rate (%)	n	Rate (%)
BBB	3	12	3.0	2	16.7^{ab}
Brahman	11	35	8.8	15	42.9^{a}
Charolais	23	91	23.0	33	36.3^{a}
Local	16	81	20.5	18	22.2^{b}
Lai Sind	33	85	21.5	40	47.1^{a}
Red Angus	25	92	23.2	33	35.9^{a}
Total	111	396	100	141	35.6

Different characters in the same column represent significant differences (P<0.05)

On the other hand, the physiological changes at the end of pregnancy, due to the large fetal compression and reduced bowel motility, caused stagnation of urine in the open cervical bladder, which was also one of the causes. Most cases of uterine inflammation are because of microorganisms, which are often present in the environment and damage the uterus or vagina when cows reproduce. In addition, when genital organs were infected during delivery perhaps due to mating and the weakness and stress of the female cattle, it can also lead to reproductive diseases (Tran Van Du, 2007).

3.3. The prevalence of reproductive diseases by parity

According to the survey on 396 beef cattle, 141 cases were recorded with the different percentages among parities which are presented in Tab 3. The rate of reproductive diseases in cows by parity was changed slightly, in which the highest proportion was found in the higher parity of 6 with 26 cases, accounting for 46.4%, followed by parity groups including 1 and 2, 3 and 4, 5 and 6 with the cases of reproductive diseases accounting for 33.1, 34.1 and 34.8%, respectively. However, this difference was not statistically significant (p>0.05). These results showed that the investigated beef cattle were at a high risk of reproductive diseases and the rate of disease appears to increase by parity. In fact, the results indicated that breed cows with many parities had a relatively high rate of reproductive diseases, which were also caused by inappropriate nutrition, and breeding techniques.

Table 3. Reproductive disease in different parity

Parity	No. of observation	No. of infection	Rate (%)
1 and 2	145	48	33.1
3 and 4	129	44	34.1
5 and 6	66	23	34.8
> 6	56	26	46.4
Total	396	141	35.6

In addition, the low conception rate was due to the uncertainty of each cow's reproductive history recording, failure to keep an eye on the herd, no detection of oestrus, or no detection at the right time. Without a careful record of the individual, there was no accurate information, which was a common mistake in households (Dinh Van Cai, 2007).

3.4. The prevalence of reproductive diseases by disorders found

Through the investigation, the reproductive diseases showed disparities between disorders found in various proportions (Tab 4). There were 141 cases of cattle related to reproductive disorders, accounting for a high proportion of 35.6%. Amongt them, 94 cows were affected by calving difficulty (23.7%), followed by metritis and retained placenta with 21 cases (5.3%), while others only occurred in sporadic proportions such as mastitis, uterine prolapse with 2 cases (0.50%) and only one cow was affected by premature calving (0.25%). The previous survey also showed a high prevalence of reproductive disorders in buffaloes (46.18%) (Rabbani *et al*., 2010). The common ground of these studies showed that the prevalence of

reproductive disorders was at a high rate with various reproductive disorders recorded were repeat breeding, anestrous, genital prolapse (vaginal and uterine), abortion, retention of fetal membranes, uterine torsion and dystocia. In cases of cows exhibiting reproductive disorders related to calving difficulty, a field survey in this study showed that cows were mainly kept in captivity, resulting in sedentary cows during pregnancy. Besides, weak contractions of the uterus were not strong enough to push out the calf, which may be one of the reasons leading to a high percentage of calving difficulty.

Table 4. Reproductive disease in different disorders

Diseases	No. of affected cows	Rate (%)
Calving difficulty	94	23.7[a]
Metritis	21	5.30[b]
Retained placenta	21	5.30[b]
Mastitis	2	0.50[d]
Premature Calving	1	0.25[d]
Uterine prolapse	2	0.50[d]
Total	141	35.6

Other causes such as a weak contraction of the uterus, a decrease in the strength of the female cattle by the illness (which can also be caused by twins or the large fetus, etc) can also lead to metritis and retained placenta (Tran Van Du, 2007) after calving. In the previous study, reproductive disorders including primiparous cows, dystocia, retained placenta, and negative energy balance had been reported to associate with metritis (Giuliodori *et al*., 2013). The prevalence of mastitis in beef cattle of this study was at a very low proportion (0.50%) when compared with the rate of clinical mastitis in dairy cattle (2.2%) which was reported by Islam *et al.* (2010) in Sirajganj, Bangladesh.

3.5. Diagnostic results on the incidence of ovarian disease in different forms

The study was conducted by a combination of clinical diagnosis and ultrasound technique to determine the status of delayed breeding associated with an ovarian disease, and the results are presented in Tab 5. Ovarian diseases accounted for a high proportion (86.3%) of cows having delayed breeding (reproductive disorders). In particular, the expression of reproductive delay associated with ovarian diseases in heifers accounted for the highest percentage (100%), and the rate of ovarian diseases in breeding cows was lower with 83.8%. The study of Su Thanh Long and Nguyen Thi Thuy (2015) has shown that the cause of the ovarian disease was mainly displayed in three forms: (i) ovarian abnormalities, (ii) ovarian cysts, and (iii) corpus luteum.

Table 5. Ovarian diseases in delayed calving

Group	n	Delayed calving		Ovarian diseases	
		n	Rate (%)	n	Rate (%)
Cows	396	105	26.5	88	83.8
Heifers	58	19	32.8	19	100
Total	454	124	27.3	107	86.3

In the present study, the ultrasound technique was applied to assess the ovarian's ability to function in cows infected with reproductive diseases associated with ovarian disease and evaluate the difference between rectal examination and ultrasound technique to detect ovarian disease proportion. The reproductive disease on the ovaries was determined by clinical diagnostic testing and ultrasound technic manifested mainly in three forms including ovarian abnormalities (31.8 and 31.8%, respectively), ovarian cysts (31.8 and 35.5%), corpus luteum (36.4 and 32.7%) with relatively high rate and accuracy in both methods (P>0.05).

Table 6. Reproductive diseases on the ovaries by the different forms

Category	Clinical diagnosis		Ultrasound		Rate difference (%)	Kappa value
	No. of cows	Rate (%)	No. of cows	Rate (%)		
Ovarian abnormalities	34	31.8	34	31.8	0.0	1.00
Ovarian cysts	34	31.8	38	35.5	+11.8	0.87
Corpus luteum	39	36.4	35	32.7	-10.3	0.93
Total	107	100	107	100	0.0	1.00

These results showed that the cause of delayed calving beef cattle was related to the forms of ovarian diseases, affecting reproductive performance and economic efficiency in cow breeding. A study by Bui Thi Diem Hang (2009) has also shown that reproductive disorders manifested mainly in 3 forms: ovarian abnormalities (37.1%), corpus luteum (33.0%), and ovarian cysts (17.0%). In addition, diagnostic results on ovary between rectal examination and ultrasound have differences with rate difference in 3 forms of ovarian diseases as followed: (i) ovarian abnormalities (0%), due to an underdeveloped ovary manifesting an often small ovary, smooth surface, it was easy to distinguish from cases on other ovaries when rectal examination; (ii) the rate difference in ovarian cysts increased (11.8%), perhaps due to confusion with the case of yellow corpuscle when examined rectally; and (iii) disparity of the corpus luteum decrease (10.3%) may be due to confusion with ovarian cysts during a manual rectal examination. However, the difference between rectal examination and ultrasound technic was not statistically significant (P>0.05). The Kappa values were 1, 0.87, 0.93 in 3 forms of ovarian diseases, showing complete agreement between clinical examination and ultrasound technic. This also proved that the rectal examination technique of the technicians was relatively accurate and effective. When comparing rectal examination with other diagnostic methods such as ultrasound, rectal examination results in an accurate diagnosis of ovarian tumors of 41-85% (Mueller, 2008).

Thus, the application of ultrasound methods gives more accurate results, helping to accurately diagnose the actual operation status of the ovaries. Since then there were appropriate treatment regimens. Therefore, the accurate diagnosis of each case of reproductive disorders was the key to success that contributes to improving reproductive productivity. However, ultrasound was very difficult and expensive to implement, and thus it was important to improve the rectal examination technique of the technicians by clinical examination.

4. CONCLUSIONS

The investigation of this study recorded results with a high rate of reproductive diseases in cattle (35.6%), of which Brahman breed had a predominant prevalence of reproductive diseases than others. The parity of cows had an effect on reproductive diseases with a high proportion in cow's higher parity 6 (46.4%). The prevalence of ovarian diseases in delayed calving beef cattle was high at 27.3%, of which the disease in ovaries was present in three forms with a high rate of 86.3%.

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THE ROLE OF EOSINOPHIL IN CLEARANCE OF *EMERIA VERMIFORMIS* IN CO-INFECTED MICE WITH *NIPPOSTRONGYLUS BRASILIENSIS*

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ABSTRACT

Interspecific interactions between *Nippostrongylus brasiliensis* and *Eimeria vermiformis* were studied by measuring relative number of eosinophils and oocysts in mice after infection with *E. vermiformis* only or concurrent infection with *Nippostrongylus brasiliensis*. Results revealed that number of eosinophils increased steadily in only *E. vermiformis* infected mice and significantly reached to the peak at day 10 post-infection. In co-infected group with *Nippostrongylus brasiliensis,* the level of eosinophils was 4-fold higher when compared to singly *E. vermiformis* infected group. The number of oocysts when infected singly with *E. vermiformis,* however, was remarkably higher than co-infected group with *Nippostrongylus brasiliensis,* along with the close correlation between eosinophils and oocysts level (R=0.848), suggesting that eosinophils might play a role on the clearance of *Emeria vermiformis* in mice co-infected with *Nippostrongylus brasiliensis*. Results of this study are very useful for vaccine development to control coccidiosis.

Keywords: *Eimeria vermiformis, eosinophils, Nippostrongylus brasiliensis.*

1. INTRODUCTION

Coccidiosis caused by Eimeria sp. is the principal disease of the intestinal tract in poultry and also in many other domestic animals. Invasion of the parasites into the epithelial cells and their subsequent destruction induces immune/inflammatory responses at the infected site. The host-parasite interactions that affect the progression and control of infection with Eimeria have been studied in much detail, but the characteristics of the immune response to co-infection with other types of parasites, commonly helminths, can be substantially different. One of the effector cells in the rejection of *N. brasiliensis* is believed to be the eosinophil (Ottolenchi *et al.,* 1975; Goven 1979a,b). Results of Upton *et al.* (1987) showed that during a single infection with *N. brasiliensis*, peripheral and bone marrow eosinophil levels were elevated when compared to the uninfected control. The role of eosinophil is well known in helminth infection. Recent study indicated that eosinophil may be involved as an effector cell in antibody-dependent, cell mediated immunity to reinfection (Glauert *et al.*, 1978). On the other hand, eosinophil is exceptionally rich in peroxidase and the enzyme is released both by soluble stimuli and by adhesion to larger opsonized targets (Butterfield *et al.*, 1993). Eosinophil peroxidase is a highly basic protein that binds avidly to negatively charged surfaces with retention of peroxidase activity which can rapidly destroy target cells such as *T. gondii, T. cruzi* (Kroegel *et al.*, 1994). However, the role of eosinophil in *Eimeria* spp. infection is not yet to be elucidated. *Eimeria* has the ability to modulate the host's immune response, its inflammatory response, or both (Al-Dahwi *el al.*, 2006). Thus, the purpose of this investigation is to clarify the effects of concurrent infection with *E. vemiformis* and *N. brasiliensis* on eosinophil levels and the influence of eosinophil on parasites in co-infected host.

2. MATERIALS AND METHODS

2.1. Experimental animals

Male ICR mice at 9 weeks of age were

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used in the experiment. All mice were housed in clean cages and given standard diet and clean water adbilitum in an air-conditioned room (23±3°C), under conventional condition with a 14:10h, light: dark cycle. All protocols were approved by the Institutional Review Board for animal experiment of the University of Miyazaki, Japan.

2.2. Parasite infection

E. vermiformis was a gift from Dr. Smith A.L., Institute for Animal Health, Compton, UK, and was passaged every 3-month intervals in mice for 4 years. Oocysts were purified and sporulated, as has been previously reported (Rose and Hesketh, 1984). After microscopical scoring of the stocks for sporulation, mice were given 100 sporulated oocysts in 100µl of water by oral gavage. During the infection, feces were collected every day. Oocysts were counted on McMaster chambers using salt flotation. One group of six mice without parasite infection served as the control. *N. brasiliensis* used in this study was maintained in Miyazaki University laboratory by serial passage in Wistar rats using subcutaneous inoculation of 3,000-4,000 third-stage larvae (L3) prepared using the charcoal culture method. Fifteen mice were infected with L3 of N. brasiliensis by subcutaneous inoculation into the flank region 20 days before worm (N. brasiliensis) transfer, then 8 days later mice were infected with E. vermiformis. Infection was confirmed by counting fecal egg output as eggs per day (EPD).

2.3. Experiment design

Mice (n=36) in the experiment were divided into three groups: Group 1: Non-infected group (control, n=6); Group 2: Mice were infected with Eimeria vermiformis singly (n=15); Group 3: Mice were first infected with N. brasiliensis and 8 days later, infected with Eimeria vermiformis (n=15).

2.4. Histopathological examinations

Sections (groups 1-3) from intestines (duodenum, jejunum and ileum) were fixed in 10% of neutral buffered formalin (pH 7.0) and embedded in paraffin wax. Sections of 4mm thickness were stained with hematoxyline-eosin (HE) stain, congo red for eosinophils. The number of eosinophils in small intestine was counted on at least 10 well-orientated crypt-villus units for each animal and expressed as the mean numbers.

2.5. Statistical analysis

Statistical analysis was performed with the statistical software package SPSS for Windows (Ver 15.0; SPSS, Chicago, IL). The significance of differences between groups was evaluated by nonparametric tests. Mann-Whitney U test was used for comparing the infected groups and the control. Results are expressed as M±SEM and $P<0.05$ was considered significant.

3. RESULTS

3.1. Eosinophil number observed in intestine of mice after infected with *Eimeria vermiformis* or coinfection with *Nippostrongylus brasiliensis*

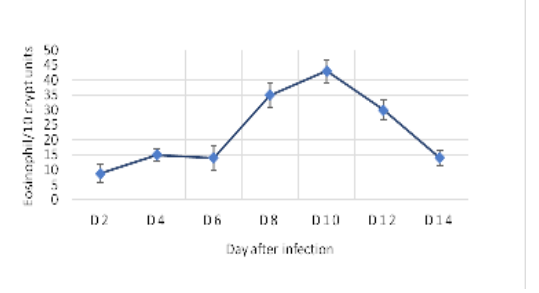

Figure 1. Number of eosinophils observed in 10 intestinal crypts of mice after infection with 100 oocysts of *E. vermiformis*. Data are the Means and SE from 15 mice ($P<0.05$)

Figure 1 showed the fluctuation of eosinophil number in crypts of mice after infection with *E. vermiformis*. About eight eosinophils were observed in 10 crypt units starting from day 2. There was a rise in the number of eosinophils from day 6 to day 10, which reached 43 eosinophils on day 10. After reaching the highest point, the figure for eosinophils started to drop down below 15 on

day 14. The related numbers of eosinophils in the experimental groups are presented in Figure 2.

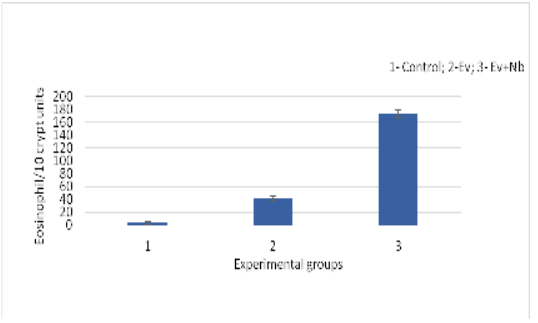

Figure 2. Relative number of eosinophils in 10 intestinal crypts of mice after infection with 100 oocysts of *Eimeria vermiformis* alone or coinfection with *Nippostrongylus brasiliensis* at day 9 post-infection. Group 1: Negative control; Group 2: single infected with *Eimeria vermiformis*; Group 3: Co-infected with *Eimeria vermiformis* and *Nippostrongylus brasiliensis.* Data are the Means and SE from 15 mice (P<0.05).

On the other hand, in group infected with *Eimeria*, eosinophil level was significantly lower than the co-infected group on day 9 post-infection. The number of eosinophils in co-infection group with *Nippostrongylus brasiliensis* was approximately four times higher than the single infected group with *Eimeria*.

3.2. Eimeria oocysts number observed in intestine of mice after infected with *Eimeria vermiformis* or coinfection with *Nippostrongylus brasiliensis*

The number of oocysts invaded the intestinal crypts of mice in the single infected with *E. vermiformis* group was 17 times higher than the co-infected group with *Nippostrongylus brasiliensis*, which showed that co-infection with helminth might suppress *Eimeria* invasion in the intestinal epithelial cells.

In addition, correlation between eosinophils and parasites level after infection *Eimeria* oocysts and *Nippostrongylus brasiliensis* was calculated and illustrated in Figure 4.

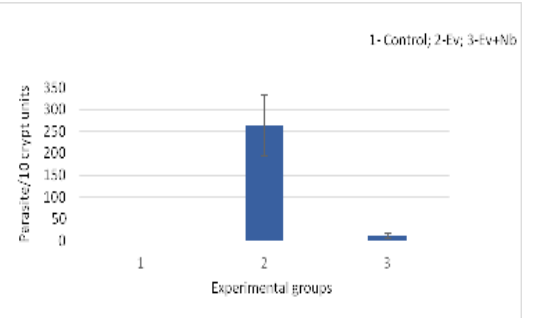

Figure 3. Number of parasites observed in of in 10 intestinal crypts of mice after infection with 100 oocysts of *Eimeria vermiformis* alone or co-infection with *Nippostrongylus brasiliensis* at day 9 post-infection. Group 1: Negative control; Group 2: single infected with *Eimeria vermiformis*; Group 3: Co-infected with *Eimeria vermiformis* and *Nippostrongylus brasiliensis.* Data are the Means and SE from 15 mice (P<0.05).

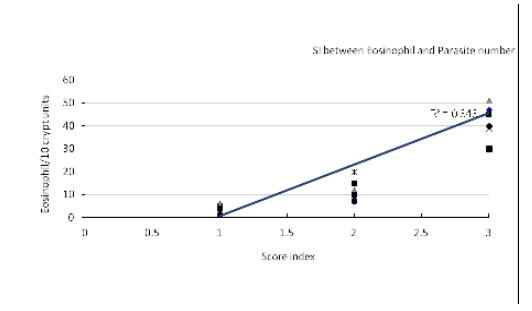

Figure 4. Correlation between eosinophil and parasites level after infection *Eimeria* oocysts and *Nippostrongylus brasiliensis* at day 9 post-infection. Data are the Means and SE from 15 mice (P<0.05).

The correlation between *Eimeria* infection and eosinophils infiltration was significantly close (0.84). The number of parasites is strongly related to eosinophils, which suggests that eosinophil might play a role in the clearance of *Eimeria vermiformis* in mice co-infected with *Nippostrongylus brasiliensis*.

4. DISCUSSION

In mice single infected with *E. vermiformis*, the eosinophil level observed was highest on day 10 post-infection before decreasing to control level, while single infected group with *N. brasiliensis* revealed a significant rise

in eosinophilia, which peaked at day 16 post-infection (Upton *et al*., 1987) as reported. In co-infected group, the eosinophil level was four times higher than that of singly infected with *E. vermiformis.* Immune response to helminth is mainly performed by the production of immunoglobin (IgE) antibodies and the activation of eosinophil, the increase of eosinophils level in helminth infection is well known to lead expulsion of nematode in intestinal tract (Makepeace *et al*., 2012).

In coinfected group, in addition, the figure for eosinophils remained high even after the appearance of *E. vermiformis.* As a result, it is likely that *E. vermiformis* does not inhibit eosinophils level induced by *N. brasiliensis*. A similar study had also shown *E. separate* does not suppress peripheral eosinophil (Ramirez *et al*., 1991), while *E. nieschuzil* has the ability to suppress peripheral eosinophil induced by *N. brasiliensis. (*Al-Dahwi *et al*., 2006). The number of oocysts invaded the intestinal crypts of mice in co-infected group, interestingly, decreased significantly as opposed to the single infected group and control group. The appearance of numerous eosinophils surrounding *Eimeria* oocysts was observed. Besides, the correlation between eosinophil and oocyst number shown is highly related *with* the reduction of *Eimeria* number during infection suggests eosinophil might play a role in the clearance of *Eimeria vermiformis* in mouse co-infected with *Nippostrongylus brasiliensis*.

Besides, in coinfected group, the quantity of eosinophils remained high even after appearance of *E. vermiformis.* As a result, it is likely that *E. vermiformis* does not inhibit eosinophil level induced by *N. brasiliensis*. A similar study had also shown *E. separate* does not suppress peripheral eosinophil (Ramirez *et al*., 1991), while *E. nieschuzil* has the ability to suppress peripheral eosinophil induced by *N. brasiliensis.* (Al-Dahwi *et al*., 2006).

Whereas eosinophilia is a characteristic feature of helminthiasis, it is not the dominant cell response to protozoan infections (Spry, 1988). Based on our result, a previous helminth infection may affect the development of Th1 responses in CD4+ T cells and protection against protozoans. The exact cause of this reduction in the number of parasites is unknown, however, it is possible that eosinophils are capable of expressing several Toll-like receptors, which are part of innate immune responses leading to TH1 acquired immune responses (Jacobsen *et al*., 2007). Another hypothesis is eosinophils have been shown to express IL-12 and IFN-γ (Lamkhioued *et al*., 2006; Grewe *et al*., 1998a). These cytokines are thought to be integral for the differentiation and establishment of protective Th1-mediated immunity, as eosinophils may play a role in switching from Th2-like to Th1-like immune responses (Grewe *et al*., 1998b; Smeltz *et al*., 2002). That might be the reason why coccidiosis was prevented from entering the intestinal mucosa since Th1-type cytokines tend to produce the immune responses responsible for killing intracellular parasites including coccidiosis (*Berger, 2000)*.

5. CONCLUSION

These findings support the paradigm of eosinophil as defender against nematode infection, yet co-infection of coccidia – nematode mice in our study had not proved clearly that eosinophil plays a key role for clearance of protozoans. Further study should clarify this point to understand all of the functions of eosinophil in response to parasitic diseases. This will be necessary for the development of vaccines and therapeutic interventions.

ACKNOWLEDGEMENT

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EFFECTS OF MINERAL SALTS AS A SUPPLEMENT TO SEMEN EXTENDER ON CHILLED CANINE SPERM MOTILITY

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ABSTRACT

The study evaluated the effects of two treatments with Tris-citric-fructose-egg yolk extender (Control) and Tris-citric-fructose-mineral salts egg yolk extender on chilled canine sperm motility during 10 days of storage. The sperm motility was performed by computer assisted sperm analysis (CASA). The results showed that although the percentage of total motility and progressive motility in Tris-citric-fructose-egg yolk and Tris-citric-fructose-mineral salts egg yolk extenders were not significantly different during the first 7 days of storage (P>0.05), the velocity straight line of sperm in Tris-citric-fructose-mineral salts egg yolk extender was notably higher than that in Tris-citric-fructose-egg yolk extender during this period (P<0.05). In particular, the sperm motility in Tris-citric-fructose-mineral salts egg yolk extender reduced quickly after the first one week and significantly lower than that in the rest extender (P<0.05). In conclusion, Tris-citric-fructose-mineral salts egg yolk extender can improve sperm motility in chilled canine sperm during 7 days of storage.

Keywords: *Canine sperm, chilled, mineral salts, extender.*

1. INTRODUCTION

The use of artificial insemination (AI) techniques is currently popular in dog reproduction. To prepare sperm for the AI technique, it must be diluted with appropriate extenders and preserved by chilling or freezing (Thomassen and Farstad, 2009). However, chilling is used more frequently than freezing for its convenience and high fertilizing capacity (Linde-Forsberg, 1991). In addition, canine sperm could be maintained at a chilling temperature without any deleterious effects before freezing (Santana *et al*., 2013). Hence, chilled sperm is more popular than frozen sperm in AI techniques.

A major problem of chilled canine sperm is the reduction of sperm quality during long periods of storage. To improve chilled sperm quality, canine sperm must be diluted with a suitable semen extender to maintain the sperm quality. In previous studies, the common semen extenders for chilled canine sperm were the Tris-citric-fructose or glucose extender with 20% egg yolk that best maintains sperm quality during chilling storage (Ponglowhapan *et al*., 2004; Verstegen *et al*., 2005; Shahiduzzaman and Linde-Forsberg, 2007; Rodenas *et al*., 2014).

In addition, seminal plasma is a complex biological fluid comprising ions (Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-), energy substrates (fructose, sorbitol, glyceryl phosphocholine), and organic compounds (citric acid, amino acids, peptides, proteins, lipids, hormones, cytokines) (Juyena and Stelletta, 2012). It has important functions in semen ejaculation and sperm survival in the female genital tract. In particular, mineral ions have essential roles for maintaining osmotic balance, forming parts of primary enzymes relating to the metabolism and function of sperm (Smith *et al*., 2018). However, canine seminal plasma is removed before diluting with semen extenders since the activity of acid and alkaline phosphatase in seminal plasma can reduce adenosine tryphosphate (ATP) concentration that lead to decrease in sperm motility (Hori *et al*., 2017). Thus, creating a new canine semen extender by adding mineral ions may improve sperm quality and increase the survival time of sperm without

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seminal plasma. Therefore, the objective of the present study was to assess the effects of mineral ions as a supplement to semen extender on sperm motility in chilled canine sperm during 10 days of storage.

2. MATERIAL AND METHODS

2.1. Animals, site, and duration of study

Sperm samples were obtained from three mature American Bullies dogs (2-5 years old) of proven fertility. All dogs were trained to ejaculate by digital manipulation for semen collection before studying.

The study was conducted from May 2019 to November 2019 at School of Animal Production Technology and Innovation, Suranaree University of Technology.

2.2. Preparation of extenders

Tris-citric-fructose and Tris-citric-fructose-mineral salts extenders added to 20% egg yolk were used to dilute canine sperm in this study. The Tris-citric-fructose-egg yolk extender (control group) (T-EY) was prepared with 3,025mg Tris, 1,700mg citric acid, 1,250mg fructose, 200mg gentamycin, 20ml egg yolk, and to 100ml distilled water. Whereas the Tris-citric-fructose-mineral salts egg yolk extender (T-M-EY) was produced from the result of canine seminal fluid composition analysis containing 900mg Tris, 500mg citric acid, 1,250mg fructose, 450mg NaCl, 60mg $KHPO_4$, 60mg KCl, 20mg $CaHPO_4$, 10mg $MgCl_2$, 200mg gentamycin, 20ml egg yolk, and to 100ml distilled water.

2.3. Semen processing and experimental design

Experimental design in this study was presented by a repeated measurement in the completely randomised design with four replicate trials.

After collection, semen from three dogs was pooled and separated into two sterile tubes. Then, the seminal plasma was removed by centrifuging (5minutes, 720×*g*). Sperm pellets were re-suspended in 2 extenders to achieve the final sperm concentration of 100 x 10^6 spermatozoa/ml. After that, extended sperm was cooled down gradually (0.3°C/min) to 5°C for up to 1 hour and stored at 5°C during 10 days. Sperm motility was analysed everyday over a period of 10 days.

2.4. Semen collection and evaluation

Ejaculates were collected once a week from each dog by digital manipulation according to the technique as described by Linde-Forsberg (1991). Sperm with the following quality criteria was used in this study: >70% progressive motility; >200×10^6 sperm/ml; <5% sperm abnormal morphology; and >90% sperm viability.

2.5. Sperm motility evaluation

Automated analysis of sperm motility was evaluated using computer assisted sperm analysis (CASA; HTR-IVOS 14.0; Hamilton Thorne, USA). The technical settings of CASA for canine sperm as the following were used in this study: frames per sec. (Hz), 60; no. of frames, 30; minimum contrast, 30; minimum cell size (pix), 7; cell size (pix), 6; cell intensity, 75; path velocity (VAP) (μm/s), 20; straightness (STR) (%), 40; VAP cutoff (μm/s), 9; and VSL cut off (μm/s), 20. Before analysing, chilled sperm was diluted with a warmed (38°C) Tris buffer at a rate of 1:1. Then, 5μL of each chilled sperm samples was mounted into a warmed (38°C) 2X-CEL counting chamber and covered by coverslips. Each sperm sample in 2X-CEL counting chamber was evaluated at least 5 randomly selected fields. The percentage of total motility, the percentage of progressive motility, velocity average pathway (VAP), velocity straight line (VSL), and velocity curvilinear (VCL) parameters were collected.

2.6. Statistical analysis

Statistical analyses were represented using IBM SPSS statistics for Windows, version 20 (IBM Corp., Armonk, N.Y., USA). All data are provided as Mean ± Standard Deviation (SD). Differences were examined

by a two-factor mixed analysis of variance (ANOVA) with interaction including time and extender as the main effects. When the results had a statistically significant interaction, the difference between groups at each level of each factor (time, extender) was determined. In this case, a modification of the repeated measurement command in the syntax was conducted by adding compare simple main effects for both time and extender factors. Pairwise comparisons were performed using a confidence interval adjustment by the Bonferroni method. A difference of $P<0.05$ was considered significant.

3. RESULTS

3.1. Sperm total motility and progressive motility

The total motility (TM) and progressive motility (PM) of sperm in the two extenders are presented in Table 1. Overall, sperm in both T-EY and T-M-EY extenders were reduced gradually in TM (from 89.4±1.9% to 62.7±1.6% in T-EY, and from 85.9±3.8% to 23.0±2.3% in T-M-EY) and PM parameters (from 66.1±3.3% to 30.2±1.6% in T-EY, and from 70.5±4.8% to 10.1±1.7% in T-M-EY) during 10 days of storage. During the first seven days, the quality of sperm in T-EY and T-M-EY were similar in TM and PM parameters ($P>0.05$). However, after day 7, the percentage of TM and PM in T-M-EY extender were decreased significantly, and had a notable difference when compared to that of T-EY extender ($P<0.05$).

3.2. Sperm velocity parameters

Besides TM and PM parameters, sperm velocity (VAP, VSL, and VCL) was alsoan important parameter in evaluating the sperm motility characteristics. The sperm velocity parameters are shown in Table 2. As TM and PM parameters, the sperm velocity parameters in T-EY and T-M-EY extenders decreased steadily during the whole experimental period (10 days). The VAP values of sperm in T-EY and T-M-EY extenders were similar during storage, while the VSL values of sperm in T-M-EY extender were significantly higher than that in T-EY extender during the first 6 days ($P<0.05$). However, from day 9, the VSL values of sperm in T-M-EY declined suddenly and was substantially lower than that in T-EY extender ($P<0.05$). In addition, there was no significantly different in the VCL values of sperm in both extenders during the first 8 days ($P>0.05$), but the T-EY extender was better than T-M-EY extender in this parameter from day 9 to day 10 ($P<0.05$).

4. DISCUSSION

The study investigated the effects of mineral salts extender on chilled canine sperm motility. The obtained results clearly demonstrated that although the percentage of TM and PM in T-EY and T-M-EY extenders were not significantly different during the first 7 days of storage, T-M-EY extender was superior to T-EY extender in VSL parameter during this period. The VSL parameter is a principal parameter of sperm velocity in the CASA system in which the average velocity of the sperm heads through a straight line connecting to the first point of the last track. In a previous study, they demonstrated that the decline in VSL was highly correlated with the outcome of fertilization *in vitro* in rat spermatozoa (Harry and Mehdi, 1996). This may be explained by the fact that T-M-EY extenders comprise several mineral cations, such as Na^+, K^+, Mg^{2+}, Ca^{2+}, which are the primary ions of canine seminal plasma. These mineral ions have principal functions to balance osmolarity and form parts of important enzymes relating to sperm metabolism and sperm function (Juyena and Stelletta, 2012; Smith *et al*., 2018). In particular, Mg^{2+} has an important function in modulating the regulation of K^+ (Na-K pump) and Ca^{2+} (Smith *et al*., 2018), and plays a crucial role in enzymatic reactions including anaerobic glycolysis and energy release from ATP for sperm activities (Wong *et al*., 2001; Asghari *et al*., 2016). Moreover, Ca^{2+}ions also

have an important function in intra-mitochondrial metabolism and energy production in cells (McCormack and Denton, 1989). Ca^{2+}can be imported from cytosol into mitochondrial matrix via the mitochondrial uniporter by mitochondria (Walsh *et al*., 2009). When the level of free Ca^{2+} increases within the mitochondrial matrix, it can activate several dehydrogenases and carriers. As a result, there is an increase in the H^+ extrusion, respiratory rate, and ATP production as well as supports energy for cell activities (McCormack and Denton, 1989; Santo-Domingo and Demaurex, 2010). Nevertheless, when the level of Ca^{2+} is overloaded, it can stimulate to open the mitochondrial permeability transition pore (PTP) and deplete ATP. This leads to mitochondrial swelling, cytochrome C release, and subsequently cell death (Demaurex and Distelhorst, 2003; Giorgi *et al*., 2008). Thus, sperm motility in T-M-EY extender is high during the former period of storage time (the first 7 days) and decreased in the last period of the storage time (after day 8).

Table 1. The effects of Tris-citric-fructose-egg yolk (T-EY) extender and Tris-citric-fructose-mineral salts egg yolk (T-M-EY) extender on sperm total motility (TM) and progressive motility (PM) of chilled canine sperm storage at 5°C during 10 days

Para	Treat	Day1	Day2	Day3	Day4	Day5	Day6	Day7	Day8	Day9	Day10
TM	T-EY	89.4±1.9^{A}	85.4±1.6^{AB}	83.4±2.7^{AB}	81.1±2.6^{AB}	80.1±3.3^{B}	77.7±4.5^{BC}	75.5±3.7^{BC}	73.9±3.9^{aBC}	69.5±5.6^{aCD}	62.7±1.6^{aD}
(%)	T-M-EY	85.9±3.8^{A}	83.7±2.4^{A}	81.6±3.1^{A}	79.1±2.7^{A}	75.7±1.8^{B}	73.5±2.8^{BC}	70.4±3.3^{C}	41.2±3.6^{bD}	29.3±2.8^{bE}	23.0±2.3^{bE}
PM	T-EY	66.1±3.3^{A}	61.8±5.0^{A}	58.5±5.8^{B}	56.7±6.5^{B}	52.0±3.0^{BC}	48.9±4.6^{BC}	46.7±5.5^{C}	41.9±3.8^{aC}	37.7±1.7^{aC}	30.2±1.6^{aD}
(%)	T-M-EY	70.5±4.8^{A}	67.3±5.0^{A}	62.3±4.1^{B}	59.5±3.3^{B}	55.6±7.5^{B}	45.5±7.9^{C}	40.2±4.2^{C}	23.3±1.9^{bD}	16.0±1.3^{bE}	10.1±1.7^{bF}

Values are Mean±SD for four replicates, each being a pool of three ejaculates. Lowercase superscript letters (a or b) in the same column indicates significant difference among extenders (P<0.05) and uppercase superscript letters (A, B, C, D, E or F) in the same row indicates significant difference within extenders with different storage time (P<0.05).

Table 2. The effects of Tris-citric-fructose-egg yolk (T-EY) extender and Tris-citric-fructose-mineral salts egg yolk (T-M-EY) extender on average pathway velocity (VAP), straight line velocity (VSL) and curvilinear velocity (VCL) parameters of chilled canine sperm storage at 5°C during 10 days

Para	Treat	Day1	Day2	Day3	Day4	Day5	Day6	Day7	Day8	Day9	Day10
VAP	T-EY	89.6±1.2^{A}	75.5±4.3^{B}	72.6±2.4^{BC}	68.9±4.0^{BC}	67.6±4.8^{BC}	65.5±6.1^{BC}	63.4±6.8^{BC}	59.8±6.6^{BC}	58.4±7.3^{BC}	54.8±7.8^{C}
(μ/s)	T-M-EY	93.2±2.7^{A}	79.0±3.2^{B}	73.3±7.2^{BC}	65.9±4.1^{C}	64.3±4.3^{C}	62.0±3.7^{C}	60.8±3.4^{C}	54.3±9.5^{CD}	47.5±6.7^{D}	43.8±6.5^{D}
VSL	T-EY	77.7±2.1^{bA}	60.6±5.6^{bB}	50.6±2.6^{bC}	45.6±6.2^{bC}	43.8±6.0^{bCD}	42.1±5.0^{bCD}	41.0±5.3^{CD}	39.5±4.0^{CD}	38.9±3.9^{aCD}	35.8±2.8^{aD}
(μ/s)	T-M-EY	87.9±3.7^{aA}	71.6±2.0^{aB}	66.9±2.6^{aBC}	60.5±4.3^{aBC}	57.0±5.1^{aBC}	51.7±4.6^{aC}	49.3±4.7^{C}	40.5±3.3^{D}	31.1±3.5^{bDE}	29.4±2.3^{bE}
VCL	T-EY	147.0±5.8^{A}	141.6±6.6^{AB}	133.8±7.0^{B}	126.2±9.4^{B}	122.8±5.2^{B}	119.3±17.5^{BC}	117.8±8.0^{BC}	115.4±7.4^{BC}	111.5±4.9^{aBC}	106.5±3.1^{aC}
(μ/s)	T-M-EY	142.7±3.6^{A}	136.8.8±2.0^{AB}	128.1±1.8^{B}	119.0±1.3^{B}	112.4±3.2^{B}	110.1±4.1^{B}	106.0±5.8^{B}	99.2±6.3^{BC}	85.6±2.6^{bCD}	79.4±3.9^{bD}

5. CONCLUSIONS

In conclusion, the results of our study presented that Tris-citric-fructose-mineral salts egg yolk extender is superior to Tris-citric-fructose egg yolk extender in sperm motility of chilled canine sperm during 7 days of storage. Further studies are necessary to evaluate more sperm quality parameters such as plasma membrane integrity, acrosome membrane integrity, mitochondrial membrane potential, DNA fragmentation and fertility ability.

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FECAL TESTOSTERONE MONITORING IN COMMON PALM CIVETS BY ENZYME IMMUNO ASSAY

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ABSTRACT

This study aims to use the Enzyme-linked Immuno sorbent assay (ELISA) method to monitor testosterone hormone in male civets that contribute to conservate species in captivity. This is also a scientific basis for similar studies on animal models, towards tracking human steroid hormone metabolites. For this purpose, we collected fecal samples from adult male civets, adult female and juvenile civets. The study demonstrated the stability of measured testosterone levels in fecal samples (fTM) exposed to ambient temperatures for up to 72hrs. Testosterone levels in the common palm civets were higher in the breeding season than in the non-breeding season. There were significant differences in fTM concentration between adult males, adult females and juvenile civets in captivity. The results clearly demonstrated that testosterone testing in faecal samples were a reliable non-invasive method to monitor testicular activity in male civets. The monitoring of the hormone testosterone in feces will create favorable conditions for the civet conservation breeding programs in Vietnam and elsewhere in the world.

Keywords: *Common palm civet, Elisa, hormone, testosterone, reproduction.*

1. INTRODUCTION

The Commom Palm Civets (*Paradoxurus hermaphroditus* Pallas, 1777) belongs to Civet (Viverridae), carnivore (Carnivora); is a rare and precious animal in group IIB. Currently, civet population structure is declining due to habitat fragmentation and indiscriminate hunting. Attempts to breed them in captivity are part of a species conservation program. As the population of the civet is declining, captivity becomes important for species management and conservation. However, in contrast to living in highly fertile natural conditions, captive individuals have low reproductive performance (Nguyen Thi Thu Hien *et al*., 2017). The analysis of the hormones of adrenal cortex and testes will improve our understanding of reproductive biology in male civets and thus provide the necessary information for livestock management to facilitate successful captivity.

In mammals, testosterone hormone is the main hormonal variable to reflect testicular activity and reproductive function in males. Testosterone is involved in physiological development and function and behavior related to male sex drive. The development of the ELISA enzyme immunoassay for monitoring fecal testosterone metabolites (fTM) is a powerful tool for assessing gonad conditions by a non-invasive method.

However, blood sampling to determine gonadal activity is often limited by limited access to research animals, especially in wildlife (Palme *et al*., 2005). In contrast, monitoring of fecal testosterone metabolites (fTM) using an enzyme immunoassay (EIA) provides a valuable method for assessing animal hormonal status (Pribbenow *et al*., 2015). Compared to plasma hormone tests based on single samples, the concentration of hormone metabolites in the faeces represents the average values of the waste excreted over a specific period of the species, and therefore less affected by the type of hormone secretion than the hormone in the blood (Goymann, 2005). Moreover, due to the non-invasive nature of this method, fTM

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levels are less affected by processes such as arrest, control, and anesthesia, which can alter circulating testosterone levels (Palme *et al*., 2005). The selection of an appropriate test plays an important role in fTM analysis, including physiological and biological confirmations to confirm that changes in testicular activity are reflected in fTM levels measured by the corresponding test. The purpose of this study is to confirm the non-invasive method using the ELISA assay to monitor testicular activity in male civets. The first objective is to study the effect of time on the stability of fTM concentration when stool is exposed to natural conditions at ambient temperature prior to collection. The second goal is to determine the testosterone change of the male civet civet season. Finally, we determined the biological values of testosterone in the palm civet by measuring the fTM levels of adult males, adult females and immature males.

2. MATERIALS AND METHODS

2.1. Materials

The common palm civets are placed in separate pens, alternating males with females, each bar attached a tag to monitor during the experiment. All individuals were considered healthy based on clinical track history. Civets are arranged into experimental formulas using a completely random arrangement.

2.2. Scope of the research

Place: Dong Nai Biotechnology Application Center in Xuan Duong commune, Cam My district, Dong Nai province.

Time: from June 2017 to May 2019.

2.3. Sample collection and analysis procedure

Faecal samples are collected at approximately 18-20hrs. Faecal samples (5g) were collected in plastic bags (size 200x140x0.04mm; Uni Pack Mark Series-G, Seisan Nippon Co., Tokyo, Japan) and stored at -20^{0}C until analysis.

After defrosting, 0.2g is weighed and placed in a glass vessel containing 2ml of 90% methanol. After shaking for 30 minutes (on the shaker HS 260-IKA, Germany), the sample was centrifuged at 1,700 cycles for 20 minutes (on the EAB 20 machine, Germany). After centrifugation, about 1ml of the solution was extracted into a 1.5ml eppendorf vial and frozen at -20^{0}C until use. The rest is put into glass jars and dried to determine the dry weight of the manure (Frederick *et al*., 2010).

2.4. Immunoassay for the ELISA enzyme

Materials: ELISA Testosterone KIT Kit (DRG International, Inc., Germany).

Procedure: The amount of testosterone is determined with the fully automated ELISA Dynex DS2 processing system (Dynex, USA). The ELISA assay procedure is preinstalled on the Dynex DS2, each run including a standard curve, including the following steps:

Add 25µl of the standard substance (0, 0.3, 1.25, 2.5, 5, 15, 40 ng/ml), control and samples with new attributable taper heads to the corresponding wells. Incubate for 5 minutes at room temperature. Small 200µl Enzyme conjugated to each well. Mix the mixture well (shake) for 10 seconds. Incubate for 60 minutes at room temperature. Wash the well 3 times with washing solution (400ul/well). Add 200µl of substrate solution to each well. Incubate for 15 minutes at room temperature. Stop the enzyme reaction by adding 100µl reaction solution to each well. Determine the absorbance (OD) of each well at 450±10nm with a micro-well tray reader.

Calculation of results: Develop a standard curve by plotting the average absorbance obtained from each standard against its concentration; absorbance value on the vertical axis (Y) and concentration on the horizontal axis (X). Use the average absorbance value for each sample to determine the corresponding concentration from the calibration curve. Concentrations of samples can be read directly from this standard curve. If the sample has

a higher concentration than the reference standard with the highest concentration, further dilution is required. To calculate the concentration, this dilution factor is taken into account.

All hormone levels are expressed in micrograms per gram of dry stools (μg/g dried faeces). The maximum testosterone (peak-peak) content is determined to be greater than the average of all remaining values from each individual civet (Frederick *et al*., 2010).

2.5. Research content

2.5.1. Study the effect of time on the stability of fTM concentration

A total of 12 fecal samples were collected from 4 adult male civets (M1, M2, M3, M4). Three new excreted excreta samples from four male civets (M1, M2, M3, M4) were collected and analyzed to check if fTM concentration was affected by storage time at ambient temperature. Each fecal sample of M1 and M2 collected was stored outdoors at ambient temperature and subsamples were taken every two hours for 48hrs. The stool samples from M3 and M4 are sub-sampled every two hours for the first 24hrs and then at 36, 48, 60 and 72hrs. The ambient temperature ranges from about 22-39^{0}C. The collected faecal samples were stored at -20^{0}C until the test.

2.5.2. Study the change of testosterone in males by the season

Collect fecal samples from 5 individual male civets (of similar age and weight), every 3 days for 12 months. The collected faecal samples were stored at -20^{0}C until the test.

2.5.3. Determine the biological value of testosterone by gender and age

Collect fecal samples from 12 aldul males, 10 mature females, and 11 juvenile males at approximately 18-20 hours every 3 days for 3 months (Apr, Jul and Dec).

2.6. Statistical analysis

All hormone levels were expressed in micrograms per gram of dried faeces (μg/g).

Statistical parameters: average value (Mean), standard deviation (SD), t-test test; ANOVA analysis of a factor with significance level of α=0.05 was processed by MS-Excel 2013 software.

The change of testosterone in faeces is calculated monthly on average in all the animals studied. Outstanding peak values are discarded if 2 standard deviations are exceeded. High values are considered peak values and the rest are considered basic values (Brown *et al*., 1999).

3. RESULTS AND DISCUSSIONS

3.1. The effect of storage time at ambient temperature on the stability of fTM concentration

The results of monitoring the influence of time on the fTM content in Figure 1 showed that the concentration of fTM in the samples from males M1, M2 did not change significantly within 48hrs, and M3 and M4 did not change within 72hrs at ambient temperature (M1: R2=0.23; F=3.11; df=12; P=9.64; regression equation Y=101.9-0.12x; M2: R2=0.59, F=15.91, df=12, P=4.68, regression equation: y=100.95-0.17x; M3: R2=0.001, F=0.015, df=16, P=1.56; regression equation: y=98.85-0,003x; M4: R2=0.48, F=13.03, df=16, P=9.06; equation the regression process: y=99.44-0.05x).

Various studies have demonstrated that the fecal hormone metabolites can vary significantly between individuals and genders due to changes in steroid metabolism, excretion and composition of intestinal bacteria (Palme *et al*., 2005). However, the concentration of steroid metabolites in faeces may also be affected by faecal sampling and storage under environmental conditions. Because in practice, the collection of fresh manure samples from free-living animals, the samples are exposed to different environmental conditions before being frozen. This may be due to the breakdown of bacteria and may therefore alter the composition of metabolites in the

stool and affect the measurements (Susanne *et al*., 2016).

In a given species, stability may vary between metabolites of different steroids and may also affect test results (Morrow *et al*., 2002). Therefore, the effect of environmental conditions prior to storage should be made in each species for each given steroid metabolite. This study found no evidence of changes in fTM concentration over time, suggesting that fTM concentrations in male civets were not affected by environmental conditions. Despite exposure to low temperatures (22-26^{0}C) or high ambient temperatures (33-39^{0}C) and direct sunlight (samples from M4), fTM concentrations remain stable for up to 72hrs (3 days). This result is similar to the study of fTM concentration in cheetahs (Ludwig *et al*., 2013), the red panda (Neema *et al*., 2016) showed stability even in low ambient temperature conditions (2-12^{0}C).

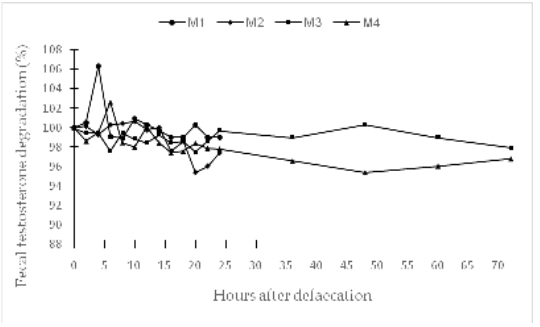

Figure 1. The relative decline of fTM (%) in the three samples of M1, M2 over a 48h period and M3, and M4 over a 72h period

The relative fTM concentration calculated relates to the reference concentration of the frozen sample (-20^{0}C) immediately after collection, accounting for 100%.

3.2. Research the biological values of testosterone by seasons

The results of changing fTM content of ancivet (M5) over time is shown in Figure 2; The graph of fTM of 5 civets (M5, M6, M7, M8, M9) studied is shown in Figure 3.

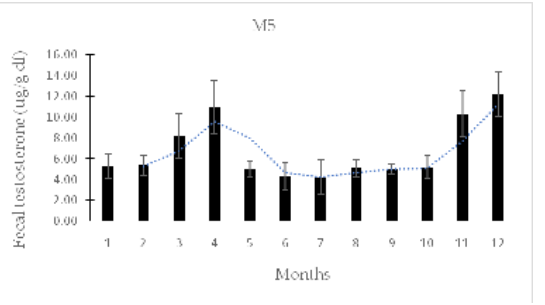

Figure 2. Changes in fTM content of civets (M5) over time (month/year)

An adult male civet (M5) were monitored for stool for 12 months. Average monthly testosterone concentrations ranged from 4.21±1.68 µg/g dried faeces (Jun) and 12.20±2.21 µg/g dried faeces (Dec). Levels of testosterone in stools were significantly higher in Mar, Apr, Nov and Dec. In captivity, the breeding season of Palm civet is Jun 4-6 and Oct 10-12 (Nguyen Thi Thu Hien *et al*., 2017). In nature, the main oestrus season is Feb to Apr (Dang Huy Huynh *et al*., 2010). Clearly, the change in testosterone over the months of the male civet is consistent with the breeding season of the species.

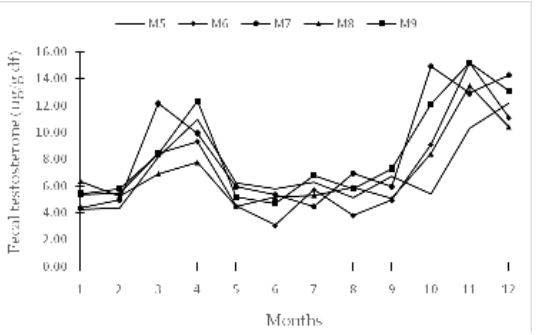

Figure 3. Testosterone content change of 5 male civets (M5, M6, M7, M8, M9) over time

Testosterone levels in the common palm civet showed a clear seasonal pattern and increased concentrations during the breeding season compared to the non-breeding season (Figure 3). In female civets, similar findings were found, high levels of Estradiol during the breeding season (Nguyen Thi Thu Hien *et al*., 2018). In red pandas (*Ailurus fulgens fulgens*),

cheetah (*Acinonyx jubatus*) all showed an increase in testosterone during the breeding season (Neema *et al*., 2016; Susanne *et al*., 2016). However, testosterone concentration of Nepal pandas was observed no clear pattern, although the values fluctuated widely over months (Spanner *et al*., 1997).

3.3. Study the biological values of testosterone by gender and age

Results of the biological value monitoring of testosterone in adult male civets, adult females and juvenile male civets in breeding season (Apr, Dec) and non-breeding season (Jul) are shown in the Figure 4.

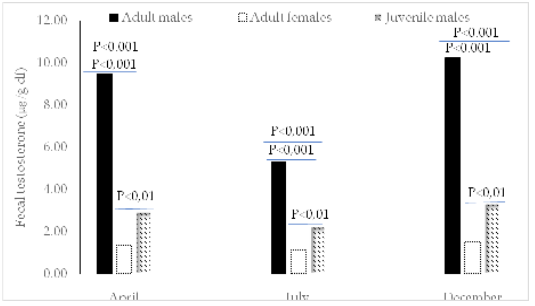

Figure 4. Testosterone levels in feces of adult males, adult females and juvenile male civets

There was a significant difference in fTM concentration between adult male civets, adult females and juvenile males (Figure 4). In adult males, the fTM concentration was 9.57±2.16 μg/g dried faeces (Apr); 5.32±1.52 μg/g dried faeces (Jul); 10.25±2.36 (Dec); in adult females ranges from 1.32±0.95 μg/g dried faeces (Apr); to 1.11±0.82 μg/g dried faeces (Jul) 1.51±0.79 μg/g dried faeces (Dec), in adult males with fTM content of 2.93±0.83 μg/g dried faeces (Apr); 2.24 μg/g dried faeces (Jul); 3.32 μg/g dried faeces (Dec). Statistical comparisons show that the adult male has a higher concentration of fTM than the adult female ($P<0.001$) or immature male ($P<0.001$); while juvenile males had significantly higher concentrations of fTM than adult females ($P=0.01$).

Testosterone (17Beta-hydroxy-4-androstene-3-one) is a C19 steroid that has an unsaturated bond between C-4 and C-5, ketones in C-3 and hydroxyl groups at Beta position at C-17. Testosterone is the most important androgen secreted into the bloodstream. In males, testosterone is mainly secreted by the testicular Leydig cells. In females, 50% of circulating testosterone is derived from the peripheral transformation of androstenedione, 25% from the ovaries and 25% from the adrenal glands. Testosterone is responsible for the development of secondary male sex characteristics and its measurements are useful in assessing testicular status (Pribbenow *et al*., 2015).

The results of a study in the newspaper showed that the biological value of testosterone is further demonstrated by the ability to distinguish between mature males, mature females and immature males (Susanne *et al*., 2016). Another study found that in cheetahs confined to North American zoos, the concentration of fTM in captive animals was lower than in free individuals (Terio *et al*., 2004).

4. CONCLUSIONS

The ELISA test provides a useful and practical tool for assessing testicular activity in male civets. Testosterone levels in the commonpalm civet showed a clear seasonal pattern and increased concentrations during the breeding season compared to the non-breeding season. FTM concentrations in adult male civets were higher than in females and juvenile males. Researchers can apply non-invasive hormone monitoring techniques to monitor and improve management in captivity, assess the impact of manipulation or treatment on research reproductive function on animal models, in order to ensure animal welfare and support for animal breeding programs.

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UNDERSTANDING OF HETEROSIS EFFECTS AND THEIR EXPLOITATION ON REPRODUCTIVE TRAITS OF MONG CAI CROSSBRED PIGS

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1. INTRODUCTION

Crossbreeding, as a component of genetic improvement, has attracted increased attention during the last few decades. Crossbreeding programs have been intensively applied to produce commercial animals in almost all species of livestock, especially in poultry and swine, where high fecundity reduces the cost of producing crossbred breeding stock.

In pigs, crossbreeding is a well-established method of improving sow productivity, mainly by lowering mortality and increasing litter size and weight. The advantage of using crossbreds is that it employs heterosis, which is a general phenomenon for most commercially important traits. Crossbreds can improve reproduction by exploiting additive effects and heterosis. Specific crossbred combinations allow maximum utilisation of heterosis and breed complementarity. Based on the fact that the final commercial products are crossbred animals, it is believed that the breeding goal should be set at the level of crossbred performance rather than purebred performance. Much experimental crossbreeding work has been done with pigs and most of it shows economically important advantages for crossbreds.

Genetic improvement, with regard to crossbreds, is characterised by the utilisation of both additive and non-additive effects. The relevant theory on analyzing crossbreeding experiments and genetic effects is well presented by Hill (1982) and Kinghorn (1982). When choosing a crossbreeding system, two essential aspects should be taken into consideration. Firstly, heterosis effects may be a linear function of the degree of heterozygosity of the corresponding genotype, and secondly, heterosis varies with the trait involved, depending on the magnitude of non-additive effects in its genotypic variance.

The use of crossbreeding by commercial swine producers is an accepted tool used to enhance productivity through the exploitation of heterosis and breed differences. Crossbreeding effects are greater when the sow herself is crossbred. In a stressful environment like Vietnam, crossbreeding might be one of the best solutions, by exploiting breed additive effects, parental effects and the benefits of heterosis. This can be achieved in any crossbred but better results can be achieved when the breeds used for crossing are less related (i.e., individuals carrying a wider range of alleles).

In addition to selecting within populations for seedstock suppliers and breeding stock, breeds and crossing systems need to be selected. The economic benefits of improved performance through crossbreeding depend largely upon the choice of breeds and crossbreeding systems. In pig breeding, it has been shown that F_1 females show about 10% advantage in overall productivity over purebreds. There is therefore, a large gain to be made by promoting the greater use of crossbreeding.

2. CROSSBREEDING COMPONENTS OF REPRODUCTIVE TRAITS

Breed means and their error (co) variances can be used in a least square analysis to estimate the crossbreeding effects.

Crossbreeding effects can be divided into two major components, additive and non-additive. The additive component of merit for a trait is the weighted averaging of merit in the parental breeds, or the product of proportion of input from each parental breed and the merit associated with each breed. The non-additive effect of crossbreeding is the heterosis, which is the amount by which merit in crossbreds, deviates from the additive component. The genetic basis of heterosis can be divided into two components, dominance and epistasis. This partitioning helps to estimate the genetic merit of any crossbred animal. The most important components of crossbreeding are direct additive (Ad), maternal additive (Am), direct dominance (Dd) and maternal dominance (Dm). Estimates of these crossbreeding effects can be used to study crosses that have been generated, and also to predict the expected genetic merit of any untested crosses under the dominance model of heterosis.

2.1. An example of crossbreeding components for NBA of northern Vietnam pigs

Data from three piggeries in northern Vietnam was available from three purebreds MC, LW and LR, as well as the crosses between these three breeds, to estimate the crossbreeding components and predict merit values for NBA in some untested crossbreds (this data set was not included in the whole data set of different analyses in this study). It was assumed the effects of paternal heterosis and epistatic recombination loss were not important effects and were ignored. Table 1 presents the crossbreeding components of MC, LW and LR breeds for NBA and the estimated merit values for some untested crossbreds of MC in northern areas.

Table 1. Direct and maternal additive and dominance effect values (second row), expected merits for NBA (last column) and degrees of expression of effects

Effects		AdMC	AdLR	AdLW	AmMC	AmLR	AmLW	Dd	Dm	Expected
Estimates	8.89	2.07	0-.26	0.00	0.05	0.27	0.00	0.55	0.30	Merit
MC	1	1	0	0	1	0	0	0	0	11.01
LR	1	0	1	0	0	1	0	0	0	08.90
LW	1	0	0	1	0	0	1	0	0	08.89
F_1(LRxLW)	1	0	0.5	0.5	0	0	1	1	0	09.31
Balanced (LR,LW)	1	0	0.5	0.5	0	0.5	0.5	0.5	0.5	09.32
F_1(LRxMC)	1	0.5	0.5	0	1	0	0	1	0	10.40
Balanced (LR,MC)	1	0.5	0.5	0	0.5	0.5	0	0.5	0.5	10.37
F_1(LWxMC)	1	0.5	0	0.5	1	0	0	1	0	10.53
Balanced (LW,MC)	1	0.5	0	0.5	0.5	0	0.5	0.5	0.5	10.38
LRx(LWMC)	1	0.25	0.5	0.25	0.5	0	0.5	1	1	10.15
LWx(LRMC)	1	0.25	0.25	0.5	0.5	0.5	0	1	1	10.35
Synthetic(LR,LWMC)	1	0.33	0.33	0.33	0.33	0.33	0.33	0.67	0.67	10.16
Rotations (LR, MC)	1	0.5	0.5	0	0.5	0.5	0	0.67	0.67	10.52
Rotations (LW, MC)	1	0.5	0	0.5	0.5	0	0.5	0.67	0.67	10.52
LRx(LRMC)	1	0.25	0.75	0	0.5	0.5	0	0.5	1	09.95
LWx(LWMC)	1	0.25	0	0.75	0.5	0	0.5	0.5	1	10.01

Where: AdMC, AdLR, AdLW, AmMC, AmLR and AmLW: Direct and Maternal Additive Effects including average mean for NBA of MC, LR and LW, Dd and Dm: Direct and Maternal Dominance Effects

Two major components of genetic differences are expressed differently in offspring and dam. These may be studied as average direct effects of the offspring, maternal genetic effects, heterosis in the crossbred progeny and dam, and recombination losses

in the offspring and dam. In this study, recombination effects were assumed to be negligible and were, therefore, dropped from the model.

2.1.1. Direct and maternal additive effects

The direct additive effects (Ad) are effects of purebreds as expressed directly in the crossbred individuals whose merit is being calculated. Direct additive effects of each breed are the proportion of genes from each breed in relation to the total genotype. In this example, these direct additive values of Ad_{MC}, Ad_{LR} and Ad_{LW} were 2.07, -0.26 and 0.00 piglets/litter, indicating the Ad_{MC} was the highest for NBA in MC crosses.

However, the maternal additive effects (Am) are the additive effects of purebreds as expressed by the dams of the crossbred individuals under consideration. The values of Am for NBA of purebreds MC, LW and LR maintained in northern Vietnam, were Am_{MC}=0.05, Am_{LR}=0.27 and Am_{LW}=0.00 piglets. This indicates that Am of LR were the highest of the breeds studied for NBA in MC crosses in northern Vietnam.

2.1.2. Direct and maternal dominance effects

Direct dominance effect (Dd) is the effect of dominance in crossbred individuals when fully expressed, for example in F_1 and three-breed or four-breed crosses. Reproductive traits seem to be the most affected by direct dominance because these traits in pigs generally have low heritabilities. The value of the Dd for NBA of this study was 0.55 piglets.

Maternal heterosis (Hm) in the dams is fully expressed in F_1 or three-breed cross dams. Reproduction traits are affected not only by direct dominance, but also by maternal dominance. Therefore, direct and maternal dominance effects are important resources in relation to NBA in pigs. The maternal dominance effect in this study was 0.30 piglets. Paternal heterosis for NBA was ignored in this study.

These estimates of crossbreeding components can be used to test or verify some crossing systems that have been used in northern Vietnam, in order to know whether this dominance model is applicable. These crossbreeding components can also be used to predict the expected genetic merit for some crossing systems under the dominance model of heterosis.

2.1.3. Calculating expected merit for NBA in different crossing systems

Until now, four main components direct and maternal additive effects, and direct and maternal dominance effects, which contribute to genetic merit, have been analysed for NBA. The following procedure can be used for testing or calculating NBA of first cross LRxMC pigs. These crossbred individuals get half their genes from the MC parent and half from the LR parent and, therefore, the expressions of AdMC and AdLR are 0.5 each. The maternal additive effect is also expressed fully in the dam of MC, therefore the AmMC component is one. These females also express full dominance as described previously, so these individuals have a full direct dominance effect (Dd), and the genetic merit estimate of these individuals must include the Dd value. No expression of maternal dominance (Dm) is present in this example. The expected genetic value of F_1 females in this cross = 8.89+0.5*(2.07+-0.26)+1*0.05+1*0.55+0*0.30 = 10.40 piglets. This estimate of performance level is close to the observed value for NBA in F_1 (LRxMC) females (10.45 piglets/litter was calculated in the same data sets of three piggeries in northern Vietnam, but not presented in this study). This indicates that the estimated genetic merit for NBA, under the dominance model of heterosis is close to the observed values in crossbreds of MC. To what extent different crossbreds express heterosis for reproduction traits under the stressful Vietnamese conditions, will be discussed in the following section.

2.2. Another example of heterosis studies for reproductive traits in MC crosses

The performance of crossbred animals is generally not the mean of the two parents due to the phenomenon of heterosis or hybrid vigour. Crossbred offspring are usually more robust and their performance is superior to what would otherwise be predicted. Hybrid vigour, the superiority of the crossbred offspring over the mean of the purebred parents, arises from increased heterozygosity. Heterosis is greatest when all pairs of alleles consist of alleles derived from two different breeds. There is an advantage in the use of crossbreeding or synthetic breeds over pure breeds, when individual and maternal heterosis is large. Heterosis can be partitioned into direct and maternal heterosis. It, therefore, the heterosis for reproductive traits of MC and exotic crosses has been analysed in this section.

2.2.1. Heterosis studies for NBA of MC and exotic crosses

The most important heterosis for NBA in swine is direct and maternal heterosis. NBA of MC crosses was used to study the different components of heterosis. The paternal heterosis could reasonably be ignored because it is very small and not significant in this study. The results of components of heterosis for NBA in MC crosses, in northern Vietnam, and exotic crosses in the whole country, are estimated by using SAS (1993) and presented in Table 2.

Table 2. Different components of heterosis (%) in MC and exotic crosses for NBA

Breed genotype	Records	Mean	Heterosis (%)		
			Direct	Maternal	Total
Pure MC	7 268	10.94	0.0	0.0	0.0
Pure LW or LR	14 657	09.08	0.0	0.0	0.0
First crosses of MC	2 103	10.75	7.4	0.0	7.4
Backcrosses of MC	971	10.20	3.3	3.6	6.9
Three-breed crosses of MC	1 143	10.39	5.2	3.7	8.9
Purebreds of exotic	15 467	09.04	0.0	0.0	0.0
First crosses of exotic	5 788	09.46	4.7	0.0	4.7
Backcrosses of exotic	3 459	09.33	1.5	1.7	3.2
Three-breed crosses of exotic	1 447	09.51	3.4	1.8	5.2

a. Direct heterosis

Direct heterosis for NBA in this study varied from 3.3% in backcrosses LRx(LRMC) or LWx(LWMC), 5.2% in three-breed crosses LWx(LRMC) or LRx(LRMC) to 7.4% in first crosses LWxMC or LRxMC pigs. However, direct heterosis values for NBA for exotic crosses were 1.5% in backcrosses, 3.4% in three-breed crosses and 4.7% in first crosses. Three-breed crosses of MC and exotic, presented higher direct heterosis than backcrosses, as expected. The values in first crosses were in agreement with the value of 6.4% in a total of 1,323 litters of F_1(LRxLW) in Nigeria, as reported by Ikeobi (1994). Direct heterosis estimates for NBA of 3.3-7.4% in MC crosses, and of 1.5-4.7% in exotic crosses in this study, were lower than estimates of 10.0% in HxLR herd reported by Baas *et al*. (1992), and this is in close agreement with the conclusion of Zhihua Jiang *et al*. (1988), that direct heterosis is the most important component for LS.

b. Maternal heterosis

Expression of maternal heterosis in this study for NBA, in both MC and exotic crosses, was similar for three-breed crosses and backcrosses, at 3.6% and 3.7% in MC crosses and 1.8% and 1.7% in exotic crosses as expected, due to maternal breed genotypes being the same. The maternal heterosis estimates for

NBA takes 53 and 41% of the total heterosis in backcrosses and three-breed crosses of MC crosses. However, these maternal heterosis estimates for NBA, takes 53 and 35% of the total heterosis in backcrosses and three-breed crosses of exotic crosses.

c. Total heterosis

Expression of total heterosis for NBA in this study, was the highest in three-breed cross females, at 8.9%, followed by first crosses (7.4%), and backcrosses was the lowest (6.9%) for crosses involving MC. In exotic crosses, the same trend was found, but was lower than MC crosses. Three-breed crosses had the highest heterosis (5.2%), followed by first crosses (4.7%), and backcrosses (3.2%). F_1 and three-breed crosses had higher heterosis than backcrosses. The heterosis of backcrosses and three-breed crosses is higher than F_1 females due to the maternal heterosis component. However, the heterosis of three-breed crosses is higher than that of backcrosses due to the direct heterosis.

Results in this example showed that first crosses using MC had a higher (7.4%) expression of heterosis than first crosses among exotic breeds (4.7%) for NBA. It was lower in backcrosses, at 6.9 and 3.2% in MC and exotic groups, respectively. In three-breed crosses, the heterosis was higher (8.9%) in MC crosses and (5.2%) in exotic crosses than in backcrosses and first crosses. These findings were higher than the values of 3.5, 4.4 and 5.9% found from 1069891 and 1045 litters in first crosses, backcrosses and three-breed crosses of MC (Duc *et al*., 1997). The reason for the high heterosis of NBA in three-breed crosses is the contribution of maternal heterosis to this trait. All reproductive traits are also better in different exotic crossbreds, but less so than in exotic with MC crossbreds, and this may be due to the longer genetic distance between exotic and MC breeds than between different exotic breeds.

Crosses between MC and exotic breeds, such as LW and LR, appeared to benefit from a high degree of heterosis for all reproduction traits, relative to that normally seen in crosses between exotic breeds. The values of heterosis in MC x exotic crosses were higher than those in exotic x exotic crosses. This can be explained when breeds used for crossing are less related. Genetic distance between MC and LW or LR is likely to be larger than that between the exotics themselves, because MC originate from Asian wild pigs, whereas LR and LW come from European wild pigs. This finding agrees with the reports of Cheng (1984) and Zhihua Jiang *et al*. (1988), who found that reproduction traits for exotic crossbreds generally had less heterosis than exotic with indigenous crossbreds. This finding was lower than that of 0.97 piglets/litter found by Bass *et al*. (1992) in the crossbred progeny HxLR.

2.2.2. Heterosis studies for reproductive traits in MC crosses

Heterosis for some reproductive traits can be expressed in the trait units (piglets/litter or kg/piglet). The direct and maternal heterosis presenting in different reproductive trait units of MC crosses, maintained in Northern Vietnamese conditions, were used for this study. The values of different types of heterosis for different reproduction traits in MC crosses are presented in Table 3.

Table 3. Different types of heterosis for reproductive traits in MC crosses

Traits	Hm	Hd	Heterosis
NBA	0.38 piglets/litter	0.44 piglets/litter	0.82 piglets/litter
NWP	0.30 piglets/litter	0.48 piglets/litter	0.78 piglets/litter
PBW	0.04 kg/piglet	0.04 kg/piglet	0.08 kg/piglet
PWW	0.37 kg/piglet	0.44 kg/piglet	0.81 kg/piglet

In MC crosses, for NBA, Hm was high (0.38 piglets/litter), explaining about 46.34% of total heterosis. However, the direct heterosis played an more important role in NBA, with

a value of 53.66%. The estimates for total heterosis in this example are in agreement with the value of 0.82 pigs per litter, reported by Jungst and Kuhlers (1984). Heterosis for NWP in MC crosses is presented in the same manner as NBA, where Hm contributed 0.30 piglets/litter to the total heterosis of 0.78 piglets/litter. It accounted for about 38.5% of total heterosis for NWP.

For weight traits in pigs, maternal heterosis was also relatively important to other types of heterosis, but not big enough (Hm=0.04 kg/piglet for PBW and 0,37 kg/piglet for PWW). This indicates that maternal heterosis is also relatively important in MC crosses for PBW and PWW, at 50 and 46% of the total heterosis, respectively. The results of this study are in close agreement with Schneider *et al.* (1982) that maternal heterosis was small for most weight traits. The heterosis of litter size and weight at birth, in MC and exotic crosses in Vietnam, was higher than at weaning which might be due to the later age of weaning.

Another example on growth traits of Du, Pi and their crossbred pigs in Vietnam, Nguyen Huu Tinh *et al.* (2015) informed that the estimates of direct additive genetic and direct heterosis were 60.3 and 31.26 g/day for ADG, 0.82 and -0,39mm for BF100, -0.12 and -0.06 for FCR. All un-tested crossbred groups of Dux(DuPi), Pix(PiDu), Du(PiDu)xDu(PiDu) possess lower predicted performance in comparison to tested crossbred groups.

3. CONCLUSION

In general, for reproduction traits, maternal heterosis also contributes an important part but smaller than direct heterosis for nearly all reproductive traits in MC crosses. The reproductive performance traits could be increased by crossbreeding, as crossbred dams have 0.82 piglets born alive per litter more than their purebreds' average. Therefore, to increase reproductive traits in MC crosses such as NBA, NWP, PBW and PWW, the benefit of maternal heterosis of MC crossbred dams may be the best policy to be used in rural areas of Northern Vietnam.

INSTITUTE OF ANIMAL SCIENCE FOR SOUTHERN VIETNAM RUMINANT RESEARCH AND DEVELOPMENT CENTER

PhD. Pham Van Quyen
Director of Ruminant Research and Development Center

1. INTRODUCTION

Ruminant Research and Development Center belongs to Institute of Animal Science for Southern Viet Nam, National Institute of Animal Science was established in 1977 under the Decision No. 211-NN-TCCB/QĐ of the Ministry of Agriculture and Rural Development. The Center with main activities: Scientific research, technology transfer, keeping the original breeds, technical consultancy, training and international co-operation in the field of ruminant husbandry. We would be appreciated to be co-operated with all individuals and organization domestically and world-wide.

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2. SCIENTIFIC

Ruminant Research and Development Center has been in performance of scientific researching, transferring about ruminant husbandry. Inheriting the traditions and research capabilities of previous generations, the Center continues to maintain and promote its capabilities and achieve many results in scientific research and technology transfer, contributing to the development of the livestock industry. Since its establishment until now, the Center has carried out many research projects at state, ministerial, sector and provincial levels and many local projects, agricultural extension, etc. With many years of experience in scientific research The Center is always ready to cooperate in researching and participating in domestic and foreign topics and projects with agencies, localities and farms in need.

3. TECHNICIANT TRAINNING

From 2003 to now, the Center has trained more than 200 courses with more than 2,000 technicians on topics such as training technicians to artificially inseminate buffaloes, cows and goats; train animal and veterinary technicians, with basic to advanced. Technicians are provided with knowledge and mastery of skills through a teaching method that combines theory and practice on cattle. With the lecturers of the Center who have PhD, MSc and doctor degree, engineers with many years of experience. In addition, the courses also have experts from institutes and universities specializing in animal husbandry and veterinary medicine of the South of Vietnam.

4. TECHNICAL ADVICE

The Center always ready to provide technical support and advice to individuals, organizations with the following contents::

- Guide to build barns.
- Train to choose breeding animal.
- Instruct to feed animal,
- Instruct to prevent and treat diseases for animal,
- Guide to planning pastures and food plants.
- Guide techniques for planting, tending and harvesting, processing, preserving and using grass and fodder crops.
- Support and promote the consumption of breed animal, beef and grass seed products.

5. ANIMAL BREEDS

With a history of more than 40 years of intensive operation in the field of large cattle breeds, the Center is assigned to keep many original breeds imported from countries with developed livestock industries such as Australia, India and Thailand. The Center constantly researches and selects in order to improve the productivity and quality of the original breed herds, produce buffalo and cow breeds that are highly adaptable to Vietnamese climatic conditions. All breeds are monitored and recorded individually before being transferred to individuals and organizations for the best quality.

Brahman
Weight: Males: 700-800kg; Females: 500-550kg
Carcass percentage: 51-56%

Drought Master
Weight: Males: 700-800kg; Females: 450-500kg
Carcass percentage: 58%

Red Angus
Weight: Males: 700-800kg; Females: 450-550kg
Carcass percentage: 60%

Senepol
Weight: Males: 800-900kg; Females: 550-650kg
Carcass percentage: 60-62%

Charolais crossbred
Weight: Males: 600-700kg; Females: 450-500kg
Carcass percentage: 58-60%

Three crossbred: Sind x Charolais x DroughtMaster
Weight: Males: 500-600kg; Females: 400-450kg
Carcass percentage: 55-60%

Sind crossbred
Weight: Males: 500-550kg; Females: 350-400kg
Carcass percentage: 48-49%

Bach Thao
Weight: Males: 75-80kg; Females: 40-45kg
Carcass percentage: 40-45%

Boer
Weight: Males: 110-135kg; Females: 90-100kg
Carcass percentage: 50-55%

Murrah
Weight: Males: 650-750kg, Females: 550-600kg
Milk: 3000kg/lactation. Fat: 6.2-6.5%

Saanen
Maturity: Males: 85kg; Females: 60-70kg
Milk yield: 838kg/264 days. **Milk fat**: 3.2%

Swamp buffalo
Weight: Males: 500-600kg; Females: 400-450kg
Carcass percentage: 45-50%

6. PLANT AND GRASS

Ruminant Research and Development Center is the only unit that keeps and develops a large number of high quality, productive grasses. Each year, the Center produces from 5,500 to 6,000 tons of green grass and 1,000-1,500 kg of grass seeds to provide for production. Currently, the Center is the first unit to produce grass seed from high-yield and high-quality seeds for the market.

Panicum maximum cv. Hamil
Yield: 220-250 tons/ha/year
Crude protein: 11-13%

Panicum maximum cv. Mombasa
Yield: 220-250tons/ha/year
Crude protein: 11-13%

Panicum maximum cv. TD58
Yield: 220-240 tons/ha/year
Crude protein: 11-13%

Panicum maximum cv. K280
Yield: 180-200 tons/ha/year
Crude protein: 11-13%

Brachiaria ruziziensis
Yield: 150-180 tons/ha/year
Crude protein: 8-12%

B brizantha x B. ruziziensis
Yield: 180 – 200 tons/ha/year
Crude protein: 12-14%

Pennisetumpurpureum x P. typhoides
Yield: 250-400 tons/ha/year
Crude protein: 7-9%

Stylosanthes guianensis Ciat 184)
Yield: 60-90 tons/ha/year
Crude protein: 16-18%

Different grass seed

7. PROVIDING VETERINARY MATERIAL AND EQUIMENT

Ruminant Research and Development Center specializes in distributing products used in artificial insemination, veterinary materials and equipment with reasonable prices, good quality and dedicated service.